Network: Genomic Mechanisms of Functional Recovery from Stroke

Project: Gene Expression Profiles of Bone Marrow-derived Cells in the Brain after Cerebral Ischemia

Josef Priller – Humboldt University Berlin, Charité Medical School - josef.priller@charite.de
Ulrich Dirnagl - Humboldt University Berlin, Charité Medical School - ulrich.dirnagl@charite.de

Introduction

After a cerebral infarction, there is an acute inflammatory response with entry of neutrophils, macrophages, and other blood elements into the ischemic zone. A large body of evidence suggests that this inflammatory response is harmful and contributes to tissue injury (Dirnagl et al., 1999). However, inflammation may be a double-edged sword, and inflammatory cells and blood elements may also be involved in reparative and restorative processes.

Bone marrow-derived cells (BMDCs) contribute to revascularization and tissue regeneration in a wide range of ischemic pathologies, including myocardial infarction (Hamano et al., 2001), limb ischemia (Iba et al., 2002), retinal degeneration (Grant et al., 2002) and stroke (Beck et al., 2003). Bone marrow is a rich source of stem and progenitor cells that can mobilize to ischemic sites. After cerebral ischemia, BMDCs rapidly infiltrate the brain, where they have been reported to give rise primarily to microglia (Priller et al., 2001; Hess et al., 2004) or endothelial cells (Zhang et al., 2002) and a limited number of cells that express astrocytic (Eglitis et al., 1999) and neuronal markers (Cogle et al., 2004; Hess et al., 2004). The recruitment of BMDCs after stroke may therefore represent an attempt at endogenous self-repair.

It is still unknown whether the gene expression profile in circulating mononuclear cells is the same or different from that of mononuclear cells invading the infarct tissue or even reactive microglia. Moreover, it is not known whether microglia/monocytic cells in angiogenic and neurogenic regions express a differential gene profile.

The current project in the National Genome research Network aims to identify expression profiles of functional gene classes in microglial cells, monocytes/macrophages and endothelial cells characterized *in situ* in the brain. A temporospatial comparative analysis of these cellular gene expression profiles in healthy versus ischemic and neurogenic versus non-neurogenic brain regions should help uncover potential functional interactions of inflammatory, angiogenic and neurogenic responses after stroke. Previous gene expression profiling studies for isolated brain endothelial and microglial cell populations have revealed characteristic messenger RNA profiles under normal and pathological conditions, including neurodegenerative disorders and electroconvulsive seizures. Functional genomic analysis of CNS remyelination has recently emphasized the role of inflammation in regeneration, providing proof of principle for the feasability of our study. Since gene regulation levels are particularly low in the brain and may require analysis of specific subregions, the approach of gene expression profiling at the cellular level is likely to reveal novel functional aspects of neurogenesis and the responses to stroke.

For gene expression array analysis both cell types are prepared from animals, in which an ischemic insult was induced and from sham-treated (or untreated) animals. In addition to C57Bl/6 mice, GFP-NLS bone marrow chimeric mice will be used. In a first step, target cells in project tissue sample sections are immunostained with different primary antibodies (IBA, F4/80, vWF). In a second step, laser microdissection of target cell types is performed and a minimum of 50 cells/sample will be collected (every section will be recorded photographically before and after laser microdissection). Thereafter RNA will be extracted and linear RNA amplification will be carried out. Microarray transcription profile analysis will be performed on amplified RNA and samples will be subjected to gene expression array analysis.

In addition to the proposed project, long term functional outcome will be evaluated in sham-operated and stroke mice using tests for spatial memory (Morris Water Maze) and associative learning (passive avoidance).

Results/Project Status

In order to specifically label target cells in tissue sample sections, cryosections of nonischemic and ischemic mouse brains were immunostained using different antibodies against microglia/macrophages (Iba1, Mac1) or endothelial cells (CD31, vWF). All immunostaining protocols were established and revealed reliable labelling of the above cells. The arrangement of a good labelling technique is an absolute requirement for the next step and allows us now to specifically collect microglial and endothelial cells.

In addition to our immunohistochemical experiments, we were able to set up a new lab for the evaluation of functional parameters. Using different tools for the assessment of motor (RotaRod, Open Field) and cognitive function (T-Maze, Morris Water Maze, Passive Avoidance) we are trying to establish useful paradigms for the evaluation of motor and cognitive deficits after cerebral ischemia. Using chimeric and transgenic mice we are planning to elucidate the role of inflammation in regeneration and associated functional outcome.

*Lit.: **1.** Dirnagl U et al. Pathobiology of ischemic stroke : an integrated view. Trends Neurosci. 1999; 22: 391-397. **2.** Hamano K et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart diesease : clinical trial and preliminary results. Jpn Circ. 2001; 65: 166-180. **3.** Iba O et al. Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. Circulation. 2002; 106: 2019-2025. **4.** Grant MB et al. Adult hematopoietic stem cells provide functional hemangioblast activity during retinal neovascularization. Nat Med. 2002; 8: 607-612. **5.** Beck H et al. Participation of bone marrow-derived cells in long-term repair processes after experimental stroke. JCBFM. 2003; 23: 709-717. **6.** Priller J et al. Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. Nat Med. 2001; 7(12): 1356-1361. **7.** Hess DC et al. Hematopoietic origin of microglial and perivascular cells in brain. Exp Neurol. 2004; 186: 134-144. **8.** Eglitis MA et al. Targeting of marrow-derived astrocytes to the ischemic brain. Neuroreport. 1999; 10: 1289-1292. **9.** Cogle et al. Bone marrow transdifferentiation in brain after transplantation: a retrospective study. Lancet. 2004; 363: 1432-1437.*