Endotoxaemia as a cause of fever in immunosuppressed patients

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SUMMARY Using a recently developed chromogenic substrate assay sensitive to 10 pg/ml *Escherichia coli* endotoxin in plasma, systemic endotoxaemia was found in 52% of 21 episodes of fever in patients with a haematological malignancy who were infected. Endotoxaemia was also found in 27% of 22 episodes of fever of unknown origin. In 45 afebrile patients neither neutropenia nor cytotoxic chemotherapy was a cause of endotoxaemia. Passage of endotoxin from portal blood into the systemic circulation can contribute to unexplained fever in immunosuppressed patients.

In man the normal bowel flora constitutes a large reservoir of bacterial endotoxin with the potential to cause portal venous endotoxaemia.¹ Any endotoxin present in portal blood is normally destroyed by peroxidases and esterases present in hepatic fixed macrophage (Kupffer) cells and thus does not reach the systemic circulation.² In cirrhosis and in Reye's syndrome, however, systemic endotoxaemia without bacteraemia has been shown^{1,3} and has been ascribed to Kupffer cell dysfunction^{4,5} or intrahepatic portal-systemic shunting of blood.⁶

In haematological malignancy systemic endotoxaemia without bacteraemia could occur as a consequence of intestinal mucosal damage by cytotoxic agents, or because of a numerical or functional defect of neutrophils, or because of impaired hepatic reticuloendothelial cell clearance of endotoxin. Episodes of fever are common in these patients and often remain unexplained despite intensive microbiological investigation. We have therefore determined the incidence of endotoxaemia, as measured by a new chromogenic substrate assay, in patients with haematological malignancy and have studied the relation between endotoxaemia and the development of fever.

Patients and methods

PATIENTS

Endotoxin assays were carried out on 27 healthy subjects (18 men, nine women) and 32 outpatients

(10 men, 22 women) with a non-malignant haematological disorder. One hundred patients (54 men, 46 women) with the following haematological malignancies were also studied: acute myeloblastic leukaemia (21 patients), acute lymphoblastic leukaemia (13 patients), multiple myeloma (20 patients), chronic lymphocytic leukaemia (26 patients), and lymphoma or macroglobulinaemia (20 patients). Sixty of these were outpatients who were not receiving chemotherapy and were not neutropenic. Several patients were studied on more than one occasion as their clinical circumstances changed.

METHODS

After cleaning the skin with 70% isopropanol, heparinised blood (25 IU/ml; Weddel Pharmaceuticals Ltd, London) was collected into sterile plastic universals (Sterilin Ltd, Teddington) for endotoxin assay. All heparin batches were checked for endotoxin contamination before use. During episodes of fever additional blood samples were taken for C reactive protein estimation, viral antibody titres, and blood cultures (aerobic and anaerobic). Urine and, where possible, sputum samples were also obtained for culture. Febrile patients underwent daily clinical examination and additional investigations (for example, bronchoscopy) were performed when clinically indicated.

Plasma endotoxin assays were carried out as described previously⁷ using a class II microbiological safety cabinet and new, endotoxin free tubes for each determination. Unseparated blood specimens

were kept at 4°C and processed within 2 h of venepuncture. Briefly, platelet poor plasma was diluted 1/10 in sterile, pyrogen free water and heated at 75°C for 5 min to remove plasma inhibitors. The diluted, heated sample was incubated with *Limulus* amoebocyte lysate ("Pyrogen" Mallinckrodt, St Louis, Missouri) for 45 min at 37°C before adding chromogenic substrate S2423 (Kabi Diagnostica, Stockholm). The chromogenic reaction was stopped after exactly 3 min and read spectrophotometrically at 405 nm. Results were reported as the optical density (OD) reading obtained after the subtraction of both test blank and reagent blank readings.⁷ C reactive protein estimations were performed by a laser nephelometric method,⁸ total leucocyte counts on EDTA blood were obtained using a Coulter Model S (Coulter Electronics Ltd, Luton), and differential counts were performed manually. Statistical analysis was carried out using the Mann-Whitney U test.

Results

The 27 healthy subjects all gave endotoxin assay values in the range 0–0.15 OD units. The same reference range was obtained for 31 of the 32 outpatients with a non-malignant haematological disorder and 58 of the 60 outpatients with a haematological malignancy who were not taking cytotoxic drugs and were not neutropenic. These three exceptions (endotoxin values in the range 0.17–0.25 OD units) are shown in Fig. 1, where the three groups have been amalgamated to give a reference group of 119 individuals. To determine the effects of neutropenia and cytotoxic chemotherapy on this reference range endotoxin assays were performed on 75 plasma specimens from 45 afebrile outpatients with a haematological malignancy, who were subdivided as follows:

Group I—currently receiving cytotoxic agents but not severely neutropenic (mean neutrophil count $3.7 \times 10^9/l$; range 1.1–6.8)

Group II—neutropenic (mean neutrophil count $0.4 \times 10^9/l$; range 0–0.9) but no cytotoxic agents given for at least three days before study

Group III—currently receiving cytotoxic agents and also neutropenic (mean neutrophil count $0.4 \times 10^9/l$; range 0–0.9)

There was no significant difference ($p > 0.1$) in plasma endotoxin values between these three groups or between any one of them and the reference group (Fig. 1). Only two results above 0.15 OD units were obtained (0.16 and 0.17 OD units in group I). Thus the reference range of 0–0.15 OD units for healthy subjects was also applicable to 98% of the 194 test results obtained for all the afebrile patients in Fig. 1.

Fig. 1 Endotoxin assay results (OD units) for reference group (119 individuals) compared with 27 specimens from non-neutropenic patients currently receiving cytotoxic agents (group I), 25 specimens from neutropenic patients not currently receiving chemotherapy (group II), and 23 specimens from patients currently receiving chemotherapy who were also neutropenic (group III). Interrupted line shows upper limit of reference range incorporating 98% of all 194 test results and 100% of results for the 27 healthy individuals.

Endotoxin assays were performed within 24 h of the onset of 43 episodes of fever (mean oral temperature 38.4°C, range 37.5–39.8°C) in 29 inpatients with haematological malignancy. In 21 episodes of fever in which there was clinical or laboratory evidence of infection, or both, 11 showed plasma endotoxin activity greater than 0.15 OD units (Fig. 2). These episodes comprised Gram negative septicaemia (5 cases), Gram positive septicaemia (2 cases), respiratory infection (2 cases), and unidentified infection causing a rise in C reactive protein concentration to greater than 150 mg/l (2 cases). An episode of unexplained fever was defined as: absence of clinical signs of infection together with negative bacteriological cultures, maximum serum C reactive protein concentration less than 100 mg/l, negative viral antibody titres, and no temporal relation to administration of blood products. Twenty two such episodes were identified and six had endotoxin assay results greater than 0.15 OD units (Fig. 2).

Serial endotoxin assays were undertaken during 12 episodes of fever showing initial endotoxin values of more than 0.15 OD units; endotoxaemia and fever failed to resolve in parallel in 11 of these episodes (Fig. 3). In the six endotoxaemic patients with unexplained fever, endotoxin assay results fell to less than 0.15 OD units within 48 h; in three of these patients the fever also resolved within 48 h but

Fig. 2 Endotoxin assay results (OD units) in 43 episodes of fever: 21 with evidence of infection (●—Gram negative septicaemia; ◐—Gram positive septicaemia; ○—other infection) and 22 unexplained. Interrupted line shows upper limit of endotoxin reference range as in Fig. 1.

Fig. 3 Persistence of endotoxaemia after resolution of fever in a patient from whom *Pseudomonas aeruginosa* was isolated from day 1 blood cultures.

in two it was sustained for five to seven days. Study of seven episodes of clinically evident infection without fever showed transiently raised endotoxin activity in five. A close temporal relation between endotoxaemia and fever was not therefore established in any of these groups.

Discussion

Most of the earlier clinical studies of endotoxaemia used the *Limulus* gelation assay, which has the disadvantages of poor specificity, subjective end point, and relative lack of sensitivity. The improved chromogenic substrate assay developed for the present study links the activation of the *Limulus* amoebocyte proenzyme to a chromogenic substrate, thus bypassing the gelation reaction. This assay is more sensitive (to 10 pg/ml *Escherichia coli* endotoxin in plasma), specific, and reproducible (within batch coefficient of variation of 4%).⁷ Contamination with extraneous endotoxin, which may have occurred in earlier studies, was minimised by performing the assay in a laminar air flow cabinet and by using new, endotoxin free tubes for each test.

Our studies in 45 afebrile patients suffering from haematological malignancy suggest that normal mechanisms for the prevention of systemic endotoxaemia usually remain intact despite neutropenia or cytotoxic chemotherapy. In febrile patients, however, an increase in endotoxin activity (range 0.17–1.59 OD units) was seen in 11 of 21 (52%) episodes of fever in infected patients and a smaller increase (range 0.17–0.43 OD units) in six of 22 (27%) episodes in patients with unexplained fever. The latter result is similar to that of a recent Dutch study, in which endotoxaemia was detected in 25% of 55 blood samples obtained during unexplained fever in children with acute lymphoblastic leukaemia.⁹ The less sensitive *Limulus* amoebocyte lysate gelation assay was used in the Dutch study, however, and it is likely that some of our patients would have shown negative results by this method.

Systemic endotoxaemia was found in two of our patients who had Gram positive bacteraemia. It has been suggested¹⁰ that non-specific activation of the *Limulus* proenzyme can occur in the presence of peptidoglycan derived from Gram positive organisms, but this has been disputed.¹¹ An alternative explanation is functional reticuloendothelial blockade induced by the bacteraemia, causing endotoxaemia as a secondary event.

Serial study of patients with endotoxaemia showed an inconstant temporal relation with fever. Possible reasons for this include the variation in pyrogenicity between different endotoxins,¹² development of host tolerance to endotoxin,¹¹ and the presence of other pyrogens. Patients with unexplained fever and endotoxaemia may have had an undiagnosed Gram negative bacterial infection. Alternatively, some other occult infection could have caused Kupffer cell dysfunction, by antigen blockade, with endotoxaemia occurring as a secondary event. Damage to Kupffer cells by cytotoxic

drugs may also cause secondary endotoxaemia in the absence of infection.

This study of a chromogenic substrate assay for endotoxin has established a reference range of 0–0.15 OD units which was representative of 98% of 194 assays performed on both healthy subjects and afebrile patients with and without a haematological malignancy. The raised plasma endotoxin concentrations found in 52% of febrile infected patients and in 27% of episodes of unexplained fever indicate that endotoxaemia is a contributory factor to fever in immunosuppressed patients.

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