Int J Leg Med (1993) 105:333–338

International Journal of
Legal Medicine

The potential role of bacterial toxins in Sudden Infant Death Syndrome (SIDS)

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Received August 31, 1992 / Received in revised form November 16, 1992

Summary. Toxigenic bacteria have been implicated in some cases of Sudden Infant Death Syndrome (SIDS). Although there is not much evidence that *Clostridia spp.* are associated with SIDS in Britain, strains of *Staphylococcus aureus* producing pyrogenic toxins have been isolated from significant numbers of these infants at autopsy. The pyrogenic toxins, produced by some strains of group A *Streptococcus pyogenes* as well as staphylococci, are powerful "superantigens" that have significant physiological effects including induction of fever $> 38°C$. In this article, interactions between genetic and environmental factors that might enhance colonization of epithelial surfaces by toxigenic staphylococci are analyzed: infant's expression of Lewis[a] antigen which acts as a receptor for some microorganisms; viral infections; the effect of mother's smoking on susceptibility to respiratory infection. Based on epidemiological findings and laboratory investigations, a hypothesis is proposed to explain how bacteria producing pyrogenic toxins might contribute to some cot deaths.

Key words: Bacterial-toxins – SIDS

Zusammenfassung. In einigen Fällen des Sudden Infant Death Syndrom (SIDS) wurde die Rolle Toxin bildender Bakterien diskutiert. Obwohl es keinen Beweis gibt, daß Clostridia spp. mit SIDS in Großbritanien assoziiert sind, sind von signifikanter Anzahl dieser Kinder bei der Autopsie Stämme von Stapylokkokus aureus isoliert worden, welche Fieber erzeugende Toxine produzieren. Die Fieber erzeugenden Toxine, welche von einigen Stämmen der Gruppe A Streptokkokus pyogenes produziert werden, wie auch von Staphylokkoken, sind mächtige „Superantigene", welche signifikante physiologische Effekte haben unter Einbeziehung der Induktion von Fieber mit mehr als 38°C. In diesem Artikel werden Interaktionen zwischen genetischen und Umgebungsfaktoren erörtert, welche die Kolonisierung epithelialer Oberflächen durch Toxin bildende Staphylokkoken steigern könnten: die Expression des Lewis[a] Antigens des Kindes, welches als Rezeptor für einige Mikroorganismen wirksam ist; virale Infektionen; die Auswirkung des mütterlichen Rauchens auf die Empfänglichkeit für Atemwegsinfektionen. Basierend auf epidemiologischen Befunden und Laboratoriumsuntersuchungen wird eine Hypothese vorgeschlagen, wie Bakterien, welche pyrogene Toxine produzieren, zu einigen plötzlichen Kindstodesfällen beitragen könnten.

Schlüsselwörter: Bakterien-Toxine – SIDS

Introduction

Epidemiological studies indicate that infectious agents might be involved in some case of Sudden Infant Death Syndrome (SIDS). At autopsy there can be evidence of minor inflammation and infection of the respiratory tract in many of these infants [1]. The deaths are more frequent during the period when maternal antibodies are declining and the immune system of the infant is immature. The risk of SIDS increases during autumn and winter months when respiratory infections are more common [2, 3]. There is often a history of minor upper respiratory tract infection in these infants [4] and histological evidence of viral infection or inflammation in the lung has been reported [5, 6]. Both smoking and passive exposure to cigarette smoke have been associated with increased risk of respiratory infection [7], and maternal smoking is one of the factors identified in the New Zealand studies of SIDS [8]. In some series, SIDS is more frequent in families in which socio-economic conditions are poorer, and smoking is also related to socio-economic status. In the United Kingdom, the proportion of women who smoke increases as social class decreases from "professional" to "partly skilled" or "unskilled" categories [9]. Breast feeding protects infants in this age range from gastrointestinal and respiratory illnesses, and

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SIDS is reported by some workers to be more frequent among bottle fed babies [8].

Microorganisms investigated for their associations with SIDS

By definition, invasive bacterial diseases are excluded from deaths diagnosed as SIDS. As a result, the role which respiratory virus infections might play in these infant deaths has been investigated [10, 11], however, there is little evidence for direct associations between viral infections and SIDS [12, 13]. Toxin-producing bacteria have been isolated from autopsy material. Infant botulism has been suggested to contribute to 4% of cot deaths in the United States and up to 16% of those in Sweden [14–17]. This pattern has not been observed in the United Kingdom [18]. Toxins of *Clostridium difficile* can produce death in monkeys with features that are pathologically consistent with SIDS [19]. These bacteria and enterotoxigenic *Escherichia coli* have been isolated from a few infants [20, 21], however, the evidence for involvement of intestinal toxigenic bacteria has been inconsistent.

One of the more recent hypotheses regarding sudden infant death syndrome (SIDS) is that nasopharyngeal colonization by toxigenic strains of *Staphylococcus aureus* might contribute to some of these cases [22]. These bacteria remain localized on the mucus membranes but their toxins disseminate into the circulation. In one series, these bacteria were isolated from the nasopharynx of approximately 40% of SIDS infants [23]. The toxic shock syndrome toxin of *S. aureus* can kill a previously healthy adult, so it might easily kill a small infant. Pyrogenic toxins are produced by some strains of *S. aureus* and also by some strains of group A beta-haemolytic *Streptococcus pyogenes*. These families of toxins are associated with staphylococcal food poisoning, toxic shock syndrome and also with the rash that accompanies scarlet fever. These substances are powerful "superantigens" that have significant physiological effects such as induction of fever (> 38°C), possibly due to direct action on the hypothalamus or through their induction of tumor necrosis factor (TNF) and interleukin 1 by monocytes (Table 1). The bacteria can produce these toxins at temperatures between 37–40°C, but the amount of toxin produced increases with increasing temperature [24].

Table 1. Properties of Pyrogenic Toxins of *S. aureus* and *Strep. pyogenes*

1. Pyrogenic, fever > 38°C
2. Mitogenic for lymphocytes
3. Induce release of TNF and IL-1 from mononuclear phagocytes
4. Non-specific suppression of immunoglobulin production
5. Enhancement of delayed type hypersensitivity
6. Alteration of liver clearance function and enhance endotoxic shock
7. Produced between 37–40°C, but in greater quantities at higher temperatures

Factors contributing to susceptibility to infectious agents

Our research group has been investigating both genetic and environmental factors affecting susceptibility to infectious agents, in particular the secretor gene *(Se)* located on chromosome 19. The gene is inherited in a Mendelian dominant pattern and there are two phenotypes, secretors and non-secretors. Secretors which comprise 75–80% of most populations, have the antigens of their respective ABO blood group determinants in their body fluids. The minority 20–25% who are non-secretors do not have these antigens in their body fluids. The secretor gene is in the same linkage group as the gene for the Lewis blood group antigens *(Le)*, and the secretor gene controls expression of the Lewis blood group antigens. Non-secretors can produce only Lewis^a while secretors produce predominantly Lewis^b and also variable amounts of Lewis^a [25]. The distribution of the ABO and Lewis antigens on cells and in body fluids of secretors and non-secretors in summarized in Table 2.

Susceptibility to bacterial diseases and superficial yeast infections appears to be associated with the non-secretor phenotype, as does asymptomatic carriage of group A streptococci [26], meningococci [27], and *Candida* species [28–30]. Susceptibility to respiratory viral diseases [31] and to acquisition of the human immunodeficiency virus (HIV) through heterosexual intercourse [32] appear to be associated with the secretor phenotype.

When we examined these phenotypes among SIDS infants, the distribution of secretors and non-secretors did not differ significantly from that observed for the general population. There was, however, a high proportion of these infants in whose secretions the Lewis^a antigen was detected, 63/89 (71%) [33]. This was not unexpected; compared with adults and children over the age of 18 months, Lewis^a antigen is detected in a much higher proportion of infants. The peak incidence for the detection of Lewis^a on erythrocytes of infants is 2–3 months (80–90%) [34], coincident with a high incidence of SIDS (Fig. 1).

The production of Lewis substances is due to the interactions between two fucosyl transferases, one coded for by the secretor gene and one coded for by the Lewis gene. Both these enzymes add fucose to the type 1 precursor chain from which most of the ABO and Lewis antigens in secretions is derived. If the secretor transferase adds fucose to the terminal sugar in the precursor chain, the Lewis enzyme can add fucose to the subterminal sugar to produce Lewis^b. If the Lewis enzyme adds

Table 2. Distribution of ABO and Lewis blood group antigens on cells and in body fluids of secretors and non-secretors

BG antigen	Secretors		Non-secretors	
	Cells	Secretions	Cells	Secretions
H (A/B)	+	+	+	−
Lewis^a	− (+)*	− (+)*	+	+
Lewis^b	+	+	−	−

* Present in variable quantities

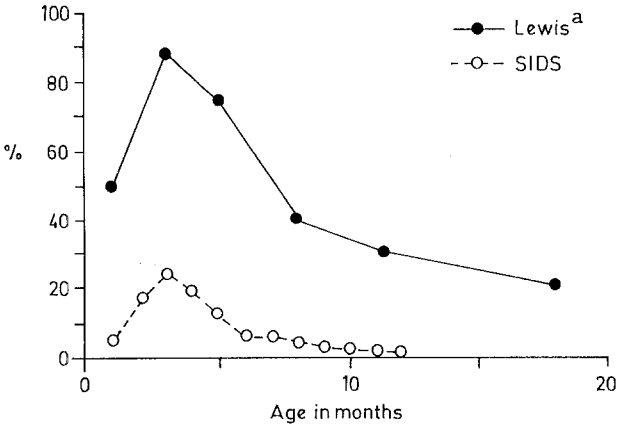

Fig. 1. Detection of Lewis^a antigen on cells of infants and incidence of SIDS

fucose to the subterminal sugar first to produce Lewis^a, the secretor enzyme cannot use this structure as a substrate and Lewis^a is the final product [35]. In infants, the Lewis enzyme is more efficient that the secretor enzyme. As a result, infants express easily detectable amounts of Lewis^a even though the amount of this antigen might be greatly reduced as the child becomes older.

One of the hypotheses proposed to explain the higher proportion of non-secretors found among patients with bacterial or yeast infections was that there are adhesins on some strains of microorganisms that can bind to the Lewis^a antigen usually present in greater quantities on epithelial cells of non-secretors [36]. This might enhance the probability of colonization by bacteria or yeasts expressing adhesins that use Lewis^a as a receptor. Evidence for this hypothesis was obtained initially from studies of *Candida* species [37–38], and there is evidence that the Lewis^a antigen is a receptor for the pertussis toxin [39].

If there are strains of toxigenic bacteria with adhesins that can bind to Lewis^a, infants might be easily colonized if they are exposed to these bacteria. In the age range in which SIDS occurs, they have little serum or secretory antibodies that might reduce colonization by these bacteria or activities of their toxins.

We have tested the hypothesis that there are adhesins on *S. aureus* that bind Lewis^a. Binding of 3 strains of *S. aureus* producing pyrogenic toxins (including one producing the TSST-1 toxin) to epithelial cells of non-secretors was significantly higher than to cells from secretors. As in the experiments with yeasts, pre-treatment of cells with monoclonal anti-Lewis^a significantly reduced the binding of the toxigenic strain tested. It also significantly reduced the binding of the non-toxigenic strain which bound equally well to cells of secretors or non-secretors [40].

This apparent discrepancy was solved by examining the amount of Lewis^a on epithelial cells of individual secretor and non-secretor donors. When assessed semiquantitatively by flow cytometry, binding of monoclonal anti-Lewis^a antibody to cells of non-secretors was uniformly high while binding of the antibody to cells from donors who lack the Lewis gene (Lewis-negative) was barely detectable [33]. Binding of the antibody to cells of secretors was highly variable, some as low as that observed for the Lewis-negative cells (i.e. Le^{a-b-}), some as high as that for non-secretors and some between these two extremes (Fig. 2). The binding of the bacteria was significantly correlated with the amount of anti-Lewis^a antibody detected on the epithelial cells regardless of secretor status [40].

Viral infections as predisposing factors for colonization by bacteria

Although there is no direct evidence for viruses causing cot deaths, they might be predisposing factors for colonization by toxigenic bacteria. Much of the work in this area has been done on influenza virus and superinfections by staphylococci or pneumococci [41]. We have also found that cells infected with respiratory syncytial

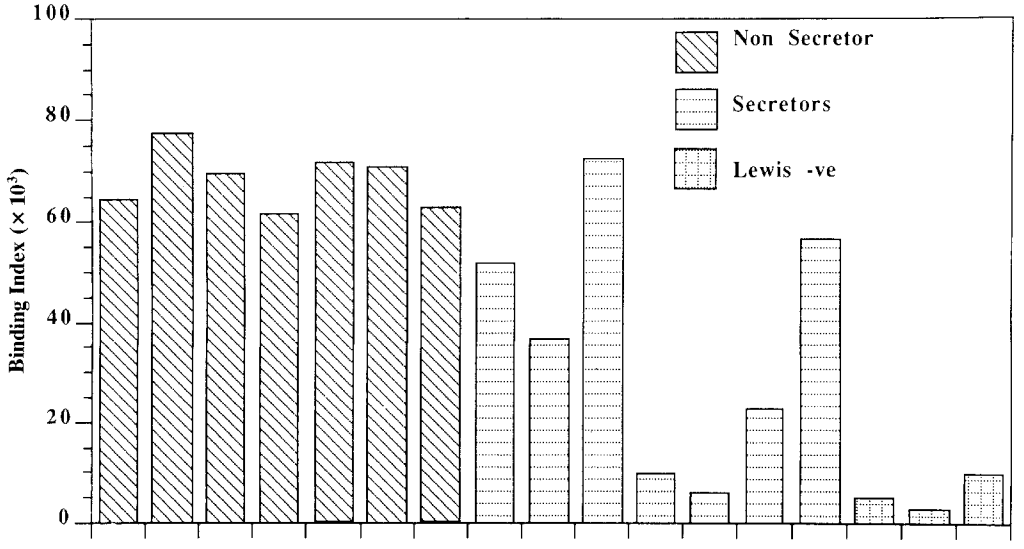

Fig. 2. Variability in binding of monoclonal anti-Lewis^a to epithelial cells of non-secretors, secretors and Lewis-negative individuals

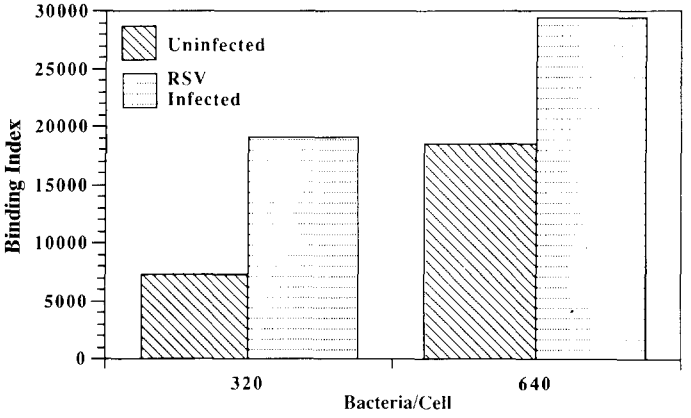

Fig. 3. Binding of *Staph. aureus* to HEp-2 cells and HEp-2 cells infected with RSV

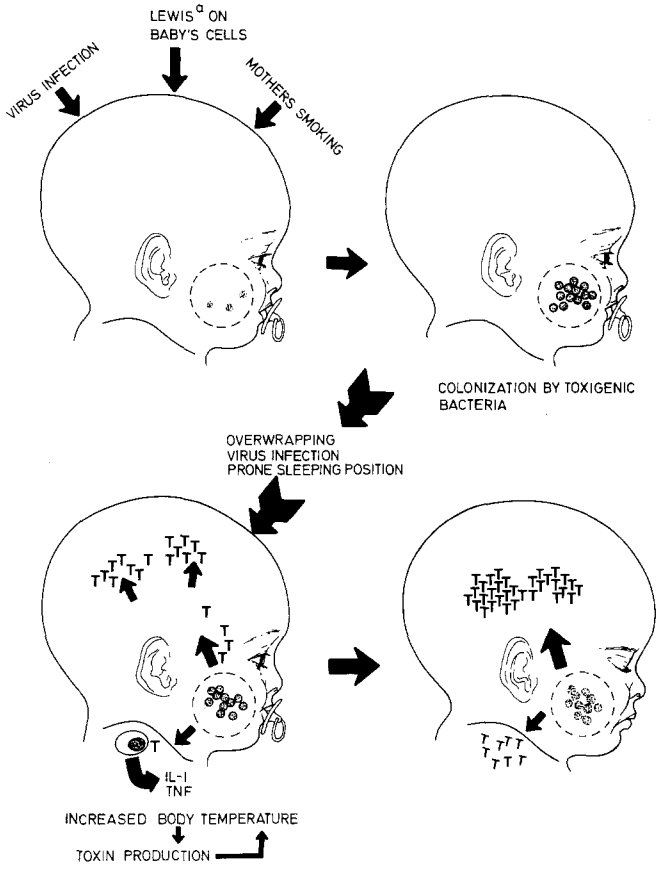

Fig. 4. Factors proposed to affect colonization of infants by toxigenic *Staph. aureus* and to precipitate events leading to SIDS

virus (RSV) *in vitro* bind significantly more meningococci and type b *H. influenzae* [42, 43]. A condition similar to toxic shock syndrome caused by staphylococci has been identified as a complication of influenza or influenza-like illness [44]. RSV is a common cause of disease among infants. Its peak prevalence occurs during the winter months [45] when SIDS is most common. By flow cytometry assays, we demonstrated that binding of a variety of bacteria including one non-toxigenic and one of the toxigenic strains of staphylococci to HEp-2 cells was significantly enhanced in RSV infected cells [40] (Fig. 3).

If toxigenic staphylococci are responsible for some cot deaths, we have identified 2 factors that might contribute to increased colonization or density of colonization by these bacteria: expression of Lewis[a] in this age range; infection with RSV. Since the majority of infants who become colonized by these bacteria suffer no ill effects, there must be additional factors that precipitate the chain of events leading to cot deaths. From our results and the epidemiological data reported, we have suggested the following hypothesis illustrated in Fig. 4.

Exposure to and colonization by staphylococci or streptococci

Mother's smoking might enhance exposure of the infant to *S. aureus*. Smokers are more frequently carriers of potentially pathogenic microorganisms [30, 46] and epithelial cells of smokers bind significantly more staphylococci than those of non-smokers [47]. Smoking also enhances susceptibility to respiratory viral infection, and epithelial cells from individuals with natural or experimental viral infections bound more staphylococci than those from uninfected controls [47, 48]. Passive exposure to cigarette smoke decreases mucociliary clearance. The effect of breast feeding on staphylococcal or streptococcal carriage is not known, however, breast fed infants are less susceptible to toxigenic strains of *Cl. botulinum* [49].

Enhancement of toxin production

If an infant becomes heavily colonized with bacteria producing pyrogenic toxins and environmental factors raise the body temperature, this might increase the quantity of toxin produced. Body temperature might be increased by concurrent minor respiratory viral infection, overwrapping or placing the infant in the prone sleeping position [50, 51]. The toxin produced diffuses into the blood stream to increase the temperature of the infant and further enhance toxin production. This synergistic effect might account for the unusually high temperatures recorded for some cases of SIDS [52]. The actual cause of death might be heat shock or increased frequency or duration and depth of sleep to induction of IL-1 which has been proposed as the link between respiratory infections and fatal sleep apnoea [53].

Acknowledgement. This work was supported by the Scottish Cot Death Trust.

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