A Study on Limulus Amebocyte Lysate (LAL) Reactive Material Derived from Dialyzers

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ABSTRACT: The amebocytes of horseshoe crab (Limulus) hemolymph contain a coagulation system highly sensitive to bacterial endotoxins. Limulus amebocyte lysate (LAL) reactive material derived from cuproammonium rayon membranes, however, is not an endotoxin and acts upon the alternative pathway in the coagulation cascade found in Limulus amebocyte lysate. This study confirmed these facts by using the coagulation system of Limulus without factor G, which is a substrate of the alternative pathway. LAL reactive material lingered in the circulation for a relatively long time. In acute hemodialysis, its plasma concentration increased by an average of 100 pg/ml with each dialysis and eventually reached a plateau of approximately 300 pg/ml. In patients with chronic renal failure under regular hemodialysis, the mean level of LAL reactive material was 330.0 ± 8.0 pg/ml before hemodialysis which increased by 70.6 ± 20.7 pg/ml after four hours of hemodialysis.

KEY WORDS: endotoxin, limulus amebocyte lysate reactive material, factor G, renal failure, hemodialysis

INTRODUCTION

Limulus amebocyte lysate (LAL) reactive material derived from the cuproammonium rayon membrane has already been reported[1-3] and due to the fact that the pyrogen tests are negative for this substance in rabbits, it is not considered to be an endotoxin. Its action and metabolism in man, however, remain unclear.[4,5] It was recently discovered that the blood clotting system of a limulus has an alternative pathyway that is activated by β-glucan, in addition to the one activated by endotoxin.[6,7] In the present study, we assayed LAL reactive material in platelet rich plasma from patients with renal failure under hemodialysis using Limulus amebocyte lysate deprived of the substrate (factor G) in this alternative pathway.

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MATERIALS AND METHODS

The subjects studied were 8 patients who had experienced severe trauma (mean age: 49.6 ± 16.1 years) and who underwent hemodialysis for acute renal failure developed in an early posttraumatic stage and 5 patients with chronic renal failure (mean age: 45.0 ± 6.4 years) who were receiving hemodialysis regularly three times a week. LAL reactive material in platelet rich plasma was measured by colorimetry of the synthetic chromogenic substrate (8; Toxicolor test®;

Seikagaku Kogyo, Japan) using both factor G-containing and factor G-deprived specimens.[9] The colorimetric curve of LAL reactive material was superimposed on the standardized concentration curve obtained for *E. coli* 0111:B4 endotoxin (Difco, USA) and the concentration of LAL reactive material was denoted by the corresponding value of the latter.

Results

Fig. 1 shows the results of a time-course assay in factor G-containing specimens from the 8 patients subjected to acute hemodialysis. Only one patient survived in this group. The level of LAL reactive material, which was less than 10 pg/ml before dialysis, increased linearly as the hemodialysis was repeated. In contrast, no relevant reaction occurred in the factor G-deprived specimens. In fact, its levels were below 10 pg/ml throughout the observation period in all cases (Fig. 1). Fig. 2 presents the clinical course and concentration of LAL reactive material in the one patient who survived. In

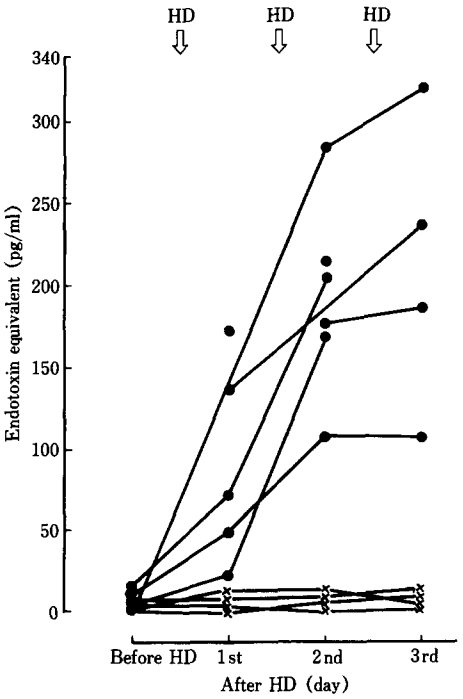

Fig. 1. The concentration of LAL-reactive material in platelet rich plasma from trauma patients with acute renal failure after hemodialysis (HD). ●—●, factor G-containing; ×—×, factor G-deprived.

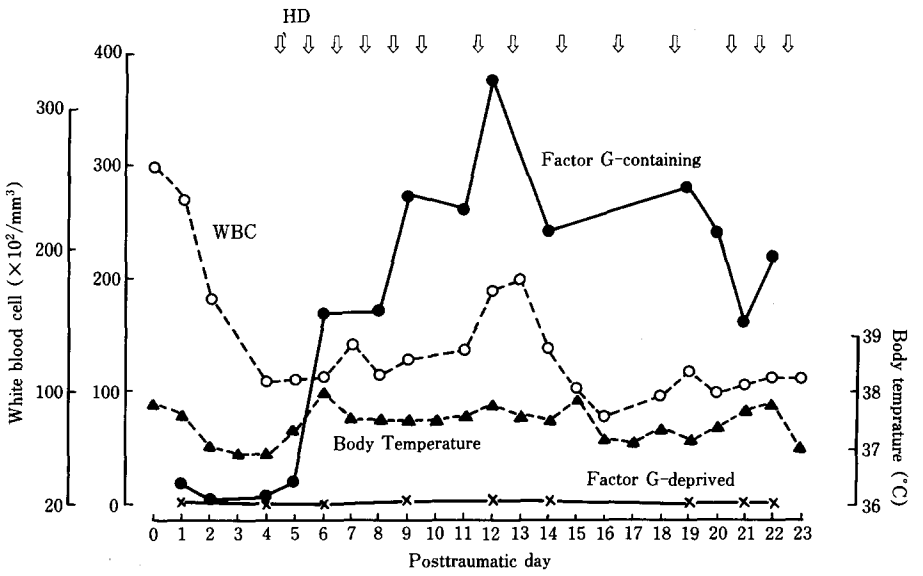

Fig. 2. Clinical course and the level of LAL-reactive material in a survival patient who underwent acute hemodialysis (HD). ●—●, factor G-containing; O---O, white blood cell; ▲---▲, body temperature (BT); ×—×, factor G-deprived.

this patient, the level of LAL reactive material increased almost linearly and reached a plateau of approximately 300 pg/ml, without any signs of infection (Fig. 2).

In the chronic renal failure group under hemodialysis with the cuproammonium rayon membrane, the mean level of the LAL reactive material was already as high as 330.0 ± 8.0 pg/ml before hemodialysis, and showed a further increase of 70.6 ± 20.7 pg/ml just after hemodialysis, although no relevant reaction occurred in the factor G-deprived specimens (Fig. 3).

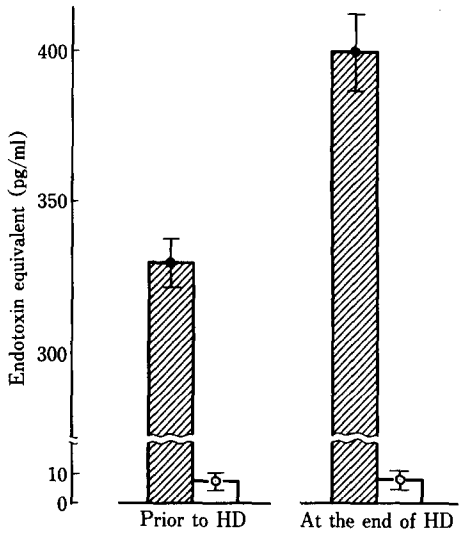

Fig. 3. The concentration of LAL-reactive material in platelet rich plasma from the patients with chronic renal failure receiving hemodialysis regularly over 2 years.

Discussion

We ascertained experimentally that LAL reactive material, which is different from endotoxin, comes from the cuproammonium rayon membrane and not from dialysate or perfusate. It was also found that it can be removed by means of washing with an excess amount of physiological saline solution.

Sequential activation of the coagulation factors in horseshoe crab hemolymph on contact with endotoxin and the mechanism of the colorimetric limulus test are shown in Fig. 4.[8] Kakinuma et al. suggested the presence of the alternative pathway, which is indicated with a thick line in the illustration,[6,7] and Tanaka et al. succeeded in isolating factor G from the extract of horseshoe crab hemolymph.[9] Those previous investigations provided a basis for the postulate that the alternative pathway is activated by LAL reactive material and the present observations substantiated this postulate. Moreover, the present study suggested that the plasma

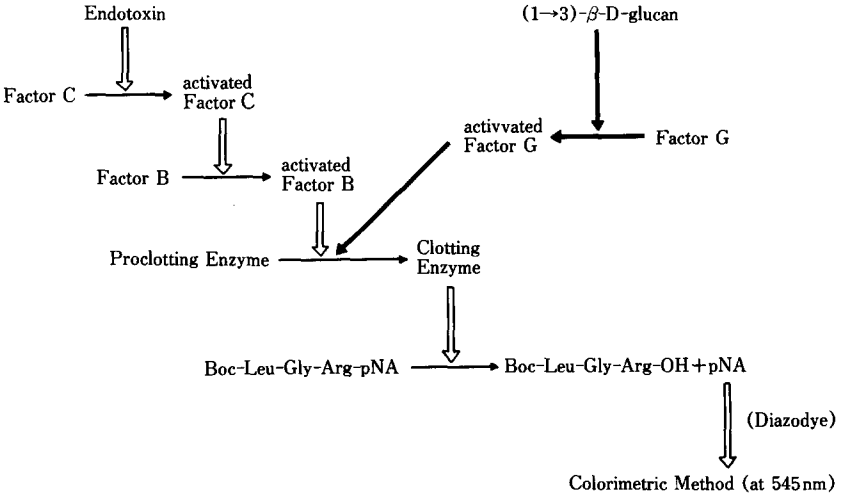

Fig. 4. The principle of colorimetry and the alternative pathway.

half-life of LAL reactive substance is long. In fact, the levels of the acute cases became elevated by an average of 100 pg/ml at each dialysis (until they reached a plateau of 300 pg/ml) and, in the chronic cases, they were approximately 330 pg/ml before dialysis which increased by about 70 pg/ml after each dialysis.

On the other hand, it was reported that LAL reactive material was found to be a cellulose-derived mixture whose elution pattern as observed by column chromatography showed three peaks: the first peak at a molecular weight of 23,000–24,000, the second at 3,000, and the last at less than 200.[4] It was further reported that, aside from endotoxin and β-glucan, synthetic nucleic acid and polysaccharides extracted from *Candida* and other bacteria had a LAL reactive substance-like action.[5]

Trauma victims often develop acute renal failure requiring hemodialysis, the clinical course of which is often complicated by infection. Gram-negative rods are commonly detected in the early stage but followed by *Candida* after the commencement of antibiotics. The diagnosis of a Gram-negative rod infection using a factor G-containing specimen would not be possible because of the presence of LAL reactive materials. With the combined use of both factor G-containing and factor G-deprived specimens, the diagnosis of a Gram-negative rod infection could be made. The diagnosis of Candidal infection in patients who have undergone hemodialysis, however, remains obscure even with factor G-containing and factor G-deprived specimens. The identity of all these LAL reactive materials thus remains to be clarified.

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