CCL2 upregulation triggers hypoxic preconditioning-induced protection from stroke.

A brief exposure to systemic hypoxia (i.e., hypoxic preconditioning; HPC) prior to transient middle cerebral artery occlusion (tMCAo) reduces infarct volume, blood-brain barrier disruption, and leukocyte migration. CCL2 (MCP-1), typically regarded as a leukocyte-derived pro-inflammatory chemokine, can also be directly upregulated by hypoxia-induced transcription. We hypothesized that such a hypoxia-induced upregulation of CCL2 is required for HPC-induced ischemic tolerance. Adult male SW/ND4, CCL2-null, and wild-type mice were used in these studies. Cortical CCL2/CCR2 message, protein, and cell-type specific immunoreactivity were determined following HPC (4 h, 8% O2) or room air control (21% O2) from 6 h through 2 weeks following HPC. Circulating leukocyte subsets were determined by multi-parameter flow cytometry in naïve mice and 12 h after HPC. CCL2-null and wild-type mice were exposed to HPC 2 days prior to tMCAo, with immunoneutralization of CCL2 during HPC achieved by a monoclonal CCL2 antibody. Cortical CCL2 mRNA and protein expression peaked at 12 h after HPC (both < 0.01), predominantly in cortical neurons, and returned to baseline by 2 days. A delayed cerebral endothelial CCL2 message expression (p < 0.05) occurred 2 days after HPC. The levels of circulating monocytes (p < 0.0001), T lymphocytes (p < 0.0001), and
granulocytes were decreased 12 h after HPC, and those of B lymphocytes were increased (p< 0.0001), but the magnitude of these respective changes did not differ between wild-type and CCL2-null mice. HPC did decrease the number of circulating CCR2+ monocytes (p< 0.0001) in a CCL2-dependent manner, but immunohistochemical analyses at this 12 h timepoint indicated that this leukocyte subpopulation did not move into the CNS. While HPC reduced infarct volumes by 27% (p< 0.01) in wild-type mice, CCL2-null mice subjected to tMCAo were not protected by HPC. Moreover, administration of a CCL2 immunonutralizing antibody prior to HPC completely blocked (p< 0.0001 vs. HPC-treated mice) the development of ischemic tolerance. The early expression of CCL2 in neurons, the delayed expression of CCL2 in cerebral endothelial cells, and CCL2-mediated actions on circulating CCR2+ monocytes, appear to be required to establish ischemic tolerance to focal stroke in response to HPC, and thus represent a novel role for this chemokine in endogenous neurovascular protection.