

Involvement of mast cells in sudden infant death syndrome

Marvin S. Platt, MD,^a John W. Yunginger, MD,^b
Adrienne Sekula-Perlman, MD,^c Anne-Marie A. Irani, MD,^d
John Smialek, MD,^a Haresh G. Mirchandani, MD,^c and
Lawrence B. Schwartz, MD, PhD^d

Baltimore, Md., Rochester, Minn., Philadelphia, Pa., and Richmond, Va.

The pathogenesis of sudden infant death syndrome (SIDS) is elusive and probably multifactorial. The occurrence of mast cell activation in SIDS was assessed in this study by measuring concentrations of tryptase, a neutral protease produced mainly by mast cells, in postmortem sera from term infants with SIDS and from age-matched control infants who died unexpectedly at home from a known cause. Tryptase levels were significantly higher in the 50 infants with SIDS than in the 15 control infants ($p = 0.0004$). Forty percent of the infants with SIDS and none of the control infants had a tryptase level greater than 10 ng/ml, the threshold chosen to indicate premortem mast cell activation. An infant with SIDS had a 20-fold higher chance of having an elevated tryptase level compared with a control infant. The postmortem interval did not influence these results. Thus mast cell-mediated anaphylaxis is likely to be the pathogenetic mechanism involved in some but not all SIDS cases. Recognition of this pathway as operative in SIDS should facilitate a more precise identification of the allergens involved, the processes leading to mast cell activation, and procedures to identify those infants at risk for anaphylaxis, and should, in time, lead to better therapeutic interventions aimed at preventing this specific cause of SIDS. (J ALLERGY CLIN IMMUNOL 1994;94:250-6.)

Key words: SIDS, mast cell, tryptase, anaphylaxis

The cause of sudden infant death syndrome (SIDS) is elusive and probably multifactorial. Its diagnosis is one of exclusion, and it was defined in 1989 by the expert panel convened by the National Institute of Child Health and Human Development as: "the sudden death of an infant under 1 year of age which remains unexplained after a thorough case investigation, including per-

Abbreviations used

PMIT: Postmortem interval

SIDS: Sudden infant death syndrome

From ^athe Office of the Chief Medical Examiner of Maryland, Baltimore; ^bthe Department of Pediatrics, Mayo Medical Center, Rochester; ^cthe Philadelphia Medical Examiner's Office, Philadelphia; and ^dthe Departments of Pediatrics and Medicine, Virginia Commonwealth University, Richmond.

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Reprint requests: Lawrence B. Schwartz, MD, PhD, Virginia Commonwealth University, MCV Station Box 263, Richmond, VA 23298.

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formance of a complete autopsy, examination of the death scene, and review of the clinical history."¹ Disorders of neurologic,²⁻⁴ cardiac,^{5, 6} and respiratory systems^{7, 8}; anaphylaxis⁹; and environmental factors such as maternal cigarette smoking¹⁰ have been proposed as risk factors, but each remains unproven as the cause. Typically, these disorders do not leave pathognomonic footprints, which is consistent with the absence of disease observed in autopsies of SIDS victims.¹¹ For example, pulmonary changes of acute emphysema and airway edema are only seen in a minority of adults who die of systemic anaphylaxis, and these changes are not specific for this cause of death.¹² Infant victims of systemic anaphylaxis may be less able to generate such changes.

Of particular interest to our investigations is the possibility that an anaphylactic event causes at

TABLE I. Characteristics of SIDS and control groups

Characteristic	SIDS group	Control group
Number of subjects	50	15
Sex		
Male	29	8
Female	21	7
Race		
White	11	3
Black	36	12
Other	3	0
Age (mo)		
Median	2	3
Range	0.5-11	1-11
Postmortem interval (hr)		
Median	26	22
Range	2-41	6-36
Resuscitation interval (min)		
Median	26	20
Range	5-40 (<i>n</i> = 12)	10-45 (<i>n</i> = 8)

least a portion of these unexplained deaths in infants. Cow's milk protein and house dust mite antigens, agents to which infants are commonly exposed, have been proposed to contain potential inciting allergens. Evidence against this hypothesis includes studies in which antigen-specific IgE levels⁹ and serum total IgE¹³ were not significantly different between subjects with SIDS and control subjects. However, the likely multifactorial nature of SIDS may mask a putative anaphylactic subgroup identified by measurement of allergen-specific IgE alone. Additional evidence against an anaphylactic cause is that no alteration in the number or histochemical staining properties of pulmonary mast cells has been detected in post-mortem tissue taken from infants with SIDS compared with normal tissue.^{14, 15} However, histochemical techniques at the level of light microscopy do not reliably distinguish between mast cells at rest and those that are activated and therefore do not rule out the premortem occurrence of anaphylaxis.

Mast cells are the predominant effector cells of anaphylactic reactions.¹⁶ Mast cell activation can be detected in vivo by measuring the serum or plasma concentration of tryptase, an enzyme selectively concentrated in mast cell secretory granules.¹⁷ Along with histamine, tryptase is released by degranulating mast cells in tissues, diffuses more slowly than histamine into the circulation, and, after reaching a maximal concentration by about 1 hour, exhibits a half-life of about 2 hours, compared with a half-life for histamine of less

than 2 minutes.¹⁸ Concentrations of tryptase in blood correlate with the clinical severity of an anaphylactic response.¹⁹ Tryptase concentrations are increased in postmortem sera from adult victims of anaphylactic reactions but not in post-mortem blood from subjects in whom the diagnosis of anaphylaxis was excluded clinically.²⁰

In this study tryptase concentrations were measured post mortem in samples of serum obtained from the left side of the heart of infants with SIDS and from control infants to determine whether mast cell activation is involved in the pathophysiology of sudden death in infants.

METHODS

Subjects

Fifty SIDS and 15 control cases (Table I) identified between March and November of 1992 were studied at the Office of the Chief Medical Examiner in Baltimore (26 SIDS and 11 control cases), and the Medical Examiner's Office in Philadelphia (24 SIDS and 4 control cases). All infants were 1 year of age or younger. For each case, a scene report or viewing by the medical investigator of the office, clinical history, complete autopsy examination, and toxicologic analyses were performed. Microscopic evaluations were completed by the pathologist responsible for the case and also reviewed by either M. Platt or A. Sekula-Perlman. Only term infants were studied to avoid the confounding variables of extreme prematurity, bronchopulmonary dysplasia, low birth weight, and apnea of prematurity. Cases in which there appeared to be abuse or neglect, trauma, infection, or intoxication were excluded as cases of SIDS.¹ Cerebrospinal fluid and blood

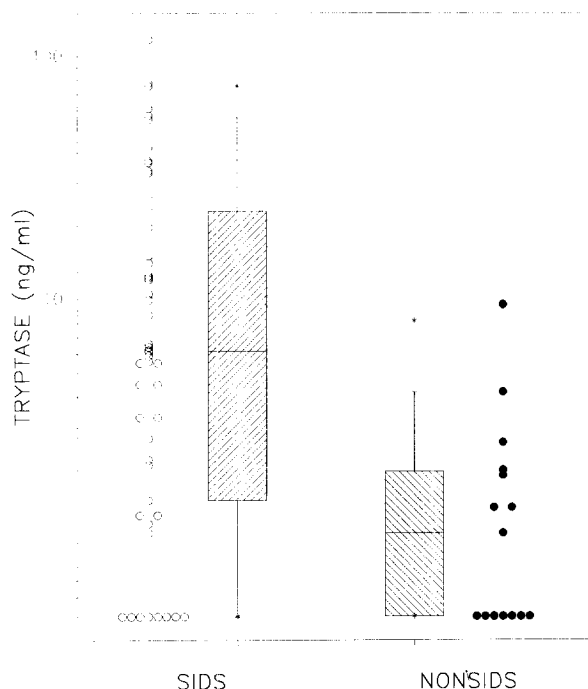


FIG. 1. Tryptase concentrations (log scale) in postmortem sera from 50 infants with SIDS (○) and 15 control infants (●). The range for infants with SIDS was 0.5 to 117 ng/ml, and the range for control infants was 0.5 to 9.5 ng/ml. Corresponding box plots show the median, 25th, and 75th percentiles (box borders); 10th and 90th percentiles (whiskers); and 5th and 95th percentiles (asterisks).

cultures were obtained in 31 of the Baltimore cases, and only bacteria thought to be contaminants were detected. All subjects in the study died at home. No infant with SIDS was receiving a medication near the time of death, which was likely to have caused drug-induced anaphylaxis. One infant with SIDS had received diphtheria-pertussis-tetanus vaccination 1 day previously, and was being given Tylenol. Control infants received cause of death diagnoses of pneumonia ($n = 7$), trauma ($n = 1$), suffocation ($n = 1$), sepsis ($n = 1$), congenital heart disease ($n = 2$), myocarditis ($n = 1$), heart tumor ($n = 1$), or smoke inhalation ($n = 1$). Although all infants were receiving milk, histories to distinguish how many were receiving cow's milk versus breast milk or soy formula were available for only 30 infants with SIDS and two control infants, each of whom was receiving cow's milk. No evidence for general or milk-related allergies, asthma, or urticaria was apparent as determined by history. The high percentage of minorities among the SIDS and control groups reflects the distribution of individuals served by the participating centers.

Serum studies

Blood was typically obtained from the left side of the heart (left ventricle, aorta, pulmonary veins). However,

tryptase levels in samples of blood taken from the right side of the heart were comparable to samples from the left side (data not shown). Also, gentle compression of the left ventricle did not increase the level of tryptase in blood from the left side of the heart (data not shown). Each sample of blood was subjected to centrifugation at 300g, and the supernatants, containing the defibrinated type of plasma found in postmortem specimens, were stored at -20°C or -70°C . Tryptase was analyzed by a radioimmunoassay,²¹ for which the lower limit of sensitivity is 1 ng/ml. Purified human lung-derived tryptase was used as standard. IgE-specific antibodies to bovine β -lactalbumin, α -lactalbumin, and κ -casein (Sigma Chemical Co., St. Louis, Mo.) and *Dermatophagoides pteronyssinus* and *D. farinae* mite extracts (Miles Allergy Products, Spokane, Wash.) were measured by solid-phase radioimmunoassay in which each allergen was coupled to CNBr-activated microcrystalline cellulose.²² The respective lower limits of sensitivity for each allergen-specific IgE were 3.7, 1.7, 0.9, 1.6, and 2 U/ml. Pooled sera from milk-sensitive or mite-sensitive subjects were used as standards, and each pool was arbitrarily assigned an allergen-specific IgE content of 1000 U/ml. A negative control serum and a low-level positive serum were analyzed for each assay to determine interassay coefficients of variation. For samples in which the concentration of tryptase or antibody was lower than the limit of detection, a value of half the lower limit was assigned for statistical calculations.

Tissue studies

Sections of lung fixed in Carnoy's fixative and embedded in paraffin were prepared as described previously and stained for the presence of MC_{TC} (tryptase⁺, chymase⁺) and MC_T (tryptase⁺, chymase⁻) types of mast cells.²³ Mast cells were examined, and tissue concentrations were determined at peribronchiolar, perivascular, interstitial, and bronchial sites in 18 infants with SIDS and eight control infants, chosen at random; data were collected in a blinded fashion. For routine histologic analyses, autopsy specimens were fixed in neutral buffered formalin, embedded in paraffin, and stained by hematoxylin and eosin.

Statistical analyses

The postmortem interval (PMIT) was estimated in each case as the time between when the child was last seen alive and the time of autopsy. Because the exact time of death typically occurs later than when the child was last seen alive, calculated PMITs likely overestimate the true PMIT in most cases. Comparisons of the tryptase, PMIT, and mast cell concentration data between SIDS and control groups were performed by the nonparametric Mann-Whitney U test for unpaired samples (STATGRAPHICS, STSC, Inc., Rockville, Md.). The relationships of IgE levels between infants with SIDS and control infants, and between those infants with SIDS with elevated tryptase levels and

those without elevated levels were analyzed by Fisher's exact t test, to account for small sample size, and by calculation of odds ratios.

RESULTS

Fifty cases diagnosed as SIDS met the standards set forth by the 1989 National Institute of Child Health and Human Development expert panel.¹ The infants in these cases were under 1 year of age, and their sudden deaths were unexplained after a thorough case investigation, including an examination or review of the death scene, review of the clinical history, and complete autopsy. The median age for the SIDS group, 2.0 months, was not significantly different than that for the control group, 3.0 months ($p = 0.43$) (Table I). In accord with most epidemiologic studies, male subjects outnumbered female subjects in this study, 1.4:1 in the SIDS group and 1.1:1 in the control group.

The median level of tryptase in serum was significantly higher in the SIDS group (6.2 ng/ml) than in the control group (1.1 ng/ml ($p = 0.0004$)) (Fig. 1). Tryptase levels in 20 (40%) of the subjects with SIDS, and none of the control subjects were greater than 10 ng/ml, a value previously recommended for adults as the concentration of tryptase best used to indicate mast cell activation premortem. One control subject had a value of 9.5 ng/ml; he was thought to have been inadvertently suffocated because he had shared the same bed with a parent, but there was no evidence of overlying. All other control subjects had tryptase values below 5 ng/ml. Infants with SIDS showed an odds ratio of 20 for having an elevated tryptase concentration compared with control infants (Fisher's exact two-tailed t test, $p = 0.003$). Living infants under 1 year of age without active allergic disease or mastocytosis typically have tryptase levels below 1 ng/ml ($n = 24$, three with detectable tryptase at 1 to 1.7 ng/ml; Platt and Schwartz. Unpublished data).

To determine whether variations in resuscitative interventions might account for the different tryptase concentrations between infants with SIDS and control infants, treatment measures were examined. When information was available and resuscitation was attempted, infants with SIDS ($n = 12$) and control infants ($n = 8$) received similar treatments. For example, there was no significant difference between the median interval of resuscitative treatment in infants with SIDS (26 minutes) and control infants (20 minutes) ($p = 0.70$) even though tryptase levels were

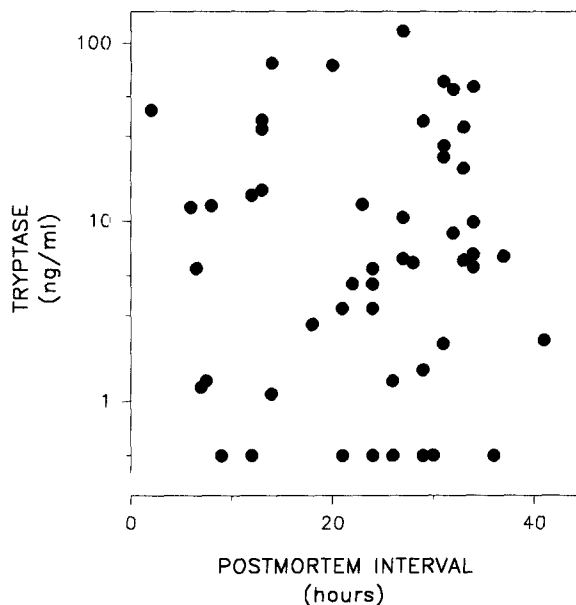


FIG. 2. Correlation of tryptase concentrations (log scale) in postmortem sera from infants with SIDS to the PMITs. Linear regression analysis showed no effect of PMIT on the level of tryptase ($r = 0.33$, $p = 0.82$).

higher than 10 ng/ml in five of the infants with SIDS. Also, both groups received similar intravenous therapies consisting of fluids, epinephrine, atropine, and bicarbonate; comparable numbers in each group underwent intubation and cardiopulmonary resuscitation. In eight infants with SIDS for whom no resuscitation was attempted, five had a tryptase level greater than 10 ng/ml; their median of 13.1 ng/ml was not significantly different from that of the SIDS subgroup that underwent resuscitation (median = 7.5 ng/ml) ($p = 0.62$). Thus the level of tryptase was not substantially influenced by treatment.

The median PMIT value for the entire SIDS group (26 hours) was not significantly different from that for the control group (22 hours) ($p = 0.15$). Also, a linear regression analysis of the PMIT values and the log of the tryptase values within the subjects with SIDS (Fig. 2) showed no correlation ($r = 0.33$, $p = 0.82$), suggesting that the tryptase level was not affected by the PMIT.

To investigate whether mite or milk allergens could be involved in the pathogenesis of SIDS, IgE-specific antibodies to bovine β -lactalbumin, α -lactalbumin, and κ -casein and to *D. pteronyssinus* and *D. farinae* mite extracts were measured in remaining serum samples. For each mite extract and for α -lactalbumin and κ -casein, no significant differences in IgE antibody prevalences and mean

TABLE II. β -Lactoglobulin-specific serum IgE in SIDS and control groups

β -Lactoglobulin specific IgE	SIDS group		Control group
	Tryptase $\geq$ 10 ng/ml	Tryptase $<$ 10 ng/ml	
Positive	9	6	1
Negative	7	16	8

levels were found between the SIDS and control groups. In contrast, for β -lactoglobulin, 15 of 38 subjects with SIDS had detectable antibody (range, 3.8 to 14 U/ml), whereas only one of nine control subjects had positive results (6.8 U/ml) (Table II). Although not significantly different according to a two-tailed Fisher's exact *t* test ($p = 0.16$), the odds ratio of 5.9 suggests a tendency for infants with detectable β -lactoglobulin-specific IgE to be at higher risk for SIDS. Within the SIDS group, there was a weak tendency for those with detectable IgE anti- β -lactoglobulin levels to have higher tryptase concentrations (odds ratio = 3.4; $p = 0.10$); nine of the 15 (60%) subjects with detectable IgE anti- β -lactoglobulin had tryptase values greater than 10 ng/ml, whereas only seven of the 23 (30%) subjects with undetectable IgE anti- β -lactoglobulin levels had a tryptase level above 10 ng/ml.

Lung sections examined for mast cells revealed no significant differences in the numbers or types of mast cells in the entire lung or in peribronchial, peribronchiolar, perivascular, or interstitial areas between the SIDS ($n = 18$) and control ($n = 8$) groups and between subjects with tryptase levels greater than 10 ng/ml ($n = 5$) and less than 10 ng/ml ($n = 13$) within the SIDS group. Furthermore, mast cell numbers were similar to previously reported values in normal adult lungs.²⁴

DISCUSSION

Although the cause of SIDS remains elusive, one hypothesis periodically begs reconsideration. Parish et al.²⁵ in 1960 and Coombs and Holgate⁹ as late as 1990 argued that anaphylactic reactions to milk protein could account for some of these deaths. Supporting evidence for this hypothesis has been lacking. In two previously published studies, no significant differences between subjects with SIDS and control subjects were found in the numbers of mast cells in sections of formalin-fixed lung,^{14, 15} consistent with the results of

this study in which lung tissue fixed in Carnoy's fluid and immunohistochemical stains that are highly sensitive and specific for mast cells were used.²³ Our group and others have been unable to demonstrate activation of mast cells by examination under light microscopy of human skin biopsy specimens taken from sites of an allergic reaction. Thus histologic procedures to identify the occurrence of mast cell degranulation in human beings are insensitive at best. Ultrastructural studies have been used to ascertain mast cell activation in tissues, but such studies require fresh specimens to avoid the confounding changes observed with postmortem tissue. Also, no differences in allergen-specific IgE levels between SIDS and control cases have emerged,¹³ consistent with the results of this study when one compares the entire SIDS group with the control group. Nevertheless, the inability to provide direct evidence for mast cell activation has been the most prominent obstacle in attempting to demonstrate an anaphylactic cause. This study, by means of a tryptase immunoassay to detect mast cell activation, provides critical new evidence that mast cell activation is involved in a substantial subgroup of infants with SIDS.

A tryptase value of 10 ng/ml had been recommended previously as a conservative threshold for the diagnosis of anaphylaxis as a cause of death in adults.²⁰ Twenty of 50 subjects with SIDS, but none of the 15 control subjects, had a tryptase value greater than 10 ng/ml. It is unlikely that such a level would result from postmortem manipulation of tissues, resuscitative effort, or the PMIT because these parameters were not different between subjects with SIDS and control subjects. Also, elevations of serum tryptase are not observed in sensitive adults after bronchial allergen challenge, even though elevated levels of tryptase were detected in bronchoalveolar lavage fluid (Schwartz. Unpublished data), suggesting that the serum tryptase level reflects systemic rather than local mast cell activation. Thus elevated levels of tryptase in at least 40% of the infants with SIDS likely result from a degree of mast cell activation sufficient to have caused anaphylaxis before death.

Low levels of IgE-specific antibody to milk β -lactoglobulin were found in 15 of 38 (39%) infants with SIDS. In 9 of these 15 (60%) infants, the tryptase level was greater than 10 ng/ml. In a previous study by Yunginger et al.,²⁶ detectable IgE antibodies to β -lactoglobulin were measured in three of 62 (4.8%) infants with SIDS and in

four of 24 (16.7%) control infants. However, the allergen-specific IgE antibody assay used in this study involved a more sensitive and quantitative technique, and the frequency with which detectable IgE antibodies to β -lactoglobulin were measured in the sera of infants with SIDS was higher than that in sera from control infants. These findings tend to support the hypothesis that cow's milk may be an offending allergen and induce an IgE-mediated anaphylactic reaction in some infants. Arguments against milk allergy being truly involved in SIDS-related anaphylaxis are the low serum levels and low prevalence of detectable IgE and the weak statistical association with SIDS noted above. However, infants may not have the capacity to generate substantial amounts of antigen-specific IgE,²⁷ such that the little IgE generated may reside primarily with high-affinity IgE receptors on tissue mast cells rather than in the circulation. Indeed, a substantial percentage of infants with positive skin test responses to egg and milk allergens have been observed to have no detectable IgE antibody in serum.²⁸ On the other hand, fatal and near-fatal cases of putative anaphylaxis caused by food ingestion failed to demonstrate elevated serum tryptase levels.²⁹ Thus milk allergy involvement in the mast cell activation observed in the infants with SIDS remains uncertain.

Mast cell degranulation may also result from non-IgE-mediated mechanisms. Complement anaphylatoxins are known to activate cutaneous mast cells and basophils and may be produced in association with immune complex formation, as seen with certain infectious and autoimmune diseases. These pathways require further study, though obtaining laboratory evidence for complement activation in postmortem samples is likely to be problematic.

The cause of SIDS is probably multifactorial and may involve other pathways not directly related to mast cell activation. Some of these factors may contribute indirectly to the development of an allergic reaction. For example, passive inhalation of cigarette smoke has been implicated as a risk factor for SIDS in some studies^{10, 30} but not in others.³¹ Total IgE levels are higher in infants whose mothers smoke than those in a nonsmoking environment,³² and the irritant effects of cigarette smoke may exacerbate the severity of allergic pulmonary reactions. However, data on cigarette smoke exposure were not obtained in this study. Also, whether the prone or supine position,³³ the number of eosinophils in lung sections,³⁴ or gas-

troesophageal reflux⁷ were factors in our subjects with SIDS was not ascertained. Recently, a protective effect of prophylactic treatment with anticholinergic agents was observed for infants at risk for SIDS.³⁵ Thus if small amounts of milk are aspirated during sleep in SIDS,³⁶ and initiate the allergic reaction, a therapeutic effect of anticholinergic agents may be to attenuate the allergic airway response.

Is elevated tryptase a specific marker for the immunopathology seen in cases of SIDS? One might argue that elevated tryptase is an epiphenomenon. For example, it may reflect hypoxemia, which is thought to be the reason for finding vitreous humor hypoxanthine³⁷ in SIDS. However, an elevated level of serum tryptase is not a marker for hypoxia because it was not found in the control infants who also died unexpectedly outside of the hospital as a result of heart disease, acute pulmonary infection, or smoke and soot inhalation. Furthermore, an elevated serum tryptase level in vivo has been shown to be a specific indicator of mast cell activation.¹⁷

In conclusion, we suggest that elevated postmortem serum tryptase levels in serum samples from the left side of the heart indicate that one cause of SIDS is anaphylaxis caused by mast cell activation. The finding warrants corroboration by others. If confirmed, substantial efforts should be made to identify more precisely any allergen involvement, the process leading to mast cell activation, and those infants at risk for anaphylaxis and to develop therapeutic interventions aimed at preventing this specific cause of SIDS.

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REFERENCES

1. Willinger M, James LS, Catz C. Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. *Pediatr Pathol* 1991;11:677-84.
2. Matthews TG. The autonomic nervous system—a role in sudden infant death syndrome. *Arch Dis Child* 1992;67:654-6.
3. Kinney HC, Filiano JJ, Harper RM. The neuropathology of the sudden infant death syndrome. A review. *J Neuropathol Exp Neurol* 1992;51:115-26.
4. Becker LE. Neural maturational delay as a link in the chain of events leading to SIDS. *Can J Neurol Sci* 1990;17:361-71.

5. Guntheroth WG. Theories of cardiovascular causes in sudden infant death syndrome. *J Am Coll Cardiol* 1989; 14:443-7.
6. Gillette PC, Garson A Jr. Sudden cardiac death in the pediatric population. *Circulation* 1992;85:164-169.
7. Bernard F, Dupont C, Viala P. Gastroesophageal reflux and upper airway diseases. *Clin Rev Allergy* 1990;8:403-25.
8. Martinez FD. Sudden infant death syndrome and small airway occlusion: facts and a hypothesis. *Pediatrics* 1991; 87:190-8.
9. Coombs RRA, Holgate ST. Allergy and cot death: with special focus on allergic sensitivity to cows' milk and anaphylaxis. *Clin Exp Allergy* 1990;20:359-66.
10. Haglund B, Cnattingius S. Cigarette smoking as a risk factor for sudden infant death syndrome: a population-based study. *Am J Public Health* 1990;80:29-32.
11. Valdes-Dapena M. A pathologist's perspective on the sudden infant death syndrome—1991. *Pathol Annu* 1992; 27:133-64.
12. Delage C, Irely NS. Anaphylactic deaths: a clinicopathologic study of 43 cases. *J Forensic Sci* 1972;17:525-40.
13. Mirchandani HG, Mirchandani IH, House D. Sudden infant death syndrome: measurement of total and specific serum immunoglobulin E (IgE). *J Forensic Sci* 1984;29: 425-9.
14. Parish WE, Richards CB, France NE, Coombs RRA. Further investigations on the hypothesis that some cases of cot-death are due to a modified anaphylactic reaction to cow's milk. *Int Arch Allergy* 1964;24:215-43.
15. Emery JL, Carpenter RG. Pulmonary mast cells in infants and their relation to unexpected death in infancy. In: Robinson RR, ed. *Proceedings of the Francis E. Camps International Symposium on Sudden and Unexpected Deaths in Infancy*. Toronto, Ontario: Canadian Foundation for the Study of Infant Deaths, 1974:7-19.
16. Schwartz LB, Huff TF. Biology of mast cells and basophils. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. *Allergy: principles and practice*. 4th ed. St. Louis: Mosby-Year Book, Inc., 1993: 135-68.
17. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987;316:1622-6.
18. Schwartz LB, Yunginger JW, Miller JS, Bokhari R, Dull D. The time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. *J Clin Invest* 1989;83:1551-5.
19. Van der Linden PG, Hack CE, Poortman J, Vивиé-Kipp YC, Struyvenberg A, Van der Zwan JK. Insect-sting challenge in 138 patients: relation between clinical severity of anaphylaxis and mast cell activation. *J ALLERGY CLIN IMMUNOL* 1992;90:110-8.
20. Yunginger JW, Nelson DR, Squillace DL, et al. Laboratory investigation of deaths due to anaphylaxis. *J Forensic Sci* 1991;36:857-65.
21. Enander I, Matsson P, Nystrand J, Andersson A-S, Eklund E, Bradford TR, Schwartz LB. A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. *J Immunol Methods* 1991;138:39-46.
22. Yunginger JW, Gleich GJ, Adolphson CR. Standardization of allergens. In: Rose NR, de Macario EC, Friedman H, Penn GM, eds. *Manual of clinical laboratory immunology*. Washington, DC: American Society for Microbiology, 1992:678-84.
23. Irani A-MA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB. Detection of MC_T and MC_{TC} types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem* 1989;37:1509-15.
24. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci USA* 1986;83:4464-8.
25. Parish WE, Barrett AM, Coombs RRA, Gunther M, Camps FE. Hypersensitivity to milk and sudden death in infancy. *Lancet* 1960;2:1106-10.
26. Clark JW, Yunginger JW, Bonnes PA, Ray CG, Saltzstein SL. Serum IgE antibodies in sudden infant death syndrome. *J Pediatr* 1979;95:85-6.
27. Zeiger RS. Development and prevention of allergic disease in childhood. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. *Allergy: principles and practice*. 3rd ed. St. Louis: The CV Mosby Co, 1988:930-68.
28. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985;60:727-35.
29. Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380-4.
30. Schoendorf KC, Kiely JL. Relationship of sudden infant death syndrome to maternal smoking during and after pregnancy. *Pediatrics* 1992;90:905-8.
31. Malloy MH, Hoffman HJ, Peterson DR. Sudden infant death syndrome and maternal smoking. *Am J Public Health* 1992;82:1380-2.
32. Magnusson CGM. Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allergy. *J ALLERGY CLIN IMMUNOL* 1986;78: 898-904.
33. Guntheroth WG, Spiers PS. Sleeping prone and the risk of sudden infant death syndrome. *JAMA* 1992;267:2359-62.
34. Roche WR. Immunopathology of SIDS. *J Clin Pathol* 1992;45(suppl):46-8.
35. Lucet V, Denjoy I, Do Ngoc D, Toumieux MC, Picard-Claudel MA, Coumel P. Effect of atropinic treatment on sudden infant death. A multicentre study on 7,851 infants at risk. *Presse Med* 1992;21:1896-1900.
36. Gaultier CL. Interference between gastroesophageal reflux and sleep in near miss SIDS. *Clin Rev Allergy* 1990;8:395-401.
37. Rognum TO, Saugstad OD. Hypoxanthine levels in vitreous humor: evidence of hypoxia in most infants who died of sudden infant death syndrome [see comments]. *Pediatrics* 1991;87:306-10.