Homeostatic Mechanisms Controlling B lymphopoiesis In Aging

Daniel Riazanov, Jonah Sachs, Daniella Baruch
Mentor: Reem Dowery, PhD candidate; PI: Prof. Doron Melamed
Department of Immunology, The Ruth and Bruce Rappaport Faculty of Medicine, Technion

Abstract

Generally, the number of peripheral B cells does not change significantly throughout life as generations of new B cells in the Bone Marrow (BM) is balanced by death of B cells in the periphery, a process referred to as **cellular homeostasis** [1]. This balance between cell input and output within the B cell compartment is important for the integrity of the organism and for mounting an effective immune response. Yet, cellular homeostasis can adapt to physiological changes.

A good example for such adaptation is the dramatic changes in the B cell compartment that occurs with aging, where B cell production declines and long-lived memory B cells accumulate in the periphery [2]. These changes in B cell homeostasis are associated with reduced responsiveness to vaccination, increased autoimmunity and increased morbidity.

So far, the mechanism by which cellular homeostasis is established is still unknown. Studies in our lab have demonstrated a feedback mechanism by which peripheral B cells suppress B lymphopoiesis in aging. In these studies we found that the removal of peripheral B cells reactivates B lymphopoiesis in the BM and rejuvenates the peripheral compartment in old mice and humans [1].

Hypothesis

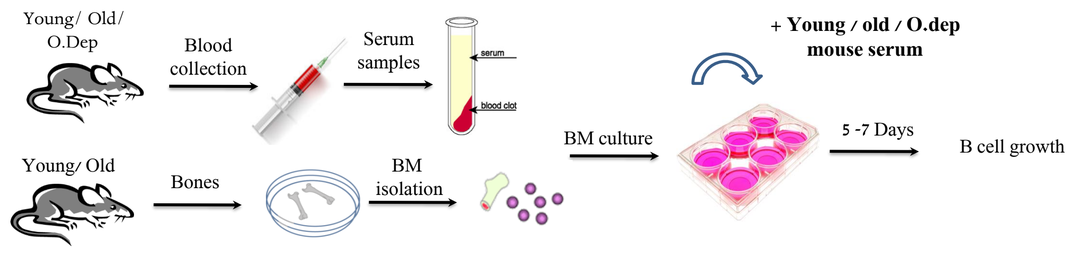
We hypothesize that cellular homeostasis in the B cell compartment is mediated by **cross-talk** between peripheral B cells and progenitors in the BM.

Aim of Research

In the present study we aim to identify a molecular mechanism and/or soluble molecules that may mediate this cross-talk. By finding these mediators, we will be able to increase B cells lymphopoiesis and therefore rejuvenate the peripheral lymphocyte compartment. Carrying out the assays below have helped confirm our mentor's previous findings and may allow for further research to be carried out on a larger scale.

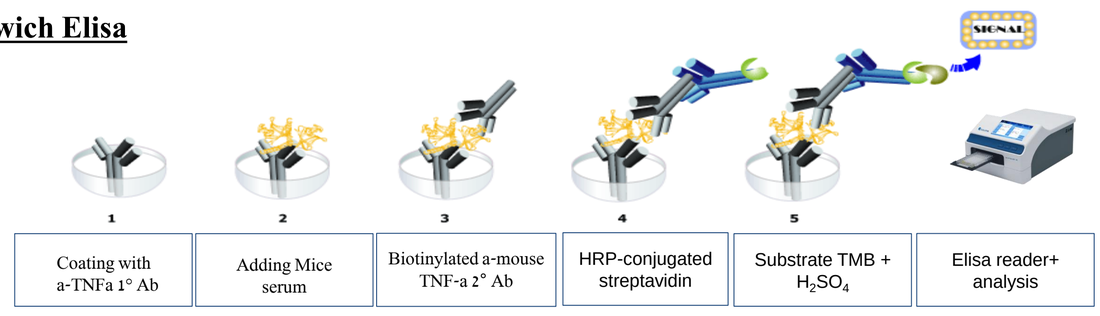
Materials & Methods

Bone Marrow culture



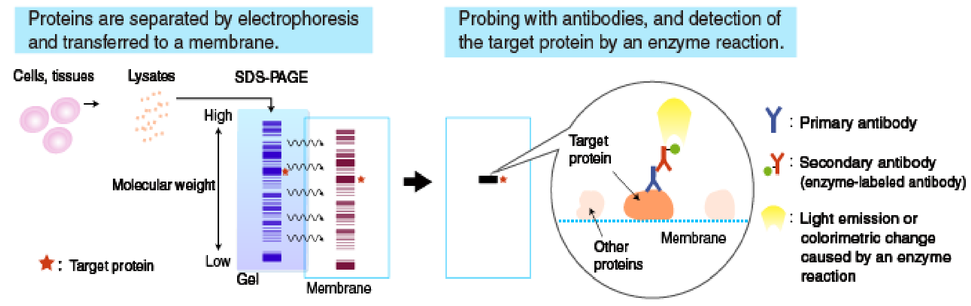
Bone Marrow cultures in the presence of fresh 1% mouse serum from young, old and old depleted mice to check inhibitory effects. After 5 days we determined the proportion of B cell generation (Fig. 1).

Sandwich Elisa



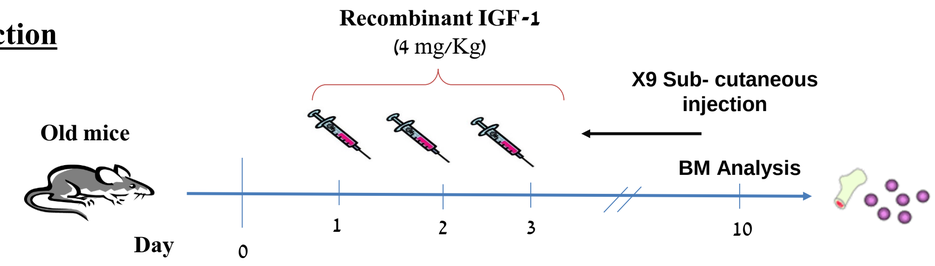
In our lab we compared TNFα concentration in serum of old, young and old depleted mice (Fig. 2).

Western Blot



Comparison the secretion of TNFα by long-lived B lymphocytes from spleen samples of Young, Old and Old depleted mice (Fig. 3).

IGF-1 Injection



In order to check the effect of IGF-1 on B cell lymphopoieses , old WT mice were subcutaneously injected with recombinant IGF-1 (2mg/kg, twice a day) for 9 days (Fig.4).

Results

Figure 1: Suppressive effect of old serum

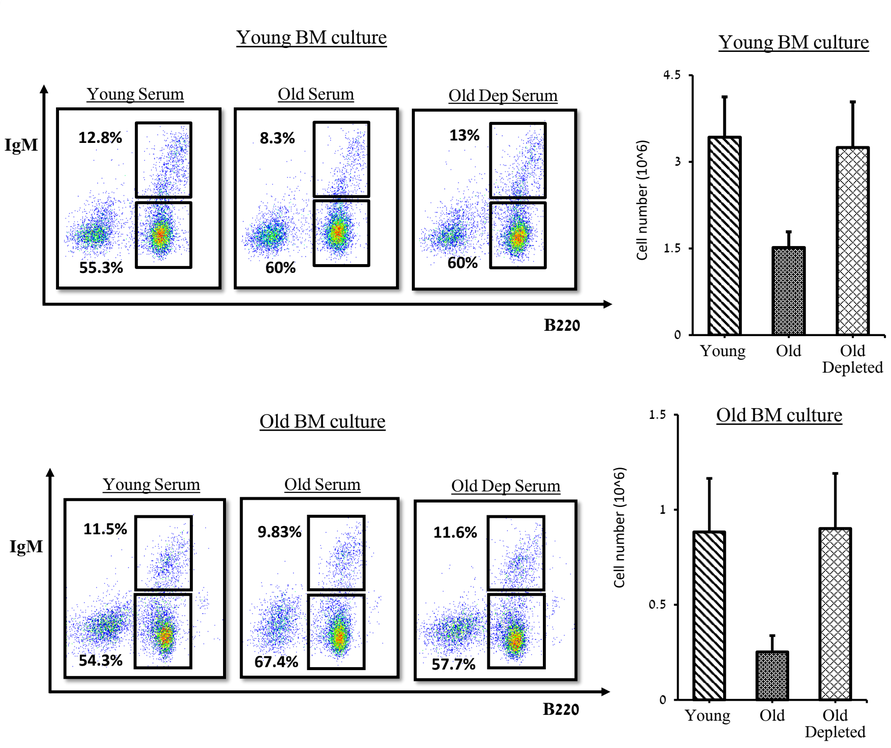
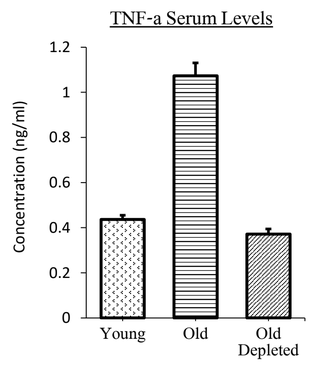


Fig. 1: Old mouse serum significantly inhibits B lymphopoiesis in both old and young BM cultures compared with young and Old depleted serum. In contrast, a strong B lymphopoiesis was detected in cultures in the presence of young and old depleted serum

Figure 2: Sandwich Elisa system



	Young	Old	Old depleted
Concentration (ng/ml)	0.436	1.073	0.371

Fig. 2: Checking the differences in total TNF-a concentration between young, old and old depleted mice [3].

As shown, young and old depleted mice have a lower concentration of serum TNF-a compared with the old mice.

Figure 3: Western Blot

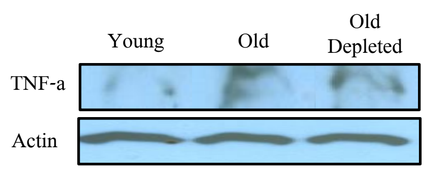


Fig. 3: We tested the secretion of TNFα by the peripheral B cells of young, old and old depleted mice samples and found that more TNFα was secreted in the older samples, corroborating our ELISA results [3].

We checked also Beta-actin, a "housekeeping" protein, as a loading control to be sure that we load the same concentrations of proteins in every sample.

Figure 4: IGF-1 Injection

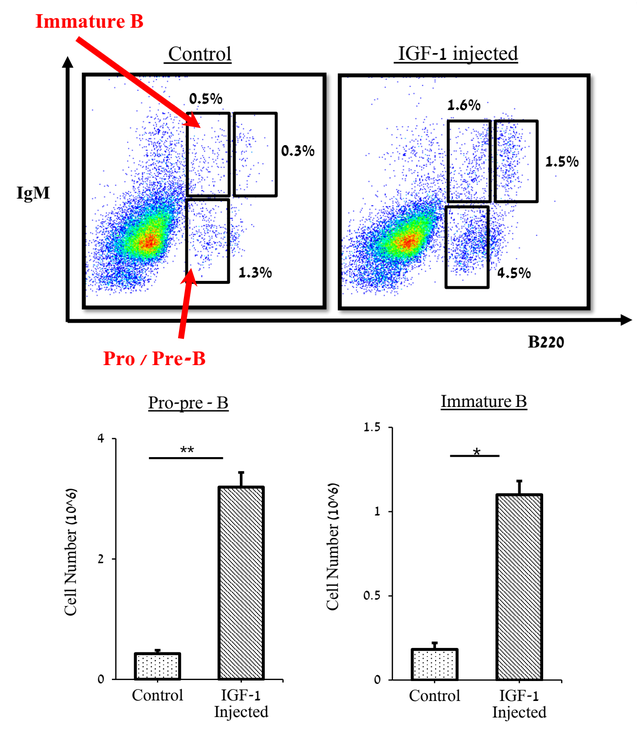


Fig. 4: A strong lymphopoiesis was detected in the old mice that were injected with IGF-1 compared with the control group [4], as revealed by cell proportion and cell number (*p<0.05, **p<0.01).

Conclusions

- Aging in B lineage is mediated by accumulation of long-lived memory B cells in the periphery which inhibit B lymphopoiesis in the BM (Fig. 1).
- Inhibition occurs by increased secretion of cytokines into the serum by long-lived peripheral B cells – as a cross talk mechanism between BM and periphery (Fig. 1).
- TNFα plays a key role in activation of inhibitory circle (Figs. 2+3).
- B cell depletion, injection of α-TNFα or of IGF-1 could restore the rate of B lymphopoiesis (Figs. 2+4).

Acknowledgments

We would like to thank Reem Dowery, PhD candidate, and Prof. Melamed for hosting and guiding us through our research in his laboratory. We would also like to thank the foundations and donors for their generous support of the SciTech program.

References

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