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Red Blood Cell (RBC) Transfusion Cause Brain Inflammation in Murine Pups with Phlebotomy-Induced Anemia

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Background: One in 10 infants born in the United States is pre-term infants and anemia is one of the common comorbidities in preterm infants in neonatal intensive care unit. Transfusions of red blood cells (RBC) remains the mainstay of treatment of severe anemia that occurs in premature and critically ill infants both due to physiologic and iatrogenic causes. RBC transfusions improve oxygen-carrying capacity to tissues, promote weight gain and growth in the preterm infants. There is correlation between anemia, blood transfusion and brain injury in clinical and animal studies. Specifically, severe anemia has been associated with hippocampal injury in neonatal animal models, but the effects of transfusion on brain inflammation after anemia has not yet been evaluated.

Objective: To identify inflammatory changes in the neonatal brain in a mouse model of phlebotomy-induced anemia and stored RBC transfusion.

Methods: C57BL/6 pups were studied in 4 groups (n=6 each): (1) naïve controls; (2) RBC transfused; (3) severe anemia (hematocrit 20-24%); and (4) anemia with RBC transfusion. Severe anemia was induced by facial vein phlebotomy on postnatal day (P)2, 4, 6, 8, and 10. RBC transfusion consisted of 20 ml/kg of leukoreduced and refrigerated stored (7 days at 4°C) packed RBCs from allogeneic (adult FVB mice) donors administered intravenously into the retro-orbital plexus to P11 pups. After 24 hours, whole brain tissue was extracted, and total protein concentration estimated by Bicinchoninic acid (BCA)-Bradford assay. Milliplex Map Mouse cytokine/chemokine premixed 32-plex multiplex assay were used to quantify the cytokines and chemokines (C-C motif ligands) levels in brain tissue homogenate of all four groups.

Results: The levels of the cytokines IL-1α (**p=0.002), IL-1β (*p=0.028), IL-6 (**p=0.010), TNF-α (*p=0.011) and IL-12p70 (*p=0.035), but not IFN-γ (p=0.641), were significantly increased in the brain of anemic transfused mouse pups compared to transfused controls. The levels of the chemokines (C-C motif ligands) MCP1 [CCL2] (***p=0.001), MIP1-α [CCL3] (**p=0.005), MIP1-β [CCL4] (**p=0.004), RANTES [CCL5] (**p=0.002) and Eotaxin [CCL11] (**p=0.004) were also significantly increased in anemic-transfused mouse pups compared to transfused control.

Conclusion: RBC transfusions increase the brain inflammatory cytokine response and monocyte chemoattractant chemokines release in murine pups with severe anemia. We have elucidated the connection between anemia and brain inflammation leading to rising inflammatory makers in whole brain that was further enhanced by RBC transfusion leading to brain injury.