

Changes in the concentration and distribution of immunoglobulin-producing cells in SIDS palatine tonsils

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Seventeen sudden infant death syndrome (SIDS) cases and 9 controls, were examined immunohistochemically with regard to the presence of IgA-, IgM-, IgD, and IgG, as well as for the subtypes IgG₁-, IgG₂-, IgG₃-, and IgG₄-immunocytes. Differences in compartmentalization were also investigated. Differences were demonstrated between SIDS and controls in total number of IgG cells per 0.1 mm² tissue area (median: 18.3, range: 12.3–30.2 versus median: 6.3, range: 2.0–14.6) ($p < 0.01$), and for IgA immunocytes (median: 3.9, range: 2.4–5.0 versus median: 1.5, range: 1.1–3.7) ($p < 0.05$), while no differences were demonstrated for IgM cells (median: 1.8, range: 1.2–3.3 versus median: 1.8, range: 0.7–5.6) or IgD cells (median: 1.9, range: 0.8–2.9 versus median: 1.6, range: 0.7–2.4). Differences were demonstrated between SIDS and control IgG plasma cells in all the four palatine tonsillar compartments; germinal centre ($p < 0.01$), mantle zone ($p < 0.05$), interfollicular area ($p < 0.01$) and reticular epithelium ($p < 0.01$). Furthermore, the number of IgA cells was higher in SIDS vs. controls in both the germinal centre (median: 1.4, range: 0.6–2.1 versus median: 0.6, range: 0.3–1.3) ($p < 0.05$) and in the interfollicular area (median: 2.2, range: 1.1–3.1 versus median: 0.5, range: 0.4–2.0) ($p < 0.01$). For IgM immunocytes, differences were demonstrated in the germinal centre (median: 1.0, range: 0.4–1.6 versus median: 0.4, range: 0.3–1.3) ($p < 0.01$) as well as in the germinal centre (median: 0.6, range: 0.5–0.8 versus median: 0.4, range: 0.3–0.7) ($p < 0.01$) and in the interfollicular area (median: 1.2, range: 0.8–1.6 versus median: 0.5, range: 0.5–0.7) ($p < 0.01$) in the IgD immunocyte group. The total number of IgG₁- and IgG₃-immunocytes were increased in SIDS (median 15.6, range: 5.3–58.9 versus median: 2.5, range: 1.5–8.4) ($p < 0.01$) and (median: 3.6, range: 0.4–8.6 versus median: 1.1, range: 0.4–1.3) ($p < 0.01$) respectively. Furthermore, significantly increased numbers of these two subclasses, were seen in all the four compartments in the SIDS cases. The palatine tonsillar immune system is stimulated in SIDS. Furthermore, the changes being predominantly in the germinal centres and interfollicular areas, are indicating a recent stimulation, and the IgG-subgroup response pattern makes a viral protein antigen the most likely stimulant.

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The observation that an otherwise healthy infant may die in the presence of a common seasonal respiratory infection (1–4), was noted long before the SIDS-definition was agreed upon in 1969 (5). SIDS is more common in colder climates and an increased incidence correlates with the season of respiratory tract infections (6). More than half of the SIDS victims have reportedly had a mild infection in the

week preceding death (7), and up to 75% have had histological or microbiological evidence of a respiratory tract infection at autopsy (8–12). Furthermore, recent research has indicated that SIDS-infants have a stimulated immune system at time of death (13–15). All the previous studies have been on the secretory immune system, of which the palatine tonsils are not a part due to the lack of secretory

Table 1. Clinical and autopsy data about the SIDS infants

Signs/ symptoms	Microbiology	Autopsy	Immune response
Premature, vomiting [#]			
More sleep, off feeds [#]			
Resp. inf.	Branhamella catarrhalis in lungs	Inflammation in larynx	IgG, IgA, IgA ₁ and IgG ₃
Resp. inf.	Klebsiella pneumonia in airways/lungs	Purulent mucus in airways	IgG, IgA, IgM and IgG ₁
Resp. inf.	Staphylococcus aureus in lungs	Inflammation in larynx and trachea	IgG, IgA, IgG ₁ and AgG ₃
Resp. inf.		Slight inflammation in airways	IgG
Resp. inf.			IgG, IgG ₁ , IgG ₂
Vomiting	Staphylococcus aureus in airways and GIT		IgG and IgA
Fever, restless and fussy*	Staphylococcus aureus in CSF, blood, liver and spleen	Necrosis in liver. Inflam. in intraventr. septum	IgG, IgA, IgM, IgD and IgG ₃
Resp. inf.*	Respiratory cyncytial virus	Strawcoloured fluid in airways	IgG, IgA, IgM, IgG ₁ and IgG ₃
Resp. inf., restless and fussy*	Abundant growth of Haemophilus influenza in airways	Inflammation in airways. Purulent mucus	IgG, IgA, IgM, IgD, IgG ₁ , IgG ₂ and IgG ₄

*Diagnosed as Borderline SIDS.

#Visited local GP within 48 h of death.

component (16). They are strategically positioned to monitor both airborne and alimentary antigens, the deep crypts make a massive antigenic and mitogenic load possible, and they contain all the cells necessary to produce an antibody response, namely antigen presenting cells, different T-cell subsets and B-cells (17). Moreover, the palatine tonsils may play an important role in "priming" the bronchus associated (BALT) and gut associated (GALT) lymphoid systems (18), and may provide for widespread dissemination of effector and memory cells to distant mucosal surfaces to provide continuous body-wide surveillance.

Our purpose was to investigate the humoral immune responses in SIDS palatine tonsillar tissue with particular interest in IgG-subgroup changes and changes in compartmentalization.

Patients and methods

Twenty-five SIDS and 16 controls were included in the preliminary study. However, on examination of these specimens, only 17 SIDS and 9 controls were accepted for the immunocyte examination due to very strict morphological criteria. All the 4 palatine tonsillar compartments; reticular epithelium, inter-follicular area, mantle zone, and germinal center had to be present in the specimen to be accepted for immunohistochemistry and cell counting. The control group was matched for age and the sex distribution was as in the SIDS population (SIDS: 9 = ♂, 8 = ♀; control: 5 = ♂, 4 = ♀). The following causes of death were represented: congenital heart defects (n = 2), head injury (n = 2), infanticide (n = 2), accidental strangulation (n = 1), CO-intoxication (n = 1), and intracranial hemorrhage (n = 1). Both the infants with congenital heart defects died during operations/investigations, while the infant with intracranial hemorrhage died within 2 h of diagnosis. All the other deaths were immediate. (It was impossible to obtain accurate data on time interval in the case of CO-intoxication.)

Sixteen of the SIDS infants were born at term (38–42 wk of gestation), while one was premature (girl, 32 wk of gestation, and did not require respiratory support).

Six of the SIDS infants had no particular clinical history and no specific findings at autopsy. Nine (53%) of the infants had definite signs and symptoms of an upper respiratory tract infection, rhinitis, cough, and/or fever. Other symptoms observed were increase in hours of sleep, restlessness and the infant described as being fussy. The average age in this group of SIDS infants with respiratory tract infections was 4.2 ± 1.9 months. Data about the SIDS infants with abnormal clinical history and/or autopsy, are given in Table 1. We decided that an immunocyte count above mean, could be considered as an immune response in this summary.

Tissue

Tissue specimens were obtained during the post-mortem examination (SIDS: mean post-mortem time: 28 h, range 10–72 h. Controls: mean post-mortem time: 24 h, 8–63 h). Sections approximately 1–2 mm thick, were paraffin-embedded after fixation in cold ethanol, either directly or after extraction in cold phosphate buffered saline (PBS) for 24 h (19) at 4°C to remove extracellular diffusible immunoglobulins.

Total IgG-, IgA-, IgM-, and IgD-immunocyte counts

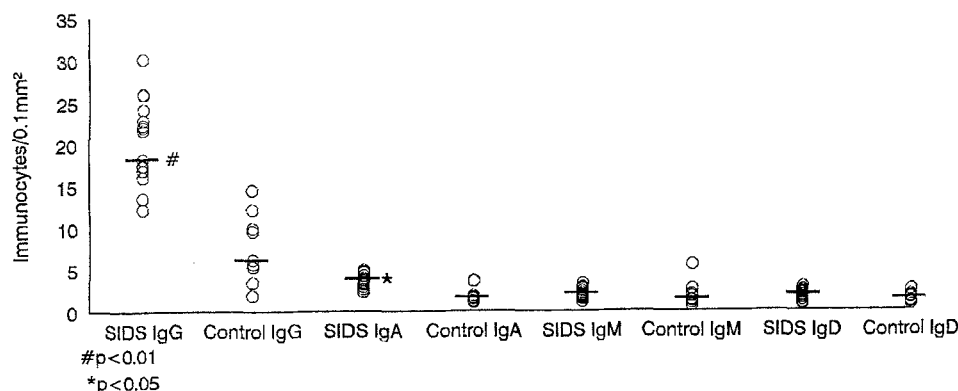


Fig. 1. The results of the total immunocyte counts given as absolute immunocyte number/0.1 mm² tissue unit. Values are given as median and range. (The IgG-subgroups have not been included).

Immunohistochemistry

Adjacent tissue sections were cut at 6 µm and investigated with direct immunohistochemical technique for the presence of IgA-, IgM-, IgG-, IgG₁-, IgG₂-, IgG₃-, IgG₄-, and IgD-producing cells (20,

21). Polyclonal rabbit reagents with specificities for human (IgG) γ-chain, (IgA) α-chain, (IgM) μ-chain, and (IgD) δ-chain respectively were all purchased from DAKO immunoglobulins A/S, Denmark. Mouse monoclonal reagents to IgG₁-, IgG₂-, IgG₃-, and IgG₄-plasma cells were purchased from The Binding Site Ltd., Birmingham B15 2SQ, England.

Fluorescence microscopy and photography

Observations were performed in a Leitz Aristoplan fluorescence microscope. Narrow band excitation and selective filtration of the fluorescence colours were obtained with a Phloem-type epiilluminator.

Quantification of IgG-, IgA-, IgM-, IgD-, IgG₁-, IgG₂-, IgG₃-, and IgG₄-immunocytes, were determined by means of an ocular grid (Leitz code No 519902) and given as cell number per grid area (0.1 mm²). Cell counting was carried out in a systematic manner throughout every lymphoid compartment; germinal center, mantle zone, extrafollicular area, and reticular epithelium. All fluorescent cells with a discernable nucleus and celllike body with a pure green or red cytoplasmic colour were included in the enumeration. Agfa 800 ASA daylight film was used in recording the findings. The inter- and intrapersonal reproducibility of the scoring system and the staining procedure was tested blindly.

Statistics

For comparison between groups, the Mann-Whitney U-test was applied and for reproducibility testing, Kappa test was used.

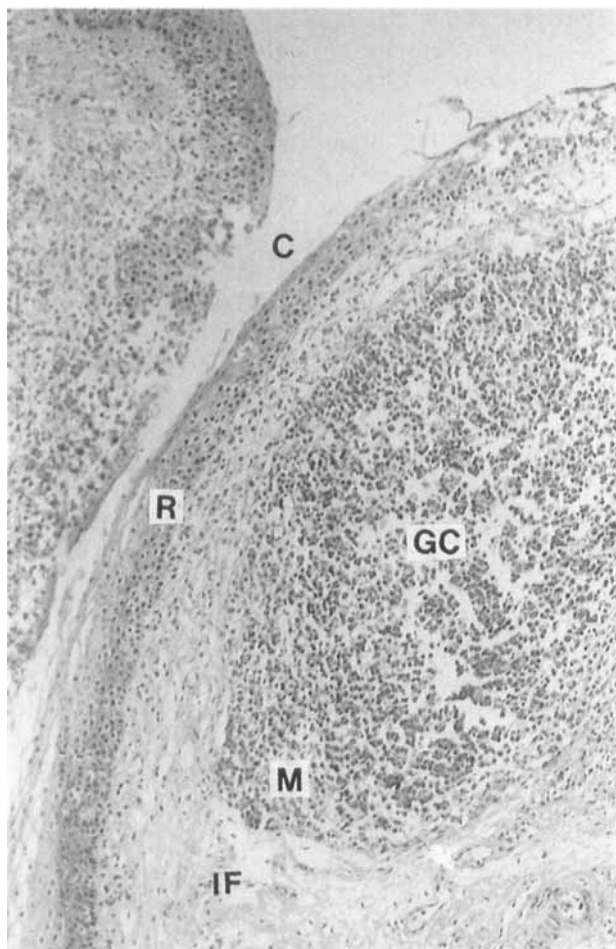


Fig. 2. Morphology of tonsillar tissue. Crypt (C), reticular epithelium (R), interfollicular area (IF), mantle zone (M), and germinal center (GC) can be seen (×100).

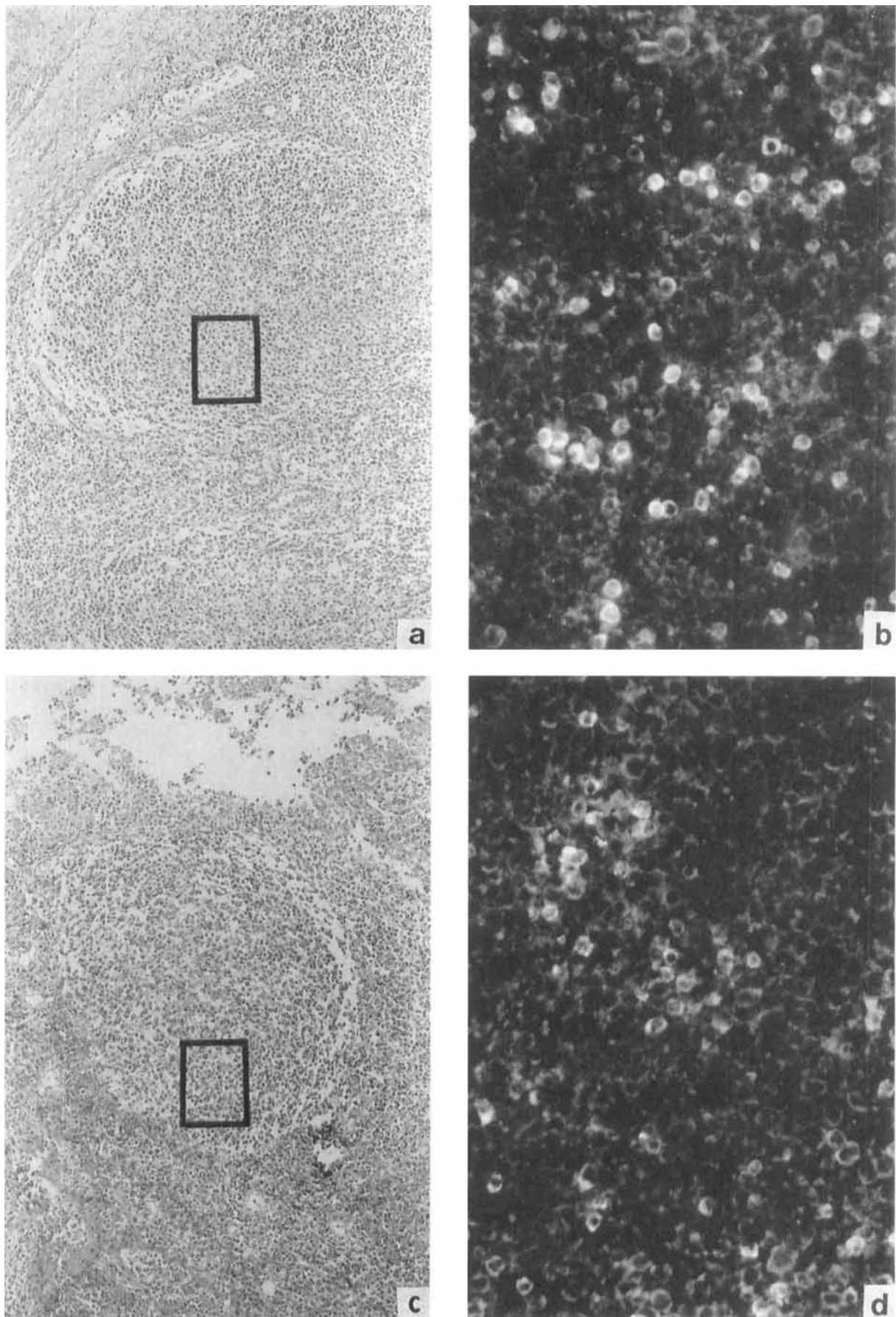


Fig. 3. IgG response. He and immunohistochemistry. a) SIDS-specimen from a boy aged 5 months. Area enlarged is marked out. b) IgG response in germinal centre ($\times 320$). c) Tonsil from a control, boy 4.5 months old. Area enlarged is marked out. d) IgG response in germinal centre ($\times 320$).

Table 2a. IgG-, IgA-, IgM-, and IgD-plasma cells in SIDS and control palatine tonsils. Values are given as median and range

		SIDS	Controls	p-value
IgG	total	18.3 (12.3–30.2)	6.3 (2.0–14.6)	<0.01
	RE	2.3 (0.1–13.4)	1.2 (0.3–3.1)	<0.01
	IF	5.2 (1.5–35.1)	3.0 (0.7–4.5)	<0.01
	MZ	0.5 (0.3–2.5)	0.2 (0.1–0.6)	<0.01
	GC	11.8 (2.0–31.6)	1.9 (0.6–8.6)	<0.01
IgA	total	3.8 (2.4–4.9)	1.5 (1.1–3.7)	<0.05
	RE	0.3 (0.0–0.5)	0.2 (0.0–0.3)	n.s.
	IF	2.2 (1.1–3.1)	0.5 (0.4–2.0)	<0.01
	MZ	0.1 (0.0–0.3)	0.1 (0.0–0.3)	n.s.
	GC	1.4 (0.6–2.1)	0.6 (0.3–1.0)	<0.05
IgM	total	1.8 (1.2–3.3)	1.8 (0.7–5.6)	n.s.
	RE	0.0 (0.0–0.4)	0.1 (0.0–0.3)	n.s.
	IF	0.8 (0.5–1.3)	0.3 (0.2–0.9)	n.s.
	MZ	0.0 (0.0–0.2)	0.0 (0.0–0.2)	n.s.
	GC	1.0 (0.4–1.6)	0.5 (0.3–1.3)	<0.01
IgD	total	1.9 (0.8–2.9)	1.6 (0.7–2.4)	n.s.
	RE	0.3 (0.1–0.4)	0.2 (0.1–0.2)	n.s.
	IF	1.2 (0.8–1.6)	0.5 (0.5–0.7)	<0.01
	MZ	0.1 (0.0–0.2)	0.1 (0.0–0.2)	n.s.
	GC	0.6 (0.5–0.8)	0.4 (0.3–0.7)	<0.01

RE = Reticular epithelium, IF = Intrafollicular area, MZ = Mantle zone, GC = Germinal centre.

Table 2b. IgG subgroup changes in SIDS and controls. Values are given as median and range

		SIDS	Controls	p-value
IgG ₁	total	15.6 (5.3–58.9)	2.5 (1.5–8.4)	<0.01
	RE	2.3 (1.3–7.6)	0.6 (0.2–1.4)	<0.01
	IF	3.2 (0.7–29.7)	1.6 (0.4–2.4)	<0.01
	MZ	0.3 (0.1–1.8)	0.1 (0.1–1.1)	<0.01
	GC	7.4 (0.7–21.3)	0.4 (0.4–5.5)	<0.01
IgG ₂	total	1.8 (0.3–8.7)	1.9 (0.4–6.0)	n.s.
	RE	0.8 (0.4–2.5)	0.6 (0.4–1.7)	n.s.
	IF	0.9 (0.4–4.4)	0.7 (0.1–2.9)	<0.01
	MZ	0.1 (0.0–0.3)	0.2 (0.0–0.5)	n.s.
	GC	1.4 (0.4–3.6)	1.1 (0.1–1.7)	n.s.
IgG ₃	total	3.6 (0.4–8.6)	1.1 (0.4–2.3)	<0.01
	RE	1.0 (0.2–2.3)	0.2 (0.1–1.3)	<0.01
	IF	1.6 (0.5–4.5)	0.3 (0.1–0.5)	<0.01
	MZ	0.3 (0.1–0.8)	0.2 (0.0–0.6)	<0.01
	GC	1.8 (0.3–7.4)	0.5 (0.1–1.6)	<0.01
IgG ₄	total	1.0 (0.4–2.3)	0.6 (0.0–3.5)	n.s.
	RE	0.3 (0.1–0.4)	0.4 (0.0–0.5)	n.s.
	IF	0.5 (0.2–1.0)	0.5 (0.2–0.9)	n.s.
	MZ	0.0 (0.0–0.2)	0.0 (0.0–0.1)	n.s.
	GC	0.2 (0.1–0.8)	0.3 (0.0–0.5)	n.s.

RE = Reticular epithelium, IF = Interfollicular area, MZ = Mantle zone, GC = Germinal centre.

Results

Reproducibility

Cell counts by two different observers (LS and ÅV); showed a fairly good degree of reproducibility (Kappa = 0.5). Reproducibility of the staining procedure and scoring system applied by the same ob-

server was even better (LS I and LS II) (Kappa = 0.7).

IgA-, IgM-, IgG- and IgD-immunocytes

The total number of IgA immunocytes per grid area, was higher ($p < 0.05$) in the SIDS victims compared to controls, median: 3.9, range: 2.4–5.0 and median: 1.5, range: 1.1–3.7 respectively (Fig. 1). Significant differences were demonstrated for the inter-follicular area and the germinal centre as well, while no differences could be demonstrated in the mantle zone or in the reticular epithelium (Table 2a).

No difference was demonstrated in the total number of IgM immunocytes in SIDS tonsils (median: 1.8, range: 1.2–3.3) compared to controls (1.8, range: 0.7–5.6). However, in the germinal center, the SIDS group had more IgM cells than the controls, median: 1.0, range: 0.4–1.6 and median: 0.5, range: 0.3–1.3 respectively ($p < 0.01$), while no differences could be demonstrated in the three other compartments (Table 2a).

The SIDS tonsils also contained higher number of IgG cells per grid area than controls, median: 18.8, range: 12.4–30.2 and median: 6.3, range: 2.0–14.6 respectively ($p < 0.01$). Furthermore, significant differences were demonstrated in all the four compartments (Table 2a).

No differences were demonstrated between the SIDS and control total number of IgD cells per grid area, median: 1.9, range: 0.8–2.9 and median: 1.6, range: 0.7–2.4 respectively. However, differences were demonstrated in the germinal centre, and interfollicular area (Table 2a).

The results of the total immunocyte-counts are given as absolute immunocyte number/0.1 mm² tissue area in Fig. 1, while detailed information about immunocyte differences in the 4 compartments, is given in Table 2a.

IgG₁-, IgG₂-, IgG₃-, and IgG₄-subgroup

A difference between SIDS and control, was demonstrated in the total IgG₁-subgroup (median: 15.6, range: 5.3–58.9 versus median: 2.5, range: 1.5–8.4) ($p < 0.01$), as well as in all the four compartments: germinal centre (median: 7.4, range: 0.821.3 versus median: 0.4, range: 0.4–5.5) ($p < 0.01$), mantle zone (median: 0.3, range: 0.1–1.8 versus median: 0.1, range: 0.1–1.1) ($p < 0.01$), interfollicular area (median: 3.2, range: 0.7–29.7 versus median: 1.6, range: 0.4–2.4) ($p < 0.01$), and reticular epithelium (median: 2.3, range: 1.3–7.6 versus median: 0.6, range: 0.2–1.4) ($p < 0.01$).

Concerning the IgG₂-immunocyte subset, differences could only be demonstrated in the interfollicular area (median 0.9, range: 0.4–4.4 versus

median: 0.7, range: 0.1–2.9) ($p < 0.01$), while no significance could be demonstrated between SIDS and control total IgG₂, germinal centre, mantle zone or reticular epithelium (Table 2b).

Significant differences could be demonstrated between SIDS and control total IgG₃ (median: 3.6, range: 0.4–8.6 versus median: 1.1, range: 0.4–2.3) ($p < 0.01$), as well as in all the compartments; germinal centre (median: 1.8, range: 0.3–7.4 versus median: 0.5, range: 0.1–1.6) ($p < 0.01$), mantle zone (median: 0.3, range: 0.1–0.8 versus median: 0.2, range: 0.0–0.6) ($p < 0.01$), interfollicular area (median: 1.6, range: 0.5–4.5 versus median: 0.3, range: 0.1–1.3) ($p < 0.01$), and reticular epithelium (median: 1.0, range: 0.2–2.3 versus median: 0.2, range: 0.1–1.3) ($p < 0.01$). Total SIDS versus control IgG as well as the differences in the four compartments, did not reach significance (Table 2b).

Discussion

Tonsils are particularly designed for direct transport of foreign material from the exterior to the lymphoid cell, in contrast to lymph nodes, which depend on an antigen delivery through afferent lymphatics. Furthermore, IgG, IgA, and IgM in that order, are transferred to the surface of palatine tonsils, particularly into the crypt lumen, by passive intercellular diffusion through the epithelium (22, 23).

Awaya has suggested that palatine tonsils are most active between the ages 4 to 10 years, with a marked decrease after the age of 20 (24). However, other researchers are of the opinion that the palatine tonsils are in a very active state in much younger children, and even in infants who have experienced no tonsillar disease (25). We have to consider the following facts, before an attempt is made to analyze and discuss our results: 1) previous investigators have shown large individual variations (26); 2) rapid changes with age (26); hence age-matched populations are of uttermost importance since a 1-month-old breastfed baby cannot be compared with a 1-year-old infant; 3) even the different lymphoid compartments may vary considerably from one specimen to the other (25).

To summarize our findings: SIDS infants had statistically significant higher concentrations of IgG, and IgA-immunocytes compared to control infants, and the common feature in all these changes were a significant difference in the interfollicular area. The increase in number of IgG immunocytes is due to increase in the subclasses IgG₁ and IgG₃. Our findings indicate a stimulated immune system in the upper aeroalimentary tract, the most likely cause being an infection. In 1988 Okamoto (27) studied tonsillar lymphocytes from 39 patients that had tonsillectomy carried out during a community outbreak

of respiratory syncytial virus (RSV) infection. The immunocytes in culture showed an IgA-, IgG₁- and IgG₃-response to RSV. Furthermore, he concluded that IgG antiviral activity in tonsillar lymphocytes seems to be largely due to IgG₁-bearing cells. Similar findings have been demonstrated in sera from infants and children less than 2 years of age undergoing primary RSV infection (28). These authors conclude that the IgG₁- and IgG₃-subgroup response is the pattern typical for viral protein antigens (28). The poor, or near absence of an immune response noted in the IgG₂ subgroup, may be due to immunological immaturity of the IgG₂ system, since it has been shown to develop at a slower pace than the IgG₁ and IgG₃ subgroups (29).

Respiratory syncytial virus have for many years been associated with SIDS (30), however, other microorganisms have to be considered as well (31, 32). Furthermore, other researchers have demonstrated that the absolute number of B-cells, were significantly greater in diseased tonsils compared to controls, and that increase in microbial load correlates with increased number of B-cells (33). Bernstein et al. (34) were able to demonstrate that both bacterial (Streptococcal) and viral (Epstein-Barr) infections tended to cause an increase in number of immunocytes in the interfollicular area and that this tendency increases with age (34).

Based on studies of death- and trigger-mechanisms in SIDS, we have suggested that SIDS might be due to an overreaction of the mucosal immune system (15, 35–38). In a vulnerable phase of both development of the immune system and the CNS, a common microbial factor might trigger a peripheral immune reaction which induce an immune response in CNS, such a response might in turn trigger a down-regulation of respiration and finally lead to coma and death. A recently published paper supports this view (39).

The tonsillar immune responses demonstrated in this paper will cause the release of several cytokines (40), and it has been suggested that interleukin-1 β (IL-1 β) may be the intermediary causing sudden infant death during a respiratory infection (41). Interleukin-1 β has been shown to appear within 1–2 h after stimulation with influenza virus and/or bacterial endotoxin (culture supernatants of human peripheral blood leukocytes) and it will reach a maximum between 16 to 20 h post stimulation (42). In the CNS it acts on the hypothalamic thermoregulatory center and other centers to cause fever and sleep and acts in many areas of the CNS to increase various neuropeptides including endorphins (43). We have recently demonstrated that intravenous and intrathecal (cisterna magna) injection of IL-1 β in piglets, prolongs apnea and modifies autoresuscitation (44), and that the duration of apnea correlates strongly

with the level of β -endorphins in the CSF ($r=0.84$) (45).

However, a viral infection by itself is not a major factor as long as the baby is lightly wrapped (46). In heavily wrapped babies sleeping prone, however, the presence of a viral infection greatly increases the risk of SIDS (47).

The possibility of a delayed CNS-maturation (48) and/or autonomic dysfunction must be considered important co-factors as well (49).

We have to increase our knowledge of the activity in the neuroimmune axis, in particular with regard to the signal trafficking from the periphery to the CNS. Further animal studies have been planned introducing interleukin-1 β antagonist, other cytokines as well as the microdialysis technique.

References

1. WERNE J. Postmortem evidence of acute infections in unexpected death in infancy. *Am J Pathol* 1942; 18: 756-61.
2. SIMPSON K. Pathology of sudden death. *Lancet* 1947; ii: 745-47.
3. WERNE J, GARROW I. Sudden apparently unexplained death during infancy: I Pathological findings in infants found dead. *Am J Pathol* 1953; 29: 633-75.
4. WERNE J, GARROW I. Sudden apparently unexpected death during infancy, II. Pathological findings in infants observed to die suddenly. *Am J Pathol* 1953; 29: 817-827.
5. BERGMAN AB, BECKWITT JB, RAY CG, eds. Proceedings of the second international conference of sudden death in infants. Seattle: Univ. of Washington Press, 1970: 17-18.
6. OSMOND C, MURPHY M. Seasonality in the sudden infant death syndrome. *Paediatr Perin Epidemiol* 1988; 2: 337-45.
7. KELLY DH, SHANNON DC. Sudden infant death syndrome and near sudden infant death syndrome, a review of the literature. 1964-1982. *Pediatr Clin N Am* 1982; 29: 1241-61.
8. UREN EC, WILLIAMS AL, JACK I, et al. Association of respiratory virus infection with sudden infant death syndrome. *Med J Aust* 1980; 1: 417-19.
9. FERRIS JAJ, AHERNE WA, LOCKE WS, McQUILLIN J, GARDNER PS. Sudden and unexpected death in infants: Histology and virology. *Br Med J* 1973; 2: 439-42.
10. STEINCHNEIDER A. Nasopharyngitis and the sudden infant death syndrome. *Pediatrics* 1977; 60: 531-33.
11. PATRICK WJA, CARRINGTON D, ARMSTRONG AA, GIBSON AAM, URQUHART GED. Eight-year study of viral isolates from cot deaths in Glasgow. *Scott Med J* 1989; 34: 462-64.
12. ANONYMOUS. Respiratory infection and sudden infant death. (Editorial) *Lancet* 1989; ii: 1191-92.
13. FORSYTH KD, WEEKS SC, SKINNER J, BRADLEY J. Lung immunoglobulins in the sudden infant death syndrome. *Br Med J* 1989; 286-23-26.
14. THRANE PS, ROGNUM TO, BRANDTZAEG P. Sudden infant death syndrome (SIDS): increased immune response in upper respiratory and digestive tract in SIDS. *Lancet* 1990; 335: 229-30.
15. STOLTENBERG L, SAUGSTAD OD, ROGNUM TO. Sudden infant death syndrome victims show local immunoglobulin M response in tracheal wall and immunoglobulin A response in duodenal mucosa. *Pediatr Res* 1992; 31: 372-75.
16. BRANDTZAEG P. Two types of IgA immunocytes in man. *Nature* 1973; 243: 142-43.
17. WATANABE T, YOSHIZAKI K, YAGURA T, YAMURA Y. *In vitro* antibody formation by human tonsil lymphocytes. *J Immunol* 1974; 113: 608-16.
18. BRANDTZAEG P. Immune functions and immunopathology of palatine and nasopharyngeal tonsils. In: Bernstein J, Ogra P, eds. *Immunology of the ear*. New York: Raven Press, 1987.
19. BRANDTZAEG P. Mucosal and glandular distribution of immunoglobulin components. *Immunohistochemistry with a cold ethanol-fixation technique*. *Immunology* 1974; 26: 731-7.
20. BRANDTZAEG P, BAKLIEN K, FAUSA O, HOEL PS. Immunohistochemical characterization of local immunoglobulin formation in ulcerative colitis. *Gastroenterology* 1974; 66: 1123-36.
21. ROGNUM TO, BRANDTZAEG P, BAKLIEN K, HOGNESTAD J. Immunoglobulin-producing cells in the "transitional" mucosa adjacent to adenocarcinomas of human large bowel. *Int J Cancer* 1979; 23: 655-89.
22. BRANDTZAEG P, KORSRUD FR. Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the J chain expressing capacity of the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue. *Clin Exp Immunol* 1984; 58: 709-718.
23. SCHMEDTJE JF, BATTS AF. Immunoglobulins and blood vessels in the tonsillar crypt epithelium. *Ann Otol Rhinol Laryngol* 1973; 82: 359-369.
24. AWAYA K. The aging of the secondary nodules in relation to the postnatal development and involution of the human palatine tonsils. *Acta Haematol Jap* 1969; 32: 127-134.
25. KORSRUD FR, BRANDTZAEG P. Immune systems of human nasopharyngeal and palatine tonsils: histomorphometry of lymphoid components and quantification of immunoglobulin producing cells in health and disease. *Clin Exp Immunol* 1980; 39: 361-370.
26. SURIAN L, BRANDTZAEG P, BERDAL P. Immunoglobulin systems and human tonsils, II. Patients with chronic tonsillitis or tonsillar hyperplasia: quantification of Ig-producing cells, tonsillar morphometry and serum Ig concentrations. *Clin Exp Immunol* 1978; 31: 382-389.
27. OKAMOTO Y, BRODSKY L, BERNSTEIN JM, OGRA PL. Characteristics of *in vitro* production of mucosal antibody to respiratory syncytial virus in tonsillar tissue lymphocytes. *Clin Immunol Immunopathol* 1988; 49: 299-507.
28. WAGNER D, NELSON DL, WALSH EE, REIMER CB, HENDERSON FW, MURPHY BR. Differential immunoglobulin G subclass antibody titers to respiratory syncytial virus F and G glycoproteins in adults. *J Clin Microbiol* 1987; 25: 748-750.
29. PABST HF, KRETH HW. Ontogeny of the immune system as a basis of childhood disease. *J Pediatr* 1980; 97: 519-534.
30. HALL CB, KOPELMAN AE, DOUGLAS JR RG, GEIMAN JH, MAEGHER MP. Neonatal respiratory syncytial virus infection. *N Engl J Med* 1979; 300: 393-396.
31. SCOTT DJ, GARDNER PS, McQUILLAN J, FERRIS JAJ. Respiratory viruses and cot death. *Br Med J* 1978; ii: 12-13.
32. TELFORD DR, MORRIS JA, HUGHES P, et al. The nasopharyngeal bacterial flora in the sudden infant death syndrome. *J Infection* 1989; 18: 125-130.
33. BRODSKY L, MOORE L, STANIEVICH JF, OGRA PL. The immunology of tonsils in children: the effect of bacterial load on the presence of B- and T-cell subsets. *Laryngoscope* 1988; 98: 93-98.
34. BERNSTEIN JM, SCHEEREN R, SCHOENFELD E, ALBINI B. The distribution of immunocompetent cells in the compartments of the palatine tonsils in bacterial and viral infections

- in the upper respiratory tract. *Acta Oto-Laryngologica* 1988; 454: 153–162 (suppl).
35. ROGNUM TO, SAUGSTAD OD. Hypoxanthine levels in vitreous humor. Evidence of hypoxia in most cases of SIDS. *Pediatrics* 1991; 87: 306–310.
 36. VEGE Å, CHEN Y, OPDAL SH, SAUGSTAD OD, ROGNUM TO. Vitreous humor hypoxanthine levels in SIDS and infectious deaths. *Acta Pædiatr* 1994 (in press).
 37. VEGE Å, ROGNUM TO, AASEN A, SCOTT H. CSF IL-6 levels in SIDS and controls. Measurements with ELISA and a biological assay. *ESPID* Nancy, France, Sept. 1992 (abstr).
 38. ROGNUM TO, SAUGSTAD OD. Biochemical and immunological studies in SIDS victims. Clues to understanding the death mechanism. *Acta Pædiatr Suppl* 1993; 389: 82–85.
 39. GLEESON M, CLANCY RL, CRIPPS AW. Mucosal immune response in a case of sudden infant death syndrome. *Pediatr Res* 1993; 33: 554–556.
 40. MINAMI A, FUJIMOTO K, OZAKI Y, NAKAMURA S. Augmentation of host resistance to microbial infections by recombinant human interleukin-1 alpha. *Infect Immunol* 1988; 56: 3116–3120.
 41. GUNTHEROTH WG. Interleukin-1 as intermediary causing prolonged sleep apnea and SIDS during respiratory infections. *Med Hypothesis* 1989; 28: 121–123.
 42. LUNDEMOSE JB, SMITH H, SWEET C. Cytokine release from human peripheral blood leukocytes incubated with endotoxin with prior infection with influenza virus: relevance to the sudden infant death syndrome. *Int J Exp Pathol* 1993; 74: 291–297.
 43. STROBER W, JAMES SP. The interleukins. *Ped Res* 1988; 24: 549–557.
 44. STOLTENBERG L, SUNDAR T, ALMAAS R, SAUGSTAD OD. Changes in apnea and autoresuscitation in piglets after intravenous and intrathecal interleukin-1BB injection. *ESPR Annual Meeting*, Edinburgh 1993 (abstr 81).
 45. STORM H, STOLTENBERG L, SUNDAR T, SAUGSTAD OD, ROGNUM TO, REICHELT KL. May apnea reflex induce sudden infant death. *ESPR Annual Meeting*, Edinburgh 1993 (abstr P42).
 46. GILBERT R, RUDD P, BERRY PJ, FLEMMING PJ, et al. Combined effect of infection and heavy wrapping on the risk of sudden unexpected infant death. *Arch Dis Child* 1992; 67: 171–177.
 47. PONSONBY AL, DWYER T, GIBBONS LE, COCHRANE JA, WANG YG. Factors potentiating the risk of sudden infant death syndrome associated with the prone position. *N Engl J Med* 1993; 329: 377–382.
 48. KINNEY HC, FILLIANO JJ, HARPER RM. The neuropathology of the sudden infant death syndrome. A review. *J Neuropath Exp Neurol* 1992; 51: 115–126.
 49. MATTHEWS TG. The autonomic nervous system: a role in sudden infant death syndrome. *Arch Dis Child* 1992; 67: 654–656.