

Light is recognized best through darkness: mast cells and Sudden Infant Death Syndrome

The Sudden Infant Death Syndrome (SIDS) by definition has no explained cause. A supine sleep position appears to reduce its incidence. However, suffocation simply from a prone sleep position provides neither a satisfactory explanation nor a precise mechanism for this syndrome. In 1960 Professor Coombs and colleagues formally proposed a relationship between anaphylaxis and SIDS, suggesting that an allergic reaction to milk might be involved [1]. Elevated levels of mast cell tryptase in the post-mortem blood of certain cases of SIDS are of interest, because such a finding implicates mast cell involvement. However, no consistent relationship between SIDS and antigen-specific IgE has emerged.

The current study by Buckley *et al.* [2] builds upon previous findings of mast cell activation without involvement of antigen-specific IgE in SIDS cases, by asking whether the total body burden of mast cells also is elevated, and by revisiting whether antigen-specific IgE is a risk factor. The strategy used to detect mast cell involvement depends upon the measurement of two different forms of tryptase; β -tryptase and total tryptase [3]. Mature β -tryptase is stored in mast cell secretory granules until released by degranulation when these cells are activated. Levels of β -tryptase are < 1 ng/mL in sera from healthy subjects and become elevated during systemic anaphylaxis. Total tryptase, in addition to mature β -tryptase, includes α -protryptase, which appears to be spontaneously released by mast cells at rest. Incompletely processed precursors such as α -protryptase, discarded by mast cells, appear to account for the tryptase detected in sera collected in the absence of an immediate hypersensitivity event. Consequently, total tryptase levels reflect the total body burden of mast cells. Total tryptase levels average 4–5 ng/mL and range from 1 to 15 ng/mL in sera from healthy subjects, but are markedly elevated (≥ 20 ng/mL) in subjects with systemic mastocytosis.

With this background in hand, the current study utilizes a now discontinued radioimmunoassay for β -tryptase (Tryptase RIACT, Pharmacia-Upjohn, Uppsala, Sweden) incorporating the G5 mouse mAb for capture and ^{125}I -labelled G4 mouse mAb for detection. G5 recognizes β -tryptase, but not α -protryptase, whereas all other antibodies under consideration recognize both forms of tryptase. A second, newly developed ELISA for total tryptase incorporates IgG

isolated from rabbits immunized with lung-derived tryptase for capture and enzyme-labelled AA5 mouse mAb for detection. Post-mortem levels of β -tryptase and total tryptase in blood obtained from SIDS cases are compared to those of explained-death controls. As reported previously, β -tryptase levels are elevated (≥ 10 ng/mL) in a greater portion of SIDS than control infants, consistent with mast cell activation occurring near the time of death in this subset of SIDS cases. However, there is no significant difference in the values for total tryptase in SIDS and control infants.

The total tryptase assay employed should have been better validated. It has not been tested with recombinant, well-defined forms of α -tryptase and β -tryptase. Serum samples from patients with biopsy-proven systemic mastocytosis have not been examined. Indeed, the one suspected case of systemic mastocytosis included in the current study has a total serum tryptase level of 5.6 ng/mL, well below the level of 20 ng/mL recommended as the threshold value for subjects with this disorder [4]. In those patients in the current study with elevated β -tryptase levels, corresponding total tryptase values are substantially lower, indicating either that β -tryptase levels are falsely elevated, or that total tryptase levels are falsely low. A potential problem of the rabbit polyclonal IgG preparation used for capture is that it may recognize multiple antigens, because it had not been affinity-purified using antigen. Some of the rabbit IgG likely competes with the AA5 mAb for binding to tryptase, potentially attenuating the dose–response curve. Different tryptase standards had been utilized for the total and β -tryptase assays, and serum samples had been subjected to the assays at different times – factors that introduce potential errors into the measurements. Thus, in the current study tryptase concentrations measured with the total tryptase assay should not be compared to the β -tryptase levels measured by radioimmunoassay, and may not be comparable to total tryptase measurements reported in other studies.

Nevertheless, there is no evidence that total tryptase levels are higher in SIDS over control sera, ranging from < 1 ng/mL in about half of the SIDS and control cases to almost 14 ng/mL [2]. These levels are within or below the normal range (1–15 ng/mL) reported for the total tryptase assay using the B12 mouse mAb for capture and enzyme-labelled G4 mAb for detection [3]. These results

are consistent with reports that failed to demonstrate mast cell hyperplasia in sections of various tissues taken from SIDS cases at autopsy [5], even though in one case a slightly elevated mast cell concentration had been noted in the peribronchial but not parenchymal regions of lung [6]. In each of these studies, eosinophil numbers in pulmonary tissue from SIDS cases are elevated.

β -Tryptase levels in serum are > 10 ng/mL in 5 of the 32 SIDS cases, and in 1 of the 31 controls by the radioimmunoassay [2]. This is consistent with previous reports in which β -tryptase levels were ≥ 10 ng/mL in 18 of 44 [7], 14 of 40 [8] and 20 of 50 [9] cases of SIDS. Another study of serum samples obtained from 51 SIDS and 13 control subjects using the same β -tryptase radioimmunoassay, found that the SIDS cases range up to 11.3 ng/mL, while the control cases range up to 3.7 ng/mL [10]. Thus, only in the SIDS group did the authors find a β -tryptase level ≥ 10 ng/mL. However, the median values for 10 SIDS (2.1 ng/mL) and 3 control (2.7 ng/mL) cases with detectable levels of β -tryptase were not significantly different from one another.

Another study of tryptase concentrations in SIDS examined 82 cases of SIDS and 19 controls [11]. However, in this case AA5 mAb had been used for capture, and radiolabelled G4 mAb for detection. Consequently, it is likely that total tryptase rather than β -tryptase levels were measured. This might explain why the median concentrations of tryptase were 6 ng/mL in the SIDS group and 4 ng/mL in the control group. Also, serum tryptase levels above 10 ng/mL were found in 12 of the 82 SIDS cases and in 1 of the 24 control cases. If 20 ng/mL had been used as a cut-off, the total tryptase threshold for systemic mastocytosis in non-acute patients, 6 of the SIDS cases and 0 of the control cases would have qualified. However, the data available from this study does not resolve whether the elevated total tryptase level reflects mast cell activation or mast cell number.

Whether an elevated level of β -tryptase in post-mortem serum serves as a diagnostic clue for anaphylaxis near the time of death has been debated. For example, some cases of clinically diagnosed systemic anaphylaxis to orally-ingested food allergens fail to show an elevated level of β -tryptase, even though abundant amounts of IgE against the ingested antigen are detected [12,13]. Basophil activation in the absence of mast cell activation is one hypothesis put forth to explain such results, but has yet to be tested. Against the diagnostic specificity of an elevated post-mortem level of β -tryptase is a study that found β -tryptase levels to be ≥ 10 ng/mL in 5 of 49 cases thought to be non-anaphylactic deaths [14]. One was a salicylate overdose (23 ng/mL). Whether this subject could have been aspirin-sensitive was not addressed. Another had a diagnosis of atherosclerotic coronary vascular disease

(33 ng β -tryptase/mL), but 10 other subjects with this diagnosis had levels < 5 ng/mL. Details regarding drugs received near the time of death, particularly those that might activate mast cells such as morphine, were not available. Three subjects with elevated levels died after trauma (20, 24 and 106 ng β -tryptase/mL), while normal levels were found in the vast majority of traumatic deaths. One related study examined post-mortem sera taken from 68 adults with unexplained deaths [15], and found that 9 had β -tryptase levels ≥ 10 ng/mL. Another study found a similar prevalence of β -tryptase elevations in unexplained deaths [8]. A precise mechanism to explain elevated levels of β -tryptase in otherwise unexplained deaths is lacking. Thus, a careful consideration of the events near the time of death is needed to best interpret post-mortem levels of β -tryptase.

Allergen

IgE-mediated activation of mast cells may not be the only mechanism for activating this cell type. For example, a case of exercise-induced anaphylaxis without an apparent relationship to food intake had been reported in which hypotension, urticaria and expiratory airflow obstruction was associated with elevated serum β -tryptase (8 ng/mL) and plasma histamine levels [16]. Another interesting case involved an infant with diffuse cutaneous systemic mastocytosis who presented with severe apneic spells, the one observed in the hospital being associated with an elevation of β -tryptase [17]. Thus, anaphylaxis in infants may present differently than in adults. Whether infants undergoing anaphylaxis typically display apnea, and whether such infants are more likely to survive when supine rather than prone, is worth considering in future studies.

The current study finds that the levels of serum IgE against an array of potential allergens are no higher or more frequent in SIDS cases vs. controls, and no higher in SIDS cases with elevated vs. normal β -tryptase levels. Other studies came to a similar conclusion [7,9,10,18]. Also, IgE-positive lymphocyte numbers were not elevated in lungs of SIDS infants [5]. Thus, atopy does not appear to be a cause or a risk factor for SIDS.

The current study confirms previous work implicating IgE-independent activation of mast cells in a portion of SIDS cases and should provoke further research into this potentially important relationship.

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