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Interleukin-2 as a neuromodulator possibly implicated in the physiopathology of sudden infant death syndrome

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ABSTRACT

Dysfunction in vital brainstem centers, including those controlling cardiorespiratory- and sleep/arousal pathophysiology, is reported in sudden infant death syndrome (SIDS). Biological mechanisms underlying SIDS, however, remain unclear. Cytokines are inter-cellular signaling chemicals. They can interact with neurotransmitters and might thus modify neural and neuroimmune functions. Cytokines could therefore act as neuromodulators. Interleukin (IL)-2 is a major immune-related cytokine. It has not been previously depicted in vital brainstem centers. We detected intense neuronal IL-2 immune-reactivity in the SIDS brainstem, namely in vital neural centers. This IL-2 overexpression might interfere with neurotransmitters in those critical brainstem centers, causing disturbed homeostatic control of cardiorespiratory and arousal responses, possibly leading to SIDS.

Sudden infant death syndrome (SIDS) is the sudden death of an infant (typically during sleep) below the age of one year, which is unexpected by history, and in which thorough postmortem examinations (including a full autopsy) and explorations of the "death-scene" fail to demonstrate an adequate cause of death [15]. SIDS remains the leading cause of postneonatal death in developed countries [15]. According to the "Triple-Risk Model" (see Refs. [3,7] for a review), SIDS is thought to result from combined intrinsic and extrinsic factors [3,7]. SIDS is therefore multifactorial, and underlying biological mechanisms remain unclear. Many studies highlighted imbalances in neural mechanisms controlling cardiorespiratory functions [11,17,21]. Besides, polysomnography (polygraphic electrophysiological records of vital functions during sleep) suggested vulnerability in homeostatic control involving autonomic central nervous system (CNS) functions during sleep/arousal [4,5,15]. Brainstem centers implicated in cardiorespiratory regulation involve a delicate balance in several neurotransmitters employed in complex neural networks [24]. SIDS victims often have preceding mild infectious/inflammatory conditions (like coryza/mild upper respiratory infections, soft stools/mild gastro enteritis, post-vaccinal fever, etc.). Such trivial affections were found to induce a hyper-tuned immune/inflammatory response including high levels of immune-inflammatory cytokines (inter-cellular signaling chemicals primarily involved in immune-inflammatory mechanisms) in biological fluids including the cerebrospinal fluid (CSF) in SIDS victims [25]. It is noteworthy here that cytokines have neuromodulatory effects whereby they can modify neurotransmission [6,18]. Intricate interactions between several cytokines and many neurotransmitters have thus been increasingly reported: acetylcholine and serotonin are among several neurotransmitters critically employed in brainstem functions. Nicotinic acetylcholine receptors' subtypes are associated with differential regulation of the production of pro- and anti-inflammatory cytokines. These receptors include the alpha-7 receptor and were shown to regulate cytokines' genes including that of IL-1 beta [2].

In our studies, on the role of cytokines in the developing human brain, we previously reported in situ (intracerebral) overexpression of interleukin (IL)-1 in vital neural centers, and we suggested that proinflammatory cytokines could have a role in SIDS [11]. IL-1 beta is involved in neuronal excitability by affecting the turnover and release of neurotransmitters, and alters synaptic transmission in rodents. We observed high IL-1 beta immunoreactivity in vital brainstem neuronal centers in SIDS victims [11], and we concluded that "this IL-1 overexpression might contribute to molecular inter-

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Table 1
Features of the study groups[a].

Patient N°	Age/sex	Main clinical and pathological features[b]
SIDS		
1	4.75 m/F	Fever/high brain weight[c]
2	3 m/M	None/minor old occipital PVL
3	4.3 m/F	Fever, mild diarrhea/ASD, high brain weight[c]
4	10 m/F	None/organs and brain congestion
5	2 m/M	Fever, coryza, sibling died of SIDS (2 d)/brain: microcalcifications
6	3.3 m/M	Esophageal reflux, sudden death/none
7	4.3 m/M	Esophageal reflux, suspected UTI/high brain weight[c]
8	4.5 m/M	Equinus varus deformity, preceding rhinitis/none
9	8 m/M	Coryza/high brain weight[c]
10	3 m/M	None/splenomegaly, respiratory mucosal congestion, high brain weight[c]
11	3.3 m/M	Coryza/suspected viral UTI, high brain weight[c]
12	5.2 m/M	Premature (7 m), twin, splenomegaly, LN enlargement?/high brain weight[c]
13	3 m/F	Acute GIT infection/high brain weight[c]
14	2.75 m/M	Twin, esophageal reflux/none
15	2 m/M	Coryza/none
16	3 m/M	Coryza/high brain weight[c]
17	7.75 m/F	Coryza/splenic follicular hyperplasia
18	4.3 m/M	Post vaccination mild diarrhea/none
Non-SIDS		
1	3 d/F	Respiratory failure/pulmonary hypoplasia, diaphragmatic hernia, cerebral hemorrhages.
2	10 d/F	Shock/CHD, post-operative acidosis.
3	3 m/M	Bradycardia-standstill/congenital combined immune deficiency, systemic CMV, vasculitis.
4	4.5 m/M	AIDS, pneumonia (CMV), coma/gastric perforation; post-operative and post-anoxic coma and brain necrosis.
5	11 d/F	CS for PMRM, congenital toxoplasmosis, hydrocephalus (8SD)/severe brain damage.
6	8 d/M	PMRM & cord prolapse; CS, perinatal asphyxia, shock/HMD, bronchopneumonia, gastric perforation.
7	8 d/F	Asphyxia (cardiac malformation-HF)/early PVL.
8	20 d/F	MOF/viral infection (IM?).
9	2.3 m/M	Metabolic disorder & congenital steato-cirrhosis/GIT & intracranial hemorrhages, brain damage.
10	10 d/M	Asphyxia (cardiac malformation), infection/bronchopneumonia, late PVL.

Abbreviations: SIDS = sudden infant death syndrome; d = day; m = month; M = male; F = female; PVL = periventricular leukomalacia; ASD = atrial septal defect; UTI = urinary tract infection; LN = lymph node; GIT = gastro-intestinal tract; CHD = congenital heart defect; CMV = cytomegalovirus; CS = caesarian section; PMRM = premature ruptured membrane (during delivery); HMD = hyaline membrane disease; HF = heart failure; MOF = multiple organs failure; IM = infectious mononucleosis.

[a] Several of the SIDS and non-SIDS brains were also included in other studies [10–12].

[b] Autopsy findings are given after the slash.

[c] "High brain weight" noticed in several of these cases was found to be not related to brain edema. This was in line with observations in a separate study (Ref. [13]) whereby we reported that the weights of the brains of infants who died from SIDS were invariably heavier in comparison with those of a group of age-matched controls issuing from the same local population. It is not known whether increased brain weight is related to cytokine up-regulation in those brains.

actions in brainstem neurovegetative centers, causing disturbed homeostatic control of cardiorespiratory and arousal responses, possibly leading to SIDS" [11].

Another cytokine, namely IL-2, is a major immune-related cytokine that was originally characterized as a T-immune cells (lymphocytes) growth factor, but was later found to have a wider spectrum of functions, targets and sources [18,19]. To our best knowledge, IL-2 has not been reported in human brainstem neuronal centers.

In the present study, we conducted neuropathological explorations and carried out in situ (intracerebral) immunohistochemical studies to investigate the possible expression of IL-2, primarily in brainstem vital centers in SIDS brains, and, we compared cytokine immunoreactivity pattern with another population of infants (non-SIDS brains) who died of severe infectious/inflammatory conditions.

We examined the brainstem from 28 autopsied infants who died before the age of one year. Ten had severe (fatal) illnesses (non-SIDS). Eighteen died unexpectedly (SIDS group). The non-SIDS population included infants who died of diverse severe pathological conditions, mainly, infectious, hemodynamic, metabolic, severe congenital, or other serious conditions. Each study group included infants of both sexes. A summary of clinical and pathological data for each infant in both study groups is provided in Table 1.

Neural tissue analysis in this study has been processed within the legal frame of complete autopsy procedures involving adequate and full neuropathological examinations. All such autopsy and postmortem procedures were conducted according to the legal and ethical rules applied in our institutions.

The brains were fixed for 3–6 weeks in 20% buffered formalin. All brains were processed in a similar way and using the same technical procedures. As part of a thorough neuropathological examination, serial sections of the brainstem were obtained from all cases in both study groups. Paraffin-embedded tissue blocks were cut, and 10 μm sections were first stained with hematoxylin–eosin and cresyl violet (Nissl stain) for routine neuropathological examination. Selected blocks from the 28 autopsied infants were further processed with in situ immuno-histochemical techniques. We applied a mouse monoclonal antibody (ImmunoKontact, Wiesbaden, Germany) directed against human IL-2, to stain the human brainstem. The antibody was used in a dilution of 1:50. In each case, 8 μm thick paraffin-embedded sections were obtained on silanized slides (SuperFrost/Plus, O. Kindler GmbH & Co., Freiburg, Germany). After deparaffinization and hydration with xylene and graded alcohol solutions, the antigen was unmasked by pressurized heating for 5 min at 120 °C in a 10 mM citrate buffer (DAKO, Carpinteria, CA) at pH 6. After immersion in a TRIS buffer at pH 7.6, the brain sections were incubated with the first monoclonal antibody for 30 min at room temperature. The immunoglobulin was diluted in phosphate-buffered saline (pH 7.4). Then, sections were rinsed with TRIS buffer (pH 7.6). Labeling was revealed using the DAKO EnVision system, AP kit according to the manufacturer's instructions. Briefly, the sections were rinsed with TRIS buffer, incubated for 30 min with an alkaline phosphatase-labeled polymer conjugated with purified goat anti-mouse immunoglobulins. The labeling was visualized with fast red substrate-chromogen applied for 10 min. Counterstaining was performed with Meyer hematoxylin. An additional set of sections was treated similarly but without the primary