

SPECIAL ARTICLE

Allergy and cot death: with special focus on allergic sensitivity to cows' milk and anaphylaxis

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Introduction

Despite a great deal of focus and research world-wide over the last 40 years' the cause (or causes) of sudden death in infants (or cot deaths) is still unknown. Our own contribution to the attempts to solve this problem has been to investigate the possible role of anaphylaxis—an allergic reaction characteristically capable of producing sudden death. This acute allergic reaction may occur in many animal species. It is well recorded in the human adult but the manifestations in the human infant and the conditions under which it could occur are not at all well envisaged or established, and even less is known about the manifestations and conditions under which it might occur in the sleeping infant. This is important in the present context as the vast majority of cot deaths occur during sleep. The clinical manifestations of acute fatal anaphylaxis in the conscious guinea-pig are very different from the picture in animals similarly sensitized but lightly anaesthetized [1] to stimulate sleep. Such animals, on challenge, go into sustained periods of apnoea without any struggle or violent gasping for breath and die quietly with the heart continuing to beat for a short period after cessation of respiration. Such a terminal event would be quite consistent with the mode of death in cot death.

This annotation focuses attention on the very real possibility than an anaphylactic reaction in infants sensitized to cows' milk underlies cot death. The hypothesis holds that the challenge is administered systemically (essential in the shocking reaction) via the lungs from small quantities of inhaled regurgitated stomach contents containing anaphylactogenic cows' milk [2,3]. Until now, it has not been possible to provide incontrovertible proof or even overwhelming evidence for the hypothesis. On the other hand, as far as we know, there is nothing published or otherwise to refute it. The circumstantial evidence favouring such an hypothesis, however, is considerable. Until recently it has been extremely difficult to propose or suggest a salient marker to look for (many hours post-mortem) which was likely to be pathognomic of anaphy-

lactic death. There is now, however, greatly increased understanding of the cellular and molecular pathways in anaphylaxis and other acute allergic reactions. There are also recently developed immunoassays for some of the more stable mediators of the anaphylactic reaction. These, against adequate control post-mortem material, could possibly afford markers for anaphylactic death and are addressed in this article.

The hypothesis

Essential to the hypothesis is that some infants develop an anaphylactic sensitivity to the proteins in cows' milk derived at some stage following the oral administration (feeding) of cows' milk. However gauged, the evidence for this anaphylactic sensitivity is overwhelming. The anaphylactic reaction or challenge which kills the sensitized infant, according to the hypothesis, occurs during sleep following regurgitation of gastric contents (containing recently ingested cows' milk) and inhalation of a little of this material into the lungs, a major shock organ for anaphylaxis in the human (and also in the guinea-pig).

As mentioned below, administration of the anaphylactinogen by the respiratory tract (i.e. down the trachea) is just as lethal as direct intravenous administration. Also, in guinea-pig experiments, light anaesthesia completely modifies the outward clinical manifestation of the lethal challenge and it is suggested that deep sleep in infants may do the same.

Experimental investigations in the guinea-pig

It was Dr A. M. Barrett, University Morbid Anatomist at Cambridge with a deep commitment to cot death investigations, who in 1956 approached one of us (RRAC) and my junior colleague, W. E. Parish and asked whether 'some sort of hypersensitivity reaction' could possibly exacerbate the inflammatory consequences in the lungs following inhalation of infant feeding material. The results of our first investigations were published in 1960 [2,3]. Guinea-pigs sensitized to cows' milk proteins by parenteral sensitization, could be shocked lethally, not only by intravenous injection but also by intratracheal

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administration. Similar intratracheal administration in unsensitized guinea-pigs was without effect. Deaths in sensitized (conscious) guinea-pigs were typically agonal and the lung pathology showed marked air trapping. However, if intratracheal challenge was performed in sensitized guinea-pigs, lightly anaesthetized before the challenge [1], death also occurred, but with two very important differences, namely, (1) death was not agonal, but was quiet, rapid and insidious with simple cessation of respiration, and (2) the morbid anatomy showed no marked emphysema but was comparable with that seen in cot death infants.

To simulate more closely the situation in infants, a large number of subsequent experiments were performed in guinea-pigs sensitized to cows' milk proteins, not by parenteral injections, but by allowing animals simply to drink cows' milk—the route of sensitization obviously operative in the human infant [4–6]. In the guinea-pig, oral administration of cows' milk for 4 days is quite adequate for anaphylactic sensitization and fatal shock on challenge [7]. In the guinea-pigs drinking cows' milk, sensitization to β -lactoglobulin and casein was stronger than that to α -lactalbumin. These guinea-pig experiments illustrate and emphasize the significance of what may be called the *latent anaphylactic state*. Guinea-pigs anaphylactically sensitized to cows' milk proteins by drinking milk, can be shown to have a potentially lethal sensitization by testing their sera in passive cutaneous anaphylaxis (PCA). After drinking milk guinea-pigs remain in perfect health. However, on placing a small amount of milk (100 μ l) into the trachea a lethal reaction ensues, as it would if given intravenously.

Clinical and latent anaphylactic sensitivity in infants to cows' milk

The clinical manifestations of cows' milk allergy in infants are varied and extensively reported. Most of the overt clinical manifestations of 'milk allergy' are poorly understood, to a large extent due to a lack of relevant objective measurements. In most situations the clinical manifestations follow unquantified allergen (antigen) challenge—with presumably small amounts over the oral mucosae and the undefined absorption through the upper intestinal mucosa. Infants manifesting exquisite sensitivity with, for example, wealing of the face skin on contact with milk, are likely to have very high IgE antibody levels to milk proteins. Infants with such an overt clinical hypersensitivity are carefully kept off all milk products and thus protected. However, low levels of sensitivity may be latent and not recognized without undergoing special investigative procedures which, of necessity, would be provocative and possibly hazardous. A relatively mild

procedure would be skin testing. As long ago as 1925 Dubois *et al.* [8], showed that 50% of infants being weaned from the breast on to cows' milk developed, over a 3-week period, immediate (Type 1) skin sensitivity to intracutaneous injection of diluted cows' milk, but in most cases this was transient with the exception of infants manifesting infantile eczema in whom the response was more persistent. With the present understanding on the cellular mechanisms of anaphylactic sensitivity, it is quite possible that alveolar and bronchial tissue with their ample complement of mast cells may well have an even greater sensitivity and potential for reaction.

Sensitivity to milk proteins in infants shown by bronchial provocation testing would give immediate credibility to the anaphylactic hypothesis, but clearly such testing would be dangerous and unethical. A less satisfactory, but ethically acceptable means of measuring generalized anaphylactic sensitivity is to measure their *ex-vivo* basophil response to cows' milk antigen challenge as described by McLaughlan and Coombs [9], using 250 μ l infant blood and addition of diluted milk. Twelve of 54 samples of infants aged 4–26 weeks released >9% of their total blood basophil-bound histamine and of these 12, six released more than 20%, the three highest releasing 63%, 42% and 36%, respectively. This procedure measures the antigen-specific reactivity of basophils in the blood as evidence of systemic sensitization. Were it possible to measure the sensitivity of the tissues of the lungs, the reactivity could be even greater.

Other relevant considerations

Method of in-vivo sensitization

This must follow the oral administration of cows' milk with the corollary that the incidence of cot death is greater in bottle-fed infants. The statistics in the literature are greatly hampered by the lack of clear definitions of breast- and bottle-feeding. Breast-fed is sometimes taken as strictly breast only, sometimes mainly breast, but occasionally cows' milk supplementation. In many cases an infant may have been totally breast-fed at home until weaning, but inadvertently given one or two bottle milk-feeds in the maternity hospital. Of considerable relevance to this aspect is the experimental finding in rats [10] that IgE sensitization occurs more easily following the administration of minute doses of parenteral allergen. Supporting this from the clinical side are the observations of Gerrard and Shenassa [11] that the more dangerous clinical allergic or anaphylactic reactions to cows' milk in infants occur most commonly in those who were breast-fed until weaning and then having this replaced totally by cows' milk, leading to the presentation of the reaction.

Ventura *et al.* [12] report clinical anaphylactic reactions in seven infants when weaned onto cows' milk in this manner. These infants had been totally breast-fed for 3 months, but had all received cows' milk in their first days of life.

It is possible that the majority of infants fed cows' milk from birth develop immunological tolerance to anaphylactic sensitization to cows' milk proteins. This phenomenon has been reported experimentally for guinea-pigs fed cows' milk from the first or second day of life [13]. Also one cannot totally exclude the possibility that infants could be anaphylactically sensitized to human milk unless made tolerant to this by early feeding. Amman *et al.* [14] report autosensitization of mothers to their own milk. It has been clearly established that cows may be anaphylactically sensitized (i.e. produce reaginic antibody) and manifest an anaphylactic reaction when their own milk is absorbed from the mammary gland into the systemic circulation [15]. The critical factor as far as our hypothesis is concerned is the nature of the last feed before the infant's death, for this must contain the offending sensitizing allergen.

Intratracheal challenge

To take account of the fact that the vast majority of cot death cases appear to die in their sleep, inhalation challenge must follow gastro-oesophageal reflux, an event which is said to be confined to periods of active rapid eye movement during sleep when the arousal threshold is raised [16]. Furthermore, Williams and Phelan [17] suggest that regurgitation and inhalation of gastric contents are common in infants. In regurgitated contents lactoglobulin is likely to be undigested and soluble, while casein is likely to be in curd form, insoluble at acid pH.

One might expect inhalation to also take place during feeding, but coughing and other reflexes, operative in the conscious or wakeful state, could well reduce the amount reaching the terminal aspects of the broncho-alveolar tree and therefore mitigate the consequences. Of considerable relevance to the effectiveness for anaphylaxis on challenge via the intratracheal route is the report by Bakhle *et al.* [18] which showed that release of eicosanoids from isolated lung preparations of ovalbumin-sensitized guinea-pigs was greater if the antigen were perfused via the trachea rather than via the pulmonary artery, thereby indicating that intratracheal administration is a perilous route of challenge.

Age incidence (peak 2-4 months)

On first consideration it is not easy to explain this peak age incidence on the basis of the anaphylactic hypothesis.

However, there is evidence that IgE antibodies are to be found selectively in the early response to food allergens sensitizing from the gut. This preferential IgE antibody response is soon altered and replaced by IgA, IgG and IgM. This period may also be the period when the swallowing reflexes are most uncoordinated, thereby increasing the frequency of inhalation.

Sleep

In relation to sleep, possibilities worthy of further study include the likelihood that deep sleep could (1) increase the entry and spread of inhaled refluxed material further into the lung, (2) suppress certain clinical manifestations of the anaphylactic reaction and so (3) modify the intrapulmonary cellular events associated with an anaphylactic reaction in the usual wakeful state. In experimental investigations in the guinea-pig, light anaesthesia was the nearest approximation to sleep—sleep being an important association of infant cot death.

Seasonal incidence

The increased incidence of cot death cases during the winter months strongly suggests an association with infection. Infection could indeed be a contributory factor in the anaphylaxis hypothesis, depending as the hypothesis does on inhalation to administer the challenging dose of antigen. Infants are preferential nose breathers as this allows simultaneous breathing while sucking at the breast. Should the mucosa of the nares or pharynx be inflamed and swollen due to infection forcing mouth breathing then the swallowing reflexes could well be maladjusted resulting in more frequent inhalation of material that would otherwise be safely swallowed.

Earlier attempts of post-mortem diagnosis of anaphylactic death

In the older literature, there are reports of post-mortems on patients dying of 'anaphylaxis'. What must be remembered is that the term anaphylaxis used then, covered what we now call both Type I and Type III reactions or serum reactions [19]. A more modern description is given by Austen [20], but even here we are told that diagnosis 'depends largely on an accurate history'. Also it would have been likely that the anaphylactic reaction would have occurred in the wakeful conscious state not modifying the marked hyperinflation of the lung seen both macroscopically and microscopically. What was noted, and is also seen in cot-death lungs and in lungs of guinea-pigs investigated experimentally, is peribronchial oedema and congestion, submucosal oedema and eosinophilic

infiltration. Interestingly, two other gross post-mortem findings are seen in anaphylaxis, in cot death and in the experimental guinea-pigs: these are fluid blood, possibly due to heparin release from degranulated mast cells, and an empty urinary bladder as a consequence of contraction of involuntary muscles.

An obvious potential marker of anaphylactic death is the state of the mast cells in the lungs and their degree of degranulation. Parish *et al.* [21] examined the mast cells of cot death lungs but the findings were too irregular to have much meaning. A subsequent and more detailed examination of mast cells in cot death infant lungs was carried out by Emery and Carpenter [22]. They concluded 'that in approximately one-third of children found unexpectedly dead there is evidence of a histamine-release mechanism taking place and that an anaphylactoid-type reaction plays some part in their terminal states'. It should be borne in mind that both these examinations were made before the discovery of the mucosal mast cells—and the special fixative and stains needed for their proper demonstration. In 1964, Parish *et al.* [21] also demonstrated cows' milk antigens in a high percentage of cot death lungs by immunological means, but it was accepted that this might have no diagnostic significance due to the common occurrence of terminal agonal aspiration. Attempts to provide evidence of bronchoconstriction 24–48 hr after death by perfusion rates through the respiratory tree were also unsuccessful [21].

Bodian Heslop lesion

This is a lesion first described by Bodian and Heslop [23] using a special post-mortem technique aimed at preserving the bronchial secretions. In two-thirds of 70 lungs of sudden infant death cases they described lesions consisting of 'an explosive shedding of respiratory epithelial cells' mixed with considerable quantities of mucus in the lumen of 'bronchi and bronchioles'. Bodian and Heslop [23] suggested that these lesions were a consequence of viral infection. Very similar lesions have also been observed in the guinea-pig model of inhalation anaphylaxis in lightly anaesthetized guinea-pigs [3]. In countering criticisms that these lesions in infant lungs were post-mortem artefacts, France [24] concluded that this was not the case as air bubbles amongst the cells indicated that the desquamation preceded death. This lesion may possibly be an important pointer and find an explanation in the release of mast cell mediators, especially proteases [25] (see below). Experimental investigations by King and Miller [26] on a comparable protease, released from antigen-stimulated mucosal mast cells in the rat, showed increased gut mucosal permeability following systemic antigen challenge and histological shedding of gut epi-

thelial cells within a few minutes of administering antigen intra lumenally. Sage *et al.* [27] showed that this enzyme had proteolytic activity against Type IV collagen in the epithelial basement membrane and was particularly effective in releasing epithelial cells from intestinal slices *in vitro*.

Returning to man, Kay *et al.* [28], examining bronchial lavage fluid from asthmatics and non-asthmatic controls, found significantly more epithelial cells in the former than latter, suggesting possibly that shedding resulted from a mast cell-mediated reaction. All these observations suggest that 'explosive shedding' of bronchial epithelial cells, often seen (possibly only using special techniques) in cot death cases, might indeed have significance. However, there is a clear need for really specific post-mortem markers diagnostic of anaphylactic death—and which can then be used to investigate the anaphylactic hypothesis.

Plausibility of the anaphylactic hypothesis and the consequences were it to be verified

To those with a knowledge and understanding of anaphylaxis, the hypothesis must have great plausibility. From the clinical side there is ample evidence of both overt and latent sensitization to cows' milk in young infants. Again, solid evidence is now available that a latent, potentially dangerous sensitization develops in guinea-pigs drinking cows' milk. The suddenness and unexpectedness of the infant deaths accord well with anaphylaxis—the infant is apparently healthy until the moment the reaction (or challenge) occurs with its cataclysmic result. In the model investigations in guinea-pigs, the animals either die very rapidly or recover and are, for all intents and purposes, perfectly normal the next day. Again, in the model studies the influence of light anaesthesia (the best approximation available for sleep) on the pattern of anaphylactic death renders the hypothesis realistic.

From our knowledge of allergic sensitization and reactions in both man and the guinea-pig, anaphylactic death should occur occasionally in infants in the circumstances envisaged. Possibly an even more pertinent question is why does it not occur more frequently? One explanation for this may lie in the phenomenon of neonatal tolerance—in this case—to the production of anaphylactic antibody to milk proteins resulting from the considerable amounts of these antigen absorbed across the intestinal mucosa during the first few days of life. Evidence for such tolerance has been reported in the guinea-pig model [13]. Another consideration is the necessary combination of factors determining a fatal reaction, namely: (1) the frequency of inhalation events, (2) the level of sensitivity, and (3) the doses or amount of

the anaphylactogen reaching the most sensitive areas of the lung.

Should an answer to the enigma of cot death eventually be found along these lines, the fear of the unknown becomes less. The explanation would certainly encourage efforts to achieve less sensitizing infant feeding formulae. It would also focus on the need to comprehend fully all the factors determining tolerance to anaphylactic sensitization to milk proteins. Again, infants thought to be at special risk could be monitored for their level of anaphylactic sensitivity and special precautions taken by going on to a totally non-allergenic feeding formula such as pregestimil [7].

It has to be made quite clear that, as yet, there is absolutely no proof substantiating the anaphylactic hypothesis, but its very plausibility makes it urgent to undertake research to discover a test or assay indicative of an anaphylactic death and which can be used post-mortem (even 24–48 hr after death). The direction that such investigations might follow are mentioned in the next section.

Projected investigations to discover specific post-mortem markers diagnostic of anaphylactic death

Clearly what is needed to support anaphylaxis as a cause of infant death is the demonstration of massive mast cell activation whether manifest systemically or in a shock organ such as the lung. During anaphylaxis, mast cells sensitized with allergen-specific IgE undergo degranulation with discharge of a wide array of inflammatory mediators into the shock organ and circulation. Unfortunately, very few of the mediators known to be associated with the secretory granules of mast cells are particularly specific for the cell or are stable once released [29]. For example, histamine, an obvious candidate, once released is rapidly metabolized both extra- and intracellularly to *N*-methylhistamine and imidazole acetic acid [30]. A great problem also arises from the large concentration of preformed histamine present within the secretory granules of basophils and platelets within the vascular compartment. The acid hydrolases such as β -glucuronidase, β -hexosaminidase and β -galactosidase while present in their tetrameric lysosomal forms within the granules of human mast cells, are also ubiquitous in other lysosomes [31]. Heparin, the unique 60 000 MW proteoglycan has recently also been described in basophils and its detection after requiring various forms of blood coagulation assay or anti-thrombin III binding either lack sensitivity or specificity.

Recently, there has been renewed interest in mast cell ontogeny with the discovery that neutral proteases distribute differently in mast cell populations isolated from

different tissues and even at different sites within a single tissue [32]. Human mast cells, irrespective of their source, contain a large amount of a 130 000 MW tetrameric enzyme called tryptase [25,33] within their lysosomal granules. The presence of this enzyme alone within the granules of mast cells at mucosal surfaces has led to their classification as M_T cells [34,35]. These mast cells have an obligatory requirement for lymphocyte factors for growth and maturation and as they are found almost exclusively at mucosal sites are represented in the rodent as the IL-3-dependent mucosal mast cell containing RMCP II as its major protease [36]. Within a connective tissue environment human mast cells acquire an additional protease referred to as chymase [37] which is present along with 3–5-fold higher concentrations of tryptase when compared to M_T cells [35]. Mast cells containing tryptase and chymase are referred to as M_{TC} cells. The counterpart of the M_{TC} cell in the rodent is the chymase-containing connective tissue mast cell which has an obligatory requirement for fibroblast growth factors [36]. The airways of adult humans contain predominantly M_T cells in the epithelium and submucosa, with M_{TC} cells being found in association with airway connective tissue elements such as the submucosal blood vessels [34].

Activation of human lung mast cells by ionophore A23187 or by reversed anaphylaxis releases tryptase in association with heparin [38,39]. In atopic asthma, challenge of the airways with allergen also releases substantial amounts of this mediator into the bronchial lumen along with histamine and other mediators [40]. In patients with the systemic form of urticaria pigmentosa (systemic mastocytosis) and in patients following bee-sting anaphylaxis, elevated serum concentrations of tryptase have been reported using a sensitive radioimmunoassay for this enzyme [41]. Therefore, this mast cell enzyme presents one possibility for detecting anaphylaxis occurring ante-mortem in blood specimens obtained post-mortem. If the shock organ in some babies with Sudden Infant Death Syndrome (SIDS) is the airway as proposed [2], then the highest blood concentrations of this mediator might be expected to be found in the right atrium and ventricle which receives the effluent of the bronchial submucosal circulation. The presence of large venous sinusoids beneath the bronchial epithelium would facilitate the removal of tryptase and other mast cell products from their sites of release.

In addition to releasing preformed mediators, activated mast cells generate substantial amounts of prostaglandin D_2 (PGD_2) from arachidonic acid mobilized from the cell's phospholipid stores [41,42]. Prostaglandin D_2 is a potent bronchoconstrictor agonist [43] and an arteriolar vasodilator agent [44]. Its increased release from human dispersed lung and bronchoalveolar lavage (BAL) mast cells with IgE- and calcium ionophore-dependent stimuli

has been well documented [41,42,45]. On account of its *de-novo* synthesis, PGD₂ release from activated mast cells lags behind that of histamine and other preformed mediators, although a close relationship between the total amount of each of the two mediator classes released has been found. Although increased concentrations of PGD₂ have been reported in BAL of asthmatic subjects following allergen provocation [46], upon reaching the circulation this prostaglandin is rapidly metabolized, predominantly to chain-shortened PGF ring products. At least 20 different PGD₂ metabolites have been detected in the urine of patients with systemic mastocytosis with many having the basic PGF ring structure [47]. Following administration into normal volunteers of [³H]PGD₂ by inhalation or by intravenous infusion there is rapid conversion to two unique metabolites, 9 α ,11 β -PGF₂ and its 13,14-dihydro-15-keto metabolite [48,49]. The initial reaction is a stereospecific reduction of the 11-keto group of PGD₂ by a specific NADPH-requiring reductase found in lung, liver and kidney but not in blood [50].

Recently, 11-keto reductase has also been described in human lung tissue [51]. Although only differing in its stereochemistry from 9 α ,11 α -PGF₂ (PGF_{2 α}), 9 α ,11 β -PGF₂ has biological properties resembling PGD₂ rather than PGF_{2 α} . Further sequential Δ^13 -reduction and 15-oxidation leads to the more stable but biologically inactive metabolite 13,14-dihydro-15-keto-9 α ,11 β -PGF₂. Although these metabolites may be identified and quantified by gas chromatography and mass spectrometry, the recent availability of specific antibodies has enabled the development of radioimmunoassay [52]. Thus the assay of these PGD₂ metabolites in serum obtained from the right ventricle of children dying of SIDS should provide a further opportunity to discover whether mast cell activation in the airways occurred ante-mortem and was the likely cause of death. One difficulty that could confound this approach is the contamination of serum samples with products from circulating basophils. However, basophils only contain trace amounts of tryptase [53] and are unable to generate PGD₂. Also, human blood does not contain the 11-keto reductase necessary to metabolize PGD₂ to 9 α ,11 β -PGF₂.

Incrimination of anaphylaxis, as a causative event in SIDS, requires the demonstration of massive mast cell degranulation in the shock organ as a primary event. Whether using electron microscopy, immunohistochemistry or measuring mast cell products in the serum, the difficulty with this approach is to be sure that intrapulmonary mast cell activation has not occurred as a secondary event, e.g. due to sudden hypoxia or neuropeptide release. In guinea-pigs, hypoxia alone is sufficient stimulus to produce widespread release of mast cell mediators in the lung. Thus any investigation along these

lines of the anaphylactic hypothesis of SIDS would necessitate the use of adequate controls, i.e. children of the same age who have died suddenly from other known causes.

Conclusions

As has recently been re-emphasized, it is most unlikely that SIDS can be accounted for by a single disease process [54]. The recent evidence of increased IgG levels in BAL of SIDS [55] suggests that immunological factors in the lung are important in a high proportion of afflicted infants. With the recent recognition that virus infections may enhance the lungs' response to inhaled allergens, it is not beyond the realms of possibility that these two common factors would interact in infancy with catastrophic end results. Thus, although anaphylaxis is unlikely to be the explanation of all SIDS, there is, as yet insufficient evidence to refute the idea as a possibility.

Thirty years have elapsed since the anaphylactic hypothesis for SIDS was first suggested without a clear answer emerging. With the development of new mediator methodology and a greater understanding of mast cell function, this hypothesis can now be tested with some degree of confidence.

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