INNERVATION OF PARTICULAR HEMATOPOIETIC CELL POPULATIONS IN THE BONE MARROW OF THE RAT

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Introduction

Mammalian bone marrow is richly innervated [1]. Various cell populations residing the bone marrow are not randomly distributed. They preferentially localize with a certain relationship to the blood vessels [2]. The majority of nerve fibers are usually wrapped around vessels. However, some of them project into the bone marrow parenchyma, release neurotransmitters and thus influence target cells equipped with appropriate receptors. Therefore, the aim of the present study was to examine whether several bone marrow cell populations are located in a defined relation to the nerve fibers.

Material and methods

The study was performed on Wistar rats. Animals were perfusion fixed and excised hindlimbs were postfixed in 4% buffered paraformaldehyde, demineralized in 10% EDTA and immersed in 25% sucrose. Tissue blocks were snap-frozen and cut on 16 µm-thick cryosections. For double immunohistochemical staining the sections were incubated with primary antibodies raised against a general neural marker (anti-PGP 9.5), marker of sensory fibers (anti-CGRP), sympathetic adrenergic fibers (anti-NPY) and against markers of certain rat marrow cell lines: mononuclear phagocytes (1C7), B lymphocytes (anti-CD45R), T-lymphocytes (anti-CD6), erythroid cells (HIS49), and megakaryocytic cells (anti-CD42d), respectively. After rinse in PBS, sections were incubated with appropriate secondary antibodies conjugated with fluorochromes (DTAF and Cy3). Finally, they were examined under an epifluorescence microscope.

Results

The number of detected cells belonging to various lines was in accordance with rats marrow counts. It was confirmed that marrow is supplied with a dense network of nerve fibers mainly those accompanying the vasculature. Some marrow cells were located in the vicinity of nerve fibres. B cells localized preferentially close to CGRP-positive fibers. Also megakaryocytes were seen in the vicinity of NPY-positive fibers.

Discussion

Although marrow cells can be cultured *in vitro* without any neural influence, in the *in vivo* setting neurotransmitters can finely tune their growth and differentiation. The actual role of marrow innervation is still debated but recent observations provided evidence that sympathetic nerves are involved in the regulation of stem cell egress from the marrow niche [3]. The possible functional role of sensory endings was also suggested. The obtained results have shown spatial relationship between hematopoietic cell populations and nerve fibers. Neurotransmitters released by nerve terminals may influence the location and function of cells in the bone marrow cavity. Our data extend the current knowledge on the location of several cell populations in the bone marrow. However, regarding vast spectrum of neurotransmitters/neuromodulators found in the peripheral nerves and various populations of marrow cells at different stages of development we are still far from understanding functional implications of such cooperation.

References

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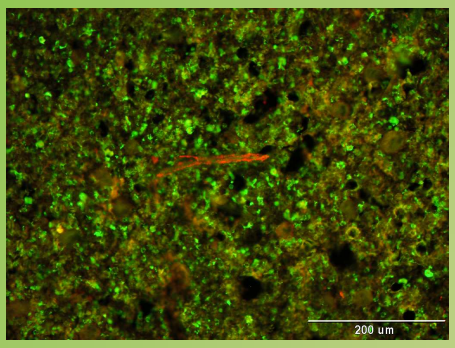
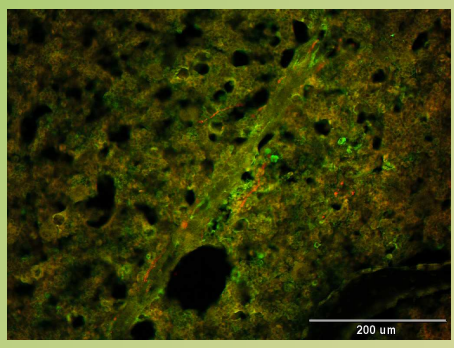
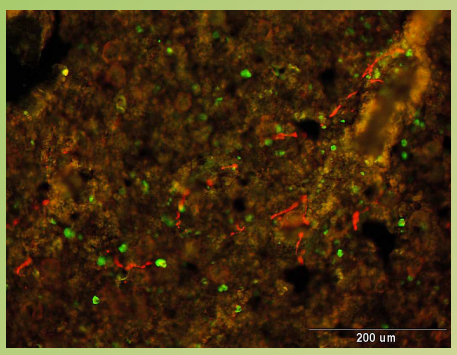
2. Naito K, Tamahashi N, Chiba T, Kaneda K, Okuda M, Endo K, Yoshinaga K, Takahasi T The microvasculature of the human bone marrow correlated with the distribution of hematopoietic cells. A computer-assisted three-dimensional reconstruction study. Tohouku J Exp Med 166, 439-450, 1992.
3. Katayama Y, Battista M, Kao W-M, Hidalgo A, Peired AJ, Thomas SA, Frenette P Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 124, 407-421, 2006.



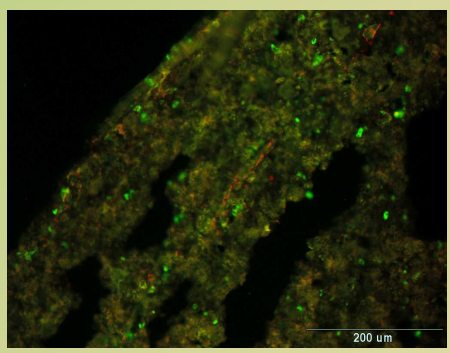
Double immunohistochemical staining for mononuclear cells (green fluorescence) and CGRP-positive sensory nerve fibres (red fluorescence).



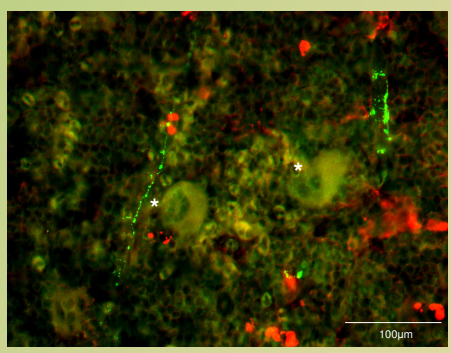
Double immunohistochemical staining for B lymphocytes (green fluorescence) and CGRP-positive sensory nerve fibres (red fluorescence) in their vicinity .



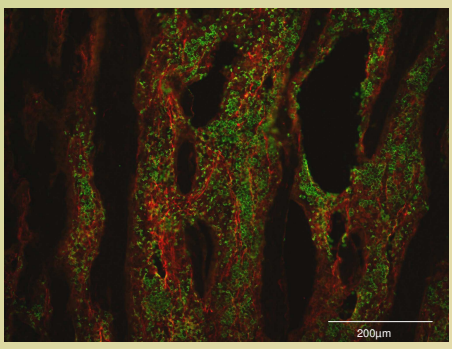
Double immunohistochemical staining for T lymphocytes (green fluorescence) and CGRP-positive sensory nerve fibres (red fluorescence).



Double immunohistochemical staining for T lymphocytes (green fluorescence) and PGP 9.5-positive nerve fibres (red fluorescence).



Double immunohistochemical staining for megakaryoblasts and NPY. Two mature megakariocytes (asterisks) also positive for NPY. One of them is in the vicinity of a NPY-positive nerve.



Double immunohistochemical staining for erythroid cells (green fluorescence) and dense network of PGP 9.5 -positive nerve fibres (red fluorescence).