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The role of interferon in the cellular response of the CNS macrophage, the microglia, during injury and inflammation.

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13. ABSTRACT *(Maximum 200 words)*

Our lab has studied the response of the CNS macrophage, the microglia to injury and inflammation. Using an *in vitro* approach, we have shown that microglia cultured from the cerebral cortices of neonatal animals (rat, mouse, hamster or human) have the same functional responses as non-CNS macrophages. That is, they demonstrate chemotaxis, express macrophage-like surface antigens and they produce a variety of cytoactive factors including proteases, interleukin-1 and reactive oxygen species (superoxide anion and nitric oxide). We found that both inflammatory and immune mediators (lipopolysaccharide and interferons, respectively) enhance the production of superoxide anion but do not directly activate the NADPH oxidase. These agents also increase nitric oxide (NO) production but in a very different time frame than that found for superoxide anion. Treatment of microglia with isopreterenol or dexamethazone depressed the microglial production of ROS. Our studies also demonstrated that human and hamster microglia do not produce NO in response to the same stimulating factors used in rat or mouse microglia. Hamster and human microglia did not produce NO except when treated with the double stranded polyribonucleotide, poly inosinic acid: poly cytidylic acid (Poly I:C). These findings have important consequences to the understanding of the response of humans to inflammation or injury.

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FINAL REPORT

GRANT #: N00014-91-J-1123 R&T CODE 4414138

PRINCIPAL INVESTIGATOR: Carol A. Colton, Ph.D.

INSTITUTION: Georgetown University Medical School

GRANT TITLE: The role of interferon in the cellular response of the CNS macrophage, the microglia, during injury and inflammation.

REPORTING PERIOD: 1 November 1993 to 31 October 1996

AWARD PERIOD: 1 November 1993 to 31 October 1996

OBJECTIVE: To investigate the response of the CNS macrophage, the microglia, to factors involved in the response to injury and infection. To investigate the role of interferon in this response and to determine potential sources of alpha/beta and gamma interferon in the CNS.

APPROACH: Primary microglia and astrocyte cultures are prepared from neonatal mouse or hamster cerebral cortices. At 14 days, microglia are separated from the underlying astrocyte layers by shaking and both cell populations, i.e., the microglia and the remaining astrocytes, are used in the experimental protocols. In some cases, primary cultures of human fetal astrocytes or human adult microglia were used in collaboration with Dr. M. DuBois-Dalq and Dr. S. Wilt, NIH.

ACCOMPLISHMENTS: Over the duration of this contract our lab has studied the response of the CNS macrophage, the microglia to injury and inflammation. Although microglia are of monocytic origin, it was unclear at the onset of the project if these cells responded in a similar fashion as other tissue macrophages. Using an *in vitro* approach, we have shown that microglia cultured from the cerebral cortices of neonatal animals (rat, mouse, hamster or human) have the same functional responses as non-CNS macrophages. That is, they demonstrated chemotaxis, express macrophage-like surface antigens which may be used to identify the cell in the CNS, and they produce a variety of cytoactive factors including proteases, interleukin-1 and notably, reactive oxygen species (superoxide anion and nitric oxide). Because these cells are found in all regions of the brain, including the hypothalmic-pituitary axis, it was of interest to examine the ability of neuroendocrine factors to modulate the activity of microglia. Such modulation serves as a bridge between the neuronal and immune systems. We found that both inflammatory and immune mediators (lipopolysaccharide and interferons, respectively) enhance the production of superoxide anion but do not directly activate the NADPH oxidase. These agents also increase nitric oxide (NO)production but in a very different time frame than that found for superoxide anion production. This difference has important consequences to the potential production (or lack of production) of peroxynitrite, a putative powerful oxidant involved in oxidative damage of cells. The stress-related hormones, namely isoproterenol, dexamethasone, corticotropin releasing hormone (CRH) and adrenocorticotropin (ACTH) also affect microglia. Our studies show that pretreatment for either 30 minutes or 24 hours with high doses of isoproterenol decreased phorbol myristate acetate (PMA)-stimulated superoxide anion production. This effect was reversed with, propranolol, a known β-adrenergic receptor blocking agent. Forskolin, an agent known to increase cAMP levels by direct activation of adenylate cyclase, also depressed PMA-stimulated superoxide anion production but only when the microglia were exposed to

forskolin for short durations. Longer exposure (i.e., 24 hour pre-treatment) had no effect on PMA-stimulated superoxide anion production. Immunoreactivity for *c-fos* and *c-jun*, products of the early response genes, was increased in both forskolin and isoproterenol pre-treated microglia.

The action of dexamethasone on PMA-stimulated superoxide anion production was also studied. Dexamethasone significantly decreased superoxide anion production and this effect was reversed by the addition of cyclohexamide, indicating that protein synthesis was essential to the inhibitory effect of dexamethasone.

A major component of these studies has been examination of the species differences in NO production. Our studies were the first to demonstrate that human and hamster microglia do not produce NO in response to the same stimulating factors used in rat or mouse microglia (). All microglia were stimulated with lipopolysaccharide (LPS), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and combinations of these factors for 1 to 5 days. The level of nitrite in the supernatants was then compared to untreated controls. For hamster microglia, no detectable nitrite level above background was found for any of the stimulation conditions or at any of the longer durations of treatment. Likewise, human microglia did not demonstrate a significant increase in nitrite levels above untreated for any stimulation conditions studied or for any duration of treatment. Human fetal astrocytes did demonstrate a significant increase in nitrite levels in cells treated with the combination of TNF-α, IL-1β and LPS compared to untreated. Our recent studies have further demonstrated that viral mediators can induce NO production ( ). Treatment of hamster microglia or human monocyte derived macrophages (MDM) with the double stranded polyribonucleotide, poly inosinic acid: poly cytidylic acid (Poly I:C)induced measurable nitrite formation which was inhibitable by N-monomethyl arginine (NMMA), a known inhibitor of nitric oxide synthase (NOS). This effect was also seen if poly I was used but not poly C and was primed by gamma or alpha interferon or other immune mediators such as interleukin 4.

In collaboration with Dr. A. Namboodiri, Department of Biology, Georgetown University, we have studied the production of quinolinic acid (QUIN)in both hamster and human microglia. The immunostaining studies indicate that QUIN is present in adult human microglia and not in human astrocytes. This level does not, however, change with stimulation using LPS or γIFN. This increase in measurable levels coincided with the presence of QUIN immunoreactivity in the cells. The level of QUIN in MDM, however, was significantly greater than that found in microglia under the same treatment conditions. Stimulated QUIN production by cultured astrocytes was not significantly increased over resting levels.
For hamster microglia, specific QUIN staining was not detectable because of a high background signal of unknown nature.

SIGNIFICANCE: Our data demonstrate that microglia function as a CNS macrophage and, as such, are part of the immune system of the CNS. The microglia are responsive to typical immune and inflammatory mediators and like other tissue macrophages, secrete a variety of cytoactive factors which kill invading organisms but also orchestrate the repair of the tissue. Because neurons are post mitotic, however, macrophage activation in the CNS is associated with neuronal death. Stress-related neuroendocrines, such as nor-epinephrine (or the closely related analog, isoproterenol) and dexamethasone inhibit microglial superoxide anion production. If a similar phenomenon occurs in vivo, the inhibition of

immune effector cell function could have important consequences to the CNS and to the immune response in the CNS. This mechanism may, however, serve a protective function potentially reducing neuronal death associated with microglial activation.

Our recent studies on human and hamster microglia further support the idea that major species differences exist in the regulation of microglia and astrocytes. Of the species studied, only rat and mouse microglia and astrocytes produce significant quantities of NO while human and hamster microglia and astrocytes do not apparently generate NO. The term "low output" NO has been applied to human macrophages while rat and mouse macrophages have been termed a "high-output" system. Only certain viral mediators activate NO production in human while mouse and rat cells respond to a variety of mediators. The dramatically different response patterns strongly suggests that caution must be used in correlating rat or mouse NO studies with human and treatment protocols based on manipulation of NO-mediated pathways.

PATENT INFORMATION: None

AWARD INFORMATION: Promoted to Full Professor

PUBLICATIONS AND ABSTRACTS:

Colton, C., Snell, J. , Chernyshev, O. and D. Gilbert, Induction of superoxide anion and nitric oxide production in cultured microglia, Ann. N. Y. Acad. Sci., 738;54-63, 1994.

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