SUPPLEMENTAL DATA

Supplemental Methods

Patients

Sixteen patients with EMC were included in the study. Inclusion criteria were: a) detectable cryoglobulinaemia with type II or type III MC after immunochemical typing and b) systemic vasculitis with at least one clinical manifestation (purpura, cutaneous ulcers or necrosis, arthralgia or arthritis, peripheral neuropathy, renal involvement, sicca syndrome and gastrointestinal involvement).

BCR clonality assessment

BCR clonality for heavy and light chain rearrangements was assessed according to the "Biomed-2 concerted action for PCR-based clonality" guidelines (van Dongen *et al*., *Leukemia* 2003; 17: 2257-317). For immunoglobulin heavy chain clonality analysis, variable framework regions VH-FR1, 2 and 3 family primers were used in combination with one reverse JH consensus primer in multiplex PCR reactions; for light chain clonality analysis, VK consensus family primers (VK1-7) were used in combination with reverse JK(1-5) consensus family primers (or with INTR/ Kde for Kde rearrangements), and VL (VL1-3) consensus family primers in combination with a reverse JL consensus primer in multiplex PCR reactions. To discriminate monoclonal lymphoid cells with identical junctional regions from polyclonal lymphoid cells with highly diverse junctional regions, heteroduplex analyses were performed; PCR products were denatured at high temperature (95° for 5 min) and then a rapid random renaturation at low temperature (4° for 1 h) resulted in the formation of many different heteroduplexes (polyclonal lymphoproliferation) detected as a smear and in homoduplexes (monoclonal lymphoproliferation) detected as a single band with a predictable size range in non-denaturing 10% polyacrylamide gels. Monoclonal bands were extracted and incubated overnight in gel elution buffer (0.5M ammonium acetate, 10 mM Mg acetate tetrahydrate, 1mM EDTA), and PCR products were purified using DNA Clean and Concentrator (Zymo Research) and directly sequenced on an ABI PRISM 377-96 instrument (Perkin Elmer, Foster City, CA, USA).

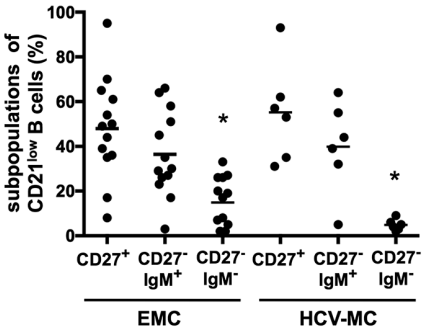
Sequence analysis of V(D)J functional rearrangements

We used the ImMunoGeneTics (IMGT) sequence alignment software to analyze the nucleotide sequences obtained and the deduced amino acid sequences from the VH, VK, VL and complementarity determining region 3 (CDR3) segments. Stereotyped CDR3 sequences were defined according to established criteria (Messmer *et al*., *J Exp Med* 2004; 200: 519-25; Darzentas N, Stamatopoulos K, *Methods Mol Biol* 2013; 971: 135-48). The amino acid sequences deduced from the VH, VK, VL and CDR3 DNA sequences

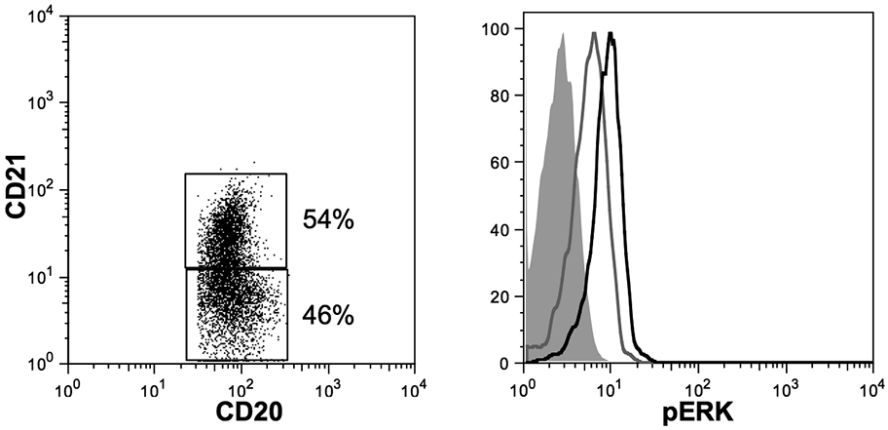
Supplemental Table 1. Demographic data and B cell functional characteristics of HD compared to patients with EMC and HCV-MC.

Variables	HD	EMC	HCV-MC
Number of patients	20	16	24
Age (y)*	66 (40-82)	74 (56-83)	68 (40-84)
Female sex	12 (60%)	8 (50%)	16 (67%)
B cells/μL**	248 ± 108	191 ± 232	529 ± 795
CD21^{low} B cells (%)**	6.9 ± 4	22 ± 15	54.5 ± 18
Proliferation**			
CpG	39 ± 6.5	27.7 ± 15.2	16 ± 14.4
CpG + a-Ig	72.6 ± 7.4	65.7 ± 24.6	27.6 ± 18.2
pERK**	3.4 ± 1.2	5.4 ± 2.4	7 ± 2.6
Apoptosis**	4.6 ± 1.1	10.2 ± 3	15 ± 11.2

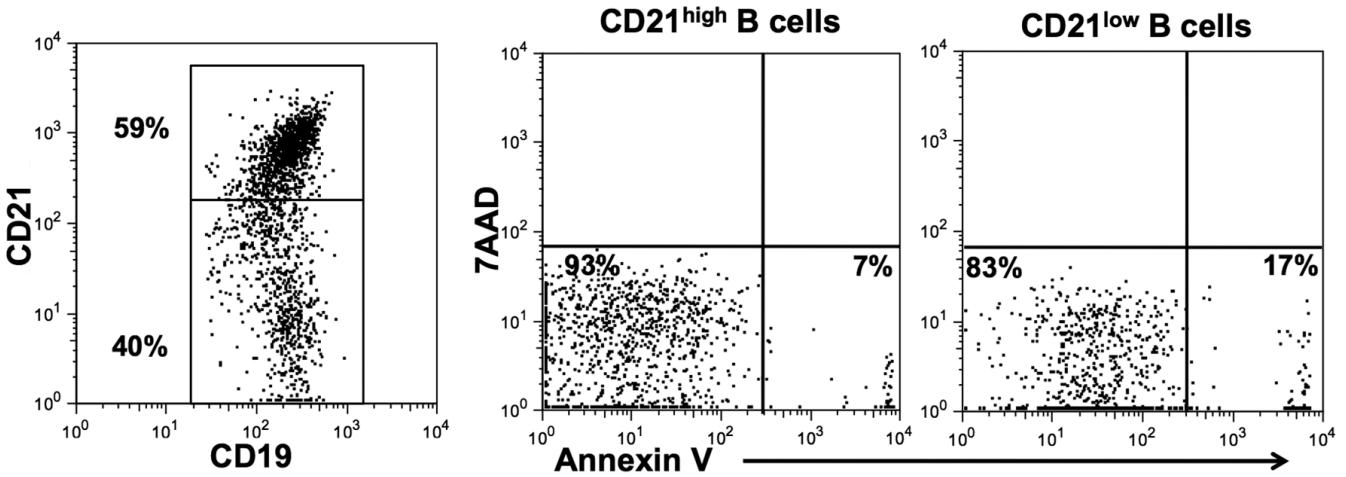
HD: healthy donor; EMC: essential mixed cryoglobulinaemia; HCV-MC: hepatitis C virus-mixed cryoglobulinaemia; pERK: phosphorylated extracellular signal-regulated kinase.
*Median (range); **mean±standard deviation (SD).



Supplemental Fig. 1. An unusual population of CD21^{low}CD27^{neg}IgM^{neg} switched B-cells is expanded in EMC and not in HCV-MC (*p=0.0475).



Supplemental Fig. 2. Representative experiment showing higher constitutive expression of phosphorylated ERK (pERK) in the CD21^{low} rather than in the CD21^{high} B-cells of a patient with EMC. Unstimulated PBMC were fixed/permeabilised and stained with CD20, CD21 and anti-pERK1/2-Alexa 488 (Becton-Dickinson) or control mouse IgG-Alexa 488, and electronically gated CD20^{+}CD21^{low} and CD20^{+}CD21^{high} cells were separately analysed. The solid histogram denotes control mouse IgG fluorescence; gray line and black line histograms denote anti-pERK1/2 fluorescence in CD21^{high} and CD21^{low} B-cells, respectively.



Suppplemental Fig. 3. Representative experiment showing accelerated spontaneous apoptosis in the $CD21^{low}$ rather than in the $CD21^{high}$ B-cells of a patient with EMC. PBMC were cultured over-night and stained with CD19, CD21, Annexin V and 7AAD and electronically gated $CD19^{+}CD21^{high}$ and $CD19^{+}CD21^{low}$ cells were separately analysed for Annexin V and 7AAD staining.

were analysed by the NCBI Basic Local Alignment Search Tool (BLAST) pairwise comparison program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find sequence homologies with rheumatoid factors from GenBank (accession numbers for IGHV: RF-WOL, 0707281c; RF-WA, AAA03467.1; RF-MR-20, AAB58436.1; RF-BOR, 1313976A; accession numbers for IGKV: RF-CUR, 1206991A; RF-FLO, 1206991B; RF-WA, AAA03468.1; RF-4C9, AAC41918.1).