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Sudden Infant Death Syndrome Victims Show Local Immunoglobulin M Response in Tracheal Wall and Immunoglobulin A Response in Duodenal Mucosa

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ABSTRACT. Twenty-two sudden infant death syndrome (SIDS) cases and 22 controls were examined immunohistochemically with regard to IgA, IgM, and IgG plasma cells in tracheal wall and duodenal mucosa. Furthermore, the presence of secretory component in tracheal surface and gland epithelium as well as in duodenal crypt and villus epithelium were evaluated. The examined specimens were obtained at autopsies. The control groups consisted of 11 infants who died of noninfectious causes and 11 who died of infections. In the tracheal wall, the SIDS group had higher IgM cell numbers than the control group that died of noninfectious causes ($p < 0.01$), whereas the SIDS victims had lower IgA and IgM cell numbers than the infectious control group ($p < 0.01$). In the duodenal mucosa, the SIDS group had significantly higher IgA cell numbers than the noninfectious control group ($p < 0.02$) but lower IgA cell numbers than the infection group ($p < 0.01$). Secretory component was present in the epithelium from all SIDS cases and controls, both in the tracheal wall glands and in the duodenal crypt mucosa. These findings indicate that the mucosal immune system is stimulated in SIDS. (*Pediatr Res* 31: 372–375, 1992)

Abbreviations

SIDS, sudden infant death syndrome
SC, secretory component
Hx, hypoxanthine

Among the Scandinavian countries, Norway has the highest incidence of SIDS, and the incidence seems to be increasing (1). Both the etiology and the pathogenesis of SIDS are at present unclear. We have, however, recently published data that indicate that hypoxia precedes death in approximately 80% of the SIDS cases (2, 3). There are several factors that might initiate respiratory inhibition and hypoxia. Because SIDS is more common in colder climates and an increased incidence correlates with the season of respiratory tract infections (4), an immune response in the respiratory tract might be one possible "trigger mechanism."

Adaptive immunity at mucosal surfaces of children and adults is mainly exerted by secretory IgA and IgM antibodies, which represent the quantitatively most important humoral immune system of the body (5–8). SC, the poly-Ig receptor, is imperative for the secretion of secretory IgA and secretory IgM (9). Increased concentrations of IgM, IgG, and to a lesser extent IgA in lung lavage fluid from SIDS victims (10) and raised numbers of IgA, IgM, and IgG producing cells in salivary glands (11) indicate a stimulation of the secretory immune system in the upper airways and lungs. Furthermore, lack of SC in the tracheal epithelium preventing the transport of dimeric IgA and pentameric IgM has been suggested as an etiologic factor in SIDS (12).

The purpose of this study was to investigate the humoral immune response and the expression of epithelial SC in the wall of the upper respiratory tract and the duodenal mucosa in SIDS.

MATERIALS AND METHODS

Tissue specimens. Tracheal wall and duodenal mucosa specimens were obtained from 22 infants who died from SIDS (15 males and seven females; median age 4 mo, range 1–7 mo). Tracheal wall samples were taken from the carina; one sample was rejected because of bacterial growth.

The control group consisted of 22 specimens from tracheal wall and from duodenal mucosa. The median age of the control group was 4 mo (range 1–12 mo). Eleven infants died of the following causes: congenital heart disease ($n = 4$), head injury ($n = 2$), infanticide ($n = 1$), CO intoxication ($n = 1$), drowning ($n = 1$), battered child syndrome ($n = 1$), and accidental suffocation ($n = 1$). None of these infants had clinical evidence of an infection. Eleven infants died of a specific infection: sepsis ($n = 4$), pneumonia ($n = 6$), or meningitis ($n = 1$). All tissue specimens were examined independently by two pathologists.

Clinicopathologic data were obtained from birth clinics and local hospitals admitting the SIDS infants.

Immunohistochemistry. Two adjacent tissue samples approximately 1 mm thick were excised from each specimen. One was fixed directly in cold ethanol 4°C, whereas the other was extracted in phosphate-buffered isotonic saline (pH 7.4) for 48 h at 4°C to remove extracellular diffusable Ig before ethanol fixation (13). Thereafter, both samples were processed for paraffin embedding.

Ig-producing immunocytes. Serial sections cut at 6 μm from the PBS-extracted samples were evaluated by paired immunohistochemical staining. Control of the staining specificity and the preparation and characterization of fluorescein isothiocyanate and tetramethylrhodamine isothiocyanate fluorochrome conjugates have been reported elsewhere (14). They were all rabbit reagents with specificities for human IgA, IgM, and IgG, and were applied in various pairs of contrasting colors. Furthermore, fluorescein isothiocyanate-labeled sheep anti-SC was combined with tetramethylrhodamine isothiocyanate-labeled anti-IgA (15).

Epithelial expression of SC. SC were demonstrated using the following procedure: One section was stained by a routine method using hematoxylin, azophloxin, and saffron (16). The

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adjacent section was first incubated for 30 min with a tetramethylrhodamine isothiocyanate-labeled sheep IgG-anti SC; its OD ratio (280 nm/515 nm) was 1.7, and its working concentration was 0.64 g IgG/L (15).

Microscopy, photography, and cell counting. A Leitz Aristoplan (Leica Microscopie und Systeme GmbH, Wetzlar, Germany) microscope was used. It was equipped with a Ploem-type vertical illuminator providing narrow-band excitation and selective filtration of red and green emission colors. Both tracheal and duodenal sections were examined with regard to the presence of IgA, IgM, and IgG plasma cells in the lamina propria and submucosa. We examined the entire cross-section of the carina. An occular grid (Leitz code no. 519902) was applied. The grid area was 0.01 mm^2. In most specimens both branches of the bronchus were intact, while in some, one bronchus was partly destroyed. In the duodenal samples, areas with preserved histologic structures were examined. Density of plasma cells was given as cell number per grid area. All fluorescent cells with a discernible nucleus and cell-like body with a pure green or red cytoplasmic color were included.

Cell enumeration and reproducibility of procedure. To obtain a representative mean cell count for each subject, it was necessary to test the number of grids that had to be counted to get a stable mean.

The cell counting was performed independently by two different observers (L.S. and T.O.R.) in 22 grid areas, and the results were subjected to correlation analyses. The ability to reproduce the whole procedure—sectioning, staining, and cell enumeration by the same observer—was tested blindly after 5 mo on 28 specimens.

Epithelial expression of SC was either judged to be present or not present. Tracheal gland epithelium and surface epithelium, as well as duodenal crypt epithelium and villous epithelium, were examined separately.

Clinical variables. Birth characteristics, weight gain, time of the year, history of respiratory tract infections, microbiologic findings, as well as pathologic findings at the autopsy, especially lung microscopy, were recorded.

Corpus vitreum Hx levels. Corpus vitreum Hx levels were determined by HPLC (2, 3) and corrected according to postmortem time (3).

Statistical analysis. The two-tailed Mann-Whitney U test was used for comparison between the two groups. For testing of interpersonal reproducibility of cell counting and for reproducibility of the whole procedure, the Pearson/correlation test was applied.

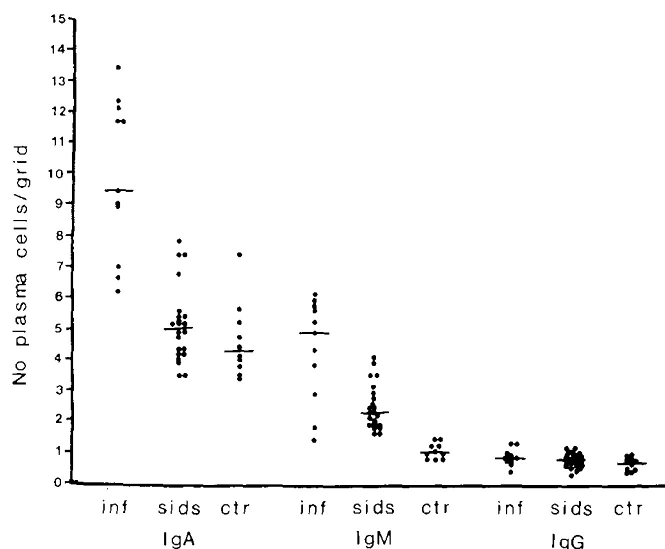

Fig. 1. Number of Ig-producing cells in the tracheal wall of subjects with SIDS (*sids*) and controls (*ctr*). SIDS tracheal lamina propria and submucosa contain significantly ($p < 0.01$) more IgM plasma cells than controls. The infectious control group (*inf*) contains significantly ($p < 0.01$) more IgA and IgM than the subjects with SIDS.

RESULTS

Representative cell enumeration. To obtain a stable mean for each subject investigated, it was necessary to count 20 grids in each section.

Reproducibility. Cell counts by two different observers were significantly correlated ($r = 0.97$, $p < 0.001$). The blind reproducibility test of the staining procedure and cell enumeration by the same observer was also significantly correlated ($r = 0.98$, $p < 0.001$).

Ig-producing cells. The tracheal lamina propria and submucosa of SIDS victims contained significantly ($p < 0.01$) more IgM plasma cells (median 2.3; range 1.6–4.0) than controls (median 0.95; range 0.8–1.5). For the IgA and IgG isotypes, no difference was found. The infectious control group had significantly ($p < 0.01$) more IgA plasma cells (median 9.4; range 6.2–13.4) than the SIDS group (median 5.0; range 3.4–7.8) and more IgM plasma cells (median 4.9; range 1.4–6.1) than the SIDS group (median 2.3; range 1.6–4.0) (Fig. 1). Duodenal mucosa from SIDS cases contained significantly ($p < 0.02$) more IgA plasma cells (median 7.0; range 4.4–11.2) than controls (median 5.0; range 3.4–7.4) (Fig. 2*A*). No such difference was seen for the isotypes IgM and IgG. The infectious control group had significantly ($p < 0.01$) more IgA plasma cells (median 12; range 6.8–18.8) than the SIDS group (median 7.0; range 4.4–11.2) (Fig. 3).

Epithelial SC. Epithelial SC was present in tracheal wall glands and duodenal crypt mucosa in all SIDS cases and all controls (Fig. 2*B*), whereas the expression of this marker was sometimes absent in the tracheal surface epithelium and always very weak in duodenal villi epithelium of both groups.

Clinical variables. Eighteen of the infants were born at term (38–42 wk of gestation), whereas four were premature and one was postmature (median 39⁴⁄₇ wk; range 33 to 42³⁄₇ wk). Ten of the SIDS victims had relatively poor weight gain, *i.e.* <50% of expected weight gain according to birth weight, length, and head circumference (17).

Twenty infants (90%) died in the period between October and April. Thirteen infants (59%) had definite signs and symptoms of an upper respiratory tract infection, rhinitis, cough, and/or fever. Nose swabs from two of these infants gave growth to *Haemophilus influenzae*; one nasopharyngeal aspirate was positive on adenovirus and another one was positive on respiratory syncytial virus. In eight of the infants with clinical symptoms of an upper respiratory infection, lung microscopy showed signs of inflammation, slight thickening of alveolar septa, and slight increase in lymphocytes and macrophages around segmental bronchi. These changes were judged to be insufficient to cause death.

Corpus vitreum Hx levels. Eighteen of 20 investigated SIDS subjects had increased vitreous humor Hx levels when compared with 11 noninfectious and 11 infectious controls ($p < 0.01$) after the values had been corrected for expected postmortem time increase. The results are given in Table 1 and are part of a previous study (3).

DISCUSSION

The presence of SC in tracheal and duodenal epithelium is in contrast to the theory put forward by Ogra *et al.* (12) in 1975 that lack of SC might represent an etiologic factor in SIDS. The most important finding of the present investigation, however, was the increased number of IgM immunocytes in the tracheal wall and of IgA immunocytes in duodenal mucosa in SIDS victims. One great problem with studies in SIDS is to have sufficient normal controls. To our knowledge, there is only one previous study on the humoral mucosal immune system of the

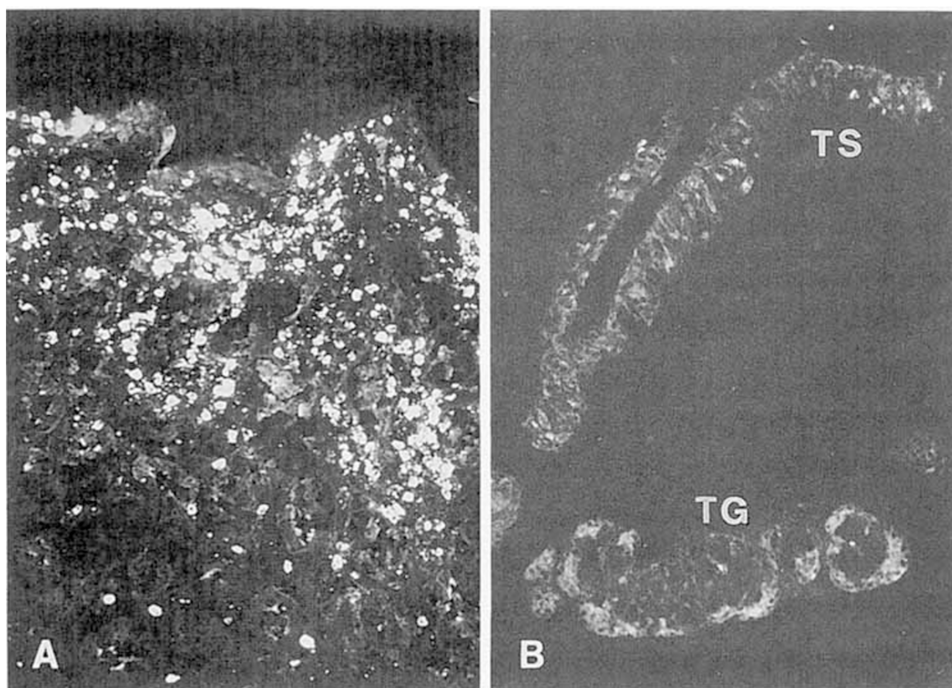

Fig. 2. *A*, Immunofluorescence staining of IgA in duodenal mucosa from a case of SIDS. Numerous IgA immunocytes are present, and the crypt epithelium is intensely IgA-positive. Magnification ×90. *B*, SC in tracheal wall in SIDS. SC is present in both tracheal gland epithelium (*TG*) and in tracheal surface epithelium (*TS*). Magnification ×90.

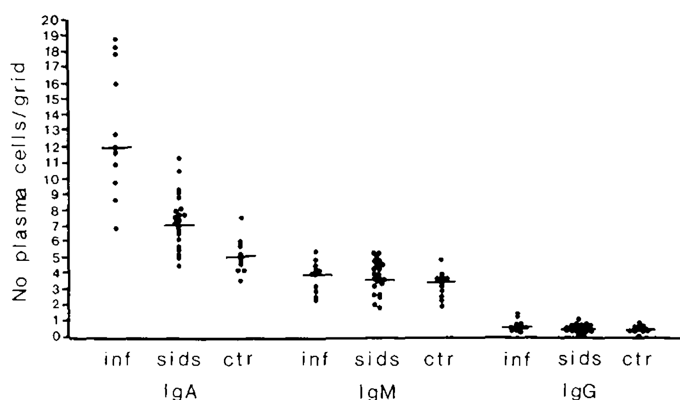

Fig. 3. Number of Ig-producing cells in the duodenal mucosa from subjects with SIDS (*sids*) and controls (*ctr*). SIDS duodenal mucosa contains significantly ($p < 0.02$) more IgA plasma cells than controls. The infectious control group (*inf*) contains significantly ($p < 0.01$) more IgA than the SIDS group.

Table 1. *Hx levels in SIDS cases and controls (μmol/L)**

	Median	Range
SIDS ($n = 22$)	253	39-603
Control ($n = 11$)	18	4–32
Infection ($n = 11$)	64	6–148

* The values are corrected for postmortem time.

respiratory tree (18). This study on segmental bronchi included nine infants from the time of birth to the age of 1 y. The authors found ratios of IgA, IgM, and IgG immunocytes that were comparable to our observations in the trachea. Additionally, our own recent study on the ontogeny of tracheal mucosal immunity gave the same results (Stoltenberg L, Thrane PS, Rognum TO, unpublished observations). The density of IgA, IgM, and IgG immunocytes in duodenal mucosa in the normal controls in the present study was similar to that observed in a previous investigation (18a).

The increased number of IgM immunocytes in SIDS tracheal lamina propria and submucosa, as well as the IgA response in SIDS duodenal mucosa indicates that both lymphoid tissue in the upper respiratory tract and gut-associated lymphoid tissue are stimulated in SIDS. These findings are in agreement with those of Forsyth *et al.* (10) on lung lavage fluid and Thrane *et al.* (11) in salivary glands. The cause of this stimulation is not known. It is, however, possible that a viral infection in the upper respiratory tract may elicit such an immune response (19). The fact that 13 (59%) of our SIDS population had definite clinical signs and symptoms of an upper respiratory tract infection before death supports this suggestion. It is well known that SIDS is more frequent during epidemics of respiratory syncytial virus and whooping cough (20).

Eight of those with symptoms (61.5%) had IgM cell numbers above the median in their carina specimens, whereas 11 (85%) had IgA cell numbers above the median in their duodenal specimens, indicating a strong relationship between the high density of these immunocyte isotypes and respiratory infections. However, the group with lethal infections had significantly higher immunocyte numbers than the SIDS infants. This difference might indicate that the immune response demonstrated in the SIDS group is by itself not lethal. The fact that the infectious control infants had significantly lower ($p < 0.02$) Hx levels in their vitreous humor than the SIDS infants suggests that the death mechanism in the former group is a different one than that in SIDS. However, other potentially predisposing factors such as astrogliosis in the brainstem (21–23) and delayed maturation of the CNS (24) have to be taken into consideration.

Brainstem astrogliosis in SIDS may indicate earlier episodes of hypoxia (20–22). Furthermore, the increased levels of Hx in the vitreous humor in 18 of the 20 investigated SIDS cases indicate that hypoxia probably was the ultimate death mechanism in these cases, but not in the noninfectious controls (Table 1). The Hx values in the infectious control group show an intermediate distribution, indicating that hypoxia may play a role in some of these cases. Other death mechanisms such as sudden cardiac arrest or ventricular fibrilation (25, 26), as well as metabolic disorders (27), must be considered in the two infants

with normal Hx values who had SIDS (two infants were not investigated). These findings support our belief that some infants are probably "at risk" already at birth and that these infants are particularly vulnerable at the age of 2-4 mo, as suggested by other researchers (23, 28).

A possible cause of autonomous dysregulation is the delayed maturation of the CNS (24), and a possible trigger mechanism for the down-regulation of respiration might be an immune response in the periphery communicated to the CNS by blood-borne cytokines or perhaps by retrograde axonal signals (29). The immune response demonstrated in the present study may thus act as a trigger mechanism for the fatal hypoxia in SIDS (2, 3). Furthermore, immune responses in organs other than those investigated in this study may act in a similar fashion (11).

A prolonged sleep apnea occuring in a predisposed child with inhibited arousal may lead to severe hypoxia, coma, and death. More studies involving measurements of various cytokines in cerebrospinal fluid and brain tissue from SIDS cases and controls as well as animal experiments may offer a better understanding of the trigger mechanisms in SIDS.

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