Hepatitis B virus reactivation after fludarabine-based regimens for indolent non-Hodgkin's lymphomas: high prevalence of acquired viral genomic mutations

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Background and Objectives. Chemotherapy can cause hepatitis flare-up through viral reactivation in patients who have had contact with hepatitis viruses. Few data are available on the genotype of the reactivated viruses.

Design and Methods. In 40 consecutive adult patients with indolent non-Hodgkin's lymphoma (NHL) receiving fludarabine-based front-line chemotherapy, we performed a prospective study on viral hepatitis reactivation and analyzed the genotype of the reactivated viruses. Before chemotherapy, 4 patients were healthy carriers of hepatitis B surface antigen (HBsAg), 2 had HB core antigen antibodies (anti-HBc), 6 anti-HBs and 6 anti-HCV; 22 were seronegative.

Results. Hepatitis flare-up occurred in the 4 HBsAg-positive patients and in 1 anti-HBc-positive patient at a median of 1 month (range 1-4) after chemotherapy, when the CD4/CD8 ratio was still inverted. HBV reactivation was documented in all 5 instances (HBV-DNA 2-8$\times 10^6$ copies/mL). Two of the 5 patients responded to lamivudine, whereas 1 died of acute liver failure and 2 had persistent severe hepatitis. HBV genome sequencing at hepatitis flare-up showed that deviation from the closest related published sequences was 1.0% and 1.1% in the 2 lamivudine-responsive patients, and 1.5%, 1.8% and 1.7% in the 3 lamivudine-resistant patients. The polymerase open reading frame (ORF) and the HBs ORF of lamivudine-resistant strains contained several novel amino acid substitutions.

Interpretation and Conclusions. These results suggest that fludarabine treatment of HBV-infected patients is frequently associated with acute hepatitis due to viral reactivation, and that lamivudine may be less effective in this situation than in other settings of immunocompromised hosts because of the emergence of resistant mutant strains.

Key words: HBV, fludarabine, NHL, viral genomic mutations.

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Roughly a third of the world's population will contract hepatitis B virus (HBV) infection during their life.[1] The outcome of HBV infection is the result of a complex, as yet not fully understood, viral-host interaction, which may give rise to a wide spectrum of clinical conditions going from healthy carrier status to acute and/or chronic liver disease.[2,3]

HBV infection is prevalent in Asia, Africa, Latin America and Southern Europe, where the percentage of persistent HB surface antigen (sAg) carriers in the general population ranges from 2% to 20%.[4,5] Thus, in these countries it is not infrequent that a HBV carrier is a candidate for antineoplastic chemotherapy. Acute hepatitis due to HBV reactivation is a complication in this setting: 14 to 55% of HBsAg-positive patients undergoing chemotherapy may experience acute liver disease related to enhanced HBV replication.[6,7] A survey of Chinese patients undergoing chemotherapy showed that the frequency of HBV reactivation was highest in patients with non-Hodgkin's lymphoma (NHL), and this was attributed to the highly immunosuppressive steroid-based regimens used.[8] The clinical consequences of HBV reactivation range from mild liver dysfunction to massive necrosis and liver failure with a 5%-12% mortality rate.[9]

Fludarabine, a nucleoside analog used as a single agent or combined with other drugs for a variety of hematologic malignancies, is very effective in the treatment of indolent NHL.[10] It exerts its cytotoxic effect by penetrating the DNA of dividing cells where it induces a wide range of helix distorting lesions that are not repaired by the nucleotide excision repair complexes.[11] However, the efficacy of fludarabine against neoplasias with a low growth fraction , such as chronic lymphocytic leukemia and indolent NHLs, indicates that other mechanisms are operating, likely determining proneness to apoptosis.[12] A well known effect of fludarabine treatment is profound and prolonged immunosuppression with a relevant decrease of $CD4^+$ and $CD8^+$ lymphocytes, which predisposes to opportunistic infections[13,14]

To our knowledge, there are no data about acute hepatitis due to viral reactivation in NHL patients treated with fludarabine-containing regimens. We evaluated the incidence, risk factors, etiology, morbidity and mortality of acute liver damage in a series of patients undergoing fludarabine-based front-line chemotherapy for indolent NHL. Additional data on viral mutation rate were obtained from the study of the genome sequence of the virus strains responsible for hepatitis flare-ups.

Table 1. Clinical features and chemotherapy protocols of the 40 patients studied.

NHL type	*n*	*Hepatitis virus serological status at NHL diagnosis*	*NHL treatment*
Follicle center	26	HBsAg = 1, anti-HBc = 1	Flu + Mito = 18
		anti-HBs, anti-HBc, anti-HBe = 5	Flu = 8
		anti-HCV = 4	
		Seronegative = 15	
Nodal small lymphocytic	10	HBsAg = 2, anti-HBc = 1	Flu = 5
		anti-HBs, anti-HBc, anti-HBe = 1	Flu + Mito = 3
		anti-HCV = 2	Flu + Ida + Cy = 2
		Seronegative = 4	
Marginal zone	4	HBsAg = 1	Flu + Ida + Cy = 3
		Seronegative = 3	Flu = 1

Flu indicates intravenous fludarabine (25 mg/m²/daily for 5 days) for 6 courses; Flu + Mito, intravenous fludarabine (25 mg/m²/daily for 3 days) and mitoxantrone (10 mg/m² for 1 day) for 6 courses; and Flu + Ida + Cy, intravenous fludarabine (25 mg/m²/daily for 3 days), idarubicin (14 mg/m² for 1 day) and cyclophosphamide (200 mg/m²/daily for 3 days) for 6 courses.

Design and Methods

Patients and study design

Over the past 4 years, we prospectively studied 40 consecutive previously untreated patients (22 women and 18 men; median age 50 years, range 40-82) affected by grade I follicular, nodal small lymphocytic or marginal zone B-cell NHL, according to the REAL/WHO classification[15] (Table 1). All patients were scheduled to receive 6 monthly courses of fludarabine-based chemotherapy regimens that did not contain steroids. Diagnostic tests were performed before the start of each course of treatment, and monthly after chemotherapy completion. Before starting chemotherapy, all patients were assessed for serum HBsAg and HBeAg, and for antibodies against HBs, HBe, HBc, HδδHCV, HAV, cytomegalovirus, Epstein Barr virus and herpes simplex virus using commercially available kits; HBV-DNA (sensitivity of the assay: >10² copies/mL) and HCV-RNA were measured by a quantitative polymerase chain reaction (PCR) procedure (Cobas Amplicor Roche Diagnostic Systems, Basel, Switzerland). The same tests were performed again in patients who developed a hepatitis flare-up (defined as a greater than 3-fold elevation of serum ALT and/or AST above the upper normal limit) together with flow cytometric assessment of CD4, CD8 and CD56 in circulating lymphocytes. Lamivudine (100-200 mg daily) alone or in combination with interferon-α (10 MU, subcutaneously three times a week) was administered to patients with hepatitis flare-up due to HBV; liver ultrasound and biopsy were performed in patients who did not show serological improvement, despite the antiviral therapy.[16]

Sequence analysis of HBV-DNA

Viral DNA was purified from 200 μL of the patient's serum, withdrawn at the time of HBV reactivation, using a QIAamp Blood Kit (Qiagen, Chatsworth,

Table 2. Primers used to amplify overlapping sequences of HBV genomes isolated from the patients' sera.

	Strand	*Sequence*	*Annealing temperature*	*Alignment to HBV reference sequence (no. X65257)*
1F	+	5'GGGTCACCATATTCTTGGG3'	50°C	2816-2834
1R	–	5'CA(A/G)AGACAAAAGAAAATTGG3'	50°C	821-805
2F	+	5'GTCTGCGGCGTTTTATCA3'	50°C	383-400
2R	–	5'GGAGTTCCGCAGTATGGATCGG3'	50°C	1284-1263
3F	+	5'GAACTCCTAGCAGCTTGTTTTGC3'	65°C	1278-1300
3R	–	5'GTGCGCCCCGTGGT3'	65°C	1531-1518
4F	+	5''TCTTTTGGAGTGTGGATTCGC3'	65°C	1263-1283
4R	–	5'CAGGTACAGTAGAAGAATAAAGCCCA3'	65°C	2513-2489
5F	+	5''TCAATCGCCGCGTCG3'	65°C	2409-2423
5R	–	5'GGATAGAACCTAGCAGGCATAATTAATT3'	65°C	2658-2631

Table 3. Clinical features and outcome of the five NHL patients who had HBV reactivation.

Pt.	Sex/Age (years)	NHL DX	NHL therapy*	Serological status at NHL diagnosis	Acute hepatitis flare-up		HBV status at reactivation		Hepatitis treatment	Hepatitis outcome
					AST/ALT peak (IU/L)	weeks†	DNA copies (¥106/mL)	sAg/eAg		
1	M/52	NSL	Flu + Ida + Cy	HBsAg+	1000/1200	9	5	+/-	Lamivudine	No improvement
2	F/60	NSL	Flu	HBsAg+	400/800	4	8	+/-	Lamivudine + α-interferon	No improvement
3	F/57	FC	Flu	Anti-HBc+	400/900	16	2	+/+	Lamivudine + α-interferon	Normalization in 48 weeks
4	F/40	MZ	Flu + Ida + Cy	HBsAg+	2000/2400	4	8	+/ -	Lamivudine	Normalization in 5 weeks
5	F/52	FC	Flu + Mito	HBsAg+	800/900	4	5	+/-	Lamivudine	Normalization in 4 weeks

*NSL indicates nodal small lymphocytic; FC, follicle center; and MZ, marginal zone. *See Table 1. †Time from NHL treatment completion.*

CA, USA). Most of the HBV genome including the complete polymerase and HBs open reading frame (ORF) was amplified by a PCR in five separate reactions using partially overlapping primer couples (Table 2). PCR was performed using 20 ng aliquots of DNA in a mixture containing 10 mM Tris HCl (pH 8.3), 2 mM $MgCl_2$, 50 mM KCl, 0.2 mM of each deoxyribonucleotide, 2.5 U of Taq polymerase and 0.5 mM of each specific primer in a final volume of 50 μL. The time/temperature profile of the amplification reactions was: denaturation at 94°C for 1 min, annealing at primer specific temperature (Table 2) for 1 min, and extension at 72°C for 1 min, for 35 cycles. PCR-amplified products were cloned in a plasmid vector (TOPOCloning, Invitrogen, Carlsbad, CA, USA) and both strands of at least five independent clones of each amplification product were sequenced using automated DNA sequencing (ABI-310, Applera, Forster City, CA, USA).

The ABI sequence editor software was used to reassemble the whole viral genome sequence of each patient. The genomic sequences were compared with the HBV sequences available in the GeneBank and EMBL databases. The predicted translations of the five viral reading frames corresponding to polymerase, surface (S, PreS1, and PreS2), core, precore and X proteins, were compared to the SWISS-PROT resources. All genomic and protein sequence analyses were performed with the Blast and the Clustalw software available at the URL *http://bioinfo.biogem.it*. We followed the HEP DART International Committee recommendations for genotype comparisons and for the nomenclature of the mutations found in the genomic sequences of viral isolates.[17]

Results

Acute hepatitis development

Of the 40 patients analyzed, pre-treatment assessment of viral serological status showed that 12 were HBV-positive, 6 were HCV-positive, and the others were seronegative for both viruses. Of the 12 HBV-positive patients, four were healthy carriers of HBsAg (normal liver function tests and serum HBV-DNA negative by PCR), two were anti-HBc-positive without anti-HBs, and the remaining 6 were anti-HBs-positive (together with anti-HBe and/or anti-HBc) (Table 1). All the anti-HCV-positive patients had histologically-documented active chronic hepatitis, and HCV-RNA sequences were detected in their serum by PCR. In addition, 25 patients were positive for anti-HAV, 20 for anti-cytomegalovirus, 18 for anti-Epstein Barr virus and 15 for anti-herpes simplex virus-1.

All patients received the scheduled 6 courses of chemotherapy; none received hemoderivatives, or antiviral prophylaxis before, during or after chemotherapy.

Acute hepatitis was diagnosed in five patients after a median of 7 months from the start of chemotherapy (1 month from the completion of chemotherapy). All episodes of acute hepatitis were due to HBV reactivation (positive conversion of serum HBV-DNA assay) and were correlated with the pre-treatment serological status (Table 3). Indeed, all the four HBsAg-positive patients and one of the two anti-HBc-positive patients developed signs of acute liver damage. There were no signs of hepatitis in the 6 anti-HBs-positive patients, in the 6 anti-HCV-positive patients or in the remaining 22

Table 4. Peripheral blood lymphocytes in the 40 NHL patients at diagnosis and during chemotherapy, and lymphocyte subpopulations of the five patients at HBV reactivation.

	N. of patients		*Peripheral blood lymphocytes* *Total*	*CD4*	*CD8*	*CD56*
Pre-treatment	40	median	1600	na	na	na
		range	(893-5628)			
After 3 courses of treatment	40	median	770	na	na	na
		range	(336-2150)			
At treatment completion	40	median	572	na	na	na
		range	(296-1500)			
At onset of acute hepatitis	5	median	1200	240	593	216
		range	(600-1600)	(132-353)	(354-810)	(108-592)

na indicates data not available.

patients (Figure 1). In particular, no hepatitis flare-ups were observed in patients who had had contact with HAV and with minor hepatotropic viruses (cytomegalovirus, Epstein Barr virus, herpes simplex virus-1). The drugs used in combination with fludarabine did not seem to influence the viral flare-up. In fact, of the five patients with HBV reactivation, two had received fludarabine as a single agent, one fludarabine plus mitoxantrone, and the remaining two fludarabine plus idarubicin and cyclophosphamide (Table 3). Interestingly, in all patients viral reactivation was observed at the time of immunological reconstitution; however, in all cases the CD4/CD8 ratio was still inverted (Table 4).

Clinical features and outcome of acute hepatitis

All five patients had a severe form of acute hepatitis. The median serum peaks were: AST 800 IU/L (range, 400-2000), ALT 900 IU/L (range, 800-2400), total bilirubin 12 mg/dL (range, 6-22) and HBV-DNA 5×10^6 copies/mL. Consequently, lamivudine was used as first-line treatment in all five. In two patients (#4 and #5 in Table 3) lamivudine rapidly induced normalization of liver function tests and disappearance of HBV-DNA from serum. HBV replication persisted in the remaining three patients. Ultrasound scan ruled out focal liver lesions in all three. Biopsies from patients #1 and #2, 32 and 40 days after acute hepatitis onset, respectively, showed marked hepatocyte lysis, moderate portal space fibrosis and inflammation, a number of portal-portal bridges, and intracytoplasmic HBsAg/HBcAg/HBV-DNA in the majority of hepatocytes, thereby confirming the viral etiology of the liver disease. Two of these three patients died. Patient #1 died of acute liver failure 7 weeks after starting lamivudine. Patient #2, who also received interferon-α, died of a second malignancy (intestinal perforation due to colon adenocarcinoma) two months after the onset of hepatitis, without any sign of liver function improvement. Interferon-α was added to lamivudine in patient #3; liver function tests normalized and serum HBV-DNA disappeared 12

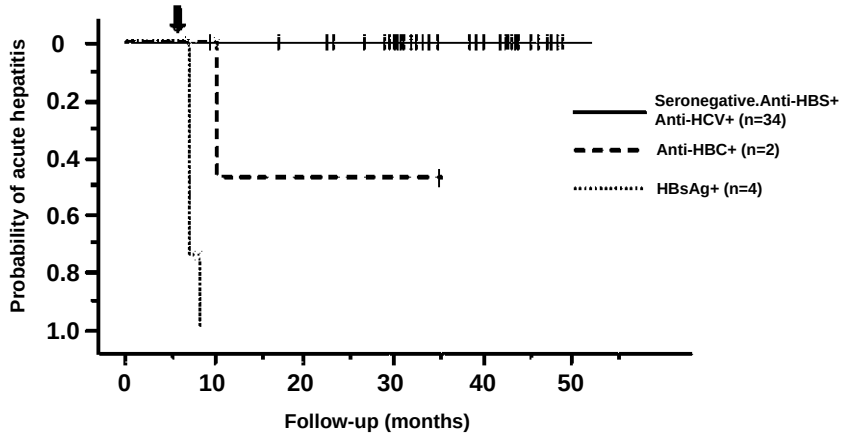

Figure 1. Actuarial risk of hepatitis flare-up in fludarabine-treated NHL patients. Patients are stratified according to the pre-treatment serological status (see text for details). The vertical arrow indicates the time of fludarabine treatment completion.

Table 5. Mutations found in the prevalent HBV strains of NHL patients at hepatitis flare-up.

Patient	*#Closest matched HBV sequences (Gene Bank no.)*	*Geno/ serotype*	*Total nucleotide mutations (%)*	*Total amino acid mutations (%)*	*Mutations at the MHL domain of HBs antigen*	*Mutations at conserved RT domains (A-F)*
1	X65257	D/ayw	1.5	2.3	Y134F	N238H
2	X65257	D/ayw	1.8	3.9	Y134F	C256T
3	V01460	D/ayw	1.7	3.7	L109Q T118K C124F Y134F S154L	
4	V01460	D/ayw	1.0	1.6	–	–
5	V01460	D/ayw	1.1	1.8	T140I	N246H

months after the onset of hepatitis.

Patients #3, #4 and #5 are currently thriving and off-therapy; they had seroconversion from HBsAg to anti-HBs and normal liver function tests at a median of 3.4 years from the onset of acute hepatitis.

HBV genomic mutations

None of the five patients with post-chemotherapy hepatitis flare-up had detectable circulating HBV genomic molecules at the time of NHL diagnosis. We were, therefore, able to analyze the genomic sequences only at the time of reactivation. Because of genetic diversity among the HBV genotypes A to F, there is no consensus sequence that could serve as standard for all comparisons. To measure the degree of variability in these sequences, we compared them to the closest related published sequence, which was then considered the reference sequence, and deviations from the reference sequence were considered to be mutations. In all five cases, the genomic sequences had the highest degree of homology with genotype D sequence, corresponding to the ayw serotype (Table 5). We found two types of HBV clones in each patient: a prevalent clone and a minor clone. The prevalent clone was considered the etiological agent of the hepatitis flare-up. The genomic sequences of the etiological clones deviated from the reference sequence by a mean of 1.42%, with a high degree of variability among the isolates. In each patient, the minor clone sequence matched the reference sequence much closer than the etiological strain. Interestingly, we found a rough correlation between the clinical response to lamivudine and the degree of genomic mutations of the corresponding etiological viral isolate. The deviation from the closest sequences was 1.0% and 1.1% for the isolates from, respectively, patients #4 and #5, who rapidly responded to lamivudine, whereas it was 1.5%, 1.8%, and 1.7% in, respectively, patients #1, #2 and #3, who did not respond to lamivudine (Table 5). When we translated the observed mutations into amino acid substitutions, we found that the difference between responders and non-responders was evident also at the polypeptide level. Indeed, the mean rate of amino acid substitutions was lower in patients #4 and #5 than in patients #1, #2, #3, both at the polymerase ORF and at the HBs ORF (2.7% vs 4.0%, and 1.25% vs 3.2%, respectively; Figure 2 and Table 5). It is noteworthy that all non-responders had the tyrosine→phenylalanine substitution at position 134 of the MHL domain of the surface ORF, and all the amino acid substitutions of the highly conserved regions were found only in the prevalent etiological clones and never in the minor clones (*data not shown*). As expected in our geographical area, we found stop codon mutations at the pre-core ORF in the isolates of four patients with HBV reactivation, which is consistent with the absence of HBeAg (Table 3).

Discussion

In four large series of HBsAg-positive patients treated with cytotoxic agents for hematologic malignancies, the rate of HBV reactivation ranged from 14% to 55%.[7-9,18] Inclusion of steroids in the chemotherapy regimens led to the highest rate of hepatitis flare-up and of fatal liver failure[3,7] probably because the HBV genome contains a glucocorticoid-responsive element that may act as an enhancer of viral replication.[19] More than 50% of hepatitis flare-ups due to HBV reactivation occur in the window between the end of chemotherapy and the complete recovery of immunocompetence.[7] After widespread viral infection of hepatocytes during immunosuppression, the rebound in T-cell function and number

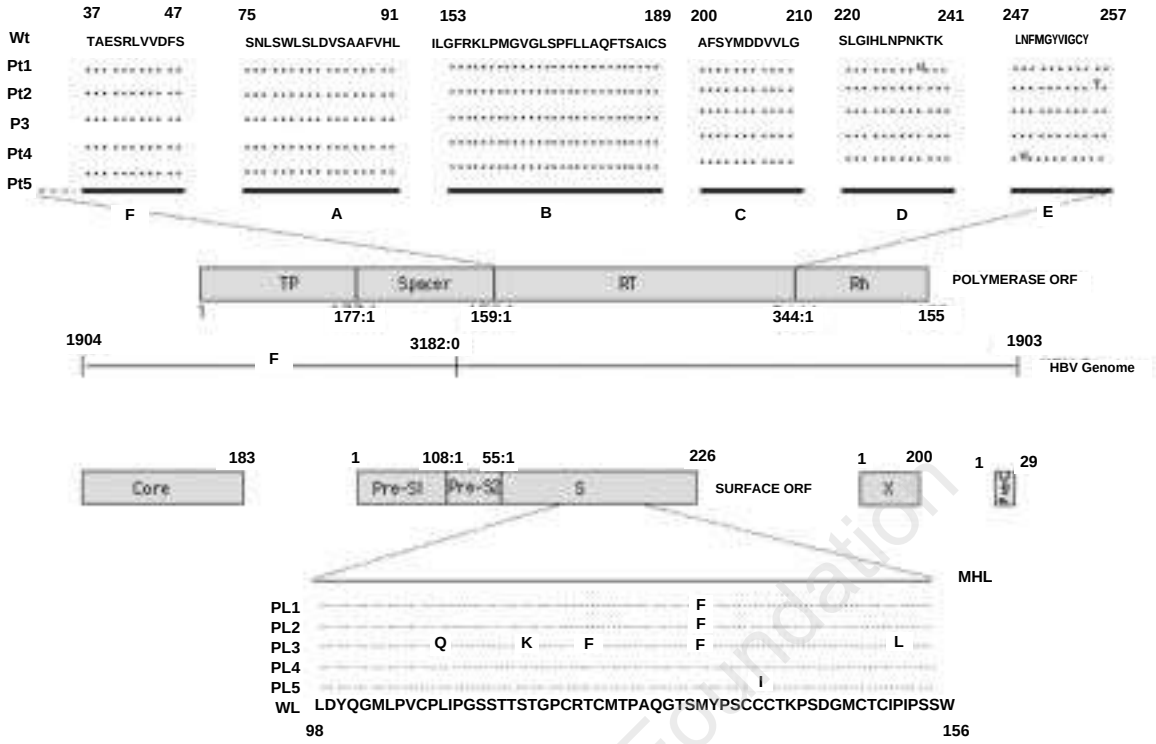

Figure 2. Amino acid mutations at the HBV conserved regions in the five NHL patients. Schematic representation of the HBV genome with its five open reading frames. The amino acid sequences of conserved regions of the reverse transcriptase and the major hydrophilic loop of the surface antigen are shown together with the amino acid substitution found in the patients. Amino acid numbering in the case of reverse transcriptase follows recommendations of the consensus proposals for HBV polymerase nomenclature.[17]

may lead to massive hepatocyte destruction.[6]

In the present study, fludarabine-based cytotoxic regimens were administered without steroids as front-line treatment in 40 consecutive patients affected by indolent NHL. Consistent with the high prevalence of hepatitis virus infections in Southern Europe, 30% of patients were HBV-positive and 15% had HCV active replication with chronic hepatitis. No hepatitis flare-up occurred after fludarabine treatment in the anti-HBs-positive patients, in the anti-HCV-positive patients or in patients seronegative for both viruses, whereas life-threatening acute hepatitis occurred in all four HBsAg-positive patients and in one of the two anti-HBc-positive patients. All five patients had normal liver function tests and no detectable levels of serum HBV-DNA before starting chemotherapy –conditions that can be defined as *healthy carrier* and *occult infection*, respectively.[20,21] This means that before chemotherapy, our patients had a very low level of circulating HBV-DNA ($\leq 10^2$ copies/mL), which was not detectable by the standard diagnostic tests. Alternatively, they could have harbored variable amounts of HBV-DNA exclusively in the liver, and this began to replicate upon the breakdown of immunosurveillance.[22,23] The clinical signs of hepatitis were manifested at reconstitution of circulating lymphocytes, but at a time when the CD4/CD8 ratio was still inverted (Table 4). Fludarabine treatment in NHL patients has been reported to cause a profound and prolonged CD4 depletion, lasting months after the termination of therapy.[24,25] Incomplete recovery of CD4 cells might explain the severity of the hepatitis observed in our patients. Although the hepatocellular injuries caused by HBV are mainly immune-mediated (CD8 cytotoxic lymphocytes directly recognize and lyse liver cells that have HBV-derived peptides on their surface), non-cytotoxic (CD4 lymphocyte-dependent) mechanisms are required for viral clearance.[5,26] The rate of spontaneous genomic mutations reported in HBV carriers is $\leq 0.7\%$.[27] Because HBV reverse transcriptase lacks proof-reading activity it has a high mutation rate, i.e., between 10^{-5} to 10^{-6} per site per year.[28] The consequent accumulation of sequence variants is called *sequence or strain evolution*. Because the virus mutation rate was higher in our patients than expected from spontaneous sequence evolution, one may suspect that fludarabine affects this mutation rate. The-

oretically, fludarabine might enter hepatic cells and, given its similarity to purine nucleotides, it could be incorporated into HBV-DNA during viral replication thereby leading to proof-reading errors and, consequently, to the accumulation of mutations. Another, not necessarily alternative, mechanism is that the profound immunosuppression induced by fludarabine might favor a high rate of HBV replication, thereby increasing the probability of spontaneous genomic mutations. Upon restoration of immunocompetence, immune system effectors might select the predominant strain because of its replication capacity; in addition, lamivudine may favor the expansion of drug-resistant mutants. In our patients, the finding of a minor clone with only a few mutations seems to support the latter model.

There was a high incidence of non-responders to lamivudine in our small series of patients. Only two patients showed prompt normalization of liver tests and HBV-DNA disappearance, whereas patient #1 died of liver failure, patient #2 died of unrelated causes without any evidence of clinical response, and patient #3 recovered from the liver disease after 10 months of combined antiviral treatment. In the three lamivudine-resistant patients, the correlation between rate of genomic sequence mutations in the prevalent strain and response to the drug was confirmed by the finding of a high number of amino acid substitutions at two highly conserved regions, namely the MHL region of the surface antigen and the conserved A to F region of polymerase.[17] In particular, two patients had an amino acid substitution at the catalytic domains of reverse transcriptase, which are highly conserved among various viruses including HIV.[29,30] Lamivudine binding to a specific target on the polymerase (a pocket within the reverse transcriptase/polymerase domain) is crucial for the drug's powerful antireplicative activity. Most mutations associated with lamivudine resistance were found to occur at the 6 highly conserved domains of reverse transcriptase.[17,31] The mutations are usually selected for during treatment, increasing progressively with the duration of treatment, ranging from 15% to 30% after 12 months of treatment and exceeding 50% after 3 years.[32,33] Patients #1 and #2 had punctiform mutations of the polymerase gene, suggesting that nucleotide replacements may lead to conformational changes of the binding site, thus preventing access to lamivudine.[34] Patient #3 had 6 amino acid substitutions at the MHL domain, which is the target of the neutralizing antibodies.[35]

In conclusion, our study indicates that among patients who harbored HBV, HBsAg-positive and anti-HBc-positive patients constitute a subset at risk of severe hepatitis due to viral reactivation after fludarabine treatment. The incidence of hepatitis exacerbations in these patients is higher than that reported for patients treated with other types of chemotherapy regimens, including those containing steroids.[7-9,18] The absence of viral reactivation in our HCV-infected patients and the high reactivation rate in HBV-infected patients indicate that the mechanisms damaging the liver are substantially different in the two diseases. Specific prophylaxis does not seem necessary to prevent HCV reactivation during and after fludarabine therapy. By contrast, lamivudine administration may be needed to prevent or to treat HBV reactivation in immunocompromised patients.[36,37] However, the high rate of genome mutations and amino acid substitutions at critical conserved domains and the prolonged CD4 depletion observed in our series could be important factors for the viral reactivation, for the severity of the hepatitis and for the lack of response to lamivudine. Clinical trials are needed to determine the efficacy of other potent antiviral drugs and of combination treatments in the management of fludarabine-related HBV reactivation.[38,39]

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Pre-publication Report & Outcomes of Peer Review

Contributions

MPi, FP, FS and BR were the main investigators who designed the study and wrote the paper. FS, FP, CQ, ADG and BdD performed the molecular analyses. MPi, ADR, MPe and RC were responsible for the clinical care of analyzed patients. All the authors gave their critical contributiuon to the manuscript. BR and FS revised the paper and gave final approval for its submission. We thank Jean Ann Gilder for editing the manuscript. Primary responsibility for the paper: FP; primary responsibility for all Tables and Figures: CQ.

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received August 6, 2003; accepted October 2, 2003.

In the following paragraphs, Professor Cazzola summarizes the peer-review process and its outcomes.

What is already known on this topic

Chemotherapy can cause hepatitis flare-up through viral reactivation in patients who have had contact with hepatitis viruses. Few data are available on the genotype of the reactivated viruses.

What this study adds

Fludarabine alone or in combination with other (non-steroid) antineoplastic drug(s) was a powerful trigger of severe hepatitis due to HBV reactivation. A high incidence of genomic mutations was found in the strains of HBV responsible for the hepatitis flare-ups, and there was a high incidence of non responders to lamivudine in this small series of patients with HBV reactivation.