Brain, Behavior, and Immunity 29 (2013) 104–112

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Inflammation-initiating illnesses, inflammation-related proteins, and cognitive impairment in extremely preterm infants

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ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form 17 December 2012
Accepted 19 December 2012
Available online 4 January 2013

Keywords:
Cognitive impairment
Necrotizing enterocolitis
Extreme prematurity
Systemic inflammatory response
Neonatal chronic lung disease
Neonatal sepsis

ABSTRACT

Neonatal inflammation is associated with perinatal brain damage. We evaluated to what extent elevated blood levels of inflammation-related proteins supplement information about the risk of impaired early cognitive function provided by inflammation-related illnesses. From 800 infants born before the 28th week of gestation, we collected blood spots on days 1, 7 and 14, for analysis of 25 inflammation-related proteins, and data about culture-positive bacteremia, necrotizing enterocolitis (Bell stage IIIb), and isolated perforation of the intestine, during the first two weeks, and whether they were ventilated on postnatal day 14. We considered a protein to be persistently or recurrently elevated if its concentration was in the top quartile (for gestational age and day blood was collected) on two separate days one week apart. We assessed the children at 2 years of age with the Bayley Mental Development Index (MDI). The combinations of NEC and ventilation on day 14, and of bacteremia and ventilation on day 14 consistently provided information about elevated risk of MDI <55, regardless of whether or not a variable for an elevated protein concentration was included in the model. A variable for a persistently or recurrently elevated concentration of each of the following proteins provided additional information about an increased risk of MDI <55: CRP, SAA, IL-6, TNF-alpha, IL-8, MIP-1beta, ICAM-1, E-SEL, and IGFBP-1. We conclude that elevated blood concentrations of inflammation-related proteins provide information about the risk of impaired cognitive function at age 2 years that supplements information provided by inflammation-associated illnesses.

1. Introduction

Extremely preterm infants (born before 28 weeks of gestation) are at a ten-fold increased risk of cognitive impairment compared to full term infants (Johnson et al., 2009). The underlying structural brain pathology is probably diffuse, but one component consists of periventricular white matter abnormalities that can be identified during the first postnatal weeks using ultrasonography or magnetic resonance imaging (MRI). The periventricular echolucent lesions correspond to histologically defined cerebral white matter necrosis (Paneth et al., 1990). These lesions, as well as MRI evidence of thinning of the corpus callosum, loss of periventricular white matter volume (Woodward et al., 2006), low hippocampal volume (Thompson et al., 2008), and increased apparent diffusion coefficient in the white matter (Krishnan et al., 2007), predict cognitive impairment evident years later.

Epidemiologic studies repeatedly link perinatal infections and inflammation with later childhood brain dysfunctions, including cerebral palsy and cognitive impairment (Dammann and O'Shea, 2008; Malaeb and Dammann, 2009; Shatrov et al., 2010; O'Shea et al., 2012), while experimental models that initiate systemic inflammation with lipopolysaccharide document later brain structure alterations and brain dysfunctions (Wang et al., 2006). Based on such models, molecular mechanisms that lead to perinatally acquired periventricular white matter damage include aberrant activation of developmentally regulated apoptotic pathways and microglial activation (Kaindl et al., 2009). Acutely, these lead to axonal damage and depletion of oligodendrocyte precursors, and subsequently to damaged subplate neurons, disordered white matter tracts, and reduced brain "connectivity."

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0889-1591/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2012.12.012

Fetuses and preterm newborns are capable of mounting a vigorous inflammatory response to intra-uterine bacteria and other inflammatory stimuli that lead to preterm birth and placenta inflammation (Fichorova et al., 2011; Hecht JL et al., 2010; McElrath et al., 2011; Leviton et al., 2011b). Inflammatory stimuli evident after delivery, such as bacteremia, necrotizing enterocolitis, and those associated with processes leading to bronchopulmonary dysplasia (i.e., ventilator-associated barotrauma and oxidative stress), also appear capable of inducing a postnatal inflammatory response (Ng et al., 2010; Bose et al., 2011; Leviton et al., 2011a; Edelson et al., 1999). Systemic inflammation during the first weeks of life, as reflected in elevations of specific inflammation proteins, is predictive of neonatal cerebral white matter injury (Procianoy and Silveira, 2012; Leviton et al., 2011b) as well as microcephaly (Leviton et al., 2011a) and cognitive impairment at two years of age (O'Shea et al., 2012).

Possible relationships among perinatal inflammation stimuli (e.g., bacteremia, necrotizing entercolitis, ventilator-induced lung injury), inflammation, as reflected by elevations of inflammation-related proteins in the blood, and brain injury are shown in Fig. 1. As illustrated, inflammation stimuli could result in brain injury via mechanisms mediated by inflammation proteins (Model 1) or via mechanisms that do not involve these proteins (Model 2), or both of these mechanisms might be operative (Model 3). Further, different inflammation stimuli could cause injury via different pathways (Model 4).

In Model 1, a stimulus leads to an inflammatory response, which in turn, damages the brain. Support for the first part of this model comes from observations that inflammatory stimuli, such as necrotizing enterocolitis (Edelson et al., 1999; Martin CR et al., 2012), sepsis (Ng et al., 2003) (Leviton et al., 2012b), and mechanical ventilation (Bose et al., 2013), are associated with increased blood levels of inflammatory cytokines in preterm newborns. Systemic inflammation, in turn, has been associated with such indicators of brain damage as ventricular enlargement seen on early ultrasound scans of the brain (Leviton et al., 2012) and microcephaly at age 2 years (Leviton et al., 2011a), as well as such indicators of brain dysfunction as low mental and motor indices of the Bayley Scales of Infant Development (O'Shea et al., 2012).

In Model 2, a stimulus capable of inducing inflammation, damages the brain by means other than inflammation. Such stimuli can contribute to damage by: (1) sensitizing the brain to later adverse exposures (Eklind et al., 2005), (2) introducing other brain damaging exposures such as neurotoxic antibiotics (Floersheim and Logara-Kalantzis, 1972), and (3) promoting coagulation, which might lead to blood vessel occlusion and resulting brain damage (Leviton and Dammann, 2004). Recurrent/prolonged acidemia, which has been identified as an antecedent of systemic inflammation (Leviton et al., 2011a), can also contribute to, or be a marker of, other processes leading to brain damage (Wajner and Goodman, 2011; Lee et al., 2008; Leviton et al., 2010).

Model 3 combines elements of Models 1 and 2, as does Model 4. Model 3 differs from Model 4 by invoking two separate stimuli.

To further our understanding of the relationship of perinatal inflammation stimuli, neonatal systemic inflammation, and brain injury, we evaluated the hypothesis that information about both inflammation-related proteins as well as neonatal clinical illnesses predict subsequent cognitive impairment. If Model 1 explains the observed links between elevations of inflammation-related proteins and brain injury, and between neonatal illnesses that induce inflammation (e.g., bacteremia, necrotizing entercolitis, ventilator-induced lung injury) and brain injury, then statistical adjustment for elevations of blood levels of inflammation-related proteins should completely mask associations between the neonatal illnesses and brain injury. To evaluate this possibility, we used multivariable methods to analyze data from the Extremely Low Gestational Age Newborn (ELGAN) Study (O'Shea et al., 2009).

The ELGAN Study is an observational and longitudinal cohort study of infants born before the 28th week of gestation. The over-arching objective is to test the hypothesis that perinatal infection/inflammation is a risk factor for later brain dysfunctions, such as cerebral palsy, cognitive impairment, autism spectrum disorder, epilepsy, and behavioral abnormalities (O'Shea et al., 2009). To assess potential initiators of inflammation, placentas were cultured for microorganisms and examined histologically, and the concentrations of 25 inflammation-related proteins were measured in neonatal blood specimens collected on postnatal days 1, 7, and 14. Clinical assessments completed close to age two years include a standardized neurological examination and assessment of gross motor function, Bayley Scales of Infant Development, and screening for autism spectrum disorders. Ongoing clinical assessments close to age 10 years include neuropsychological assessments for intelligence and executive functioning, gold-standard assessments for autism spectrum disorders, an evaluation for epilepsy, assessments of language and communication abilities, and a behavioral assessment.

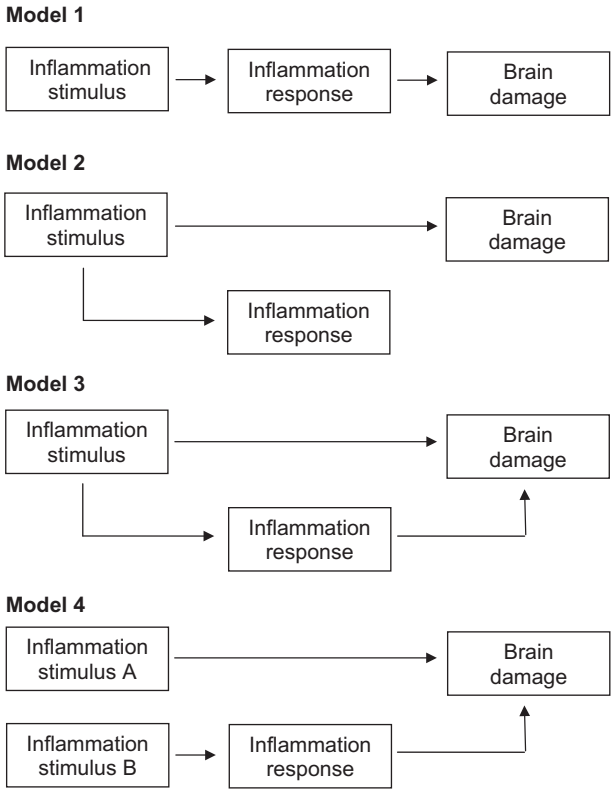

Fig. 1. Four models of the interrelationships among an inflammation stimulus (one or more inflammation-related illnesses), and inflammation response, and an indicator of brain damage.

2. Materials and methods

2.1. The ELGAN study

During the years 2002–2004, women delivering before 28 weeks gestation at one of 14 participating institutions in 11 cities in five states were asked to enroll in the study. The enrollment and consent processes were approved by the individual institutional review boards.