Impact of viral hepatitis therapy in multiple myeloma and other monoclonal gammopathies linked to hepatitis B or C viruses

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Abstract

Subsets of multiple myeloma (MM) and monoclonal gammopathies of undetermined significance (MGUS) present with a monoclonal immunoglobulin specific for hepatitis C virus (HCV), thus are presumably HCV-driven, and antiviral treatment can lead to the disappearance of antigen stimulation and improved control of clonal plasma cells. Here we studied the role of hepatitis B virus (HBV) in the pathogenesis of MGUS and MM in 45 HBV-infected patients with monoclonal gammopathy. We analyzed the specificity of recognition of the monoclonal immunoglobulin of these patients and validated the efficacy of antiviral treatment (AVT). For 18 of 45 (40%) HBV-infected patients, the target of the monoclonal immunoglobulin was identified: the most frequent target was HBV (n=11), followed by other infectious pathogens (n=6) and glucosylsphingosine (n=1). Two patients whose monoclonal immunoglobulin targeted HBV (HBx and HBcAg), implying that their gammopathy was HBV-driven, received AVT and the gammopathy did not progress. AVT efficacy was then investigated in a large cohort of HBV-infected MM patients (n=1367) who received or did not receive anti-HBV treatments and compared to a cohort of HCV-infected MM patients (n=1220). AVT significantly improved patient probability of overall survival (P=0.016 for the HBV-positive cohort, P=0.005 for the HCV-positive cohort). Altogether, MGUS and MM disease can be HBV- or HCV-driven in infected patients, and the study demonstrates the importance of AVT in such patients.

Introduction

Multiple myeloma (MM) is characterized by clonal expansion of transformed plasma cells in the bone marrow. MM is preceded by pre-malignant stages including asymptomatic monoclonal gammopathy of undetermined significance (MGUS) and/or smoldering myeloma (SMM).[1-5] A minority of MGUS evolves over time toward overt MM. Unfortunately, despite great advances in the understanding and treatment of this disease, MM remains incurable.

The primary function of plasma cells is to produce immunoglobulins (Ig) that mediate humoral immunity against infection. When there is an infection, Ig-secreting plasma cells differentiate from the naïve B cells that recognize foreign antigens. This process takes place in the germinal centers of secondary lymphoid organs where B cells undergo proliferation and somatic hypermutations, followed by the selection of B cells with high antigen affinity. In MGUS, SMM and particularly MM, clonal plasma cells secrete large quantities of a single Ig (monoclonal Ig),

which serves as a marker of the disease and triggers many of the symptoms.[1-6]

Latent infection and chronic antigen stimulation are now recognized as initial pathogenic events leading to chronic lymphocytic leukemia (CLL) and lymphoma.[7-11] B-cell receptor (BCR) signaling is central to the specific recognition of Igs, suggesting that specific antigens could be involved in the development of CLL. For instance, CLL may express stereotypic BCR specific for β-(1,6)-glucan, a major component of yeasts and fungi of the microbiota.[8] BCR stimulation from these antigens results in proliferation and increased cell survival.

Several studies support chronic antigenic stimulation as a pathogenic mechanism in subsets of MGUS and MM.[12-22] Associations between MM and viral infection, particularly hepatitis C virus (HCV), human immunodeficiency virus (HIV), or Epstein-Barr virus (EBV) were reported.[12-20] In addition, Nair *et al*. identified glucosylsphingosine (GlcSph) as the target of monoclonal Ig in the context of Gaucher's disease and also in sporadic gammopathies.[21,22] This pathogenic model opened new possibilities of treatment for MGUS, SMM and MM: if the target of a patient's monoclonal Ig is identified and can be eliminated, chronic antigen-stimulation disappears, leading to the control of clonal plasma cells. The efficacy of target antigen reduction therapy has been proven, first, for MGUS and SMM linked to GlcSph, and recently, for MGUS and MM linked to HCV.[23-25] In HCV-infected patients whose monoclonal Ig reacted against HCV, treating the HCV infection improved MGUS and MM disease.[25]

In the present study, we first explored the role of hepatitis B virus (HBV) in patients infected with HBV and diagnosed with monoclonal gammopathy. We analyzed the reactivity of the monoclonal Ig of patients against HBV, and the efficacy of treating the viral infection on MGUS and MM disease outcome. We found that for 36.7% of the 30 HBV-infected (HBV^+) patients for whom the monoclonal Ig could be analyzed, the target was HBV, suggesting that HBV infection initiated the clonal gammopathy. The efficacy of antiviral therapy was confirmed in a second set of studies performed on large cohorts of MM patients with a history of HBV or HCV infection prior to the diagnosis of MM.

Methods

Patients

A first cohort included 45 patients with monoclonal gammopathy who had been infected with HBV. In this cohort, different assays were performed with the aim of identifying the target of the monoclonal Ig of patients. Thirty patients were followed at the Centres Hospitaliers Universitaires (CHU) of Paris Saint Antoine (n=26) or Tours (n=4), France, and 15 patients at the Hospital 12 de Octubre, Madrid, Spain. Most patients were diagnosed with MGUS (n=14) or MM (n=29). Biological and clinical data were available at the time of diagnosis for 31 patients (8 MGUS, 23 MM) (*Online Supplementary Table S1)*. The studies were approved by our Institutional Review Boards and the patients provided written informed consent in accordance with the Declaration of Helsinki. MGUS or MM disease status was monitored by the quantification of monoclonal Ig or total Igs or free kappa/lambda light chain levels in serum. Protein and monoclonal Ig levels were visualized by serum protein electrophoresis and/or immunofixation electrophoresis.[25,26]

A second and a third cohort included patient data obtained from TriNetX, LLC (TriNetX) including data from subjects from healthcare organizations (HCO) all over the world with an MM diagnosed between the last 3 to 20 years from now, after a diagnosis of HBV or HCV infection, who had or had not received antiviral treatments. Cohorts were queried in TriNetX on March 22nd, 2022, and two large cohorts of MM patients found to have been infected by HBV (HBV^+, n=1367) or HCV (HCV^+, n=1220) were collected. Patients were classified into four groups: infected MM patients who received antiviral treatment (against HBV, n=175; or against HCV, n=179), and those who did not (n=1192 for HBV^+ patients; n=1041 for HCV^+ patients). The characteristics of these patients are summarized in Table 1, and the flowchart of the study is represented in Figure 1A.

Separation of monoclonal Ig from non-clonal Ig

For each patient, agarose gel electrophoresis and purification of the monoclonal Ig from other Ig present in serum samples were performed as published previously[17-20,27] and detailed in the *Online Supplementary Appendix*.

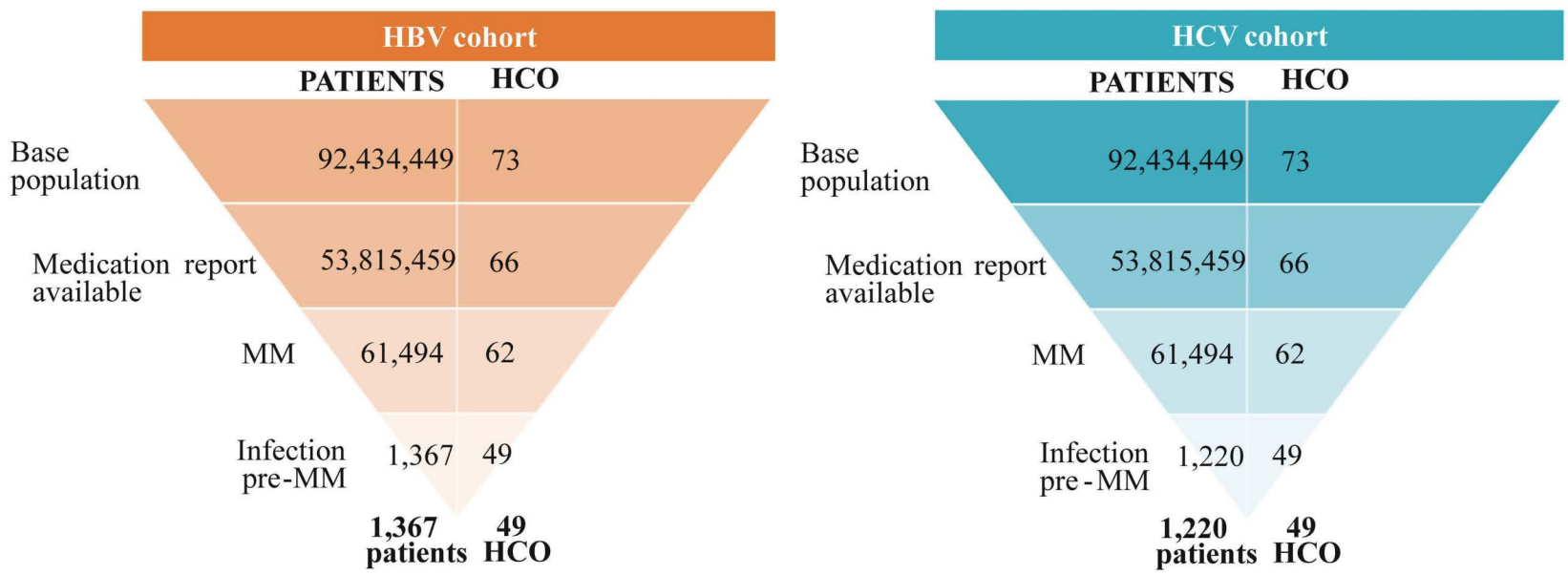
Analysis of the specificity of antigen recognition of monoclonal Ig

We and others previously reported that GlcSph, a glucolipid, and at least eight infectious pathogens could be the targets of monoclonal Ig from MGUS and MM patients.[12-22] Thus three different assays were used to determine the target of monoclonal Ig of HBV^+ patients, after purification of each monoclonal Ig : i) a GlcSph immunoblot assay, to determine whether a monoclonal Ig recognizes GlcSph; ii) the multiplex infectious antigen microarray (MIAA) assay, to determine whether purified monoclonal Ig recognize one infectious pathogen among different pathogens; and iii) dot blot assays designed to confirm that HBV or other pathogens, proteins or Ag, were recognized by the purified monoclonal Ig.[17-20,27,28] All assays are detailed in the *Online Supplementary Appendix*.

Statistical analysis

Data analysis was performed by GraphPad Prism 6.01 software and for the large HBV^+ and HCV^+ MM cohorts with

A



B

		Patients in cohort	Patients with outcome	Survival probability at end of time window
HBV cohort	Global MM post HBV infection with **AVT**	175	36	77.91%
	Global MM post HBV infection **no AVT**	1192	329	68.41%
	Log-Rank Test	**χ2** 5.786	**df** 1	***P*** 0.016
HCV cohort	Global MM post HCV infection with **AVT**	179	33	80.46%
	Global MM post HCV infection **no AVT**	1041	253	70.78%
	Log-Rank Test	**χ2** 8.026	**df** 1	***P*** 0.005

C

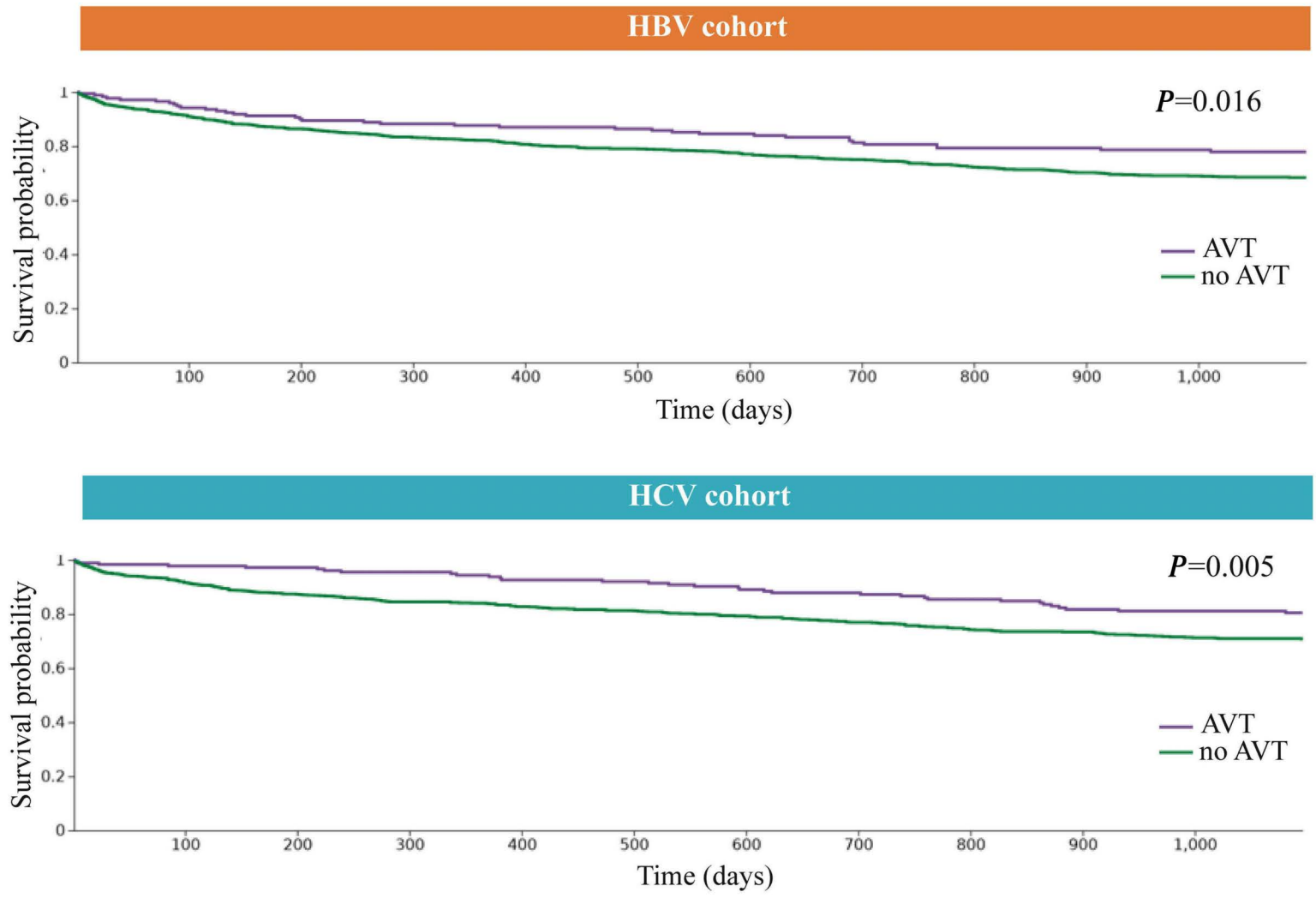


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Figure 1. The effect of antiviral treatment in hepatitis C virus- and hepatitis B virus-infected multiple myeloma cohorts. (A) Study design and flowchart of hepatitis B virus (HBV)$^+$ (orange) and hepatitis C virus (HCV)$^+$ (blue) cohorts. A total of 1,367 (HBV$^+$) or 1,192 (HCV$^+$) patients from 49 out of the 73 healthcare organizations (HCO) of the TriNetX network were included in the cohort of patients diagnosed with multiple myeloma (MM) after HBV or HCV infection. Anti-viral treatments (AVT) were tenofovir disoproxil, lamivudine, peginterferon alfa-2a, interferon alfa-2b, tenofovir alafenamide, entecavir. Anti-HCV treatments were elbasvir, grazoprevir, glecaprevir, pibrentasvir, sofosbuvir, velpatasvir, voxilaprevir, or combinations of these drugs. (B) Survival analysis of the HBV$^+$ and HCV$^+$ cohorts. Number of patients and log-rank test in each cohort, with outcome and survival probability at the end of time window in HBV or HCV cohorts. df: degree of freedom. (C) Kaplan-Meier plots comparing overall survival since the time of MM diagnosis of patients with HBV or HCV infection who received AVT (purple) or not (green). Since no differences in age or sex among groups of AVT treated *versus* untreated patients were observed, analyses were performed without propensity score matching (HBV$^+$ patients: P=0.270; HCV$^+$ patients: P=0.466).

Table 1. Characteristics of patients diagnosed with multiple myeloma post hepatitis B virus or hepatitis C virus infection, depending on receiving or not receiving antiviral therapy.

	HBV$^+$ Cohort		HCV$^+$ Cohort	
	AVT	No AVT	AVT	No AVT
Sex				
N	175	1,192	179	1,041
Male/female (%)	73.7	60.1	65.9	64.8
Age at diagnosis				
N	175	1,192	179	1,041
Mean ± SD	59.8 ± 10.4	61 ± 13.1	59.5 ± 8.9	60.2 ± 12.7
Leukocytes (x10^9/L)				
N	155	1,077	168	882
Mean ± SD	6.46 ± 5.58	12.6 ± 13.4	6.26 ± 4.79	13.6 ± 16.8
Hemoglobin (g/dL)				
N	164	1,125	175	946
Mean ± SD	11.4 ± 3.08	11.1 ± 2.67	11.4 ± 2.57	10.9 ± 2.75
Platelets (x10^9/L)				
N	166	1,101	176	916
Mean ± SD	158 ± 96.8	179 ± 102	161 ± 87.9	174 ± 100
Calcemia (mmol/L)				
N	159	1,091	175	890
Mean ± SD	2.25 ± 0.23	2.25 ± 0.21	2.27 ± 0.20	2.23 ± 0.23
Creatinine (μmol/L)				
N	164	1,117	175	937
Mean ± SD	173 ± 214	260 ± 906	250 ± 933	247 ± 715
Bone lesions				
N	175	1,192	179	1,041
N, with lesions	61	449	78	379

N: number of patients; SD: Standard Deviation; AVT: antiviral treatment; HBV: hepatitis B virus; HCV: hepatitis C virus.

the TriNetX Platform. All analyses are described in the *Online Supplementary Appendix*.

Results

Determination of the target of purified monoclonal Ig of hepatitis B virus-positive patients

Serum samples were collected from 45 HBV$^+$ patients with a monoclonal Ig (13 MGUS, 1 POEMS syndrome, 30 MM, 1 plasma cell leukemia [PCL]) and 3 control MM patients vaccinated against HBV. MGUS monoclonal Ig were 10 IgG, 1 IgA and 2 IgM. MM monoclonal Ig were 20 IgG, 8 IgA, 1 IgD, and light chains in one case. The POEMS patient had a monoclonal IgA and the PCL patient, a monoclonal IgG. The characteristics of HBV$^+$ patients are presented in *Online Supplementary Table S1*. We were able to purify 30 monoclonal Ig (27 IgG, 2 IgA, 1 IgM) from 8 MGUS and 22 MM HBV-infected patients (*Online Supplementary Figure S1*).

The specificity of recognition of the purified monoclonal Ig was analyzed using the GlcSph, MIAA and dot blot assays. These studies identified the target of 20/30 (66.7%) monoclonal Ig (18 IgG, 1 IgA, 1 IgM) from 18 HBV-infected patients and 2 patients vaccinated against HBV. Six of these patients were diagnosed with MGUS, and 14 with MM. The targets of monoclonal Ig were infectious pathogens for 18 patients (6 MGUS, 12 MM) and GlcSph for 2 MM patients (Table 2). These results were confirmed by

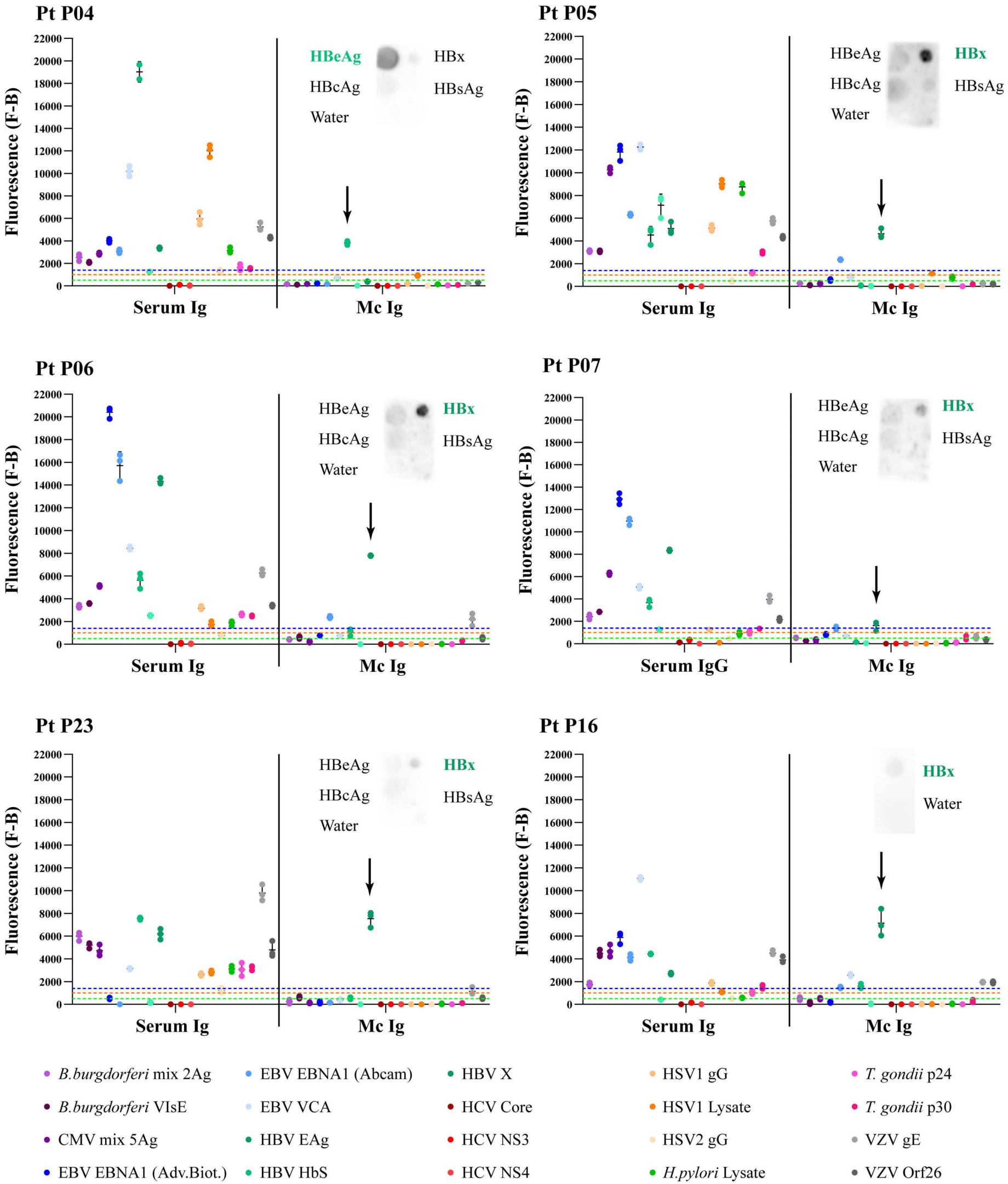

Figure 2. Results of the MIAA and dot blot assays of six hepatitis B virus-specific monoclonal Ig. For each patient (Pt), results obtained in parallel with the unseparated serum IgG (serum, left) and the patient's purified monoclonal IgG (Mc IgG, right) using the MIAA assay are represented; results are shown as fluorescent intensity (FI). The FI values shown for each pathogen, Ag, protein or lysate, were obtained after subtraction of the fluorescent background (F-B) of each pathogen protein or lysate. Dotted

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lines show thresholds of specific positivity defined for each viral pathogen or protein: 1,400 for Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus (VZV), hepatitis B virus (HBV) and *B. burgdorferi* (blue); 1000 for herpes simplex virus (HSV)-1 and HSV-2 (orange); 500 for hepatitis C virus (HCV) and *T. gondii* (green). Dots may be superimposed; horizontal bars represent means of results obtained for a pathogen (Ag, lysate). Experiments were performed in triplicate, repeated at least once. Inserts show the results of dot blot assays performed with purified recombinant HBV proteins (HBsAg, HBeAg, HBcAg, HBx) and water as control. The assays showed that the Mc IgG (or IgM, Pt M13) recognized a single HBV protein. Experiments were performed at least twice.

Table 2. Identified targets of purified monoclonal Ig from HBV-infected or -vaccinated patients with monoclonal gammopathy.

Patients	Diagnosis	Mc Ig type	Anti-HBs/HBc/HBe Ab in serum	Target of Mc Ig	AVT	Decrease in Mc Ig	Disease progression
Infected							
Pt M02	MM	IgGλ	Anti-HBc, Anti-HBs	HSV-1	No	-	Yes
Pt M13	MGUS	IgMκ	ND	HBx	Yes (entecavir)	No	No
Pt M14	MM	IgGκ	Anti-HBc	*H. pylori*	No	-	Yes
Pt M18	MGUS	IgGλ	Anti-HBc, anti-HBe, HBsAg	HBcAg	Yes (entecavir)	-	No
Pt M25	MGUS	IgGκ	Anti-HBe*	EBV EBNA-1	Yes (entecavir)	No	Yes
Pt P01	MM	IgGλ	Anti-HBc, anti-HBe*	GlcSph	No	-	-
Pt P04	MGUS	IgGκ	Anti-HBe, Anti-HBc	HBeAg	No	-	-
Pt P05	MM	IgG	Anti-HBc	HBx	No	-	-
Pt P06	MM	IgG	Anti-HBs, anti-HBc, anti-HBe*	HBx	No	-	-
Pt P07	MM	IgGκ	Anti-HBs	HBx	No	-	-
Pt P08	MGUS	IgGλ	Anti-HBe*	EBV EBNA-1	Yes (unknown)	-	-
Pt P11	MM	IgG	Anti-HBs,* anti-HBe*	EBV EBNA-1	No	-	-
Pt P12	MM	IgGκ	Anti-HBe*	HSV-1 gG	No	-	-
Pt P14	MM	IgGκ	Anti-HBc, anti-HBe*	HBx	No	-	CR
Pt P16	MM	IgGκ	Anti-HBc, anti-HBe*	HBx	No	-	Yes
Pt P23	MM	IgGκ	Anti-HBe*	HBx	No	-	-
Pt P24	MM	IgGκ	Anti-HBc	HBcAg	No	-	CR
Pt P29	MGUS	IgGλ	Anti-HBs,* anti-HBe*	HBeAg	No	-	-
Vaccinated							
Pt P13	MM	IgGκ	Anti-HBs	GlcSph	NA	-	-
Pt P17	MM	IgAκ	Anti-HBs	Coxsackievirus VP1	NA	-	-

Information on the presence of anti-HBs/HBc/HBe Ab in serum was obtained from hospital laboratories, except when labelled * (data obtained from the MIAA assay). Ab: antibodies; AVT: antiviral therapy; CR: complete remission of MM; MM: multiple myeloma; MGUS: monoclonal gammopathy of undetermined significance; Mc Ig: monoclonal immunoglobulin; HBV: hepatitis B virus; EBV: Epstein Barr virus; EBNA: Epstein-Barr nuclear antigen; HSV-1: herpes simplex virus 1; *H. pylori: Helicobacter pylori*; GlcSph: glucosylsphingosine; ND: no data; NA: not applicable; Pt: patient.

immunoblot or dot blot assays (Figure 2, Figure 3, *Online Supplementary Figures S2* and *S3*). To summarize, four infectious pathogens were recognized by monoclonal Ig from HBV-infected patients: HBV in 11 cases (4 MGUS, 7 MM), EBV in 3 cases (2 MGUS, 1 MM), *H. pylori* in one MM case, and HSV-1 for 2 MM patients. Thus, HBV was the most frequent target of monoclonal Ig from HBV-infected patients (11/30 analyzed, or 36.7%). The HBV proteins targeted were protein X (HBx) (n=7), antigen e (HBeAg) (n=2), and the core protein (HBc) (n=2). For other HBV-infected patients, the targets of monoclonal Ig were EBV EBNA-1 (n=3), HSV-1 gG (n=2), *H. pylori* (n=1), and GlcSph (n=1). In addition, the purified monoclonal Ig from 3 MM patients (P13, P17, P18) vaccinated against HBV were also analyzed. The targets were GlcSph (P13), Coxsackievirus VP1 protein (P17), or unknown (P18). Altogether, the monoclonal Ig of 2 MGUS and 8 MM patients lacked an identified target after these analyses (*Online Supplementary Figure S4*). The targets of the monoclonal Ig of the PCL and POEMS patients were also not identified. As reported,[19,20] the percentage of monoclonal Ig of unknown specificity was higher for MM (10/22 or 45.5%) than for MGUS (2/8 or 25%) patients, but in this cohort the difference was not significant (P=0.3118 by χ^2 test).

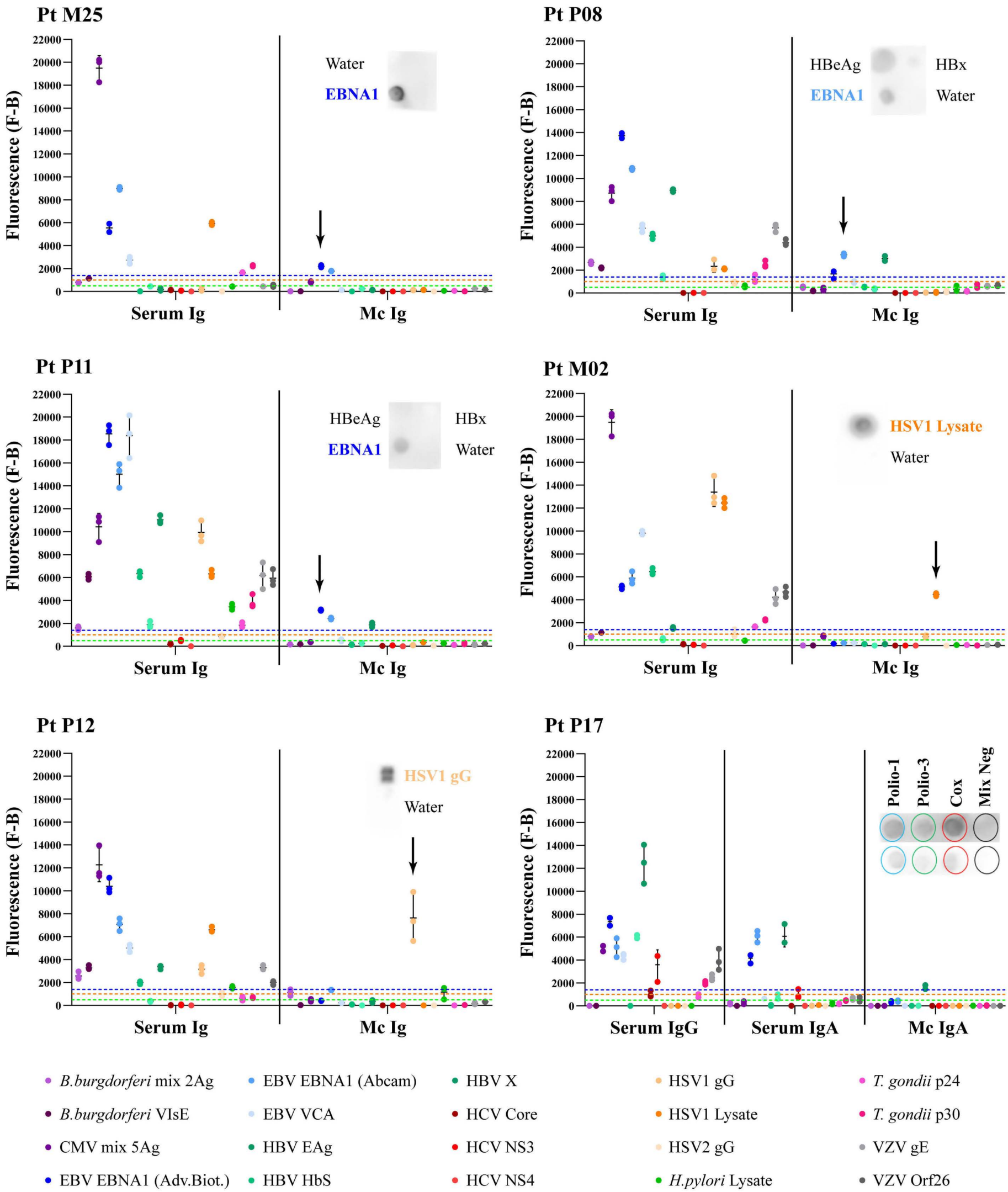

Figure 3. Results of the MIAA and dot blot assays of monoclonal Ig that target an infectious pathogen other than hepatitis B virus. For each patient (Pt), results obtained in parallel with the unseparated serum IgG (serum, left) and the patient's purified monoclonal IgG or IgA (Mc IgG or Mc IgA, right) using the MIAA assay are represented; results are shown as fluorescent intensity (FI). The FI values shown for each pathogen, Ag, protein or lysate, were obtained after subtraction of the fluorescent background

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(F-B) of each pathogen protein or lysate. Dotted lines show thresholds of specific positivity defined for each viral pathogen or protein: 1,400 for Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus (VZV), hepatitis B virus (HBV) and *B. burgdorferi* (blue); 1000 for HSV-1 and HSV-2 (orange); 500 for hepatitis C virus (HCV), *H. pylori* and *T. gondii* (green). Dots may be superimposed; horizontal bars represent the means of results obtained for a pathogen (Ag, lysate). Experiments were performed in triplicate, repeated at least once. Inserts show the results of dot blot assays performed with either purified recombinant proteins from EBV, herpes simplex virus (HSV)-1 or Enterovirus VP1, and water or PBS used as control. Experiments were performed at least twice.

Table 3. Summary of the characteristics of the cohorts in the hepatitis B virus analysis before and after matching.

Cohort		Mean ± SD	N of patients	% Cohort	*P*
1	Age at Index	59.8 ± 10.4	175	100	0.270
2	Age at Index	61.0 ± 13.1	1,192	100	

Cohort 1 is Global multiple myeloma (MM) post hepatitis B virus (HBV) infection with antiviral therapy (AVT), and cohort 2 is Global MM post HBV infection without AVT. SD: Standard Deviation.

Table 4. Summary of the characteristics of the cohorts in the HCV analysis before and after matching.

Cohort		Mean ± SD	N of patients	% Cohort	*P*
1	Age at Index	59.5 ± 8.9	179	100	0.466
2	Age at Index	60.2 ± 12.7	1,041	100	

Cohort 1 is Global multiple myeloma (MM) post hepatitis C virus (HCV) infection with antiviral therapy (AVT), and cohort 2 is Global MM post HCV infection without AVT. SD: Standard Deviation.

Evolution of monoclonal gammopathies of undetermined significance and multiple myeloma disease in patients with a hepatitis B virus-specific monoclonal Ig

Patient data and disease evolution were analyzed according to the target of the monoclonal Ig: HBV, other target, unknown target. For MGUS patients, HBV infection was anterior to the diagnosis of MGUS in all cases, and confirmed by the presence of antibodies against HBc, HBe or HBs (Table 2). MGUS patients P04 and P29, who presented with a monoclonal Ig specific for HBeAg, were both young (27 and 42 years old); unfortunately, follow-up information was not available for these patients. MGUS patients M13, M18, M25 and P08 received antiviral treatment. No progression was observed for patients M13 and M18, who both had a monoclonal Ig specific for a HBV protein (HBx for Pt M13, HBcAg for Pt M18). MGUS patients M25 and P08 had a monoclonal Ig specific for EBV EBNA-1, and anti-HBV treatment had no effect on their gammopathy. Patient M25 showed a strong increase in the amount of monoclonal Ig, and patient P08 progressed toward MM, now in third relapse.

Seven MM patients had a HBV-specific monoclonal Ig. Those patients for whom information was available presented at diagnosis with a low burden of MM disease (<15% plasma cells in bone marrow, β2-microglobulin <3.5 mg/L, hemoglobin (Hb) ≥10 g/dL, creatinine <100 μmol/L, ISS stage I-II), compared to patients with a monoclonal Ig of unknown specificity (*Online Supplementary Table S2*); however, differences were not significant. Unfortunately, none of these 7 patients had received antiviral treatment. After MM therapy, 2 patients (P14, P24) achieved complete remission (CR) for at least one year, and one patient (P16) progressed.

Overall survival of multiple myeloma patients who received anti-hepatitis B virus treatment

We then analyzed the TriNetX cohort of 1367 HBV$^+$ MM patients. The main characteristics of patients treated (or not) with antiviral drugs are summarized in Table 3; there was no significant difference between the treated and untreated cohorts. We analyzed the evolution of MM disease in patients post HBV infection: those who received antiviral treatment (tenofovir disoproxil, lamivudine, peginterferon alfa-2a, interferon alfa-2b, tenofovir alafenamide, entecavir) had an overall survival probability at 3 years of 77.91%, compared to 68.41% for patients who did not receive antiviral treatment (Figure 1B, C); this difference was statistically significant by log-rank test (P=0.016). Since we did not observe differences in age or sex among the two groups of patients (P=0.270), analyses were performed without propensity score matching.

Overall survival of multiple myeloma patients who received anti-hepatitis C virus treatment

For comparison, we analyzed the evolution of MM disease in 1,220 HCV-infected MM patients. Table 4 shows patients' characteristics; again, there was no significant difference between patients treated or not with antiviral drugs. MM patients who received anti-HCV treatments (elbasvir, gra-

zoprevir, glecaprevir, pibrentasvir, sofosbuvir, velpatasvir, voxilaprevir, or combinations of some of these drugs) had a high overall survival probability at 3 years (80.46%) compared to patients who did not (70.78%) (P=0.005 by log-rank test) (Figure 1B, C). Analyses were performed without propensity score matching since there was no difference in age or sex among the two groups of patients (P=0.466).

Discussion

For significant subsets of monoclonal gammopathies, including MM, the patient's monoclonal Ig specifically recognizes an infectious pathogen, which implies that the clonal gammopathy was initiated by chronic stimulation by an antigen from this pathogen.[12,13,17-20,29] Moreover, patients with HCV-driven MGUS or MM (when the monoclonal Ig targeted HCV) benefited from AVT: MGUS or MM disease evolution improved after anti-HCV therapy, including a refractory MM for whom antiviral therapy led to long-term complete remission.[25] The present study strengthens and extends these findings to MGUS and MM linked to HBV: for over a third of HBV$^+$ patients whose monoclonal Ig could be studied, the monoclonal Ig targets HBV, which implies that HBV initiated the gammopathy in these individuals. Moreover, we demonstrate that AVT improves the survival of HBV$^+$ MM patients. Our study also confirms in a large cohort that antiviral treatment improves MM outcome for HCV-infected patients.[24,25]

The targets of monoclonal Ig from HBV$^+$ MGUS and MM patients could be studied only in the first cohort of 45 patients, for whom 30 monoclonal Ig could be purified and analyzed. For 23.3% (7/30) HBV$^+$ MGUS and MM patients, the target of the purified monoclonal Ig was EBV, HSV-1, *H. pylori* or GlcSph; thus the gammopathy of these patients was not linked to HBV. For 36.7% (11/30) HBV$^+$ patients (4 MGUS, 7 MM), the monoclonal Ig specifically recognized a HBV protein, implying a role for HBV in the initiation of the gammopathy. These findings support early prescription of AVT, at the MGUS stage whenever possible. In western Europe, HBV-initiated gammopathies may not represent a large fraction of MGUS and MM, but the implication of HBV in MM pathogenesis may be more frequent in parts of the world where HBV infection is endemic (Eastern Europe, Asia, the Middle East, sub-Saharan Africa, South America).[30]

Previous studies showed that ≥80% monoclonal Ig of HCV$^+$ patients target HCV, especially the strongly immunogenic and oncogenic core protein. Regarding HBV, patients with controlled, inactive HBV infection present with antibodies directed at HBe, HBc, and HBs. Consistent with ancient, controlled HBV infection, at the time of MGUS or MM diagnosis patients had polyclonal antibodies against HBV (anti-HBe, HBc and/or HBs) in serum. However, monoclonal Ig of HBV$^+$ MM patients frequently targeted a lesser known viral protein, non-structural HBx. The genome of HBV, a DNA virus, contains four overlapping open reading frames (ORF) named S, P, C and X. The S ORF produces envelope proteins including HBsAg; the P and C ORF encode the HBV polymerase, core protein (HBc), and the HBeAg; and X ORF encodes Hbx.[31,32] HBx is commonly detected in hepatocytes of HBV-infected patients, and its expression correlates with viral replication.[33] Indeed, Hbx plays a central role in the initiation and maintenance of HBV replication, but also inhibits the host's immune response to the virus, and facilitates the development of hepatocellular carcinoma (HCC).[34,35]

The presence of anti-HBx IgG in the serum of individuals with chronic HBV infection is relatively frequent, and associated with the presence of anti-HBc IgG.[36,37] Several groups reported anti-HBx IgG in the serum of as many as 40.6% HBV-infected patients with liver cirrhosis (LC), and 5-34.4% of HBV-infected patients with HCC.[36,37] Thus anti-HBx IgG can be considered as markers of viral replication and HBV-mediated development of LC and HCC.[38] Chronic production of a large amount of clonal anti-HBx antibody could help control HBV infection. In this study, biological and clinical information was available for 3 MM patients with HBV-specific monoclonal Ig: at the time of MM diagnosis, none presented signs of active liver disease, and they had a mild form of MM disease (β2-microglobulin <3.5 mg/L, Hb ≥10 g/L, calcemia <2.6 mmol/L, no bone lesions, ISS I-II). Unfortunately, the evolution of these patients was unknown. More studies are necessary to establish whether the production of large amounts of anti-HBx IgG has beneficial effects on HBV infection and hepatic disease.

The second part of our studies was designed to investigate the effect of AVT on MM disease for HBV-infected MM patients, compared to HCV-infected MM patients. In both cohorts, individuals who received AVT fared significantly better after 1,000 days of evolution than MM patients whose HBV or HCV infection was not treated. The best effect of AVT was observed for HCV$^+$ patients, possibly because the anti-HBV treatments in this retrospective study do not totally eliminate HBV. HBV covalently closed circular DNA (cccDNA) remains in hepatocytes, and cccDNA persistence represents a therapeutic barrier in curing HBV infection.[39-41] Another reason is that MM is frequently driven by HCV in HCV$^+$ patients (with >80% of monoclonal Ig targeting HCV), whereas MM is presumably HBV-driven in "only" 36.7% HBV$^+$ patients. One limitation of this study is the lack of serum samples for the TriNetX cohorts, which made identifying the target of the monoclonal Ig in these cohorts impossible.

Altogether, clonal gammopathies of HBV-infected patients are frequently HBV-driven. This study demonstrates the importance of antiviral treatments for patients with HBV- or HCV-driven clonal gammopathies, including MM. Anti-

viral therapy should be prescribed as early as possible in the development of HBV- or HCV-linked clonal gammopathies, ideally at the MGUS stage. Patients with virus-initiated MGUS or MM can be identified via the analysis of the target of their monoclonal Ig.

Disclosures

No conflicts of interest to disclose.

Contributions

ARG, ML, JML, SH and EBC designed the research, analyzed data, and wrote the initial manuscript draft. NM, LB, SAM, EBC and ARG performed experiments and edited the manuscript. EP, LG, GHI, MLM, IM, DR and JML contributed patient samples and clinical data, and edited the manuscript. SH, ML and JML provided resources and financial support, and edited the manuscript. All authors gave final approval of the version to be submitted to publication and agreed to be accountable for all aspects of the work.

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Data-sharing statement

All data generated or analyzed during this study are included in this published article and its Online Supplementary Appendix. All source data are available on request to the corresponding authors.

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