Antibodies to endotoxin core in sudden infant death syndrome

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Abstract

To assess the possible role of endotoxaemia in the pathogenesis of sudden infant death syndrome (SIDS), antibodies to endotoxin core (EndoCAb), which have previously been shown to be depressed by systemic endotoxaemia, were measured.

IgG and IgM EndoCAb and total serum IgG and IgM were measured in serum samples from 25 children who had died from SIDS and 164 control children under 1 year of age. Twelve (48%) of the 25 children who had died from SIDS had no detectable IgG EndoCAb compared with 28 (17%) of the 164 control children, and this difference was concentrated in children aged less than 3 months. There was no significant difference between the two groups in the percentage of children with no IgM EndoCAb, nor in the total IgG and IgM concentrations. For IgM EndoCAb, the younger children who had died from SIDS had higher concentrations than the controls.

These results suggest that, in children who have died from SIDS, due to either unusually early or severe exposure to endotoxin, maternal IgG EndoCAb have been depleted and early IgM EndoCAb triggered.

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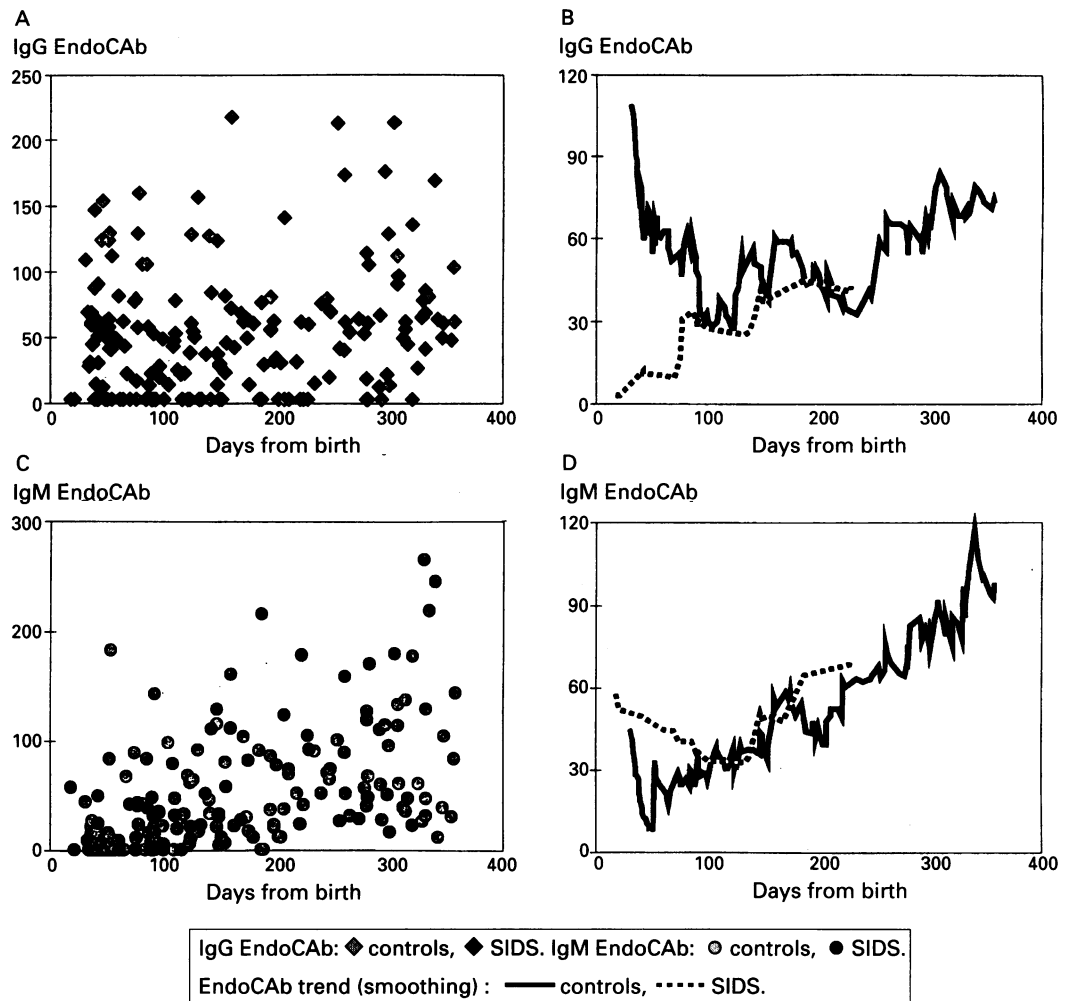G R Barclay

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Sudden infant death syndrome (SIDS) is the leading cause of postperinatal infant mortality in the United Kingdom. Although many theories have been proposed to explain the syndrome, the causes are still obscure. One consistent and characteristic feature of SIDS, however, is its age incidence, with deaths peaking at 2–3 months of age and then decreasing rapidly so that the disease is uncommon after 6 months.[1] This timing coincides with the period during which maternal IgG concentrations decrease and when the infant's own immunity is not yet fully established. Overwhelming infection has been suggested as a cause of SIDS, but in most cases evidence of invasive infection is not found.[2]

As the picture of sudden collapse and death in some ways resembles endotoxic shock, absorbed endotoxins or other bacterial toxins have been suggested as possible mediators of SIDS.[3] Antibodies to endotoxin core (EndoCAb) have been studied and have been shown to be depressed by systemic endotoxaemia, apparently by consumption by the endotoxin[4 5] and in some instances this

indicates a poor prognosis.[6] Moreover, decreasing or decreased concentrations of EndoCAb have been observed in the absence of detectable endotoxaemia, and may be a more sustainable indicator of endotoxin exposure than circulating lipopolysaccharide, which may appear only transiently.[6] For these reasons, measurement of IgG and IgM EndoCAb was thought to be a possible useful indicator of endotoxaemia in SIDS.

Methods

Measurements were performed on 25 serum samples taken at necropsy from children who had died from SIDS, diagnosed by detailed gross and histological examination of all major organs where alternative causes of death had been excluded. As controls for the technique of measuring EndoCAb on samples taken after death, 18 serum samples were taken at necropsy from adults. The SIDS serum samples were compared with 164 consecutive stored serum samples from children under 1 year of age, which had been submitted to a virology laboratory for a variety of investigations.

IgG and IgM EndoCAb were measured by coating enzyme linked immunosorbent assay (ELISA) microplates with lipopolysaccharide complexed with polymyxin B as previously described.[7 8] Rough lipopolysaccharide from a rough submutant strain of each of *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes* was used in a cocktail, where each rough lipopolysaccharide had been selected for the expression of complete endotoxin inner core but not enough endotoxin outer core to bind core type specific antibodies. Equimolar proportions of each of the four lipopolysaccharide-polymyxin complexes were used. Alkaline phosphatase antibody conjugates specific for gammaglobulin heavy chains of human IgG or IgM were used to develop the ELISA. Results were expressed in relation to a standard reference serum sample previously calibrated against adult normal ranges for EndoCAb, which were determined on 1000 healthy adult blood donors. The units are expressed as a percentage of the median of the adult ranges for IgG or IgM EndoCAb, hence median units or MU. The 10th to 90th centile adult ranges are 32·7–240·7 (IgG) and 39·7–263·1 (IgM). Total serum IgG and IgM were measured by immunoturbidimetry.

Simple χ^2 tests, or Fisher's exact tests where appropriate, were used to compare the proportion of children in the SIDS and control groups

A
IgG EndoCAb

B
IgG EndoCAb

C
IgM EndoCAb

D
IgM EndoCAb

IgG EndoCAb: ◈ controls, ◆ SIDS. IgM EndoCAb: ⊙ controls, ● SIDS.
EndoCAb trend (smoothing) : ━━━ controls, ••••• SIDS.

Peripheral blood IgG EndoCAb (A) and IgM EndoCAb (B), and age related trends in IgG EndoCAb (C) and IgM EndoCAb (D) derived using Brown's exponential smoothing method.

who had no detectable concentrations of IgG and IgM EndoCAb. Comparisons of actual specific concentrations of IgG or IgM EndoCAb between the two groups were made using the non-parametric Mann-Whitney U test.

Table 1   *Percentage (No/total) of subjects with no detectable antibody concentrations*

	SIDS	Controls	p Value
IgG EndoCAb*			
All ages	48 (12/25)	17 (28/164)	0·001
<9 months	46 (11/24)	20 (25/127)	0·013
3–9 months	33 (4/12)	24 (18/76)	0·48
<3 months	58 (7/12)	14 (7/51)	0·003
IgM EndoCAb			
All ages	20 (5/25)	16 (26/164)	0·57
<9 months	21 (5/24)	20 (26/127)	1·0
3–9 months	8 (1/12)	7 (5/76)	1·0
<3 months	33 (4/12)	41 (21/51)	0·75

Table 2   *Concentrations of IgG and IgM EndoCAb*

	SIDS		Controls		
	No of children	Median (interquartile range)	No of children	Median (interquartile range)	p Vaue
IgG EndoCAb					
<9 months	24	41·7 (0–68)	127	43·6 (15–64)	0·34
3–9 months	12	49·5 (0–68)	76	39·3 (14–61)	0·75
<3 months	12	0 (0–58)	51	53·4 (23–70)	0·096
IgM EndoCAb					
<9 months	24	42·0 (24–58)	127	21·8 (6–52)	0·075
3–9 months	12	55·6 (28–105)	76	35·2 (20–74)	0·14
<3 months	12	40·4 (0–44)	51	6·9 (0–14)	0·065

Age and total IgG or IgM concentrations were analysed by two sample t tests carried out on the $\log_e$ transformed values (which followed an approximate normal distribution). Multiple logistic regression analysis was used to compare the proportion of children in the SIDS and control groups with particular categories for values of IgG and IgM EndoCAb, adjusting for age. The relations between age and IgG or IgM EndoCAb were assessed using Spearman's correlation coefficients. Statistical significance was set at the conventional 5% level. Trend analysis was performed using Brown's exponential smoothing method with the statistical package Statgraphics.[9]

Results

The SIDS group, whose ages ranged between 18 and 280 days, were significantly younger than the controls, who were aged between 31 and 357 days (geometric mean 96 days v 130 days, t (187)=1·98; p=0·049). If children aged more than 9 months are omitted from the study groups, there is no significant difference in age (geometric means 91 (24 children) and 101 (127 children) days respectively; t (149)=0·70; p=0·48).

Twelve (48%) of the 25 children who had died from SIDS had no detectable IgG

EndoCAb compared with 28 (17%) of the 164 control children (χ^2 (1)=10·6; p=0·001). This absence of antibody was not due to the technique of taking samples after death, as all of the 18 adult serum samples taken at necropsy had detectable concentrations of EndoCAb which were not significantly different from healthy adults (IgG median 133, 95% confidence interval (CI) 68 to 208; IgM median 96, 95% CI 39 to 192).

Table 1 shows that the significantly higher percentage of SIDS infants with no IgG EndoCAb remains when comparisons are restricted to children aged less than 9 months (46 v 20%). It is also apparent that the difference between the two groups is concentrated on the younger children (ages less than 3 months), in whom 58% of the SIDS group had no detectable IgG EndoCAb compared with 14% of the control group; Fisher's exact test, p=0·003. There was no significant difference between the two groups in the percentage of children with no detectable IgM EndoCAb. The results for IgG and IgM EndoCAb for individual control and SIDS subjects, together with their associated smoothed trend curves, are shown in the figure.

Table 2 gives the summary statistics relating to the actual concentrations of IgG and IgM EndoCAb. For IgG, no statistically significant difference was found. Children aged less than 3 months in the SIDS group tended to have lower values of IgG EndoCAb (p=0·096), this result reflecting the higher percentage of children with no detectable IgG concentrations. For IgM EndoCAb, the younger SIDS children (aged less than 3 months) had higher levels than the control children. This difference was of borderline significance (median values, 40·4 v 6·9 respectively, p=0·065).

There was no significant difference between the two groups (for children aged less than 9 months) in either total IgG antibody concentrations (geometric means 3·9 (SIDS; 23 children) and 4·2 (control; 117 children); p=0·26) or total IgM antibody concentrations (geometric means: 0·49 (SIDS; 23 children) and 0·53 (control; 123 children); p=0·63). Restricting the comparison to children aged less than 3 months again showed no significant difference.

For children aged less than 9 months a multivariate analysis was then carried out which assessed the differences between the SIDS and control groups, adjusting for age and for values of IgG and IgM EndoCAb. In this analysis categories of concentrations of IgG EndoCAb (0, <55, 55+), and IgM EndoCAb concentrations (0, <40, 40+), were chosen to give approximately equal numbers of control children in the latter two classes. A significant relation between SIDS and category of IgG EndoCAb was found (χ^2 (2)=11·5; p=0·003). Sudden infant death syndrome was more likely if a child had no IgG EndoCAb compared with values of IgG EndoCAb in the range 1 to <55. A significant relation was also found between the SIDS and category of IgM EndoCAb (χ^2 (2)=6·9; p=0·03). Sudden infant death syndrome was more likely if a child had values in the range 40+ compared with values in the range 1 to <40.

Concentrations of IgG and IgM EndoCAb increased significantly with increasing age in the control group, but only for those older children aged 3–9 months (IgG: r=0·26, p=0·024; IgM: r=0·32, p=0·005). For the SIDS children the only significant association with age was found in the 3–9 month age group for specific IgM EndoCAb (r=0·63; p=0·029).

Discussion

EndoCAb are directed to the inner core (lipid A/ketodeoxyoctonic acid/heptose) region of the endotoxin lipopolysaccharide and are cross reactive with a wide range of endotoxins from Gram negative bacterial species.[4] [10] Antibodies with similar specificities arise when rabbits (G R Barclay, unpublished data) or mice[11] are repeatedly immunised with different endotoxins, and monoclonal antibodies specific for endotoxin inner core (not lipid A) can be produced which have wide endotoxin cross reactivity and endotoxin neutralising properties.[11] [12] It is probable that human natural EndoCAb arise by repeated exposure to different Gram negative bacterial endotoxins, which will be present in the host as soon as the gut is colonised by Gram negative bacteria. The maturation of EndoCAb in the control infant group (figure) indicates that these antibodies approach adult median concentrations by as early as 1 year of age.

The controls used in this study were not age matched healthy subjects, but rather infants for whom serum samples had been submitted to a virology laboratory for a variety of investigations. A proportion of these must therefore have been infected at the time the blood was taken. Nevertheless, for serum immunoglobulins and EndoCAb, the results show the expected trends, with significant amounts of maternal IgG at 1 month, decreasing to low levels by 3 months, by which stage IgM starts to increase.

In contrast, concentrations of IgG EndoCAb from children who had died from SIDS are low in the first 3 months, whereas IgM concentrations are increased. These low concentrations of IgG EndoCAb, mainly reflecting the large number with no detectable antibodies, cannot be explained on the basis of the samples being taken after death as this phenomenon was not found in the control group of adult necropsy specimens. It is also not due to an overall deficiency of total serum IgG as there was no significant difference in total immunoglobulins in the two groups of infants, a finding which has been reported previously by other workers.[13]

The most likely interpretation of this pattern of EndoCAb response is that the maternal IgG EndoCAb which might have been expected to persist have been depleted earlier than usual, and an unusually early response of IgM EndoCAb has been triggered. These results suggest that, as a group, the children who died from SIDS have had an unusually early or

more severe exposure to endotoxin than other infants of a similar age.

If systemic endotoxaemia is an important factor in SIDS, this could tie in with a number of findings and hypotheses surrounding the syndrome. High body temperatures have been reported in a number of children who died from SIDS[14] and endotoxin is a well known stimulus for pyrexia. On the other hand, if hyperpyrexia due to overheating is the primary event, endotoxins have been shown to play an important part in the pathophysiology of heatstroke.[15-17]

Further studies are required to confirm the finding of depletion of IgG EndoCAb in larger series of children who died from SIDS, and to prove that this is due to depletion of antibody rather than maternal IgG antibodies not being transmitted. If a consistent picture emerges it will be important to elucidate the reasons why this group are exposed to endotoxin and to investigate possible preventive measures.

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