marrow failure syndromes (eg, congenital amegakaryocytic thrombocytopenia), splenectomy will not result in clinically significant improvement. However, it is possible that for certain individuals a small change in circulating platelet number may improve hemostasis. For example, affected males with *GATA1* mutations in one family reported diminished frequency and severity of bleeding episodes in adulthood after splenectomy despite persistent thrombocytopenia.[1] It is not clear whether this change was due to splenectomy. I believe that splenectomy should be reserved for those rare individuals for whom hematopoietic stem cell transplantation is unavailable and the potential impact of a small absolute increase in circulating platelets is clinically significant.

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References

1. Mehaffey MG, Newton AL, Gandhi MJ, Crossley M, Drachman JG. X-linked thrombocytopenia caused by a novel mutation of *GATA-1*. Blood. 2001:98:2681-2688.

To the editor:

Association of HCV-related mixed cryoglobulinemia with specific mutational pattern of the HCV E2 protein and CD81 expression on peripheral B lymphocytes

Interaction of the hepatitis C virus (HCV) envelope (E) 2 protein with the cellular receptor CD81 leads to B lymphoproliferation in vitro, a major characteristic of mixed cryoglobulinemia (MC).[1] Within E2, 2 CD81 binding sites have been described in vitro comprising the hypervariable region 1(HVR1) and HVR2.[2,3] In patients with chronic hepatitis C, CD81 expression on peripheral B lymphocytes is increased.[4] We hypothesize, that specific amino acid (aa) sequences within E2 and CD81 expression on B lymphocytes may act as determinants for the development of MC in vivo.

There were 58 consecutive patients with chronic hepatitis C tested for MC. Cryoprecipitates were detectable in 14 (24.0%) of

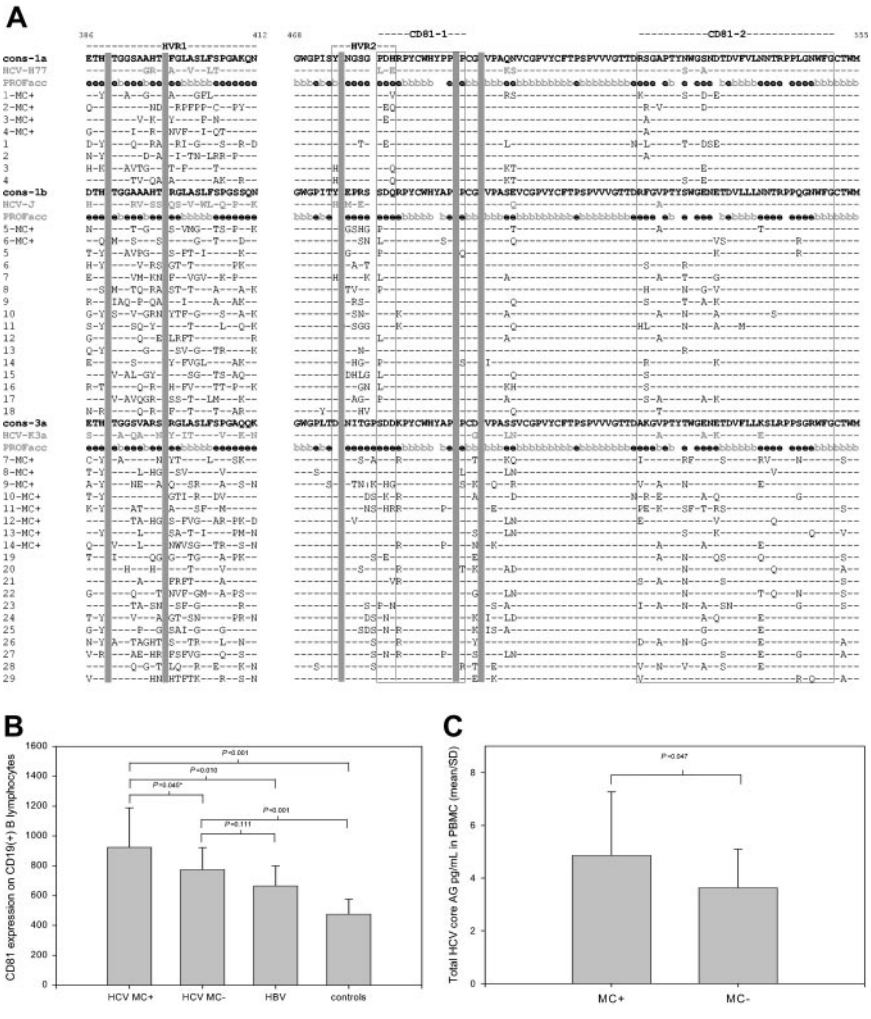

Figure 1. E2 amino acid mutations, CD81 expression, and HCV core antigen in patients with mixed cryoglobulinemia. (A) Sequence alignment of combined E2 aa residues 386 to 412 (HVR1) and 468 to 555 (including HVR2/CD81 binding sites) of isolates of HCV-1a– and HCV-1b–infected patients (numbering according to HCV-J) and aa residues 468 to 556 of HCV-3a–infected patients (numbering according to HCV-K3a) (n = 43). HVR1 was amplified by nested reverse transcriptase–polymerase chain reaction in all patients (n = 58), and HVR2/CD81 binding sites were amplified in 14 of 14 patients with MC and 29 of 44 patients without MC. Residues are indicated by standard single-letter codes. Dashes indicate residues identical to the consensus sequence calculated from all isolates investigated (cons-1a, cons-1b, and cons-3a). Vertical lines indicate the HVR2; uncolored boxes, the CD81 binding sites CD81-1 and CD81-2; and MC+, patients with mixed cryoglobulinemia type II. The prototype sequences for HCV-1a (HCV-H77), HCV-1b (HCV-J), and HCV-3a (HCVK3a) subtypes are shown as reference sequences. | indicates deletion at this position. Prediction of solvent accessibility (exposed [e], buried [b], unknown [gap]) shown on the basis of complete E2 aa sequence of HCV prototype HCV H77, HCV-J, and HCV K3a (www.embl-heidelberg.de/predictprotein). Gray boxes indicate aa positions identified by statistical learning classification algorithms that allow prediction of MC (www.csie.ntu.edu.tw/~cjlin/libsvm, and Durbin et al[6]), and prediction based on HVR1 aa sequences (n = 58) and HVR2/CD81 binding site sequences (n = 43) of the present study. There were 21 HVR1 sequences of MC-positive patients[7] found in the European Molecular Biology Laboratory database added for analyses (accession nos. AJ406073-AJ406149). (B) CD81 expression on CD19$^+$ B lymphocytes in relative fluorescence units (mean and upper SD error bar) measured by FACS analyses shown for all patients with (MC+) and without (MC−) mixed cryoglobulinemia, chronic hepatitis B (n = 7), and healthy controls (n = 10). *One-tailed *P* value. (C) Total HCV core antigen detection on PBMCs (pg/mL) measured by enzyme-linked immunosorbent assay (Quantitative Ortho trak-C assay; Ortho-Diagnostics, Neckargemünd, Germany) shown for MC-positive (MC+) and MC-negative (MC−) patients. Error bars represent means and upper SD.

58 patients and immunofixation showed MC type II in all patients. MC-positive patients did not differ from those without MC regarding age, alanine aminotransferase level, baseline viremia, and estimated duration of infection.

The E2 regions including HVR1 and HVR2/CD81 binding sites were sequenced and the obtained E2 aa sequences were analyzed according to physicochemical properties.[5] The hydrophobic pattern of the E2 regions was widely conserved and allowed no differentiation between patients with and without MC. Analyses of the solvent accessibility showed that the majority of mutations were located within the HVR1 and HVR2/CD81 binding sites at residues predicted on the surface of E2 (exposed), underlining the functional relevance of these regions (Figure 1A).[3] Furthermore, we evaluated the predictability of MC based on the E2 sequences and used adjusted classification algorithms composed of statistical learning procedures, probabilistic models, and empiric likelihood ratio tests.[6,8] These classification algorithms account for mutational frequencies and dependencies between different aa positions. Classification rates (CRs) and Matthews correlation coefficients (MCCs) were calculated using a nested cross-validation procedure, and the importance of individual aa positions was expressed as differences in MCCs in comparison with random permutations at this position. There were 2 HVR1 positions (389 and 398; CR, 67%, MCC, 0.32) and 3 HVR2/CD81-binding site positions (474, 493, and 497; CR, 72%, MCC, 0.29) correlated with the presence of MC (Figure 1A). The identified positions give reason for site-directed mutagenesis studies to evaluate their influence of the E2 binding affinity to CD81.

Fluorescence-activated cell sorter (FACS) analyses showed that CD81 expression on $CD19^+$ B lymphocytes, representing the vast majority of B lymphocytes, was significantly higher in patients with MC (923.7 ± 264.8) compared with those without MC (774.5 ± 147.3; $P = .045$, one-tailed), patients chronically infected with hepatitis B virus (665.4 ± 132.3; $P = .010$), and healthy controls (476.0 ± 100.5; $P < .001$; Figure 1B). These results underline the concept that CD81 exerts a specific role in the host interaction with HCV.[4] Higher CD81 expression was notably found in MC-positive patients infected with HCV-1a isolates possessing high binding affinity to CD81 in vitro ($P = .024$, data not shown).[3] B lymphoproliferation is thought to be driven by HCV-specific antigen in vitro.[9] Accordingly, HCV core antigen levels of peripheral blood mononuclear cells (PBMCs) were higher in MC-positive patients (4.86 ± 2.41 pg/mL) compared with those without MC (3.63 ± 1.46 pg/mL; $P = .047$; Figure 1C).

In conclusion, classification algorithms provide information about specific E2 positions correlated with MC. CD81 expression and HCV core antigen levels in PBMCs are increased in patients with MC.

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References

1. Cocquerel L, Kuo CC, Dubuisson J, Levy S. CD81-dependent binding of hepatitis C virus E1E2 heterodimers. J Virol. 2003;77:10677-10683.
2. Flint M, Maidens C, Loomis-Price LD, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J Virol. 1999;73:6235-6244.
3. Roccasecca R, Ansuini H, Vitelli A, et al. Binding of the hepatitis C virus E2 glycoprotein to CD81 is strain specific and is modulated by a complex interplay between hypervariable regions 1 and 2. J Virol. 2003;77:1856-1867.
4. Zuckerman E, Kessel A, Slobodin G, et al. Antiviral treatment down-regulates peripheral B-cell CD81 expression and CD5 expansion in chronic hepatitis C virus infection. J Virol. 2003;77:10432-10436.
5. Penin F, Combet C, Germanidis G, et al. Conservation of the conformation and positive charges of hepatitis C virus E2 envelope glycoprotein hypervariable region 1 points to a role in cell attachment. J Virol. 2001;75:5703-5710.
6. Durbin R, Eddy S, Krogh A, Mitchison G. Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids. Cambridge, United Kingdom: Cambridge University Press; 1998.
7. Gerotto M, Dal Pero F, Loffreda S, et al. A 385 insertion in the hypervariable region 1 of hepatitis C virus E2 envelope protein is found in some patients with mixed cryoglobulinemia type 2. Blood. 2001;98:2657-2663.
8. Cai CZ, Wang WL, Sun LZ, Chen YZ. Protein function classification via support vector machine approach. Math Biosci. 2003;185:111-122.
9. Quinn ER, Chan CH, Hadlock KG, et al. The B-cell receptor of a hepatitis C virus (HCV)-associated non-Hodgkin lymphoma binds the viral E2 envelope protein, implicating HCV in lymphomagenesis. Blood. 2001;98:3745-3749.

To the editor:

Induction of imatinib metabolism by hypericum perforatum

Hypericum perforatum (St John Wort) is an herbal preparation commonly used by cancer patients for mood elevation and is known to induce intestinal and hepatic cytochrome P450 3A4 (CYP3A4) and P-glycoprotein.[1] CYP3A4 is the major isoenzyme responsible for imatinib metabolism, making imatinib potentially susceptible to drug interactions with numerous compounds, including hypericum perforatum.

We evaluated the impact of hypericum perforatum on imatinib pharmacokinetics in an open label, fixed sequence, complete crossover study. There were 10 volunteers who received single doses of imatinib (400 mg) prior to and following 2 weeks of hypericum perforatum (300 mg) thrice daily. Imatinib plasma concentrations were determined at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after dosing by liquid chromatography (LC)–tandem mass spectrometry (MS/MS). Free imatinib concentrations were measured at each 3- and 24-hour sample by ultrafiltration.

Imatinib pharmacokinetics were significantly altered by hypericum perforatum (Table 1). The median area under the curve ($AUC_{0-\infty}$) decreased by 32% (28.9 vs 19.7 μg × h/mL), with maximum concentration (Cmax) and half-life reduced by 29% and 21%, respectively. Imatinib was approximately 95% protein bound

Table 1. Pharmacokinetics of imatinib when administered alone and with hypericum perforatum in 10 subjects

Period	Tmax, h	Cmax,* μg/mL	Half-life,* h	$AUC_{0-\infty}$,* μg × h/mL	C_{last},* μg/mL	Protein binding, %
Imatinib	2.5 (1-4)	1.80 (0.88-2.6)	13.5 (10.8-18.1)	28.9 (13.4-36.7)	0.11 (0.05-0.17)	95.0 (90.3-97.7)
Imatinib + hypericum perforatum	2.5 (1-8)	1.28 (0.92-2.0)	10.7 (9.1-13.5)	19.7 (10.7-25.0)	0.05 (0.03-0.12)	95.1 (90.9-97.3)

Data presented as median (range). Tmax indicates time of Cmax; C_{last}, imatinib concentration at 48 hours.
*$P < .005$ by paired *t* test on logarithmically transformed data.