filter and reassaying the concentrate. This time the inhibition was comparable with that by the whole organism or the sonicate.

The finding of profound acid inhibition by a bacterium could possibly be due to non-specific bacterial inhibition by a ubiquitous substance such as bacterial lipopolysaccharide. To examine this possibility we tested C. jejuni, which is an organism of comparable morphology, contains cross-reacting antigens, and is an intestinal pathogen. Since this organism had negligible effect on acid secretion we judge this factor.

Of the extent of bacterial infection is the most likely and will require mapping studies of the gastric distribution of the organism and its quantitation. This is consistent with the concentration needed to see an effect with bacterial supernatants.

The demonstration of a protein or protein-containing acid inhibitor produced by a bacterium raises several questions. Why should the effect of the inhibitor eventually disappear in most patients, despite continued presence of the organism? Possible explanations include reduction in the bacterial population, a genetic switch-off in the organism itself, and an immune response to the inhibitor. The last is unlikely in view of the long interval (5–250 days) between infection and return to normal acid production. A reduction of the extent of bacterial infection is the most likely and will require mapping studies of the gastric distribution of the organism and its quantitation.

Presumably, the reduction of acid secretion helps C. pylori to colonise the stomach. If produced in the local environment of the epithelial cell the toxin would not need to be secreted in large quantities or be highly potent. The site of activity of the inhibitor could be on the apical cell membrane, possibly involving the potassium hydrogen ATPase.

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REFERENCES

References continued at foot of next column

ENDOTOXAEMIA, PULMONARY COMPLICATIONS, AND THROMBOCYTOPENIA IN LIVER TRANSPLANTATION

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Summary Plasma endotoxin was measured in 64 patients undergoing primary liver replacement. Endotoxin concentrations increased during the anhepatic phase of the operation, and remained high for several days. Although the severity of endotoxaemia did not correlate with duration of the anhepatic phase, there was a correlation between endotoxaemia and the need for perioperative platelet transfusions, ventilator dependency postoperatively, and one-month case-fatality.

Introduction

Common complications after orthotopic liver transplantation (OLT) are coagulopathy, cardiovascular instability, respiratory distress syndromes, and multiple organ failure; these abnormalities also occur in animals with endotoxaemia that have not had a transplant. Using a quantitative blood endotoxin assay, we have shown a correlation between endotoxaemia and pulmonary failure in man. We have now used this assay to see whether there is an association between endotoxaemia after OLT and the development of pulmonary complications or thrombocytopenia.

Patients and Methods

Patients

36 men and 28 women (mean age 46 years, range 18–66) had primary OLT between March 7 and July 31, 1988. None of the patients had had preoperative bacterial infections or massive intraoperative bleeding due to technical mishaps. Samples of systemic venous blood were collected preoperatively, at the end of the anhepatic phase of the operation but before platelet transfusion, and on the 1st, 3rd, and 7th postoperative days. The 64 recipients were divided into two groups: group A consisted of patients whose endotracheal tube could be removed within 5 days (n = 32); group B

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consisted of those who needed longer ventilatory support (30) or who died within 5 days (2). 6 patients in group B had a retransplantation within 7 days. The two groups did not differ with patients in group B than to those in group A—mean acid, amoebocyte lysate from Tachypleus tridentatus, and graphic were on ventilators preoperatively.

Platelet replacement during OLT was guided by thrombelastographic monitoring. In most cases, 1 unit of platelets was given with each unit (200 ml) of fresh frozen plasma and 250 ml of crystalloids. Platelets were not given until the end of the anhepatic phase.

Endotoxin Assay

Systemic venous blood was drawn in a pyrogen-free disposable syringe, anticoagulated with heparin (10 units/ml), centrifuged immediately at 1000 g for 10 min to remove platelets, and stored at −80°C. For the colorimetric limulus assay, 0·32 mol/l perchloric acid, 0·18 mol/l sodium hydroxide, and "Toxicolor" (lyophilised amoebocyte lysate from Tachypleus tridentatus and synthetic chromogenic substrate Boc-Leu-Gly-Arg-p-nitroanilide; Seikagaku Kogyo, Tokyo, Japan) with "tris"-hydrochloric acid buffer (pH 8·0) were used. The standard curve was plotted with Escherichia coli 0111:B4 endotoxin (Westphal type; Difco Laboratories, Detroit, Michigan) in distilled water. The value of plasma endotoxin concentrations from 24 healthy volunteers was always less than 10 pg/ml.

Student's t test was used to analyse the data.

Results

The mean preoperative endotoxin concentrations of the two groups were similar and were within the normal range. Intraoperative endotoxaemia developed at the end of the anhepatic phase in most of the patients of both groups (fig 1). The severity of endotoxaemia did not correlate with the duration of the anhepatic intervals (fig 1)—range 41–173 min, mean 104·8 (SEM 5) for group A and 108·5 (3·7) for group B. Endotoxaemia was more severe in group B patients who eventually required prolonged ventilatory support (fig 2). Significant differences between groups A and B were maintained for the next 3 days as the endotoxin concentration gradually decreased (fig 2).

43 patients received platelet transfusions because of coagulopathy and thrombocytopenia. More transfusions were given (intraoperatively and during the next 24 h) to patients in group B than to those in group A—mean 13·3 units (1·8) vs 4·6 (1·0), p < 0·01. There was a positive correlation between the number of units transfused and endotoxin concentrations at the end of the anhepatic phase (fig 3). 8 of the 64 patients died within a month; 7 could not be extubated until death. The other patient died suddenly on the 11th postoperative day from rupture of a splenic artery aneurysm: convalescence up to this time had been satisfactory. Diffuse pulmonary infiltrates developed in 6 of the group B patients before death.

Discussion

Most of the endotoxin that enters the blood stream is detoxified in the liver. During the anhepatic phase of liver transplantation endotoxin entering the circulation from intestinal bacterial flora or infectious foci can accumulate in the blood stream. In laboratory animals endotoxaemia leads to a rapid decrease in the number of platelets in peripheral blood. Thus, the positive correlation between the volume of platelet transfusion needed perioperatively and the endotoxin concentrations at the end of the anhepatic phase in the present study was not surprising. Similarly, the correlation of high endotoxin with pulmonary complications was as expected from animal experiments and our previous report in human beings.

The role of endotoxin in human disease is poorly understood, partly because the lethal dose even in the target organs varies with the animal species. Additionally, there have been difficulties with the qualitative assay that was
previously available.\textsuperscript{19} The chromogenic substrate method developed by Iwanaga et al.\textsuperscript{20} in 1978 paved the way to a sensitive quantitative assay of endotoxin.\textsuperscript{19} Study of patients with liver transplants may indicate how endotoxin triggers such diverse processes as pulmonary oedema,\textsuperscript{1,3} and also shock\textsuperscript{2} and coagulopathy.\textsuperscript{4}

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REFERENCES


Preliminary Communication

**CULTURED COMPOSITE SKIN GRAFTS: BIOLOGICAL SKIN EQUIVALENTS PERMITTING MASSIVE EXPANSION**

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**Summary** After assessment in mice, a biological skin equivalent consisting of cultured fibroblasts in a collagen gel overlain with cultured keratinocytes was applied to three patients who had had tattoos excised. In all patients the grafts took well with good cosmetic results and little or no contracture. A biopsy at 4 weeks showed that the central graft area had a fully differentiated epidermis and a mature dermis without adnexae.

**INTRODUCTION** Skin defects are commonly resurfaced with split-thickness autografts, which can be expanded in area four-fold by meshing\textsuperscript{1} or twenty-fold by dicing into small fragments.\textsuperscript{2} However, harvesting of the split skin leads to donor site morbidity, and the skin obtained may be inadequate for resurfacing extensive burns. By contrast, culture of epidermal keratinocytes to obtain sheets of epithelium\textsuperscript{3} permits almost unlimited expansion. This technique has been used successfully in patients with extensive burns,\textsuperscript{4} but epithelial grafts are not stable in the long term and may give rise to contour defects.\textsuperscript{5} The 4-week lag period between donor skin sampling and the availability of large sheets of keratinocytes may be eliminated by use of allografts. There is no need to harvest any skin from the recipient if allografts are used, and donor site morbidity is therefore eliminated. However, several groups report that donor cells do not survive more than a week.\textsuperscript{5-8} Healing improves but these grafts are simply acting as sophisticated dressings.

To combine the advantages of massive expansion permitted by cultured keratinocyte sheets with the inherent stability and cosmetic acceptability of split-thickness skin grafts, we have created a skin equivalent. This is a composite of cultured keratinocytes and a dermal component which displays many features of a normal dermis but is without the constituents that would elicit a pronounced immune response. The technique was initially assessed in mice, and was then applied to patients.

**MATERIAL AND METHODS**

**Preparation of Skin Equivalent**

Skin biopsy samples 4 mm in diameter were taken under local anaesthesia from healthy volunteers and the epidermis and dermis were separated with trypsin (2.5 mg/ml) in phosphate buffered saline with 0.5 mmol/L EDTA overnight at 4°C. The keratinocytes were cultured on a feeder layer\textsuperscript{4} and the fibroblasts were grown by the method of Ham.\textsuperscript{9} The culture medium was Dulbecco’s modification of Eagles’ medium with 10% fetal calf serum (FCS), kanamycin 100 µg/ml, amphotericin 2.5 µg/ml, epidermal growth factor 17 ng/ml, hydrocortisone 11 µg/ml, and cholera toxin 0.1 nmol/l. The dermal equivalent was prepared by neutralisation of a 5% solution of rat tail collagen\textsuperscript{10} with a mixture of concentrated Eagle’s basal medium (× 10) and 0.4 mmol/L sodium hydroxide (2:1), and the fibroblasts (10^5/ml) were added immediately before gelling. Keratinocytes were layered onto the surface and the cultures were maintained in vitro at 37°C for 4–7 days. Both fibroblasts and keratinocytes were derived from the same donor. The HLA class I and class II (DR) status of the cultured keratinocytes and fibroblasts was assessed with W6/32 and OKT-6 monoclonal antibodies, respectively; cells were examined on coverslip cultures with an indirect immunoperoxidase method.\textsuperscript{11}