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R. C. M. Stephens
K. Fidler
P. Wilson
G. R. Barclay
M. G. Mythen
G. L. J. Dixon
M. W. Turner
N. J. Klein
M. J. Peters

Endotoxin immunity and the development of the systemic inflammatory response syndrome in critically ill children

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M.W.T. and N.J.K. both act as scientific consultants for NatImmune, a Danish company exploring the therapeutic potential of MBL. M.G.M. is involved in the development of an anti-endotoxin vaccine

R. C. M. Stephens (✉) · M. G. Mythen
Institute of Child Health, Critical Care Group, Portex Unit,
30 Guilford Street,
WC1N 1EH London, UK
e-mail: r.stephens@ich.ucl.ac.uk
Tel.: +44-207-9052382
Fax: +44-207-8298634

K. Fidler · G. L. J. Dixon · N. J. Klein
Institute of Child Health, Infectious Diseases & Microbiology Unit,
30 Guilford Street,
WC1N 1EH London, UK

P. Wilson · M. J. Peters
Great Ormond Street Hospital for Children NHS Trust, Paediatric Intensive Care Unit,
Great Ormond Street,
WC1N 3JH London, UK

G. R. Barclay
Scottish National Blood Transfusion Service, Western General Hospital, John Hughes Bennett Laboratory,
Edinburgh, UK

M. W. Turner
Institute of Child Health, Immunobiology Unit,
30 Guilford Street,
WC1N 1EH London, UK

Abstract *Background:* The systemic inflammatory response syndrome (SIRS) may be triggered by endotoxin. Humans have antibodies directed against the core of endotoxin (endotoxin core antibodies, EndoCAb) that appear to be protective following surgery and in sepsis. We hypothesised that children with elevated antibodies to endotoxin core would be less likely to develop SIRS in their initial period on intensive care. Because of the existing literature we defined two sub-groups according to the primary reason for ICU admission: infection and non-infection. *Methods:* We recruited 139 consecutive patients admitted to a paediatric intensive care unit (PICU) with more than one organ failure for longer than 12 h as part of another study. Patients were classified on admission to PICU as having an infectious or a non-infections diagnosis. The occurrence of SIRS within 48 h of admission was recorded along with detailed clinical and demographic data, EndoCAb concentration and the potential confounding variables C-reactive protein and mannose-binding lectin. *Results:* In the 71 patients admitted without infection (primarily post-operative and head injured) IgG EndoCAb was significantly lower in patients who developed SIRS than those who did not (72 vs. 131 MU/ml), independent of potential confounding variables. In patients with infection there was no significant difference in IgG EndoCAb between children developing SIRS and those who did not (111 vs. 80 MU/ml). *Conclusion:* Head injured and post-operative patients admitted to PICU who develop early SIRS have significantly lower serum IgG EndoCAb levels than those who do not.

Keywords Critical illness · Endotoxin · Endotoxin core antibodies · Immunity · Paediatric · Systemic inflammatory response syndrome

Introduction

The systemic inflammatory response syndrome (SIRS) can be caused by a variety of clinical scenarios, including trauma, major surgery, infections, cerebral haemorrhage, burns and pancreatitis [1]. SIRS may lead to organ dysfunction. However, only a proportion of patients who are subjected to similar procedures, illnesses or insults

develop SIRS. Furthermore, whilst the prevalence of SIRS is high, not every patient with SIRS to incurs measurable organ dysfunction [1]. Common genetic polymorphisms which influence the concentrations of key mediators, including Mannose-binding lectin (MBL) and tumour necrosis factor, have recently been implicated in determining the incidence and severity of systemic inflammation in critically ill patients [2, 3, 4].

Endotoxin, found in the outer membrane of Gram-negative bacteria, is an important trigger of SIRS. With co-factors, endotoxin binds to Toll-like receptor 4, one of a family of transmembrane proteins expressed on key cells which recognise 'pathogen-associated molecular patterns' as part of the innate immune response [5]. This initiates a complex series of intra-cellular signalling events resulting in the production of inflammatory mediators including cytokines and adhesion molecules. Experimentally endotoxin can initiate a systemic inflammatory response and is found in large quantities in the colonised human gut [6, 7]. Critically ill patients as well as those undergoing surgery may be exposed to endotoxin from leakage into the systemic circulation via an impaired gastrointestinal barrier, Gram-negative infection or as a result of bowel manipulation during surgery.

All adult humans have antibodies directed against the core of endotoxin (EndoCAb) although observed levels vary within populations by more than 80-fold [8]. Immunoglobulin G (IgG) EndoCAb is present at birth and is probably maternal in origin (trans-placentally acquired). Immunoglobulin M (IgM) EndoCAb is almost absent in the first month of life but increases to approximately adult levels within a year [9]. In adults higher preoperative levels of IgM EndoCAb are associated with a good outcome following surgery, whilst higher IgG EndoCAb levels have been linked to survival in sepsis [10, 11, 12, 13, 14]. It is not known whether this is a causal association, with EndoCAb acting to modulate systemic inflammation, or whether high EndoCAb titres are simply a marker of a favourable immune state in patients at risk of systemic inflammation.

Our hypothesis was that children who develop SIRS in their initial period on ICU would have lower levels of antibodies to endotoxin core. Because of the previous literature on EndoCAb we defined two sub-groups according to the primary reason for ICU admission: infection and non-infection. The analyses were performed on the combined group and the individual sub-groups.

Methods

Local Research Ethics Committee approval and parental informed consent were obtained. Consecutive admissions to our tertiary multi-disciplinary paediatric intensive care unit (PICU) were recruited as part of a study into the role of mannose-binding lectin over a 6 month period in 2002 [2]. On enrollment, cases were assigned to one of two groups, infection or non-infection, according to the principal reason for PICU admission as documented for audit purposes by independent PICU physicians not involved in the study. The group admitted for non-infectious indications (post-operative management, after head injury or with other non-infectious conditions) included 71 children and the group admitted with infection (localised infection, sepsis or septic shock) 68; their characteristics, organ failures and site of surgery are shown in Tables 1, 2, and 3. Within each of these groups, patients were subdivided into those who did or did not develop SIRS within the first 48 h of admission. MBL levels from the previous study were recorded as a potential confounder of any effect of EndoCAb.

Subject selection

Inclusion criteria were: age under 17 years and the presence of at least one organ system failure for more than 12 h (or death within the first 12 h). The following exclusions were applied: presence of multiple congenital abnormalities, known congenital immunodeficiency, known central neurological or neuromuscular disease (all considered to represent major risk factors for PICU admission resulting from infection), persistent pulmonary hypertension of the newborn, weight less than 2.2 kg, informed consent not available, suspected non-accidental injury, repeat PICU admission during the study period, surgery requiring cardiopulmonary bypass, lack of intravenous or intra-arterial access and anticipated short stay (less than 24 h) on the PICU.

Clinical measurements

Infection was considered as 'confirmed' if a causative organism was isolated from a normally sterile site and 'presumed' in those with a history and examination consistent with an infection, for example, fever, cough and coryza combined with chest radiographic changes consistent with pneumonia. Diagnoses of SIRS, sepsis and septic shock were made according to 1992 ACCP/SCCM guidelines modified for age [15]. In brief, SIRS was determined by the presence of two or more of the following: central temperature below 38.0 °C or above 36.0 °C, white cell count less than 12×10^9/l or less than 4×10^9/l and a heart rate outside age specific ranges (newborn to 3 months: 95–145 bpm, 3–12 months 110–175, 1–3 years 105–170, 3–7 years, 80–140, 7–10 years 70–120, >10 years 60–100). Respiratory rate was not

included as a diagnostic criterion because of the very high proportion of cases receiving mechanical ventilation. Cases meeting these criteria for SIRS with confirmed or presumed infection were classified as 'sepsis' whilst septic shock was diagnosed in cases of sepsis who were hypotensive, defined against age-specific values for mean blood pressure after fluid resuscitation requiring treatment with inotropes and/or vasopressor therapy [2]. Our electronic patient charting system (Care Vue, Hewlett-Packard) was reviewed daily, maximum and minimum ventilator settings and physiological parameters for each 24-h period were recorded prospectively onto a Microsoft Access database. Microbiological, biochemical and haematological information was recorded from our PICU and the referring hospital. Paediatric Logistic Organ Dysfunction (PELOD) scores were calculated daily [16], and all patients in the PICU receive pain relief as determined by an age-related protocol.

Laboratory measurements

Serum samples were taken within 48 h of admission, spun, and separated and the serum stored in aliquots at –80 °C until analysed. The investigators performing the endotoxin-core antibody (EndoCAb) serum levels (R.S., K.F.) and MBL serum levels (K.F.) were blinded to the diagnosis of SIRS. Similarly, the clinician acquiring the clinical data (P.W.) was blinded to the laboratory data.

EndoCAb and MBL

Polystyrene microplates (precoated with an equimolar mixture of incomplete core, rough, mutant endotoxins from each of four species of Gram-negative bacteria complexed with polymyxin B) were used to measure IgM and IgG EndoCAb concentrations using an enzyme-linked immunosorbent assay described previously [17, 18]. An eight-point standard curve was constructed using doubling dilutions of a pooled-serum calibrated in EndoCAb median units, where 100 was the median value for 1,000 healthy adults' IgG or IgM, respectively. Test and control samples were diluted 1:200 with dilution buffer and 100 μL of each sample to be assayed added in duplicate to the precoated plate and incubated for 1 h at 37 °C. After washing three times with wash buffer (sodium chloride, 0.138 M; phosphate, 0.01 M; pH 7.4 containing 0.10% [v/v] polyoxyethylene sorbitan monolaurate), 100 μL of a diluted alkaline phosphatase conjugated goat antihuman IgG or IgM antibody (Sigma-Aldrich, Poole, UK) was added to each well. After incubation for 1 h at 37 °C the plates were washed three times with wash buffer then once with distilled water and blotted dry. Substrate (180 μL per well) comprising 1 mg/ml disodium *p*-nitrophenyl phosphate dissolved in 1 M diethanolamine buffer with 0.5 mM magnesium chloride, was added and the plate incubated at room temperature in the dark for 20–30 min. The reaction was stopped with 50 μL per well of 2 M sodium hydroxide and read at 405-nm wavelength with an automated plate reader (Dynatech MRX, Va., USA). Results from the whole plate were rejected and repeated if predetermined characteristics were met. MBL levels in serum were determined by a symmetrical sandwich ELISA using commercial kits from Antibody Shop, Copenhagen, Denmark according to the manufacturer's instructions [2].

Statistics

As EndoCAb levels are not normally distributed, we report medians and interquartile ranges. Non-parametric analytical statistics (Mann-Whitney *U* test) were used apart from log transformed data for regression analysis. Normal distribution of the transformed data was confirmed using the Kolmogorov-Smirnov test. All statistical calculations and analyses were conducted using SPSS (version 11.5, Chicago, Ill., USA).

Results

After informed consent we were able to obtain serum from 139 suitable patients: their characteristics, organ failure and site of surgery are shown in Tables 1-3. 71 children were admitted for non-infectious indications (postoperative management, after head injury or with other non-infectious conditions) whilst 68 were admitted with infection (localised infection, sepsis or septic shock). In the overall series of patients ($n = 139$) IgG EndoCAb levels were lower in those who had early SIRS, but the difference did not reach statistical significance; SIRS median 99 MU/ml, (IQR 44–198) vs. non-SIRS 119 MU/ml (59–229; $p = 0.215$). There was no difference in IgM levels between the two groups [87 MU/ml (44–152) vs. 86 MU/ml (45–189), $p = 0.588$] (Fig. 1). There was a weak correlation between EndoCAb IgG and EndoCAb IgM levels (Spearman's rank correlation coefficient, $r = 0.311$, $p \leq 0.001$) but not between EndoCAb IgG and MBL level ($r = 0.071$, $p = 0.406$). There was no significant relationship between PELOD-predicted mortality and absolute IgG EndoCAb ($r = 0.59$, $p = 0.49$) nor any evidence of a 'threshold' effect of IgG EndoCAb on PELOD-predicted mortality for the unselected cases (Mann-Whitney test for IgG greater/lower than 57 MU/ml, $p = 0.67$). Of the potential confounding variables considered (age, sex, ethnicity, PELOD score, C-reactive protein, MBL) only MBL level was significantly associated with the development of SIRS on univariate analysis (Table 1).

Table 1 Characteristics of the 139 study patients (*SIRS* systemic inflammatory response syndrome, *IQR* interquartile range, *PELOD* Paediatric Logistic Organ Dysfunction, *EndoCAb* endotoxin core antibody, *MBL* mannose-binding lectin)

All patients	All ($n = 139$)	SIRS ($n = 82$)	Non-SIRS ($n = 57$)	p^a
Age, median (months; IQR)	26 (9–121)	25 (9–108)	34 (10–130)	0.389
Initial PELOD score, median (IQR)	12 (10–21)	12 (11–21)	12 (2–21)	0.182
Diagnosis				
Septic shock	18.7 (26%)	31.7 (26%)	0	
Sepsis/severe sepsis	17.3 (24%)	29.3 (24%)	0	
Infection	12.9 (18%)	0	31.6 (18%)	
Post-operative	23.7 (33%)	13.4 (11%)	38.6 (22%)	
Head injury	23.7 (33%)	19.5 (16%)	29.8 (17%)	
Other	3.6 (5%)	6.1 (5%)	0	
Ethnic group				
White	69 (96%)	67.1 (55%)	71.9 (41%)	
Arab	5 (7%)	8.5 (7%)	0	
Asian	3 (4%)	2.4 (2%)	3.5 (2%)	
Black Caribbean/African	10 (14%)	11 (9%)	8.8 (5%)	
Other	13 (18%)	11 (9%)	15.8 (9%)	
CRP, median (mg/l, IQR)	75 (35–137)	84 (41–157)	66 (30–120)	0.149
IgM EndoCAb, median (MU/ml, IQR)	86 (45–159)	87 (44–152)	86 (51–200)	0.588
IgG EndoCAb, median (MU/ml, IQR)	101 (46–209)	99 (44–198)	119 (59–229)	0.215
MBL, median (ng/ml, IQR)	1851 (525–3886)	754 (325–2516)	2831 (993–4284)	<0.001
Non-infection	All ($n = 71$)	SIRS ($n = 32$)	Non-SIRS ($n = 39$)	p^a
Age, median (months; IQR)	57 (18–151)	57 (24–139)	64 (12–153)	0.959
Initial PELOD score, median (IQR)	12 (10–21)	12 (11–12)	12 (10–21)	0.939
Diagnosis				
Septic shock	0	0	0	
Sepsis/severe sepsis	0	0	0	
Infection	0	0	0	
Post-operative	46.5 (33%)	34.4 (11%)	56.4 (22%)	
Head injury	46.5 (33%)	50 (16%)	43.6 (17%)	
Other	7.0 (5%)	15.6 (5%)	0 (0%)	
Ethnic group				
White	64.8 (46%)	56.2 (18%)	72 (28%)	
Arab	4.2 (3%)	9.4 (3%)	0 (0%)	
Asian	4.2 (3%)	6.2 (2%)	2.6 (1%)	
Black Caribbean/African	7 (5%)	12.5 (4%)	2.6 (1%)	
Other	19.7 (14%)	15.7 (5%)	23 (9%)	
CRP, median (mg/l, IQR)	73 (34–120)	75 (37–147)	62 (34–120)	0.408
IgM EndoCAb, median (MU/ml, IQR)	98 (57–179)	99 (72–158)	95 (54–208)	0.826
IgG EndoCAb, median (MU/ml, IQR)	100 (49–198)	72 (43–153)	131 (80–210)	0.009
MBL, median (ng/ml, IQR)	2091 (633–4174)	1185 (408–3860)	2518 (624–4344)	0.054
Infection	All ($n = 68$)	SIRS ($n = 50$)	Non-SIRS ($n = 18$)	p^a
Age, median (months; IQR)	14 (3–74)	13 (4–81)	17 (2–29)	0.956
Initial PELOD score, median (IQR)	12 (11–21)	16 (11–22)	11 (1–21)	0.106
Diagnosis				
Septic shock	38.2 (26%)	52 (26%)	0 (0%)	
Sepsis/severe sepsis	35.3 (24%)	48 (24%)	0 (0%)	
Infection	26.5 (18%)	0 (0%)	100 (18%)	
Post-operative	0	0	0	
Head injury	0	0	0	
Other	0	0	0	
Ethnic group				
White	73.5 (50%)	74 (37%)	72.2 (13%)	
Arab	5.9 (4%)	8 (4%)	0 (0%)	
Asian	1.5 (1%)	0 (0%)	5.6 (1%)	
Black Caribbean/African	13.2 (9%)	10 (5%)	22.2 (4%)	
Other	5.9 (4%)	8 (4%)	0 (0%)	
CRP, median (mg/l, IQR)	76 (41–147)	89 (41–169)	70 (27–83)	0.315
IgM EndoCAb, median (MU/ml, IQR)	79 (30–138)	60 (30–131)	83 (23–141)	0.9
IgG EndoCAb, median (MU/ml, IQR)	106 (43–229)	111 (49–218)	80 (31–264)	0.518
MBL, median (ng/ml, IQR)	947 (293–3284)	682 (163–2379)	3715 (1078–4107)	0.001

[a]SIRS vs. non-SIRS (Mann-Whitney U test)

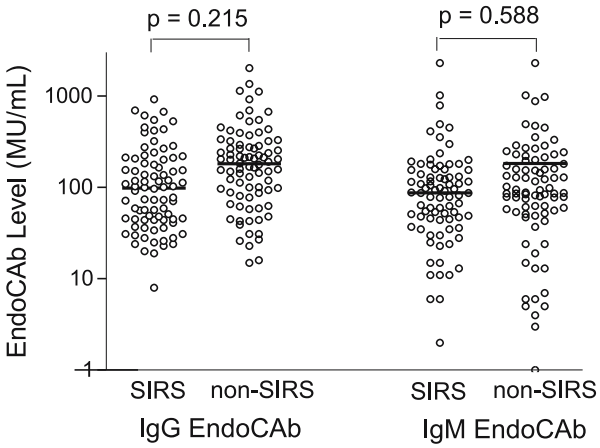

Fig. 1 EndoCAb and SIRS in the 139 patients. IgG (*left*) and IgM (*right*) EndoCAb levels of the 139 critically ill children and the subsequent development of SIRS in the first 48 h following admission to PICU. Statistical significance was calculated using the Mann-Whitney *U* test. No significant differences were seen in either IgG or IgM EndoCAb between patients with and without SIRS. *SIRS* Systemic inflammatory response syndrome; EndoCAb endotoxin core antibody; IgM immunoglobulin M type; IgG immunoglobulin G type

Non-infected sub-group

The characteristics of the 71 children without infection are shown in Table 1. IgG EndoCAb levels were significantly lower ($p = 0.009$, Mann-Whitney *U*) in those children developing SIRS, but there was no difference in IgM EndoCAb levels between the two groups ($p = 0.710$ Mann Whitney *U*; (Fig. 2a). Log_{10} IgG EndoCAb remained independently associated with the development of SIRS in this population in a binary logistic regression analysis after correction for the effects of age, sex, initial PELOD scores, C-reactive protein and MBL.

The likelihood ratio for development of SIRS if the IgG EndoCAb is below 57 MU/ml is 3.65 (95% confidence interval 1.48–8.9) compared to those patients with an IgG EndoCAb above 57 MU/ml (Table 4). There was no significant relationship between PELOD-predicted mortality and absolute IgG EndoCAb ($r = 0.005$, $p = 0.97$) nor any evidence of a 'threshold' effect of IgG EndoCAb on PELOD-predicted mortality for the unselected cases (Mann-Whitney test for IgG greater/lower than 57MU/ml, $p = 0.55$).

Table 2 Sites of surgery for the 33 surgical patients

Surgery site	Total	SIRS	Non-SIRS
Abdominal	6	3	3
Abdomino-thoracic	3	1	2
Airway/facial	7	2	5
Gastro/oesophageal	4	3	1
Neurosurgery	4	1	3
Spinal	7	1	6
Thoracic	2	0	2

Table 3 The frequency of organ failures in the study patients. The numbers add up to more than 139 as some patients had more than 1 organ failure. Organ failure definitions were taken from the first International paediatric sepsis consensus conference 2002 [40]

Organ failure	*n*	%
Respiratory	130	93.5
Cardiovascular	50	35.9
Hepatic	14	10.1
Renal	5	3.6
Haematological	1	0.7

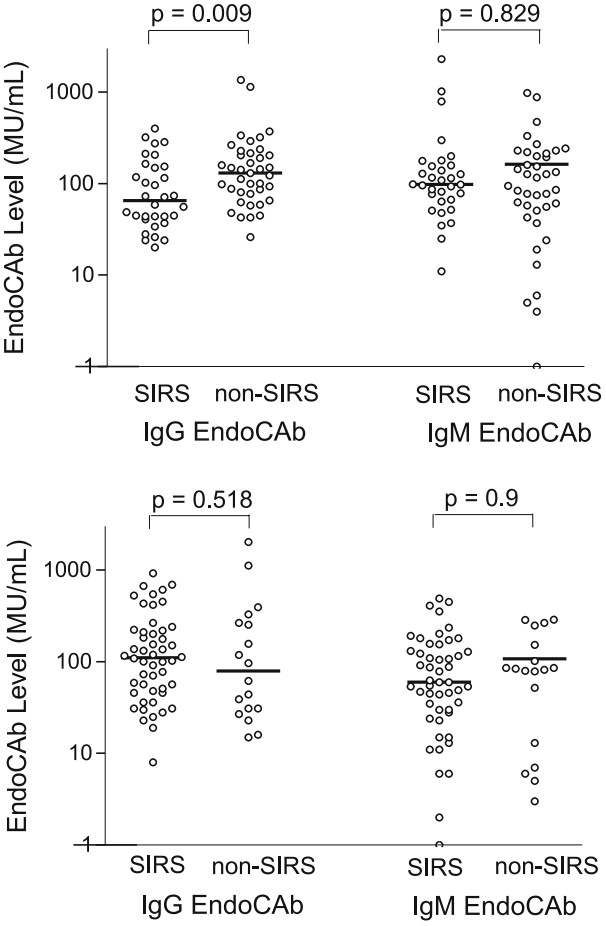

Fig. 2a,b EndoCAb and SIRS in the non-infected patients. IgG (*left*) and IgM (*right*) EndoCAb levels in the 71 non-infected patients (**a**) and the 68 infected patients (**b**) according to whether they did or did not develop SIRS in the first 48 h following admission to PICU. In non-infected patients there was a significant difference in IgG EndoCAb between patients with and without SIRS ($p = 0.009$, Mann-Whitney *U* test); in infected patients no significant differences were seen in either IgG or IgM EndoCAb between patients with SIRS and without SIRS

Table 4 SIRS and IgG EndoCAb. The occurrence of SIRS in the first 48 h following admission to PICU in the non-infected group according to a 'threshold' of IgG EndoCAb: above and below 57 MU/ml. The likelihood ratio for SIRS is 3.65 (95% CI 1.48–8.9) if the IgG EndoCAb <57 MU/ml

	≤ 57 MU/ml	>57 MU/ml
Non-SIRS	5 (25%)	34 (66.7%)
SIRS	15 (75%)	17 (33.3%)
Total	25	51

Infected sub-group

The characteristics of the 68 children with infection are shown in Table 1. There were no significant differences in IgG or IgM EndoCAb levels between those children developing SIRS and those not [111 vs. 80 MU/ml ($p = 0.518$) and 60 vs. 83 MU/ml, ($p = 0.906$), respectively; Fig. 2b]. There was no significant difference in the serum IgM and IgG EndoCAb levels in patients who had increasing severity of infection (localised infection vs. sepsis vs. septic shock, $p = 0.4$, analysis of variance and Kruskal-Wallis test) for both EndoCAb IgG and IgM (data not shown). Of the potential confounding variables (age, sex, ethnicity, initial PELOD score, C-reactive protein and MBL) only low MBL levels were associated with the development of SIRS in this group ($p = 0.008$) as previously reported [2].

Discussion

This is the first study to investigate the levels of antibodies against endotoxin core in a mixed population of critically ill children. We have demonstrated broadly similar levels to those seen in large adult studies [10, 12, 14, 19]. In addition, we have shown that cases admitted following trauma or surgery (or for other non-infectious reasons) who go on to develop an early SIRS have lower IgG EndoCAb levels than do those who do not develop SIRS. These data are consistent with observations that lower levels of IgG antibody to endotoxin core have also been associated with an increased number of febrile episodes in children with acute lymphoblastic leukaemia [20].

Higher titres of EndoCAb exhibit anti-inflammatory effects in vitro, for example, IgG antibodies to endotoxin core increase the uptake of endotoxin by macrophages, opsonise bacteria, attenuate tumour necrosis factor α production [21, 22] and are protective in a lamb *Escherichia coli* model of sepsis [23]. Furthermore increasing levels of IgG or IgM EndoCAb (although independent of total IgG and IgM) is associated with increasing ability to 'neutralise' endotoxin, as judged by the limulus amebocyte lysate assay [19, 24].

High preoperative levels of EndoCAb are consistently associated with reduced morbidity following major surgery particularly involving cardio-pulmonary bypass [11, 13, 25, 26, 27, 28]. These effects can be understood if antibodies to endotoxin core are binding to and inactivating a proportion of the endotoxin that is translocated either during reduced gut perfusion or from other sources [29]. The pro-inflammatory consequences of endotoxin are dose-dependent and hence high EndoCAb could be viewed as increasing the threshold endotoxin level for an excessive pro-inflammatory response [30]. Indeed following coronary artery bypass surgery, lower preoperative endotoxin antibodies are associated with greater rises in measured endotoxin [31].

This simple view must be considered with caution because levels of antibodies to endotoxin core fall in the presence of endotoxin [27, 32, 33]. Therefore low EndoCAb levels may simply reflect recent infection or exposure to endotoxin from other sources such as on-going poor gut perfusion, i.e. it may fall further in patients with SIRS, although the lack of difference in EndoCAb levels between the infected SIRS/non-SIRS groups suggests this may not be the case. Of course, low EndoCAb may be a non-specific marker of illness rather than high EndoCAb being protective per se. Data are conflicting on this point as Mythen et al. [34] showed that higher *preoperative* IgG EndoCAb levels were associated with better peri-operative gut perfusion whereas others have found EndoCAb levels to be independent of measures of general well-being including the POSSUM surgical risk score [11]. The situation is made more complicated by the fact that endotoxin exposure may lead to reduced gut perfusion rather than result from it [27, 35]. While the mechanisms underlying these relationships remain unclear, it is probably appropriate to consider patients with low titres of EndoCAb as having a 'reduced reserve' against further exposure to endotoxin.

This study's observations that the risk of SIRS is increased in critically ill children following trauma or surgery with lower EndoCAb provide some support for the idea that high EndoCAb is protective rather than low EndoCAb reflecting a prior poor condition, as a high proportion of these patients were previously healthy trauma victims.

The similarity that we observed in the EndoCAb levels in the infection sub-group between patients who went on to develop SIRS or not is also in keeping with the findings of studies in adults with sepsis that observed no clear relationship between initial EndoCAb and outcome [12, 14, 36]. The initial IgM EndoCAb concentrations in 146 adults with sepsis were higher in survivors in one study although this may have been explained by other factors [12]. A subset of patients with very low initial IgG EndoCAb (<10%) had increased mortality. In 205 ICU adults with sepsis a clear relationship was seen between low IgM EndoCAb and progression to septic shock, whilst rising IgG EndoCAb values were associated with a positive outcome [37]. The results in our study are

difficult to interpret because EndoCAb, especially IgM, are known to fall in the presence of endoxaemia before recovering to baseline levels or even beyond [31]. As we did not standardise the sample collection time more precisely than the first 48 h on the ICU, we can speculate that EndoCAb levels varied widely from the pre-morbid levels. A study employing repeated sampling throughout episodes of infection (including mild cases that do not require intensive care) is required to define the relationship between EndoCAb and progression to SIRS/severe sepsis in children.

Our study has several other limitations. Firstly, we did not collect data on the administration of plasma products to our patients. As these contain antibodies in the same range as the donor population, this is a potential confounding factor that would tend to reduce the magnitude of any measured differences [38, 39]. Plasma is not administered on our unit other than in the presence of established coagulopathy. This might account for the reduced effect of EndoCAb in the infected group, who are more likely to have coagulopathy and hence receive plasma products. More difficult is the fact that we did not correct for the volume of resuscitation fluid administered to each individual. High volume administration of colloid or crystalloid is more likely to be required in patients developing SIRS and is expected to reduce EndoCAb by a simple dilutional effect. This is unlikely to be a major factor as on closer inspection of the data in the infected subgroup there was a trend for *higher* EndoCAb concentrations in patients meeting the criteria for systemic inflammation. In addition, EndoCAb levels vary with age in children: IgM and IgG EndoCAb climb from 3 months towards adult values by 1 year [18]. In this study there are no significant differences in age between those who developed SIRS and those who did not. Furthermore, in the multivariate analysis of the non-infected group age did not alter the effect of IgG EndoCAb on the development of SIRS. We did not measure endotoxin levels because levels can change quickly, and we would have had to have an unacceptable sampling frequency in these children to ensure we had a 'peak' level, otherwise a one off measurement might be meaningless. Patients undergoing laryngeal surgery and those with acute severe asthma or catecholamine-resistant septic shock will have received steroids. Lastly, our numbers are too small to directly investigate an effect on mortality.

Many other factors influence this risk of developing systemic inflammation including the nature and extent of the insult. Recently the effect of host factors, including common genetic polymorphisms influencing the plasma levels of an important molecule in innate immunity, mannose-binding lectin, on the risk of SIRS has been reported [2]. Our data suggest that the effect of EndoCAb is independent of these factors.

The hypothesis that EndoCAb has a direct protective role by 'mopping-up' endotoxin is appealing but is far from proven. This study opens up the possibility of immunotherapy aimed at reducing systemic inflammation even after the patients have entered the ICU. Many questions remain including defining the factors that determine EndoCAb levels in an individual and the relevance on subsequent outcomes of a diagnosis of SIRS.

Conclusion

This study shows that children admitted to the intensive care after head injury, surgery or for other non-infectious reasons, who suffer from the systemic inflammatory response syndrome early in their ICU course have low levels of IgG EndoCAb. Whilst this does not indicate causation, this information provides a potential mechanism by which elevated EndoCAb levels may protect against the systemic effects of endotoxin and may lead to new therapies such as passive immunisation with EndoCAb hyperimmune plasma. That the protective effect of EndoCAb occurs in children as well as adults is further evidence that these antibodies may inhibit endotoxin-induced inflammation.

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