

# **Respondent's Exhibit LL**



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Dr. Thomas L. Kemper, MD  
Boston, Massachusetts

Dear Dr. Kemper,

As per our conversation last year, I would like to clarify some of the concepts regarding the role of neuroimmune response in the brain of patients with autism and the potential significance of such findings in the pathogenesis and pathobiology of the disorder.

As you well know, our neuropathological studies demonstrated that in patients with autism, both microglial and astroglial activation appeared to be one of the most consistent findings at ages ranging from 5 to 44 years. These are likely responses associated with the neuro-innate immune response rather than an adaptive immune response. It was very clear from our studies, that there were no specific T cell or B cell responses in such tissues. It is very possible that some of the neuroglial reactions observed in these brains are associated with the neuronal and cortical abnormalities that have been well described by your original neuropathological observations and they may perpetuate during life, a phenomenon that has been well established in the setting of other neurodevelopmental abnormalities. It is important to note, that the astroglial response is quite prominent and characterized by an active morphological plasticity in which glial processes spread to different portions of the gray and white matter. In our cases, there was no decrease in the population of astrocytes; rather there was an active process of plastic response of glial processes. This was confirmed by immunocytochemical studies with glial fibrillary acidic protein (GFAP) and immunoblotting techniques that demonstrated clearly an increase in the amount of GFAP protein in all brain regions studied. These findings are inconsistent with the hypothesis of a potential toxic effect on astrocytes by neurotoxins or toxic material.

One important issue that also needs to be clarified is the use of the term neuroinflammation. In our paper on neuroinflammation in autism (Vargas et al., 2005) we mentioned this term and clarified that this neuroinflammatory response is specifically associated with innate immune responses and in no case did we observe adaptive immune responses such as infiltration by inflammatory cells from the periphery (T or B cells) or antibody deposition in brain tissues. As also mentioned in our paper, along the immunomorphological findings of neuroglial activation, presence

of both pro-inflammatory and anti-inflammatory cytokines were observed in brain tissues and spinal fluid from patients with autism. These observations are quite interesting, as we now know that both cytokines and chemokines not only have immune properties but also facilitate the neurobiological processes such as neuronal migration, synaptic stability and function, and processes that are fundamental for maintaining the homeostasis of the central nervous system. So the presence of these immune modulators is not exclusively for immune function in the brain but rather other complex interactions of the brain-immune system that facilitate homeostasis.

Another issue that is important to clarify is the notion that neuroinflammatory responses mediated by innate responses and neuroglial activation are directly associated with injury. At present, we are not able to conclude that these neuroglial reactions are deleterious for the central nervous system. These reactions may invoke anti-inflammatory and repairing processes as demonstrated in our study in which one potent anti-inflammatory cytokine, the transforming growth factor  $\beta$ -1 (TGF $\beta$ 1) was significantly present in all areas of the brain. Other members of the same family of proteins (TGF $\beta$ 2) were also present in the cerebrospinal fluid. So, it is erroneous to assign an exclusive deleterious or injury effect of these neuroglial responses in the CNS.

An observation that may be also derived from our experiments with terbutaline exposure (Zerrate et al., 2007) is the effect of environmental factors on brain development and the increased innate immune response. Again, this model showed that neuroglial activation was prominent and this change resulted likely from prenatal and early exposure rather than late exposure.

I hope these comments clarify some of the misconceptions about the observations described in our paper on neuroinflammation and autism.

Sincerely,



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#### Reference List

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* 57:67-81.

Zerrate MC, Pletnikov M, Connors SL, Vargas DL, Seidler FJ, Zimmerman AW, Slotkin TA, Pardo CA (2007) Neuroinflammation and behavioral abnormalities after neonatal terbutaline treatment in rats: implications for autism. *J Pharmacol Exp Ther* 322:16-22.