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Intraoperative cytokines production during orthotopic liver transplantation

J. Pirenne, F. Noizat-Pirenne, D. De Groote, Y. Vrindts, M. Lopez, R. Gathy, P. Damas, M. Meurisse, N. Jacquet, P. Honoré, and P. Franchimont

Departments of Surgery, Transplantation, and Anesthesiology, and Laboratory of Radioimmunology, University of Liège, CHU Sart Tilman, 4000 Liège, Belgium. Medgenix Diagnostics, 6220 Fleurus, Belgium

Abstract. In summary, we established that a significant production of the monokines interleukin-6, tumor necrosis factor apha, and interleukin-1 occurred during orthotopic liver transplantation whereas the lymphokines interferon gamma and interleukin-2 were not detected. Levels of interleukin-6 reached their maximum values before and especially at the end of the anhepatic phase. They remained high after the anhepatic phase, i. e. after reperfusion of the new livers. Tumor necrosis factor alpha and interleukin-1 reached their maximum values after the anhepatic phase. Not only were interleukin-6, tumor necrosis factor apha, and interleukin-1 present in the serum but they could also be detected in the bile produced by these new livers. Mechanisms of monokine production during orthotopic liver transplantation is multifactorial in origin and further studies will have to evaluate the relative contribution of the various factors involved. The possibility of an association between peroperative monokines and transplant outcome and their potential clinical implication will have to be elucidated.

Key words: Cytokines – Interleukin-1 beta – Interleukin-6 – Tumor necrosis factor alpha – Liver transplantation

The liver plays a pivotal role in the cytokine network; cytokines act on the liver, liver cells produce cytokines, and the liver is a major clearance organ for circulating cytokines [1]. Orthotopic liver transplantation (OLTx) is a major abdominal surgical procedure in which a diseased liver is removed and a new liver is transplanted. Operative induced tissue injury, endotoxinemia, and kupffer cell activation after liver graft reperfusion are various conditions encountered during OLTx [2, 9, 11, 14]. Since each of these conditions has been shown to stimulate cytokine synthesis, OLTx should thus be accompanied by a significant production of cytokines. Moreover, the absence of hepatic clearance during hepatectomy and during the anhepatic phase is likely to interfere with cytokine elimination [1]. OLTx should thus impose a considerable impact on both cytokine production and metabolism.

The aims of this study were twofold. The first was to determine peroperative cytokine production during OLTx by measuring cytokines in the serum during the different phases of OLTx. The second was to assess whether cytokines could be detected in the bile produced by the new liver.

Materials and methods

Patients. This study included 7 recipients of hepatic allografts. Indications for OLTx were as follows: hepatocellular carcinoma, $n = 2$, primary graft nonfunction, $n = 2$, alcoholic cirrhosis, $n = 1$, secondary biliary cirrhosis, $n = 1$, and posthepatitic cirrhosis, $n = 1$.

OLTx. The principles of OLTx were removal of the diseased liver and placement of the graft followed by circulation reestablishment and biliary tract reconstruction. During hepatectomy and placement of the graft, a venovenous bypass was routinely used.

Collection of serum samples and bile. Serum samples of systemic venous blood were collected preoperatively in the right atrium through a swan ganz catheter immediately before the anhepatic phase (< AP), at the end of anhepatic phase (AP), after reperfusion of the transplanted liver (> AP), and at day 1 posttransplant. Bile was recovered from the transplanted liver > AP, and at day 1 posttransplant.

Cytokine immunoassays. The cytokines interleukin-1 beta (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF) were measured in the serum and bile of all patients. Moreover, the lymphokines interleukin-2 (IL-2) and gamma interferon (IF) were measured in four patients. These cytokines were measured by using specific commercially available immunoassays from Medgenix Diagnostics (Medgenix Diagnostics, Fleurus, Belgium). The methods used, type of tracer sensitivity, precision, reproducibility, and accuracy are described in Table 1.

By performing recovery tests, we established in an earlier study that cytokines can be detected in the bile, using similar immunoassays (Cytokines measurement in the bile, manuscript in preparation).

Offprint requests to: Jacques Pirenne, M.D., M.S., CHU Sart Tilman, 4000 Liège, Belgium

Table 1. Characteristics of cytokine immunoassays

Cytokine	Type of assay	Tracer[a]	Sensitivity	Precision	Reproducibility	Accuracy	Int. Std. origin	Correspondence weight/units
IL-1	IRMA 1 step	Mab-I^{125}	4 pg/ml	3.2%	7.2%	98%	MRC:86/552	1 ng/150 IU
IL-6	IRMA 2 steps	Mab-I^{125}	5 pg/ml	5.6%	7.5%	89%	MRC:88/514	1 pg/0.25 IU
TNF	IRMA 1 step	Mab-I^{125}	5 pg/ml	6%	7%	101%	MRC:87/650	1 pg/24 mIU
IL-2	RIA sequential saturation	Mab-I^{125}	0.5 U/ml	8%	11%	82%	BRMP:ISDP 841	100 pg/1 IU
IF	IRMA 1 step	Mab-I^{125}	0.2 U/ml	3.8%	9.9%	97%	NIH:Gg 23/901/530	50 pg/1 IU

IRMA, Immunoradiometric assay. 1 step, standards or samples + tracer incubated together. 2 steps, standards or samples incubated in a first step; washing and incubation of the tracer in a second stepRIA, classical radioimmunoassay
[a] By a modification of the chloramine T method

Table 2. Preoperative values as compared with control values. Control sera were obtained from normal healthy volunteers

	Control Values	Preoperative values
IL-1 (pg/ml)	< 15 (n = 40)	Range: 0–3 Mean: 0.5
IL-6 (pg/ml)	0 (n = 49) 3–8.5 (n = 29) 24 (n = 1) 72 (n = 1)	Range: 0–205 Mean: 78
TNF (pg/ml)	3–20 (n = 72) O (n = 8)	Range: 7–30 Mean: 20

Results

A. Preoperative values of IL-6, TNF, and IL-1

Preoperative values of IL-6 and TNF were increased as compared with a group of 80 normal healthy volunteers (Table 2). Preoperative values of IL-1 were not increased as compared with 40 normal sera.

B. Peroperative production of monokines IL-6, TNF, and IL-1

Serum. Peroperative production of monokines IL-6, IL-1, and TNF are shown in Fig. 1 a–c. These three monokines were elevated during OLTx as compared to preoperative values. IL-6 reached its maximal value at the end of AP. IL-6, however, was already elevated before AP and continued to display high levels after AP, i. e. after reperfusion of the new liver. TNF reached its maximal values after AP, i.e. after reperfusion of the new liver. IL-1 had a production pattern similar to TNF with maximum values reached after AP, i. e. after reperfusion of the new liver. The levels reached, however, were low. The levels reached by all these three monokines had returned to preoperative values by day 1 posttransplant.

Bile. High levels of IL-6, TNF, and low levels of IL-1 were detected in the bile produced by the new livers (Fig. 2 a–c). Except for IL-1, biliary levels of these cytokines had significantly decreased by day 1 posttransplant.

C. Perioperative production of IL-2 and IF

IL 2 and IF were not detected in serum in bile during OLTx.

Discussion

There were high serum levels of the cytokines IL-6, and TNF, and low serum levels of IL-1 during OLTx. IL-6, TNF, and IL-1 were also detected in the bile produced by the newly transplanted liver. We believe that this cytokine production was triggered by intraoperative events occurring *during* OLTx procedure as these cytokines reached their maximal value during OLTx and subsequently returned to preoperative values by day 1 posttransplant. A similar observation has already been made by Tono et al. who found a transient but significant elevation of IL-6 in the bile soon after OLTx in rats [13]. The lymphokines IL-2 and IF were detected in the serum in the bile during OLTx. This demonstrated that the cytokine production we observed reflected merely a major stimulation of monokine synthesis by monocytes/macrophages in the absence of lymphocyte activation and detectable lymphokine production at this early stage of recipient immune activation.

At present, there is evidence supporting four possible mechanisms of monokine production during OLTx. These involve the stimulation of cytokine production by (1) tissue trauma, (2) endotoxinemia, (3) lack of hepatic clearance, and (4) kuppfer cell activation. Tissue trauma induced by surgery can lead to monokine synthesis which in turn can initiate the acute phase response that is generally observed after surgical operations [11]. The significance of both monokine production and acute phase response have been shown to correlate with the significance of the surgical operation. Since OLTx is a major abdominal surgical procedure, it should trigger considerable monokine production. Endotoxinemia has been shown to

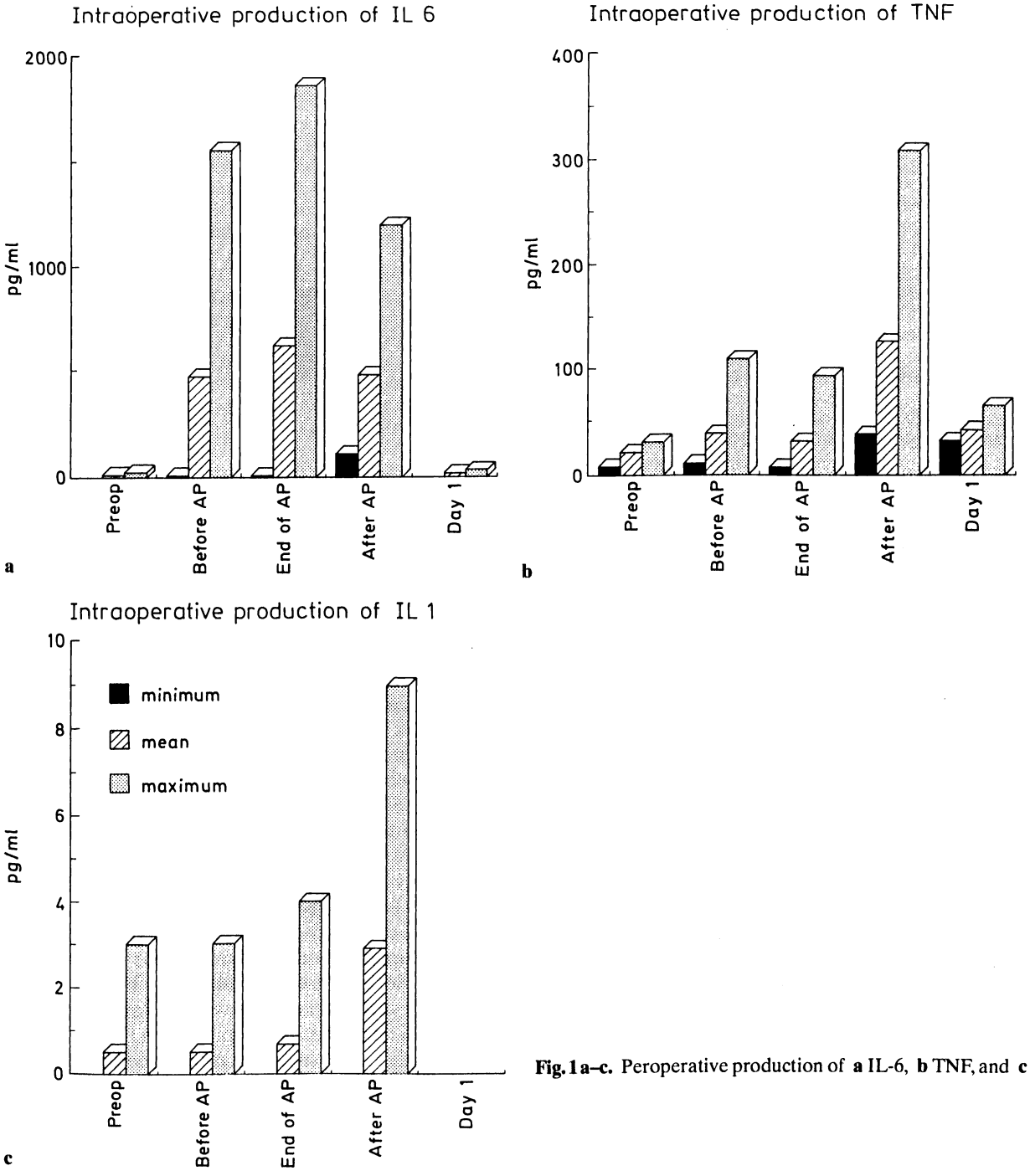
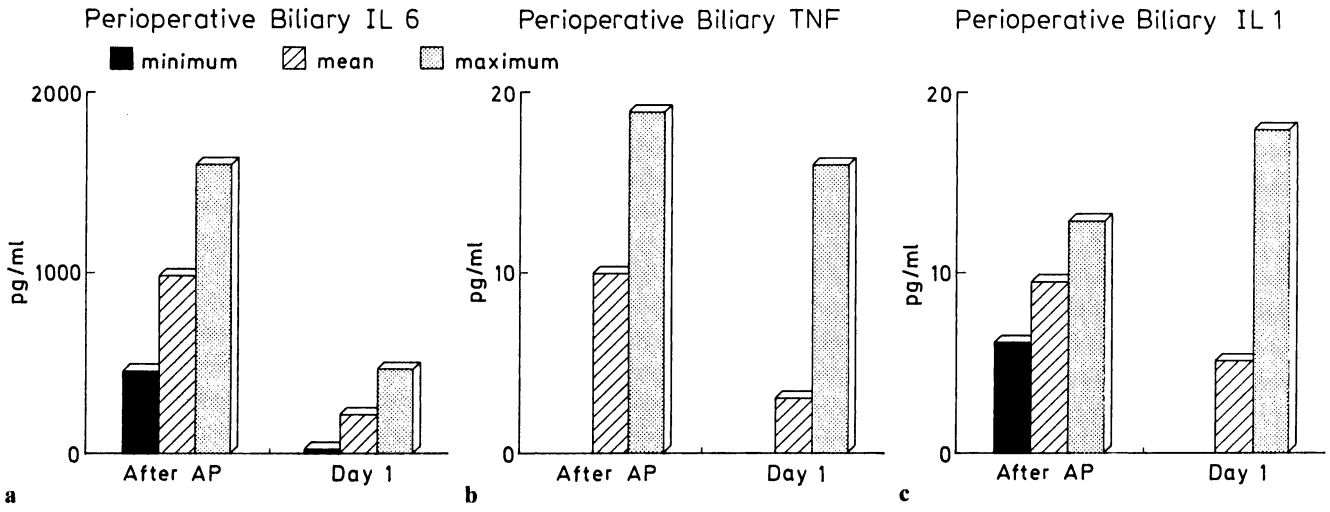


Fig. 1a–c. Peroperative production of **a** IL-6, **b** TNF, and **c** IL-1

occur during OLTx, especially at the end of AP [9, 10, 14]. Monokines are among the various biologically active substances that are released following monocyte exposure to endotoxin. Thus, endotoxinemia would explain, at least partly, the high levels of monokines that we observed during OLTx, in particular IL-6 whose serum levels reached their maximal value at the end of AP.

A third hypothesis needs to be taken into account. The liver is the principal clearance organ of circulating cytokines [1]. Hepatic clearance is obviously absent during AP, and is already severely compromised before AP due to both surgical manipulation of the diseased recipient liver and to the presence of the venovenous bypass that we routinely use. Lack of hepatic clearance might account for impaired cytokine elimination and the increased serum levels that are observed before the new liver is functioning. Again, this would be, true mainly for IL-6 whose levels reached their maximum value before and especially at the end of AP. Reduction of hepatic clearance in patients with liver failure might also account for the observation that preoperative serum levels of IL-6 and TNF are increased when compared to normal values [1].

Maximum levels of the monokines IL-1 and particularly TNF were detected after AP, i.e. by the time the newly transplanted livers were revascularized. Interestingly, these monokines were present in the bile produced


Perioperative Biliary IL 6
minimum
mean
maximum
2000
1000
0
pg/ml
After AP
Day 1
a
Perioperative Biliary TNF
20
10
0
pg/ml
After AP
Day 1
b
Perioperative Biliary IL 1
20
10
0
pg/ml
After AP
Day 1
c

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