Detection of Immunoglobulin Heavy Chain Gene Rearrangement and Bcl-2-JH Translocation [t (14 ; 18)] in Patients with Chronic Hepatitis C Virus Infection.

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ABSTRACT

The mechanism of lymphomagenesis by HCV is still obscure. The present study was carried out on 64 untreated patients previously diagnosed as having chronic liver disease due to HCV infection, 30 patients with HCV negative chronic liver disease (CLD) and 30 healthy controls. Serum cryoglobulins were tested in all subjects. In addition, the presence of immunoglobulin heavy chain gene (IgH) rearrangement and Bcl-2-JH translocation in peripheral blood mononuclear cells (PBMC) were assessed by seminested and nested polymerase chain reaction (PCR), respectively. Percutaneous liver biopsies were performed in 61 of the 64 patients with HCV related CLD and 26 of the 30 patients with HCV negative CLD to determine the severity of chronic liver injury. None of the patients received immunomodulatory drugs or had hepatocellular carcinoma, lymphoma or other malignancies. Cryoglobulinaemic and non-cryoglobulinaemic chronic HCV infected patients had significantly higher rates of monoclonal IgH rearrangement than patients with HCV negative CLD (P=0.006 and 0.047, respectively) and healthy controls (P=0.001 and 0.005, respectively). There were no statistically significant differences between chronic HCV infected patients with and without monoclonal IgH rearrangement with respect to age, sex, mean ALT and AST levels. Moreover, the frequency of monoclonal IgH rearrangement in PBMC did not differ significantly according to histologic severity of chronic liver injury. On the other hand, cryoglobulinaemic and non-cryoglobulinaemic chronic HCV infected patients had significantly higher rates of Bcl-2-JH translocation than non HCV infected CLD patients (P=0.0002 and 0.001, respectively) and healthy controls (P=0.0002 and 0.001, respectively). There were no statistically significant differences between chronic HCV infected patients with and without Bcl-2-JH translocation with respect to age, sex, mean ALT and AST levels. Moreover, the frequency of Bcl-2-JH translocation in PBMC did not differ significantly according to histologic severity of liver injury. Interestingly, the frequency of coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation was significantly higher in cryoglobulinaemic than non-cryoglobulinaemic chronic HCV infected patients (P=0.05), HCV negative CLD patients (P=0.009) and healthy controls (P=0.009). We concluded that patients with chronic HCV infection are more liable to develop monoclonal IgH rearrangement or Bcl-2-JH translocation in PBMC. Moreover, coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation is a frequent finding in cryoglobulinaemic patients with chronic HCV infection suggesting that these aberrations may be involved, at least in part, in the complex multistep mechanisms occurring in HCV infected patients ending in B cell lymphoproliferative diseases (LPD). Further studies are needed to establish whether determination of these aberrations in PBMC of chronic HCV infected patients could be useful as non invasive molecular markers for the predisposition to acquire cryoglobulinaemia and/or other B cell LPD.
INTRODUCTION

Hepatitis C virus (HCV) is a major health problem worldwide. The World Health Organization (WHO) estimated in 1997 that about 3% of the world population has been infected by HCV. The population of Egypt has a high prevalence of HCV infection. HCV is a major etiological agent of chronic viral hepatitis worldwide and may be complicated by cirrhosis and hepatocellular carcinoma. In addition to being hepatotropic, HCV is also a lymphotropic virus. Within this context, an association between chronic HCV infection and a variety of lymphoproliferative disorders, including cryoglobulinaemia and B cell non-Hodgkin lymphoma (NHL) has been demonstrated.

Ferri et al. reported HCV seropositivity and viraemia in a high percentage of cases of NHL (30% and 32%, respectively) and suggested a causal role for HCV in this disease. More recently, epidemiologic and molecular observations have suggested that HCV may be the causative agent of some B cell NHL. Based on these results, it has been hypothesized that HCV may play a role in the pathogenesis of clonal proliferation of B cells which may evolve into malignant lymphoma. However, the mechanism of malignant transformation is still obscure. A possible mechanism may involve clonal proliferation of B cells induced by the virus, inhibition of apoptotic cell death of the infected lymphocytes, or both.

During B cell development, somatic recombination of antibody genes takes place to create V region genes encoding functional immunoglobulin (Ig) heavy and light chains. For the heavy chain, 1 in about 100 to 200 VH genes rearrange to 1 in about 30 DH genes rearranged to 1 in 6 JH genes. A K light chain V region gene is generated by rearrangement of 1 in about 70 VK genes to 1 in 5 JK genes. If no functional VK region was created either by introduction of a stop codon or by loss of the correct reading frame in the process of V gene rearrangement, the pre-B cell rearranges one of its V, DH and J genes, the imprecise joining, the exonuclease activity that can remove several nucleotides from the ends of the rearranging V, DH and J genes, and the addition of a variable number of non-germline encoded nucleotides or N-sequences that can be added especially on the heavy chain, an enormous V region diversity is created. For the human Ig heavy chain, a potential to generate more than 10^11 different VH region genes has been estimated. Interestingly, the distance between the most 3’ VH gene and the JH cluster comprises about 70 kb in unarranged form. By rearrangement the rearranged VH gene is brought close to one of the six JH genes. The rearranged VH region is subdivided into a leader region, four framework regions (FRI-IV) and three complementarity determining regions (CDR I-III).

The rearrangement of the heavy and light chain genes can be used to determine clonality of B cells. With the availability of the polymerase chain reaction (PCR), several groups established PCR-based methods for detection of monoclonal immunoglobulin heavy chain (IgH) rearrangement particularly for diagnosis of NHL. For amplification of the rearranged VH genes, either a primer specific for the FR-III region of most human VH genes or a set of family-specific primers for the FRI region of VH genes were used together with a consensus JH primer.

The antiapoptotic Bcl-2 oncogene product is overexpressed in the majority of cases of follicular NHL and in many patients with diffuse NHL mostly as a consequence of reciprocal t (14; 18) translocation where the Bcl-2 gene is moved from its normal chromosomal location 18q21 and joined to the immunoglobulin heavy chain locus at 14q32 under the control of its powerful promoter and enhancer elements, resulting in deregulation of expression and inappropriately high levels of Bcl-2 mRNA and protein, ending as part of a multistep process in malignant transformation of B cells to lymphoma.

This study aimed at evaluating the occurrence of monoclonal immunoglobulin heavy chain gene rearrangement and Bcl-2-JH translocation [t (14; 18)] in peripheral blood mononuclear cells (PBMC) from patients with chronic HCV infection in comparison with patients with HCV-negative chronic liver disease (CLD), and healthy controls.

SUBJECTS and METHODS

The present study was conducted on the following groups:

1. **Group I:** Sixty four patients previously diagnosed as having chronic HCV infection who did not receive antiviral treatment.
2. **Group II:** Thirty patients with HCV negative CLD: thirteen patients with chronic hepatitis, 14 with cirrhosis and 3 with fatty liver.
3. **Group III:** Thirty healthy controls.

All patients attended the Hepatology Department, National Liver Institute, Menoufiya University. Patients receiving immunosuppressive treatment and those with schistosomal periportal fibrosis, hepatocellular carcinoma, lymphoma or other malignancies were excluded from the present study. No patient had systemic disease nor had been treated with steroids, interferons, anticancer agents or any other immunomodulatory drugs. Sixty one
of the group I patients and 26 of the 30 group II patients were subjected to percutaneous liver biopsy to determine the severity of chronic liver injury.\(^{26}\) HCV infection was previously diagnosed in group I patients by positivity for serum HCV antibodies by third generation enzyme immunoassay (Murex) and HCV RNA by quantitative reverse transcription PCR (COBAS AMPLICOR HCV Monitor test, version 2.0). These patients were negative for hepatitis B surface (HBs) antigen (Biochem) and hepatitis B virus (HBV) DNA by PCR.\(^{27}\)

Group II patients were negative for HCV antibodies by third generation enzyme immunoassay (Murex) and negative for HCV RNA by nested reverse transcription PCR\(^{28}\) and had proven CLD (histologically confirmed in 26 patients) including chronic hepatitis in 13 patients, cirrhosis in 14 patients and fatty liver in 3 patients. CLD was HBV related (HBs antigen positive and HBV DNA positive by PCR)\(^{29}\) in 16 patients, due to autoimmune hepatitis in 6 patients, drug hepatitis in one patient, cryptogenic cirrhosis in 4 patients and fatty liver in 3 patients.

Group III subjects (healthy controls) were negative for serum HCV antibodies by third generation immunoassay (DIA-PRO), HCV RNA by nested reverse transcription PCR,\(^{28}\) HBs antigen (Biochem) and HBV DNA by PCR.\(^{27}\) They had normal blood chemistry profile (including hepatic function parameters and blood counts).

All subjects were tested for cryoglobulins in serum as previously described elsewhere\(^{4, 29}\) as well as for the presence of immunoglobulin heavy chain gene rearrangement and Bcl-2-JH translocation \([t (14 ; 18)]\) in PBMC. In addition to thorough history taking, complete physical examination, abdominal ultrasonography and routine investigations, a liver function panel was evaluated in all patients including prothrombin time, hepatic function parameters and blood counts.

Detection of immunoglobulin heavy chain gene (Igh) rearrangement:\(^{4, 33}\) Igh (FR3/JH) rearrangement was detected by a seminested PCR approach. The reaction mixture (25 \(\mu\)L) contained 5 \(\mu\)L of DNA extract, 20 picomoles (2 \(\mu\)L) of each of the 2 primers, 3.5 \(\mu\)L nuclease free water and 12.5 \(\mu\)L of Taq PCR master mix (Qiagen). Taq PCR master mix is a premixed solution containing Taq DNA polymerase, PCR buffer and dNTPs. The PCR mixture provides a final concentration of 1.5 mM MgCl\(_2\) and 200 \(\mu\)M of each dNTP. To avoid evaporation, 2 drops of mineral oil were poured on top of the reaction mixture. Amplification was performed in Hybaid thermal cycler. The first round of amplification was performed using the 5’ primer FR3 (5’ACACGGCTCTGATTACTCT-3’) and the 3’ primer JH out (5’ACCTGAGGAGACGGTGACCAGGT-3’). Reaction mixtures were heated at 95°C for 4 minutes followed by 30 cycles of 60 seconds at 94°C, 90 seconds at 55°C, 60 seconds at 72°C with a 5 minutes final extension at 72°C. A second round of amplification using 5 \(\mu\)L of 1/500 dilution of the initial reaction was performed under similar conditions using the 5’ primer FR3 and the 3’ primer JH in (5’GTGACCAGGGTCTGGGCCCAG-3’) for 20 cycles only. Primers were synthesized by a DNA synthesizer (Qiagen Operon). PCR amplified material (10 \(\mu\)L) was electrophoresed in 2% agarose gel in Tris borate EDTA (TBE) buffer containing 0.5 \(\mu\)g/mL ethidium bromide at 100 volts for 90 minutes and the DNA was visualized over an ultraviolet transilluminator. For each experiment, a negative control (PCR mix without DNA extract) was included and one well was reserved for the PCR DNA marker (MBI Fermentas) which gives 14 bands ranging in size from 100 to 3000 bp. The specific band of IgH rearrangement is 80-110bp long. A discrete monoclonal band indicates monoclonal IgH rearrangement while a broad smear of polyclonal amplified DNA indicates polyclonal IgH rearrangement.

Detection of Bcl-2-JH translocation \([t (14 ; 18)]\) by nested PCR:\(^{4, 34}\) The reaction mixture (25 \(\mu\)L)


The present study included the following groups:

(1) **Group I**: Sixty four patients previously diagnosed as having chronic HCV infection who did not receive antiviral treatment. They were 48 males and 16 females with a mean age of 45.8±7.6 years. They were divided into the following 2 subgroups:

(a) **Subgroup Ia**: Nine patients with cryoglobulinaemia. They were 6 males and 3 females with a mean age of 44.8±5.1 years. After histopathological examination, 8 patients were diagnosed as having chronic hepatitis C of mild activity and one as chronic hepatitis C of moderate activity.

(b) **Subgroup Ib**: Fifty five non-cryoglobulinemic patients. They were 42 males and 13 females with a mean age of 46±8 years. Fifty two of these patients were subjected to liver biopsy and classified histopathologically, into the following subgroups:

(i) **Subgroup Ib-1**: Twenty four patients with chronic hepatitis C of mild activity (CAH-mild): 75% were males and 25% females. Their mean age was 45.3±8.4 years.

(ii) **Subgroup Ib-2**: Fifteen patients with chronic hepatitis C of moderate activity (CAH-mod): 87% were males and 13% females. Their mean age was 45.8±8.3 years.

(iii) **Subgroup Ib-3**: Three patients with chronic hepatitis C of severe activity (CAH-sev): They were all males with a mean age of 48±7 years.

(iv) **Subgroup Ib-4**: Ten patients with hepatic cirrhosis (70% were males and 30% females). Their mean age was 45.1±6 years.

(2) **Group II**: Thirty patients with HCV negative CLD (thirteen patients with chronic hepatitis, 14 with cirrhosis and 3 with fatty liver). CLD was HBV related in 16 patients, due to autoimmune hepatitis in 6 patients, drug hepatitis in one patient, cryptogenic cirrhosis in 4 patients and fatty liver in 3 patients. All were non-cryoglobulinemic. They were 24 males and 6 females with a mean age of 45.9±6.7 years.

(3) **Group III**: Thirty healthy controls; 22 males and 8 females with a mean age of 43.3±6.5 years. All were non-cryoglobulinemic.

The frequency of monoclonal IgH rearrangement in PBMC from patients and controls are shown in table (1). Chi square test was done and it was statistically significant (P=0.0006). Fisher’s exact test was used for comparison between each 2 groups. Cryoglobulinaemic chronic HCV infected patients had a significantly higher rate of monoclonal IgH rearrangement than patients with HCV negative CLD (P=0.006) and healthy controls (P=0.001). Similarly non-cryoglobulinaemic chronic HCV infected patients had a significantly higher frequency of monoclonal IgH rearrangement than non HCV infected CLD patients (P=0.047) and healthy controls (P=0.005). However, no statistically significant difference in the frequency of IgH rearrangement monoclonality was detected between cryoglobulinemia and non-cryoglobulinaemic chronic HCV infected patients (P=0.204) and between HCV negative CLD patients and healthy controls (P=1.0). The specific band of IgH rearrangement of 80-110 bp is demonstrated in figure (1).
As shown in table (2), among untreated patients (cryoglobulinaemic and non-cryoglobulinaemic) with chronic HCV infection, the frequency of monoclonal IgH rearrangement in PBMC did not differ significantly according to histologic severity of liver injury (P=0.675).

There were no statistically significant differences between all chronic HCV infected patients with and without monoclonal IgH rearrangement with respect to age (t=0.49, P=0.625), sex ($\chi^2=0.726$, P=0.394), mean ALT level (t=0.48, P=0.636) and mean AST level (t=1.71, P=0.093).

Table (1): Frequency of monoclonal immunoglobulin heavy chain gene rearrangement in PBMC from the studied groups.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>No. of subjects</th>
<th>Monoclonal IgH rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Group Ia</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Group Ib</td>
<td>55</td>
<td>11</td>
</tr>
<tr>
<td>Group II</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>Group III</td>
<td>30</td>
<td>zero</td>
</tr>
</tbody>
</table>

$\chi^2=17.32$, P=0.0006

Fisher’s exact test $\chi^2=0.204$, group Ia vs. group Ib: P=0.006, group Ia vs. group II: P=0.001, group Ia vs. group III: P=0.005, group Ib vs. group II: P=1.0

Table (2): Frequency of monoclonal IgH rearrangement in PBMC from untreated patients with chronic HCV infection according to histologic severity of liver injury.*

<table>
<thead>
<tr>
<th>Histologic groups</th>
<th>No. of subjects</th>
<th>Monoclonal IgH rearrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>CAH-mild</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>CAH-mod</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>CAH-sev</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

$\chi^2=1.53$, P=0.675

* Three patients belonging to group Ib were not subjected to liver biopsy.

Figure (1): Detection of immunoglobulin heavy chain gene rearrangement by FR3/J H seminested PCR in PBMC from patients with chronic HCV infection. Lanes 2, 8 and 10 show the discrete monoclonal band (80-110 base pairs long) of amplified DNA. Lanes 1, 3, 4, 5, 7, 9, 11 and 12 show the broad smear of polyclonal amplified DNA. A PCR DNA marker was included (lane 6).

As shown in table (3), among untreated patients (cryoglobulinaemic and non-cryoglobulinaemic) with chronic HCV infection, the frequency of Bcl-2-JH translocation in PBMC did not differ significantly according to histologic severity of liver injury (P=0.397).

Table (3) shows the frequency of Bcl-2-JH translocation in PBMC from patients and controls. Chi square test was performed and it was statistically significant (P=0.00007). Fisher’s exact test was used for comparison between each 2 groups. Cryoglobulinaemic chronic HCV infected patients had a significantly higher rate of Bcl-2-JH translocation than non HCV infected CLD patients (P=0.0002) and healthy controls (P=0.0002). Likewise, non-cryoglobulinaemic chronic HCV infected patients had a higher frequency of Bcl-2-JH translocation than HCV negative patients with CLD (P=0.001) and healthy controls (p=0.001).

However, no statistically significant difference in the frequency of Bcl-2-JH translocation was observed between cryoglobulinaemic and non-cryoglobulinaemic chronic HCV infected patients (P=0.11). The specific band of Bcl-2-JH translocation of 200-280 bp is demonstrated in 3 patients in figure (2).

Table (3): Frequency of Bcl-2-JH translocation in PBMC from the studied groups.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>No. of subjects</th>
<th>Bcl-2-JH translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Group Ia</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Group Ib</td>
<td>55</td>
<td>14</td>
</tr>
<tr>
<td>Group II</td>
<td>30</td>
<td>zero</td>
</tr>
<tr>
<td>Group III</td>
<td>30</td>
<td>zero</td>
</tr>
</tbody>
</table>

$\chi^2=26.44$, P=0.00007

Fisher’s exact test $\chi^2=0.011$, group Ia vs. group Ib: P=0.0002, group Ia vs. group II: P=0.0002, group Ia vs. group III: P=0.0002, group Ib vs. group II: P=0.001, group Ib vs. group III: P=0.001

Figure (2): Detection of t (14 ; 18) translocation by MBR Bcl-2-JH nested PCR in PBMC from patients with chronic HCV infection. Lanes 4, 8 and 10 represent patients positive for t (14; 18). A negative control (lane 5) and a PCR DNA marker (lane 6) were included.

As shown in table (4), among untreated patients (cryoglobulinaemic and non-cryoglobulinaemic) with chronic HCV infection, the frequency of Bcl-2-JH translocation in PBMC did not differ significantly according to histologic severity of liver injury (P=0.397).
There were no statistically significant differences between chronic HCV infected patients with and without Bcl-2-JH translocation with respect to age (t=1.33, P=0.188), sex (x^2=0.225, P=0.636), mean ALT level (t=0.16, P=0.877) and mean AST level (t=0.57, P=0.568).

Table (4): Frequency of Bcl-2-JH translocation in PBMC from untreated patients with chronic HCV infection according to histologic severity of liver injury.*

<table>
<thead>
<tr>
<th>Histologic groups</th>
<th>No. of subjects</th>
<th>Bcl-2-JH translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAH-mild</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>CAH-mod</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>CAH-sev</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

x^2=2.96, P=0.397  
* Three patients belonging to group Ib were not subjected to liver biopsy.

As shown in table (5), the frequency of coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation was higher in cryoglobulinaemic (33.3%) than non-cryoglobulinaemic chronic HCV infected patients [(7.3%), P=0.05], HCV negative CLD patients [(zero%), P=0.009] and healthy controls [(zero%), P=0.009]. However, no statistically significant differences were observed between non-cryoglobulinaemic chronic HCV infected patients and non HCV infected patients with CLD (P=0.292) and healthy controls (P=0.292).

Table (5): Frequency of coexisting monoclonal heavy chain gene rearrangement and Bcl-2-JH translocation in PBMC from the studied groups.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>No.</th>
<th>Coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Ia</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Group Ib</td>
<td>55</td>
<td>4</td>
</tr>
<tr>
<td>Group II</td>
<td>30</td>
<td>zero</td>
</tr>
<tr>
<td>Group III</td>
<td>30</td>
<td>zero</td>
</tr>
</tbody>
</table>

χ^2=16.82, P=0.0007  
Fisher’s exact test: group Ia vs. group Ib: P=0.05, group Ia vs. group II: P=0.009, group Ia vs. group III: P=0.009, group Ib vs. group II: P=0.292, group II vs. group III: P=0.292

**DISCUSSION**

Recently, HCV has been recognized as an agent that may possibly play a role in the multifactorial process of lymphomagenesis particularly B cell non-Hodgkin lymphoma. Although HCV is a hepatotropic virus, the HCV genome and its replicative intermediates have also been detected in PBMC and in lymphoid tissues of chronically infected patients.(23, 36) When B lymphocytes undergo clonal proliferation, they can secrete monoclonal proteins including cryoglobulins which are complexes of immunoglobulin that precipitate on exposure to cold. Cryoglobulins may cause a variety of pathogenic conditions all related to the formation of immune complexes and the attendant inflammation and coagulation disorders. An association between cryoglobulinaemia and HCV infection has been reported.(37, 38)

The mechanism of lymphomagenesis by HCV is still obscure. However, it is possible that HCV may induce clonal proliferation of B cells and/or inhibit apoptotic cell death of the infected lymphocytes by a Bcl-2 dependent approach.(39)

In the present study, patients with chronic HCV infection with and without cryoglobulinaemia had significantly higher frequencies of monoclonal IgH rearrangement than patients with HCV negative CLD (P=0.006 and 0.047, respectively) and healthy controls (P=0.001 and 0.005, respectively). However, no statistically significant difference in the frequency of IgH rearrangement monoclonality was observed between cryoglobulinaemic and non-cryoglobulinaemic patients with chronic HCV infection (P=0.204).

Likewise Franzin et al.(40) detected clonal immunoglobulin gene rearrangement in 8 (88.9%) of the 9 cryoglobulinaemic patients with chronic HCV infection and also at a high frequency (24%) in the HCV infected patients without cryoglobulinaemia. A polyclonal pattern was present in all 15 normal individuals and the 16 patients with HCV-negative alcoholic liver disease which were investigated as control groups.

Furthermore, Zuckerman et al.(41) observed a significantly higher rate of B cell clonality as detected by immunoglobulin heavy chain gene rearrangement in cryoglobulinaemic patients with chronic HCV infection than in patients with CLD not related to HCV. However, although patients with HCV infection without cryoglobulinaemia had a higher rate of B cell clonality than patients with CLD not related to HCV infection, it did not reach statistical significance.

In the present study among all chronic HCV infected patients (cryoglobulinaemic and non-cryoglobulinaemic) in whom biopsy was performed the rate of monoclonal IgH rearrangement in PBMC did not differ according to histologic severity of liver injury (P=0.675). In addition, no association was found between the presence of a clonal B cell expansion and age, sex or levels of serum aminotransferases. Similar results were obtained by Franzin et al.(40)
The present study demonstrated highly significant increased rates of Bcl-2-JH translocation in PBMC from patients chronically infected with HCV with cryoglobulinemia (55.6%) and without cryoglobulinemia (25.5%) compared with none of the patients with CLD not related to HCV (P=0.0002 and 0.001, respectively) and the healthy control group (P=0.0002 and 0.001, respectively). However, no statistically significant difference in the frequency of Bcl-2-JH translocation in PBMC was observed between cryoglobulinemic and non-cryoglobulinemic patients with chronic HCV infection (P=0.11).

In accordance with our results, Zignego et al. detected t (14 ; 18)-positive PBMC in 18/57 patients with chronic HCV infection and 3/85 HCV-negative patients with CLD or chronic rheumatic disorders. Furthermore, Zuckerman et al. reported that patients chronically infected with HCV with and without cryoglobulinemia had significantly higher rates of Bcl-2 translocation than patients with CLD not related to HCV (P=0.001 and 0.014, respectively).

In the present study, among all chronic HCV infected patients (cryoglobulinemia and non-cryoglobulinemia) in whom biopsy was performed the rate of Bcl-2-JH translocation in PBMC did not differ significantly according to histologic severity of liver injury (P=0.397). In addition, no association was found between the presence of Bcl-2-JH translocation and age, sex or levels of serum aminotransferases. Similarly, Zignego et al. found no significant correlation between t (14 ; 18) and the patients’ age or the severity of liver disease.

Although Bcl-2-JH translocation presence does not necessarily correspond to a malignancy, it represents an important step predisposing to lymphomagenesis, as indicated by the observation that mice transgenic for Bcl-2-JH translocation develop lymphoid hyperplasia progressing to malignant lymphoma.

The t (14 ; 18) most likely occurs during early IgH gene rearrangements at the pre-B cell stage and a JH gene segment is frequently involved. However, it may take place as a consequence of particularly strong antigenic stimulation. The chronic stimulation of the B cell compartment by HCV, amplified by high viral variability and possibly also by HCV infection of lymphocytes themselves may explain the highly significant differences in the detection of Bcl-2-JH translocation between chronically infected patients with HCV (cryoglobulinemia and non-cryoglobulinemia) and those with HCV negative CLD and healthy controls observed in this and other studies.

It is possible that HCV stimulates the proliferation of monoclonal B cells via their HCV specific receptor (BCR) on the cell surface. Binding of the HCV envelope proteins to a cellular ligand, CD81, may also enhance this antigen driven process. This may lead to increased genetic aberration frequency of VDJ rearrangements in antigen reactive B cells.

A recent report on regression of splenic marginal zone lymphoma after antiviral treatment with interferon and ribavirin has significantly strengthened the effect relationship between HCV infection and lymphoma. Further studies should determine whether BCRs expressed on HCV-associated lymphoma, particularly those that regress in response to antiviral therapy, bind HCV antigens that stimulate their proliferation.

In the present study, the frequency of coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation was significantly higher among cryoglobulinemia than non-cryoglobulinemia patients chronically infected by HCV (P=0.05), patients with HCV negative CLD (P=0.009) and healthy controls (P=0.009). However, no statistically significant differences were observed between non-cryoglobulinemia patients chronically infected by HCV and patients with HCV negative CLD (P=0.292) and healthy controls (P=0.292). To the best of our knowledge, apart from the present study, no other reports exist in the literature regarding the rate of coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation among patients with chronic HCV infection.

Because HCV RNA genomic sequences cannot be integrated into the host genome, an indirect mechanism of B cell clonality and malignant transformation must be hypothesized. The persistence of HCV in PBMC may result in chronic stimulation of B cells, leading to polyclonal and later monoclonal expansion of these cells which are more susceptible to Bcl-2-JH translocation leading to overexpression of Bcl-2 which inhibits apoptotic death of these cells. The increased survival of clonal B cells may be a predisposing factor for further genetic aberrations including mutational activation of proto-oncogenes and/or inactivation of tumor suppressor genes ending in malignant transformation.

The restricted repertoire of V, D, J genes used to assemble the B cell receptor and the frequent occurrence of certain gene combinations observed in HCV-associated B cell non-Hodgkin lymphoma suggest a common antigen binding specificity of those clones and supports the role of chronic
antigenic stimulation in eliciting monoclonal B cell proliferation in HCV infected patients.

The Bcl-2-JH translocation constitutes the most common chromosomal translocation in human lymphoid malignancies. Approximately 85% of follicular and 20% of diffuse B cell lymphomas possess this translocation. A molecular consequence of this translocation is the placement of Bcl-2 gene in the same transcriptional orientation as the Ig heavy chain locus giving rise to chimeric mRNAs resulting in increased expression of Bcl-2. However, translocation does not interrupt the protein encoding region so that normal and translocated alleles produce the same sized, 25-kDa protein. T (14 ; 18)-bearing B cells have inappropriately elevated levels of the Bcl-2-Ig fusion mRNA. This increased steady state mRNA reflects both increased transcription as well as a processing advantage for the Bcl-2-Ig fusion allele. Follicular lymphoma often presents as a low-grade malignancy composed of small resting B cells. Over time, conversion to an aggressive high grade lymphoma with a diffuse large-cell architecture frequently occurs in these patients associated with genetic aberrations of other proto-oncogenes including c-myc. (44, 45)

Interestingly, in the current study, coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation was significantly more frequent in cryoglobulinaemic than non-cryoglobulinaemic patients chronically infected by HCV, patients with HCV negative CLD and healthy controls suggesting that determination of monoclonal IgH rearrangement and Bcl-2-JH translocation in PBMC could be used as non invasive markers for the predisposition to acquire cryoglobulinaemia and/or other B cell lymphoproliferative disorders.

We can come to the conclusion that patients with chronic HCV infection are more liable to develop monoclonal IgH rearrangement or Bcl-2-JH translocation in PBMC. Moreover, coexisting monoclonal IgH rearrangement and Bcl-2-JH translocation is a frequent finding in cryoglobulinaemic patients with chronic HCV infection suggesting that these aberrations may be involved, at least in part, in the complex multistep mechanisms occurring in HCV infected patients ending in B cell lymphoproliferative diseases. Further studies are needed to establish whether determination of monoclonal IgH rearrangement and Bcl-2-JH translocation in PBMC could be useful as non invasive molecular indicators for the likelihood to acquire cryoglobulinaemia and/or other B cell lymphoproliferative disorders.

REFERENCES

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