

ENDOGENOUS ENDOTOXIN-CORE ANTIBODY (EndoCAb) AS A MARKER OF ENDOTOXIN EXPOSURE AND A PROGNOSTIC INDICATOR: A REVIEW

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INTRODUCTION AND BACKGROUND

The EndoCAb ELISA was originally devised by us to screen blood donor plasma for high-titre antibodies to endotoxin core which are cross-reactive with endotoxins of a number of Gram-negative bacterial species and strains, and was then applied to a variety of clinical studies. The assay was devised by studying large numbers of healthy adults (blood donors) using a variety of R-LPS and S-LPS alone and in different combinations in ELISA. The final form of what we have called the *EndoCAb* ELISA was comprised of an equimolar cocktail of an incomplete-core R-LPS from each of four species (*E.coli*, *P.aeruginosa*, *K.aerogenes* and *S.typhimurium*). Each R-LPS preserved an intact inner core but did not express complete outer core (i.e. an Rc or Rb LPS where available). Each R-LPS was complexed with polymyxin B, and the cocktail was coated on a brand of polystyrene microplate selected for optimal EndoCAb ELISA characteristics, as previously described (Scott & Barclay, 1987; Barclay & Scott, 1987; Scott & Barclay, 1990; Scott, et al. 1990).

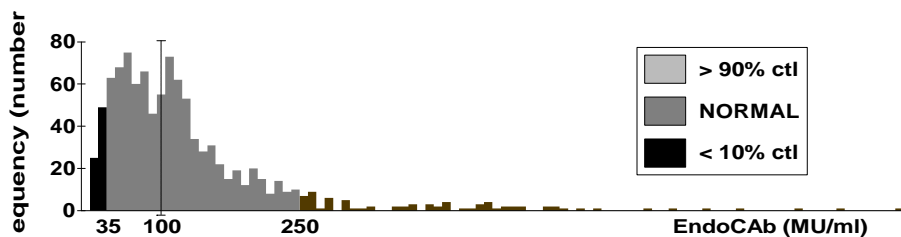


Figure 1. IgG EndoCAb range determined in 1000 healthy adults.

Endotoxin-core antibody (EndoCAb)

Initial studies of IgG EndoCAb levels in healthy adult populations (volunteer blood donors) were carried out to determine the range of EndoCAb levels (Figure 1). Values were determined in relation to a standard comprised of a pool of high-titre donor sera. Since no units were available for EndoCAb, values were expressed as a percentage of the range median, in median units (MU) per ml. The same principle was applied to IgM and IgA EndoCAb measurement, taking the medians of the respective ranges as 100 MU. The respective absolute values of the MU of each Ig class is different and of the order GMU>MMU>AMU. Median units have an effect of normalising the ranges around the medians, and the resulting 10th to 90th percentile range of each Ig class are very similar, at approximately 35 MU/ml to 250 MU/ml (Figure 1).

We have used the 10th and 90th percentiles as arbitrary markers for low and high EndoCAb levels, respectively, in clinical studies. For selection of plasma for preparation of hyperimmune intravenous gammaglobulin we used a cutoff at 400 GMU/ml, approximately the 95th percentile. This gammaglobulin is 10- to 12-fold higher in EndoCAb than normal gammaglobulin and was highly protective in an animal model (Hodgeson, et al., 1993), and raised depleted EndoCAb levels in septic shock patients (unpublished).

PREVALENCE OF EndoCAb

IgG EndoCAb is present at birth, and is probably maternal (trans-placental). This gradually diminishes over the first three months, then endogenous IgG EndoCAb begins to increase (Figure 2A). The IgM EndoCAb (endogenous) is virtually absent in the first month

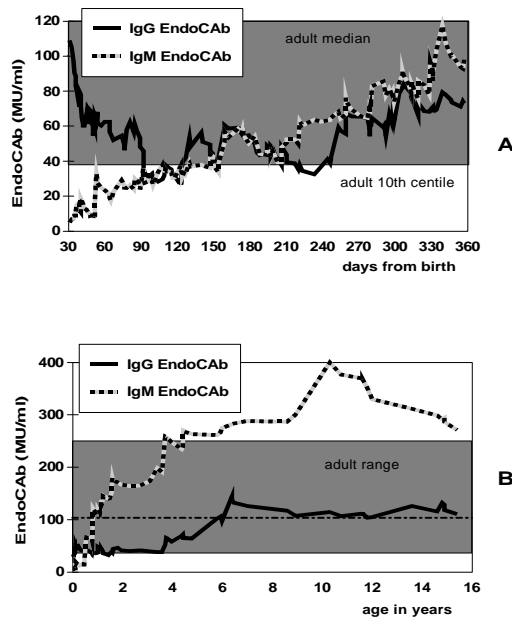


Figure 2. Development of IgG and IgM EndoCAb in (A) infants and (B) children (trend analysis using Brown's exponential smoothing method).

but increases gradually to around adult median levels by one year. In sudden infant death syndrome (SIDS or cot death) the pattern in the first 3 months is reversed, with apparent depletion of maternal IgG EndoCAB and an early appearance of endogenous IgM EndoCAB (Oppenheim, et al., 1994). While this could be interpreted as evidence of endotoxin exposure in the infant, preliminary studies indicate such infants may have low IgG EndoCAB at birth, i.e. low maternal EndoCAB may be a predisposing factor (Oppenheim BA, Crawley BA, Barclay GR, unpublished).

EndoCAB continues to develop in children (Figure 2B). By 6 to 7 years old the IgG EndoCAB stabilises at the adult median. The IgM EndoCAB rises to above the adult range by 5 years, reaches a peak around 12 years, and descends towards the upper adult range by 16 years (Barclay GR, Heyderman RS, unpublished). Studies of EndoCAB levels in children need to take this pattern into consideration, e.g. we have seen such high IgM EndoCAB values in children with meningococcal septicaemia, and these may not be a disease-related feature (unpublished).

CHANGES IN EndoCAB IN PATIENTS WITH ENDOTOXAEMIA

Early studies of IgG EndoCAB indicated perturbation by endotoxaemia (Barclay, et al., 1989; Barclay, 1990). In most cases changes in EndoCAB were reflected in changes in antibodies detected on most individual LPS. However, in some cases different patterns of change were found with different LPS. In such cases, as in the example in Figure 3, changes

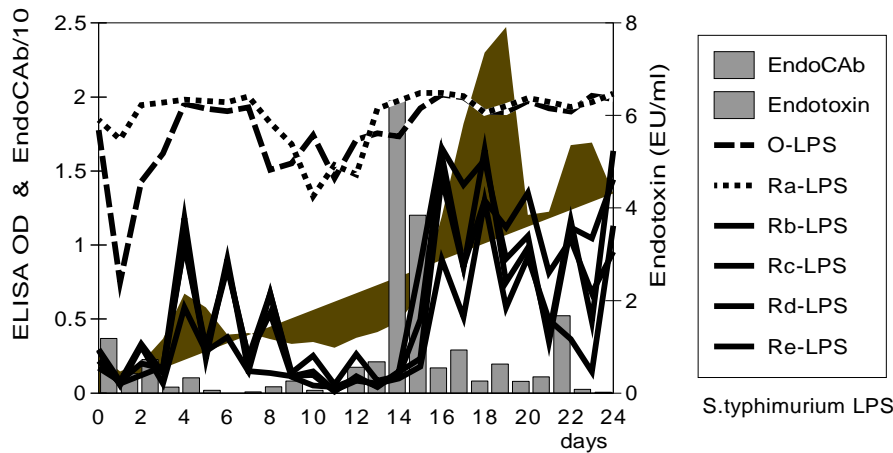


Figure 3. Changes in IgG EndoCAB and in IgG antibodies to different size LPS in relation to changing endotoxaemia in a fatal case of septic shock.

in EndoCAB reflected changes in antibodies detected on incomplete-core R-LPS but not the changes in complete-core (Ra LPS) or smooth LPS. The results are typical of patterns of change found with a range of other *Salmonella*, *E.coli*, *Klebsiella* and *Pseudomonas* R- and S- LPS for this patient (not shown).

Endotoxin-core antibody (EndoCAB)

In most individuals EndoCAB appears almost monoclonal, and can be absorbed out on e.g. a single R-LPS together with most antibodies detected on different LPSs. However, some individuals evidently express multiple endotoxin cross-reactive specificity ranges as in the example shown (Scott & Barclay, 1990; Scott, et al., 1990).

Changing IgG and IgM EndoCAB in sepsis

In healthy individuals EndoCAB levels remain relatively stable, e.g. enabling recruitment and retention of a panel of high-titre IgG EndoCAB blood donors for plasmapheresis who appear to retain their antibody levels indefinitely (unpublished). In contrast, systemic endotoxin perturbs EndoCAB homeostasis in patients who develop sepsis. In general both IgG and IgM EndoCAB are depleted by the initial endotoxaemia. However, the humoral anamnestic EndoCAB response may be triggered and EndoCAB levels can rise rapidly.

Often when patients enter studies, e.g. on admission to intensive care, there may be a history of prodromal sepsis which has already perturbed endogenous EndoCAB, and the levels and patterns of change may be difficult to interpret. The case below (Figure 4) is an example of changes in IgG and IgM EndoCAB where prodromal sepsis was minimal since

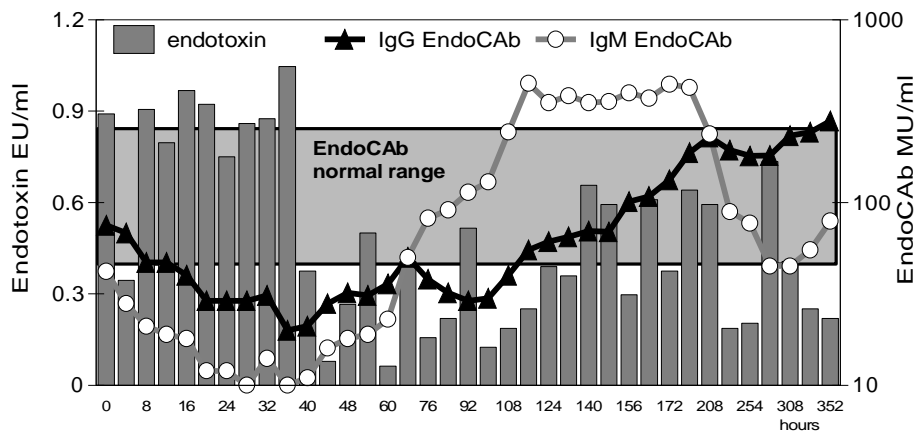


Figure 4. Endotoxaemia and changes in IgG and IgM EndoCAB in a patient developing sepsis following trauma from a road-traffic accident (Barclay GR & Willatts S, unpublished)

the patient was admitted to study within hours of experiencing trauma. In this case the rising IgM anamnestic response was short-lived while the IgG anamnestic response was more delayed but was also more protracted. The late-phase high IgG and low IgM EndoCAB levels of this patient are typical of many single sample studies of convalescent sepsis patients or patients with chronic sepsis exposure. Some examples of single-sample studies where significant differences between patients' and healthy adults' IgG and/or IgM EndoCAB have been found are shown in Table 1.

Reduction of EndoCAB has been found in cases of infection with Gram-negative

species whose LPS is not present in the EndoCAb ELISA, such as in cases of meningococcal septicaemia (Figure 5). This may indicate that the cross-reactivity of endogenous EndoCAb extends to these species' endotoxins. Alternatively, falling EndoCAb in these cases may indicate release of endotoxin from endogenous sources such as the gut as a secondary manifestation of the disease.

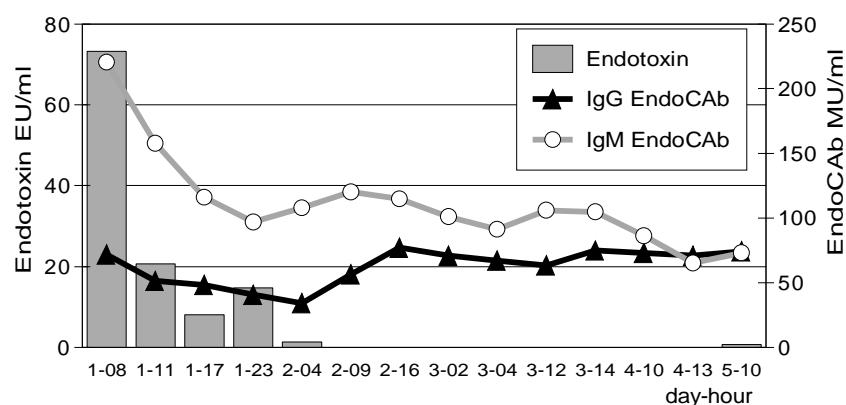


Figure 5. Endotoxaemia and EndoCAb in a case of meningococcal septicaemia in an adolescent patient (treated with plasma exchange on day 2).

CONDITION	IgG EndoCAb	IgM EndoCAb
Crohn's Disease [1]	high	normal
Ulcerative Colitis [1]	normal	normal
Renal Stones / UT infection [2]	high	low
Obstructive Jaundice [3]	high	normal
Haemolytic Uraemia [4]	low	low
Cot Death (SIDS) [5]	low*	high*

Table 1. Clinical conditions in which EndoCAb has been shown to differ significantly from healthy adults (* or age-matched controls). [1] Gardiner, et al., 1991, 1992. [2] Barclay GR, Rao PN, Oppenheim BA (unpublished). [3] McCrory, et al., 1992. [4] Heyderman, et al., 1994. [5] Oppenheim, et al., 1994.

Endotoxin-core antibody (EndoCAb)

Falling EndoCAb following clinical intervention as evidence of systemic endotoxin release.

Significant falls in IgG and IgM EndoCAb have been recorded in (i) lithotripsy for renal stone disruption (Nielson D, Rao PN, Oppenheim BA, Crawley BA, Barclay GR, unpublished); (ii) surgery for obstructive jaundice (McCrorry et al., 1992); (iii) cardiopulmonary bypass, as in the example in Figure 6 (Barclay GR, McCartney AC, unpublished);

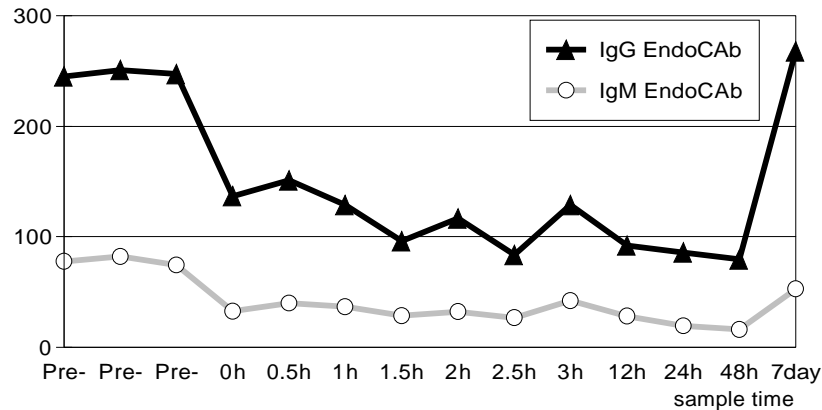


Figure 6. Changing IgG and IgM EndoCAb in a patient during cardiopulmonary bypass.

(iv) abdominal aortic aneurysm repair (Halliday et al., 1994); (v) major surgery (Mythen et al., 1993); and (vi) cardiac valve repair (Hamilton-Davies et al., 1994). In cases (i) and (ii) the physical manipulations may have directly resulted in endotoxin release from the sites manipulated, while in the other cases translocation of gut endotoxin may have occurred following gut ischaemia. Evidence from tonometry indicates that failure to maintain gastric mucosal pHi (Mythen et al., 1993) or sigmoid colonic pHi (Halliday et al., 1994) is associated with lower preoperative levels of IgG EndoCAb, falls in postoperative IgG EndoCAb, and development of postoperative multiple organ dysfunction syndrome.

EndoCAb as a Prognostic Indicator

The significant differences (above) in preoperative IgG EndoCAb between patients who maintain gastric or colonic pHi during surgery and those whose pHi falls, indicating ischaemia, are prognostic. Low or markedly falling IgG EndoCAb also indicates a poor prognosis in acute pancreatitis (Windsor et al., 1993). Low IgM EndoCAb is an indicator of a poorer prognosis (i) in cardiac valve replacement surgery (Hamilton-Davies et al., 1994), and (ii) in trauma or perforated bowel patients investigated for their risk of developing ARDS (Donnelly S, Barclay GR, et al., manuscript in preparation).

We have interpreted differences in expression of IgG and IgM EndoCAb as follows. Normally 20% of total IgG is located in the peripheral circulation at any one time and the

remaining 80% is in the interstitial fluid and can be regarded as a reservoir. IgM is a much larger molecule and is probably predominantly found in the peripheral circulation with a much smaller reservoir with which to equilibrate. When peripheral EndoCAB is depleted by an acute endotoxaemic episode, both IgG and IgM EndoCAB fall. However, peripheral IgG EndoCAB will tend to recover by equilibration and it will require a much more profound and sustained endotoxaemic episode to deplete the interstitial IgG EndoCAB. Sustained depletion of IgG EndoCAB may be regarded as a marker of a more severe endotoxaemic episode: sustained depletion of IgM EndoCAB occurs more readily and is a more sensitive marker of a recent episode of endotoxaemia.

A STUDY OF EndoCAB LEVELS IN SEPSIS SYNDROME

(Scottish Sepsis Intervention Group [SSIG] Study 1992-94: manuscript in preparation)

Patients admitted to intensive care fulfilling diagnostic criteria for sepsis syndrome were monitored for 10 days by a range of tests and clinical scores. Patients were categorised according to the source of sepsis, and mortality was scored at 30 days. A preliminary report of this study has been presented (Goldie et al., 1993). While clinical scoring remained the best prognostic indicator, of the laboratory-based measurements only EndoCAB showed any significant associations with outcome.

Of 146 patients entered into the study, sources of sepsis were attributed as abdominal 74; respiratory 39; and other (including trauma) 33. Overall mortality at 30 days was 70 (48%). The abdominal sepsis patients represented the largest and probably most homogeneous subgroup, and will be discussed in more detail.

		numbers	LOW IgG	p	LOW IgM	p	Endotoxaemia	p
TOTAL	<i>Alive</i>	76 (52%)	8 (11%)	0.014	25 (33%)	0.056	53 (70%)	ns
	<i>Dead</i>	70 (48%)	18 (26%)		33 (47%)		43 (61%)	
Abdominal	<i>Alive</i>	36 (49%)	1 (3%)	0.001	15 (42%)	ns	28 (78%)	ns
	<i>Dead</i>	38 (51%)	12 (32%)		20 (53%)		25 (66%)	
Respiratory	<i>Alive</i>	24 (62%)	5 (21%)	ns	5 (21%)	ns	14 (58%)	ns
	<i>Dead</i>	15 (38%)	4 (27%)		6 (40%)		6 (40%)	
Others	<i>Alive</i>	16 (48%)	2 (13%)	ns	5 (31%)	ns	10 (63%)	ns
	<i>Dead</i>	17 (52%)	2 (12%)		7 (41%)		12 (71%)	

Table 2. The frequencies of low (< 10th centile) IgG and IgM EndoCAB and of endotoxaemia in all (total) sepsis syndrome patients and in subgroups of patients by sepsis source (probability determined by Fisher's exact test).

Endotoxin-core antibody (EndoCAb)

Frequencies of low EndoCAb.

Mortality levels and frequencies of endotoxaemia and of low IgG and low IgM EndoCAb are shown in Table 2. The frequency of low IgG EndoCAb overall was significantly greater in non-survivors than in survivors ($p=0.014$). However, when sub-grouped by sepsis source the frequency of low IgG EndoCAb in non-survivors compared to survivors in patients with abdominal sepsis ($p=0.001$) was more distinctly greater. These differences between outcomes in patients with other sources of sepsis were not apparent, so that the pattern for abdominal sepsis influenced the study overall. There was a greater frequency overall of low IgM EndoCAb than of low IgG EndoCAb in these patients. The frequency of low IgM EndoCAb was greater for non-survivors than for survivors in each sepsis subgroup, but only approached significance when all subjects were considered together ($p=0.056$). The frequency of endotoxaemia was high and not significantly different between survivors and non-survivors overall or for any subgroup.

The differences in mortality in the abdominal sepsis subgroup according to IgG

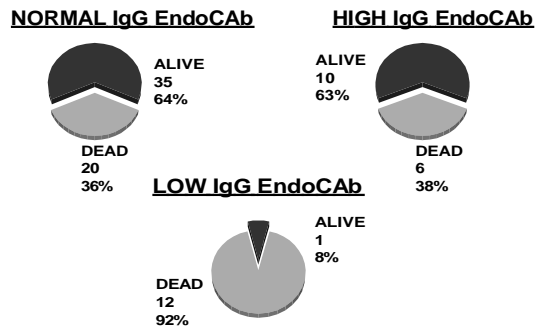


Figure 7. Frequencies of mortality according to IgG EndoCAb level in sepsis syndrome patients with sepsis of abdominal origin.

EndoCAb range (high, normal or low) are shown in Figure 7. The mortality in patients with high or normal IgG EndoCAb was similar at 38% and 36% mortality respectively. Mortality in patients with low IgG EndoCAb was 92%, with only 1 out of 13 patients surviving. Low IgG EndoCAb appears to indicate an at-risk group within the abdominal sepsis subgroup of sepsis syndrome patients.

Differences in EndoCAb levels

All sepsis patients show significant depletion of IgM EndoCAb compared to healthy adults (Table 3). Overall both IgG and IgM EndoCAb are significantly lower in non-survivors than in survivors. In the abdominal sepsis subgroup the non-survivors show significantly lower IgG EndoCAb than the survivors, but the IgM EndoCAb is depressed in both survivors and non-survivors and does not distinguish between them.

				<i>p</i> <i>alive</i> <i>v</i>	<i>p</i> <i>alive</i> <i>v</i>	<i>p</i> <i>dead</i> <i>v</i>
All patients:	<i>ALIVE</i>	<i>DEAD</i>	<i>[Normal]</i>	<i>dead</i>	<i>normal</i>	<i>normal</i>
IgG EndoCAb	104.1	78.4	[100]	0.04	ns	0.04
IgM EndoCAb	58	43.5	[100]	0.005	<0.001	<0.001
Abdominal sepsis:						
IgG EndoCAb	127.5	60	[100]	0.002	0.04	0.017
IgM EndoCAb	49.4	32.9	[100]	ns	<0.001	<0.001

Table 3. Median EndoCAb levels in sepsis syndrome patients by outcome (alive or dead) and in normal adults. Probabilities (p) determined by Mann-Whitney and/or Kolmogorov-Smirnov tests.

CONCLUSIONS

The EndoCAb ELISA is a robust and practical test which can be used as a marker for endotoxin exposure which can convey information which may be of clinical advantage. Changes in EndoCAb may indicate endotoxin exposure even where endotoxin can not be measured, and in many clinical situations EndoCAb levels may have prognostic value. The association between depressed EndoCAb and poor clinical outcome in different clinical conditions may support a case for therapeutic intervention with appropriate anti-endotoxin immunotherapy as a replacement for depleted EndoCAb. The clinical studies are supported by a variety of laboratory based animal studies (Clements, et al., 1993; Dolan et al., 1993; Neilly et al., 1993).

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