Supplementary information

Methodology

Mice and experimental in vivo procedures – B cell depletion is achieved in hCD20Tg mice upon intraperitoneal injection of 1mg/mouse of purified monoclonal mouse anti-hCD20 (clone 2H7) [1]. Three days after injection, B cell depletion was confirmed by flow cytometry analysis of peripheral blood cells. In some experiments, purified splenic B cells ($30X10^6$ cells/mouse) were adoptively transferred to B-cell depleted mice by intravenous injection. For inducible ablation of recombination activating gene 2 (RAG-2) in some experiments, we used Rag-2fl/fl mice [2], crossed with Mx-cre transgenic mice [3], where ablation of the floxed alleles *in vivo* is obtained upon administration of poly(I)-poly(C) on days 0, 3 and 7 [4]. In some experiments, mice were injected subcutaneously with recombinant hIGF1 (2mg/kg) or hGH (0.36mg/kg) twice a day for 10 days. Control group received PBS only. Mice were maintained at the Animal Care-certified facility at the Faculty of Medicine, Technion (ISRAEL) and all studies were approved by the institute's committee for the supervision of animal experiments.

Flow Cytometry and cell stain for mouse and human cells - Single-cell suspensions, following red blood cell lysis, from mouse BM and spleen were stained with fluorescently labeled antibodies for surface proteins to identify specific B cell subsets. Antibodies used were anri-CD93-APC (AA4.1), anti-B220-PB (RA3-6B2), anti-CD23-PE (B3B4), anti-CD43-PE-Cy7 (S11) (all from BioLegend), anti-IgM-FITC (μ-chain specific) from Jackson and anti-CD21-BV421 (7G6) from eBioscience. Human PBMCs were isolated by Ficoll® density gradient centrifugation per standard protocol and stained with the following antibodies: anti-CD38-PE (HB7), anti-CD24-APC (ML5) (from BioLegend) anti-CD19-FITC (4G7) (from BD). Data was acquired on LSRFortessa (BD Pharmingen) and analyzed using FlowJo software (Tree Star).

Human blood collection and clinical study details – For the clinical study IRB 0643-15 RMB to determine the role of TNFα/anti-TNFα treatments in regulating B lymphopoiesis in humans, we recruited patients with inflammatory joint diseases (IJD): rheumatoid arthritis (RA), psoriatic arthritis (PsA), and spondyloarthropathy (SPA). Exclusion criteria included another inflammatory joint disease or immunomodulatory conditions such as positivity to hepatitis C, hepatitis B or HIV. Blood samples were taken from patients naive to anti-TNFα therapy (IJD-NB) and from patients undergoing anti-TNFα therapy for at least three months (cross-sectional group, IJD-TNF). A subset of patients was analyzed upon initiation of anti-TNFα therapy and three months later (longitudinal group) and therefore are represented in the both, IJD-NB and IJD-TNF groups. The control group

consisted of patients with no inflammatory conditions; healthy volunteers or patients with degenerative osteoarthritis were included. Patients enrolled in the cross-sectional analysis belong to the groups control, IJD-NB, and IJD-TNF. Patients enrolled in the longitudinal, prospective cohort study also belong to the groups IJD-NB and IJD-TNF. These patients serve as their own controls as their blood samples were analyzed before and at least three months after initiation of anti-TNFα therapy. After collection, fresh blood samples were immediately transferred to the immunology laboratory and analyzed to quantify transitional B cells and soluble molecules in plasma.

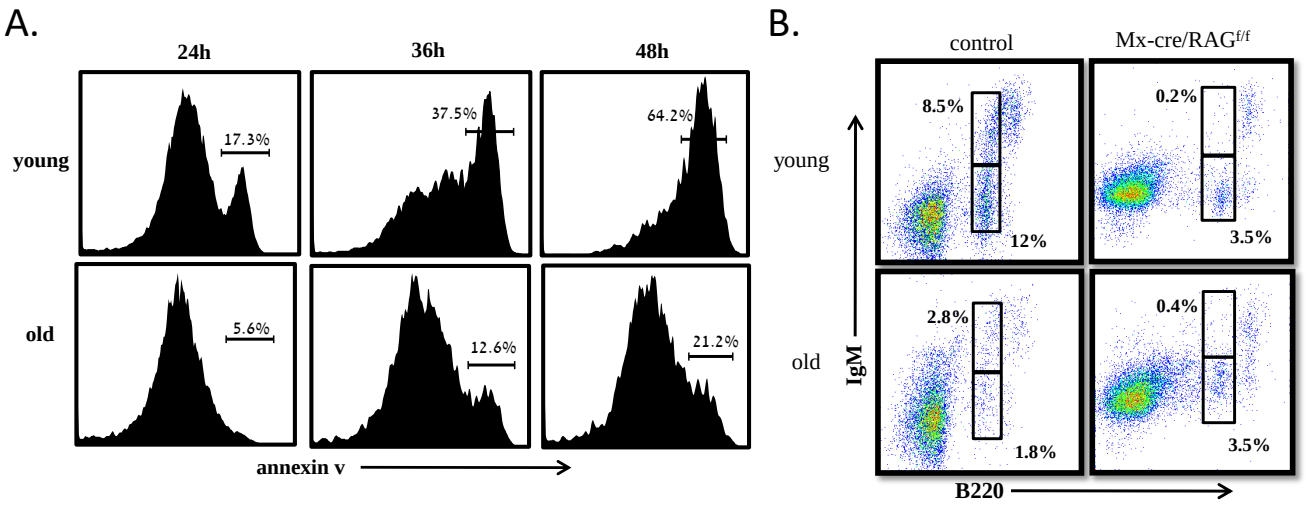
Quantitative PCR - Expression of TNFα mRNA in B cells was determined by quantitative PCR using Sybr Green MasterMix (BioRad) and a BioRad CFX connect Real-Time PCR [22]. Samples were normalized with GAPDH gene amplification and DATA were analyzed using the CFX Manager Software. The primer sequences are:

	Primer name	Sequence	Length
Mouse	TNFα- FW	CCCTCACACTCAGATCATCTTCT	23
	TNFα- REV	GCTACGACGTGGGCTACGA	19
	GAPDH - FW	TGACCACAGTCCATGCCATC	20
	GAPDH - REV	GACGGACACATTGGGGGTAG	20
Human	TNFα FW	GAGGCCAAGCCCTGGTATG	19
	TNFα REV	CGGGCCGATTGATCTCAGC	19
	GAPDH - FW	ATGGGGAAGGTGAAGGTCG	19
	GAPDH - REV	GGGGTCATTGATGGCAACAATA	22

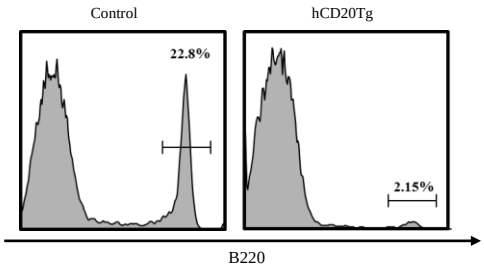
Serum and plasma analysis for IGF1, IGFBP-1 and TNFα:

Levels of IGF1, IGFBP-1 and TNFα in mouse sera and human plasma were determined by enzyme-linked immunosorbent assay (ELISA). For human and mouse IGF1, a sandwich ELISA was set using capture antibodies (Goat Anti-Murine IGF1, or Rabbit Anti-Human IGF1 (1µg/ml)) and detecting antibodies (biotinylated anti-Murine IGF1, or biotinylated Anti-Human IGF1), according

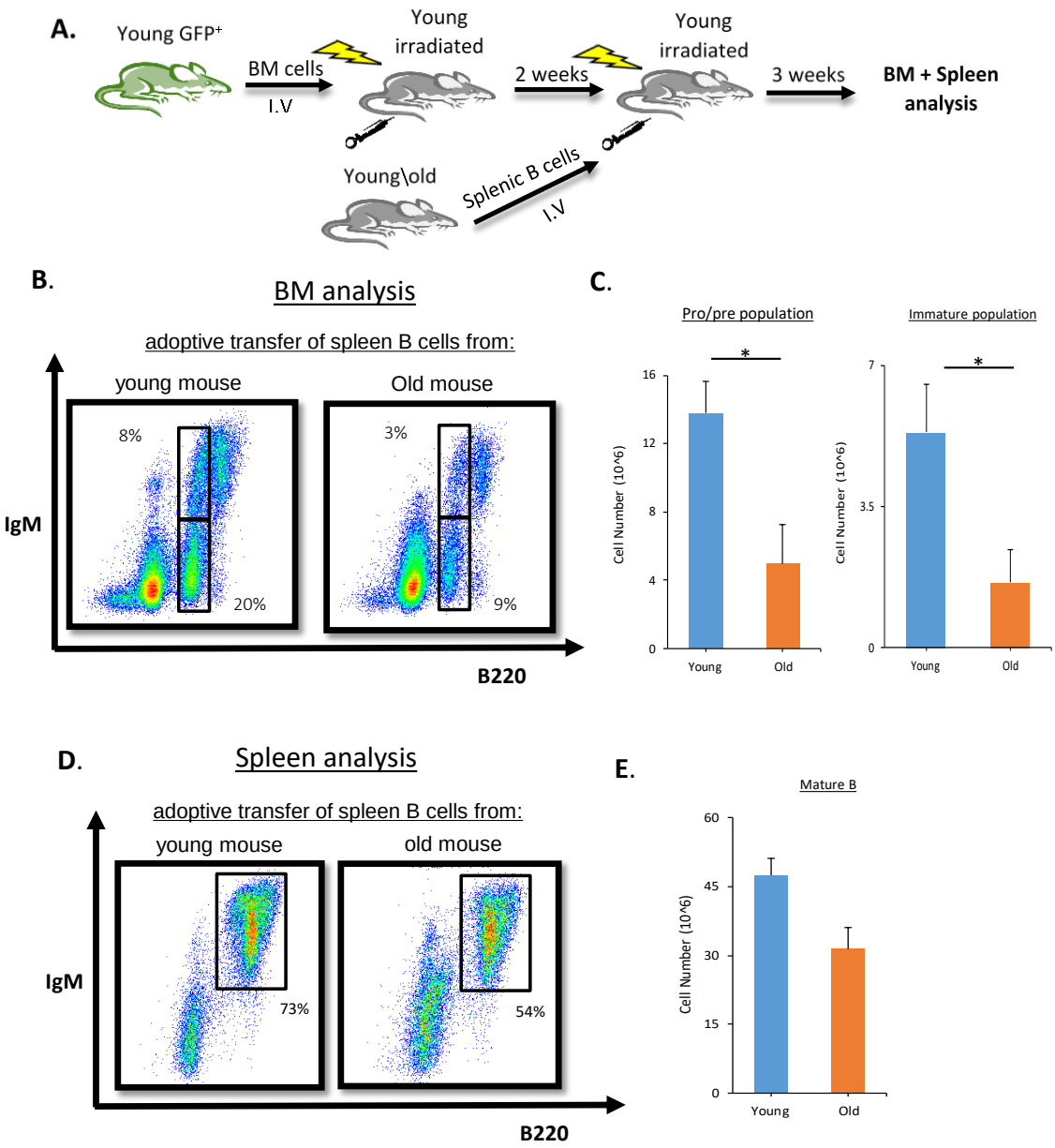
the manufacturer protocol (Peprotech). For human and mouse TNFα in we used Mini ELISA development kit for murine TNFα (Peprotech 900-M54) or mini TMB EDK kit for human TNFα (Peprotech 900-TM25). For human and mouse IGFBP-1 we used mouse IGFBP-1 PicoKine™ ELISA Kit (BOSTER EK0383) or mini ABST EDK Human IGFBP-1 kit (Peprotech 900-M315). Total IGF1, TNFα and IGFBP-1 concentrations were calculated using a reference standard curve of purified recombinant TNFα, IGF1 or IGFBP-1.



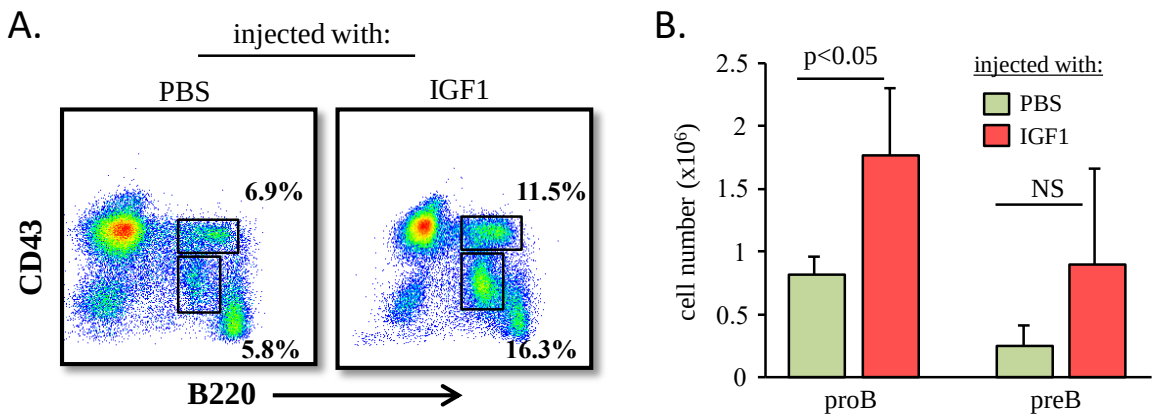
Supplementary Figure 1 – Survival of peripheral B cells. (A) – splenic B cells from the indicated mice were cultured untreated for the indicated time intervals, collected and analyzed for apoptosis by annexin V stain. Shown are representative kinetic measurements of accumulated spontaneous apoptosis for a single experiment out of three independent experiments. (B) – Mice with the indicated genetic background, old or young, were treated with poly(I)(C) to ablate RAG-floxed alleles. Seventeen weeks later BMs of the indicated mice were analyzed for B lymphopoiesis with gates set to identify pro/pre and immature B cells. Shown are representative plots for individual mice with the indicated genotypes (n=5 in each group).



Supplementary Figure 2 – Peripheral B cell depletion. The indicated mice were injected intraperitoneal with mouse-anti-human CD20 monoclonal antibodies to deplete peripheral B cells. Three days after injection, mice were bled and B cell depletion was confirmed by flow cytometry analysis of peripheral blood cells. Results shown are representative of at least 20 individual mice in each group.



Supplementary Figure 3 – Peripheral B cells from old mice suppress B lymphopoiesis. Young WT mice were lethally irradiated, followed by reconstitution with total BM cells from young GFP transgenic mice. After two weeks the GFP-chimeric mice were intravenously injected with $15X10^6$ splenic B cells isolated from young or old WT mice. Three weeks later BM and spleens of recipient mice were analyzed for B lymphopoiesis. (A) Experimental scheme. (B and C) – Analysis of BM cells (GFP+) for the indicated mice was performed by FACS with gates set for pro/pre and immature B cells. Shown are representative plots for a single mouse from each group (B), and absolute cell numbers (C). Graph depicts mean from 3 mice in each group ±SE. (D and E) Analysis of spleen cells for the indicated mice with gates set to quantify newly-generated host B cells (GFP+) as B220+/IgM+. Shown are representative plots for individual mice from each group (D), and absolute cell numbers (E). Graph depicts mean from 3 mice in each group ±SE.



Supplementary Figure 4 - B lymphopoiesis in aging is regulated by IGF1. Old mice were subcutaneously injected with hIGF-1 for 10 days. Control mice were injected with PBS. One day after last injection we analyzed BM of the mice for B lymphopoiesis with gates set to identify proB and preB cell subsets. Shown are representative plots for a single mouse from each group (A), and absolute cell numbers for proB and preB cell populations (B). Graph depicts mean from 5 mice in each group ±SE.

1. Ahuja A, Shupe J, Dunn R, Kashgarian M, Kehry MR, Shlomchik MJ. Depletion of B cells in murine lupus: efficacy and resistance. *J Immunol*. 2007;179(5):3351-3361.
2. Hao Z, Rajewsky K. Homeostasis of Peripheral B Cells in the Absence of B Cell Influx from the Bone Marrow. *The Journal of Experimental Medicine*. 2001;194(8):1151-1164.
3. Berg DJ, Leach MW, Kuhn R, et al. Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses. *J Exp Med*. 1995;182(1):99-108.
4. Avivi I, Zisman-Rozen S, Naor S, et al. Depletion of B cells rejuvenates the peripheral B-cell compartment but is insufficient to restore immune competence in aging. *Aging Cell*. 2019;18(4):e12959.