Brief report

A role for bone marrow–derived cells in the vasculature of noninjured CNS

Francesco Galimi, Robert G. Summers, Henriette van Praag, Inder M. Verma, and Fred H. Gage

The contribution of hematopoietic cells to the formation of blood vessels is currently the focus of intense scrutiny. Bone marrow–derived endothelial progenitor cells are thought to generate endothelial cells in many tissues, including myocardium, muscle, and certain tumors. In the central nervous system (CNS), however, the possible role of bone marrow–derived angiocompetent cells remains unclear. Here we have investigated the long-term involvement of bone marrow–derived cells in the maintenance of endothelial structures in the brain, spinal cord, and retina. Using hematopoietic chimeras stably expressing green fluorescent protein (GFP) in bone marrow–derived tissues, we found large numbers of hematopoietic cells closely associated with vessels in the CNS. None of these cells, however, showed an endothelial phenotype. They were positive for monocytic and microglial surface markers and demonstrated active phagocytosis of neighboring endothelial elements. Bone marrow–derived, vasculature-associated cells in the noninjured adult CNS are distinct from endothelial cells, but play an active role in vascular structures. (Blood. 2005;105:2400-2402)

Study design

Lethally irradiated (1100 cGy) C57BL/6 mice received 1 to 3 million unfractionated bone marrow cells obtained from GFP-transgenic animals.6 At 10 to 12 months after transplantation, animals were humanely killed, and the online version of the article includes a data supplement.

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Fluorescent samples were evaluated using a multiphoton-equipped MRC1024UV confocal imaging system with a 40× oil objective. Digital image reconstruction and analysis were performed with MetaMorph software version 6.1 (Universal Imaging, Downingtown, PA).

Results and discussion

Large numbers of bone marrow–derived (ie, GFP-positive) cells were observed in the central nervous system of all mice that underwent transplantation. In the brain and spinal cord, they were localized throughout the white and gray matter, without a specific regional pattern. The morphology of the GFP-positive cells varied greatly. In addition to the expected macrophages and microglial cells7 (not shown), we identified a large number of cells tightly associated with large/medium-size vessels and with the microvasculature. To explore the possible endothelial identity of these vessel-associated cells, we performed a systematic confocal microscopy analysis of their immunophenotype and 3-dimensional localization in relation to endothelial structures.

Of hundreds of candidate cells analyzed in different areas of the CNS, in more than 90 animals that underwent transplantation, we could not identify any BM-derived cell that expressed endothelial-specific markers at the cell surface. Most BM-derived cells showed an extracellular positivity for lectin, which is known to stain both endothelial cells (ECs) and microglial cells in the CNS8 (Figure 1A and Video S1, available on the Blood website; see the Supplemental Videos link at the top of the online article). CD11b and CD45, which are monocytic and panleukocytic lineage markers, respectively, were expressed at variable levels (Figure 1M,O). Endothelium-associated, BM-derived cells were always in a subendothelial location and showed a variable number of cellular processes, ranging from a classic microglial morphology to an elongated cell shape (Figure 1A-B,D; Videos S1 and S2). Cells with a microglial appearance were typically lectin positive at the cell surface, whereas elongated cells were usually lectin dim or negative. Interestingly, the vast majority of the BM-derived, endothelium-associated cells showed a very intense cytoplasmic positivity for lectin and CD31. The signal was concentrated in spherical “hot spots” that could be identified as intracytoplasmic vacuoles by...
3-dimensional confocal analysis, suggesting that the BM-derived cells were actively participating in the remodeling of neighboring endothelial sheets by phagocytosis of endothelial cells (Figure 1B-C-I; Video S2). BM-derived perivascular cells were negative for α-smooth muscle actin (α-SMA) and therefore distinct from smooth muscle cells and pericytes.9 The GFP-positive cells were invariably localized on the abluminal side of SMA-positive cells surrounding the vessel (Figure 1H-J).

In the retinas of the analyzed animals, we could identify BM-derived, vessel-associated cells in close relationship to the astrocytes that surrounded retinal microvasculature (Figure 1F,K), in addition to BM-derived microglial cells located in the inner and outer plexiform layers (not shown). The BM-derived, vessel-associated cells did not show abundant phagocytosis and were only weakly positive for CD11b (not shown). In contrast to other regions of the CNS, BM-derived cells in the retina were always located on the outside of the astrocyte sheath surrounding the vessels (Figure 1F,K-L).

It was recently demonstrated4 that self-renewing hemangioblasts from the bone marrow are capable of full hematopoietic reconstitution and generation of endothelial cells in non-CNS peripheral organs. Our data confirm that CNS vasculature has peculiar biologic features in its relationship with bone marrow progenitors in adult life.

We previously documented that endothelial cells constantly turn over in the hippocampus, indicating a continuous remodeling of hippocampal vasculature.10 In our current experiments, we showed that BrDU incorporation is not limited to the hippocampus but is common to ECs throughout the CNS (Figure 1G). In spite of the intense endothelial proliferation and the large number of BM-derived cells entering the CNS, the generation of endothelial elements from BM is apparently not occurring, or is an exceedingly rare phenomenon, at least under physiologic conditions. Rather, BM contributes a population of subendothelial mononuclear cells that actively phagocytize endothelial components, a critical step in the remodeling of endothelial sheets. Whether these cells can be considered a morpho-functional variant of microglia or pericytes, or should be considered a separate cell population, is a classification issue that goes beyond the scope of the present report and will require the identification of differentially expressed markers.

Radiation is known to affect the central nervous system,11,12 and the interference from long-term radiation toxicity in our animals cannot be ruled out. Although mononuclear subendothelial cells can be detected in normal brains (Figure 1N; Thomas13; and Guillemine and Brew14), their dynamics cannot be easily studied unless a bone marrow transplantation is performed.

In vitro experiments have recently been reported, where the vast majority of circulating EPCs are actually expressing mononuclear markers, and their angiogenic functions are most likely indirectly mediated by secretion of angiogenic cytokines (reviewed in Schmeisser et al).15 Our experimental system did not allow us to directly test this hypothesis, but a tempting scenario can be envisioned wherein BM-derived subendothelial cells in the CNS control both endothelial proliferation, by paracrine secretion of proangiogenic factors, and endothelial degradation, by actively participating in endothelial cell dismantling. While our data rule out BM as a source of endothelial cells in the noninjured CNS, experiments in animal models of brain damage will be necessary to establish the situation following injury. Previous studies16 addressing this issue have relied on Tie-2 as an endothelial-specific marker, but recent reports have shown that Tie-2 is expressed also by some perivascular hematopoietic cells.17 Careful 3-dimensional analysis is required for positive assessment of endothelial cell identity.

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References

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