

A MONOCLONAL ANTI-ENDOTOXIN-CORE ANTIBODY USED TO EXCLUDE BACTERAEMIA AS THE CAUSE OF ENDOTOXAEMIA IN A FATAL CASE OF SEPTIC SHOCK.

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ABSTRACT

In patients with sepsis syndrome and endotoxaemia the origin of circulating endotoxin cannot readily be determined. When Gram-negative bacteraemia is also present, endotoxaemia is assumed to derive from the bacteria isolated from peripheral blood. Using the LAL assay for endotoxin and a broadly cross-reacting anti-endotoxin-core monoclonal antibody (WN1 222-5), we show that it is feasible to dissociate the source of endotoxin from bacteraemia. In a fatal case of septic shock, *Citrobacter freundii* was the only Gram-negative isolate from blood cultures. MAb WN1 222-5 did not bind to heat-treated *Citrobacter freundii* or its LPS, and did not neutralise their LAL activity. In contrast, MAb WN1 222-5 binds to similar preparations from *Escherichia coli*, and neutralises their LAL activity. Since MAb WN1 222-5 neutralised the LAL activity of patient's plasma collected at the time of *Citrobacter* isolation, we suggest that in this case the circulating endotoxin was not derived from *Citrobacter*, and may have derived from *E.coli* in the gut.

TEXT

In patients with septic shock the relationship between endotoxaemia and bacteraemia is often obscure. Endotoxins are important mediators of the syndrome, and endotoxaemia frequently occurs in the absence of Gram-negative bacteraemia^{1,2}. Peripheral blood endotoxaemia is measured by the limulus amoebocyte lysate (LAL) test, which is highly sensitive to endotoxin but cannot distinguish between sources of endotoxin. A broadly cross-reacting antibody (MAb WN1 222-5) with specificity for lipopolysaccharide (LPS) core, which binds mainly but not exclusively to *E.coli* and *Salmonella* LPS³, and which has LAL-inhibiting activity for these LPS, was used with the LAL test to differentiate circulating endotoxin activity from the endotoxin of blood-culture isolate bacteria in a fatal case of septic shock.

METHODS

Endotoxin was measured by a kinetic microplate modification of a chromogenic LAL test kit (Coatest-ET, KabiVitrum Diagnostica). Test samples were compared to a standard endotoxin preparation (*E.coli* O111:B4, 1EU=83pg, KabiVitrum Diagnostica) in a 12-point serial doubling-dilution curve, from 2ng/ml downwards, in 10% v/v heat-treated normal plasma in pyrogen-free water. Plasma samples were diluted 1/10 in pyrogen-free water and heat treated at 75°C for 10 minutes before testing. To determine LAL inhibition, samples were preincubated at 37°C with purified WN1 222-

5 MAb or pyrogen-free water on microplates for 3 hours before addition of LAL test reagents. Rates of reaction were measured at 37°C in a Maxline reader (Alpha Laboratories) which recorded increments in optical density at 405nm in relation to a 650nm reference. Readings were processed using Softmax software (Alpha Laboratories) which fitted rates of reaction (V_{MAX}) to the standard curve by log:log kinetics, from which tests sample values were calculated in pg/ml. Inhibitions were calculated from quantitative (pg/ml) values.

MAb WN1 222-5 binding was determined by ELISA using previously described methods^{4,5}.

CASE HISTORY

A 39-year-old, previously well woman receiving hydrocortisone for acute colitis, developed the features of severe septic shock and suffered a cardiac arrest. Initial management was ventilation, fresh frozen plasma, cryoprecipitate, hydrocortisone 200 mg and adrenaline. Inotropic support was adjusted according to invasive haemodynamic measurements. Cefotaxime, metronidazole and flucloxacillin were commenced, the only bacteriological information at this time being a recent throat swab positive for staphylococcus aureus. HA-1A (Centoxin) was administered 30 minutes after admission to ITU. Three hours after admission to the ITU laparotomy was performed: no perforation was found, but both colon and stomach appeared grossly ischaemic. Total colectomy was performed, the appearance of the stomach improving during the procedure. 24 hours later she was still hypotensive and in acute renal failure. She underwent a second laparotomy at which time subtotal gastrectomy and splenectomy were performed. On the fourth day she had a further severe septic episode, failed to respond to therapy and died. At post mortem examination the resected colon showed active ulcerative colitis.

The only blood cultures to grow bacteria were those taken at the time of transfer to ICU, from which *Citrobacter freundii* and *Staphylococcus aureus* were isolated, both from a single bottle. Endotoxin levels in blood were 41 pg/ml on entry to ICU, coinciding with *Citrobacter* isolation from the blood. By the second morning endotoxin levels had fallen to 17 pg/ml, but rose later to 628 pg/ml before resolving to undetectable levels. Subsequent samples were negative except for one of 545 pg/ml late on the third day.

RESULTS

Similar patterns of binding and LAL inhibition were found in both crude (heat-killed bacteria) and purified LPS⁶ preparations from the patient's *Citrobacter* isolate and from blood-culture isolate *E.coli* strains: the results with purified LPS are shown in the table. The ELISA results confirmed previous studies with MAb WN1 222-5, namely that it bound well to *E.coli* but not to *Citrobacter*. LAL activity of appropriate dilutions of endotoxins from *E.coli* were inhibited by MAb WN1 222-5 while endotoxin from the patient's *Citrobacter* was not.

Endotoxin source	ELISA	LAL-inhibition
Patient's plasma	not tested	++
<i>E.coli</i> O12 LPS	++	++
<i>E.coli</i> O18 LPS	++	++
<i>Citrobacter freundii</i> LPS (patient's isolate)	-	-

Binding (ELISA) and endotoxin-neutralising (LAL inhibition) activities of anti-LPS-core monoclonal antibody WN1 222-5 on patient's plasma endotoxin and purified LPS from blood culture isolate Gram-negative bacteria.

(ELISA: ++ OD>1.5; - OD<0.1
LAL-inhibition: ++ inhibition > 85%; - inhibition < 5%)

The LAL activity of the patient's plasma, from a sample taken at the time when *Citrobacter* was isolated, was inhibited by MAb WN1 222-5. It is therefore unlikely that the endotoxin in this sample was derived from *Citrobacter*, and probably derived from *E.coli* in the gut.

We also tested MAb HA-1A (Centoxin) which had been used to treat this patient, but could not demonstrate specific binding in ELISA or LAL inhibition of crude endotoxin by MAb HA-1A under identical conditions to those used with MAb WN1 222-5. MAb HA-1A was not tested on the purified LPS.

These results indicate that it cannot be assumed that circulating endotoxin is always derived from Gram-negative bacteria in the circulation, and therefore that the types of endotoxin causing sepsis may not reflect the species of Gram-negative bacteria isolated during bacteraemia. The species origin of circulating endotoxin should be examined directly when sensitive discriminatory techniques become available. This has important implications for the specificities of anti-endotoxin antibodies which might be used for passive immunotherapy of sepsis.

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