Th1-like and Th2-like cytokines in patients undergoing open versus laparascopic cholecystectomy

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Abstract

The advantages of laparoscopic (LC versus, open cholecystectomy (OC) seems to be related to minimal invasive procedure and to the moderate infiammatory response. The aim of this study is to define the involvement of Th1 (IFN-γ) and Th2 (IL-4, IL-6, IL-10, IL-13) cytokines production in vivo and in vitro in patients undergoing OC or LC.

In 42 patients undergoing LC (n=22) and OC (n=20) Th1-like and Th2-like was evaluated before operation and at 6, 24 and 48 hours after operation for white blood cell counting and cytokines (IL-4, IL-6, IL-10, IL-13, IFN-γ, TNF-α) in the sera and in the supernatants from circulating mononuclear cells stimulated with phytohemagglutinin or lipopolysaccharide.

The acute phase response cytokine, IL-6, appeared significantly increased following OC than after LC. All other cytokines did not very significantly. In vitro data shows a reduction of IFN-γ and increase in Th2-like cytokines in OC patients compared with the basal value. In LC subjects we observed an high production of IFN-γ associated to an increase of Th2-like cytokines, like IL-10 and IL-13, even though IL-4 and IL-6 were unmodified.

In contrast to OC, LC did not significantly affect immunocompetence, maintaining a moderate infiammatory response and an adequate balance between Th1 and Th2 cytokine. Furthermore, the strong activation of cells producing Th1-like cytokines in LC patients following mitogen activation indicated a consistent anti-microbial activity, that was not detectable in OC patients, that showed after activation only aTh2 response.

Key words: Th1/Th2 cytokines after cholecystectomy.

Introduction

Laparoscopic cholecystectomy (LC) has become the gold standard technique over the open procedure for the treatment of the non-neoplastic disease of the gall bladder (1,2).

Advantages of LC over the traditional open cholecystectomy (OC) are small skin incision, less pain, more rapid post-operative recovery associated with a reduced postoperative morbidity, early return to normal activity (3, 4). In the last years several studies have been published indicating as possible causes of the favourable clinical outcome of LC the reduction of neuroendocrin stress and acute phase response and a better preservation of immune response (5-9). Immune system, abnormalities have been described in association with OC like the depression of lymphocyte proliferation against mitogen (8, 9), the impairment of natural killer (NK) cell cytotoxicity (8), and the alteration of cytokine network involved in the regulation of the activation and proliferation of lymphocytes and accessory cells (10). The most of studies have clearly demonstrated that interleukin (IL)-6 (IL-6), a pivotal cytokine of acute phase response (11), is constantly and significantly increased in OC compared with LC (6, 7, 9, 12, 13). Tumour necrosis factor-alpha (TNFα) and IL-1 were significantly higher after cholecystectomy compared with basal value, but their levels did not differed significantly between LC and OC

Riassunto

LE CITOCHINE PRODOTTE DALLE CELLULE SIMIL TH1 E TH2 NEI PAZIENTI SOTTOPOSTI A COLECISTECTOMIA LA-PAROTOMICA E LAPAROSCOPICA

I vantaggi della colecistectomia laparoscopica (LC) rispet -

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to alla colecistectomia tradizionale (OC) sembrano esse - re correlati alla minima invasività della metodica. In questo studio abbiamo valutato l'alterazione delle cito - chine (IL) prodotte dalle cellule simil Th1 (IFN-γ) e Th2 (IL-4, IL-6, IL-10, IL-13) in vivo ed in vitro nei pazienti sottoposti ad OC ed LC.
La produzione delle citochine Th1 e Th2 nei pazienti sot - toposti ad OC (n.=20) ed LC (n.=22) è stata valutata prima dell'intervento chirurgico e a distanza di 6, 24 e 48 ore nel siero e nel supernatante dalle cellule mononu - cleate periferiche stimolate con fitoemoagglutinina o lipo - polisaccaride.
Dopo OC è stato osservato un significativo incremento dei valori di IL-6 rispetto ad LC, mentre tutte le altre citochine non variavano significativamente. In vitro i dati mostrano una riduzione di IFN-γ ed un incremento del - le citochine Th2 nei pazienti OC rispetto ai valori basa - li. Nei pazienti LC è stata osservata un elevata produ - zione di IFN-γ associata ad un incremento delle cito - chine Th2 (IL-10, IL-13) mentre IL-4 ed IL-6 non han - no subito modificazioni.
Rispetto ad OC, dopo LC, si verifica una moderata rispo - sta infiammatoria ed un adeguato bilancio tra le cito - chine Th1 e Th2. Infatti l'elevata produzione di cito - chine simil Th1 dopo stimolo con mitogeni nei pazien - ti LC, indica una consistente attività antimicrobica che non è osservabile nei pazienti OC. In questi invece è stata osservata solamente una risposta di tipo Th2.
Parole chiave: Citochine, cellule simil Thl e Th2, cole-cistectomia.

(5, 12). Recently, it has been devoted attention to evaluate the modifications induced by cholecystectomy in cytokine secretion by T-helper cells (12, 14). These CD4+ lymphocytes are not homogenenous and are distinct into two major subsets, Th1 and Th2, that are strongly involved in the regulation of cell-mediated and antibody-mediated immune responses (10, 15). Th1-like cells secrete IL-2 and interferon gamma (IFN-γ) and activate macrophage functions and cytotoxic T cells, Th2-like lymphocytes produce IL-4, IL-5, IL-6, IL-10 and IL-13 and are mainly responsible of inhibition of macrophage function and stimulation of antibody production (10, 15). Furthermore, IL-4, IL-10 and IL-13 are able to inhibit proinflammatory cytokines (IL-6, IL-1α, TNFα).
In this study we tested the involvement of Th1 (IFN-γ) and Th2 (IL-4, IL-6, IL-10, IL-13) cytokines in patients undergoing LC and OC. Furthermore, we evaluated the modifications of TNFα, a key cytokine driving Th1 cell development and inducing production of IFN-γ from NK and T cells (17).

Materials and methods

Patients

Forty-two patients (age range 25-60) with symptomatic gallstone disease were included in this study. All of them were without gall-bladder complications (acute cholecystitis, choledocholithiasis), metabolic, endocrine, hepatic or renal diseases.
Furthermore, nobody assumed steroid, neither nonsteroid anti-infiammatory drugs, or received any transfusion. One group of patients was under the care of a surgeon of the team of the first Division of Surgery Department from University who routinely carried out LC (n = 22) and the other under the cure of surgeon of the second Division of the same Surgery Department who performed OC (n = 20). Patients gave written informed consent and the local ethics committee approved the study. Preoperatively, all patients received enoxaparin sodium (20 mg) and antibiotic prophylaxis (1 g of intramuscolar cefuroxime). Both study groups received the standard anaesthetic procedures using thiopental sodium for induction, vecuronium for neuromuscular blockade, isofluorane and fentanyl citrate for analgesia. OC were carried out through an 8 cm subcostal incision. LC by the four trocars techniques (18) using a $C0_2$ pneumoperitoneum maintained at 12-14 mmHg. Patients were classified as either grade I, II or III according to American Society of Anaesthesiologists (ASA) grading system (19). Age, gender, anesthesiologic grading, duration of operation and body weight index are given in Table I. As indicated, the operation time with both the techniques was similar. None of the patients included in this study developed postoperative infectious complications. LC patients left the hospital 2-3 days after the operation, whereas OC patients 4-5 days after surgery. Peripheral venous bloods samples were collected 24 hours prior to surgery and than 6, 24 and 48 hours postoperatively. Fifteen healthy controls were included in this study.

Materials

RPMI 1640, supplemented with glutamine (2mM) and antibiotics, and foetal calf serum (FCS) were from Euroclone Ltd (Devon, UK). Ficoll-Hypaque (Nycomed Pharmas, Oslo, Norway) was used to isolate peripheral blood mononuclear cells (PBMC).

Leukocyte counts

Blood counts were performed by using a ADVIA 120 (Bayer Diagnostics, Munich, Germany) which performed absolute numbers and percentages of leukocyte count.

Serum samples

Serum samples were separated from blood within 30 min of venipuncture by clotting and centrifugation at 400 g for 10 min at rt. Samples were aliquoted and frozen at -70° C until assayed.

Cell and culture conditions and in vitro *stimulation of cytokine production*

PBMC were separated by Ficoll-Hypaque density gradient centrifugation, resuspended in RPMI 1640 complete medium supplemented with 10% heatinactivated FCS. PBMC (10^6/ml) from patients and healthy controls were tested for cytokine production by stimulation with PHA (5 μg/ml) or lipopolysaccharide (LPS) (1 μg/ml) for 24 h (20). Supernatants were collected, filtered through 0.22 μm Millex Filters (Millipore S.A., Molshiem, France), and stored at -70° C until tested.

Assay for cytokine determination

Commercially available ELISA kits were used to determine the concentration of different cytokines in sera and in culture supernatants: IFN-γ (CLB, Amsterdam); IL-4, IL-6, IL-10, TNFα, IL-13 (EuroClone, LtD, UK). The principle of the assays was a sandwich enzyme-immune-assay which utilises a monoclonal antibody (MoAb) immobilised on a solid phase to capture antigen from the test specimen, and a peroxidase-conjugated monoclonal added to bind the antigens captured by the first antibody, as previously used (21).

Statistical analysis

Standard deviation (SD) and standard error (SE) were calculated and statistical significance analysed by Student's *t*-test Variance analysis was determined by ANOVA. Spearman rank correlation test was used to correlate different variables. Differences between OC and LC groups were calculated by the Mann Whitney U test. The Wilcoxon signed rank test was used to evaluate paired data. P <0.05 was considered statistically significant.

Results

Age, gender, anestesiologic grading, duration of operation and body weight index were no significant difference between the two groups (Tab. I). All patients showed an uncomplicated *intra* and post-operative course. *Levels of IL-6, IFN-γ, IL-13, IL-4, TNFα and IL-10 in the sera from LC and OC patients*

TABLE I - PATIENTS CHARACTERISTICS

		OC	*LC*
Age (years)		47 ± 15	52 ± 25
Gender (f/m)		14/6	18/4
Body Mass Index (Kg/m^2)		26 ± 5	24 ± 7
Anesthesiologic Grading			
(ASA)	I	6	8
	II	8	6
	III	6	6
Duration of the operation (min)		62 ± 28	66 ± 30

Data are expressed as mean ± standard deviation P > 0.05 all parameters.

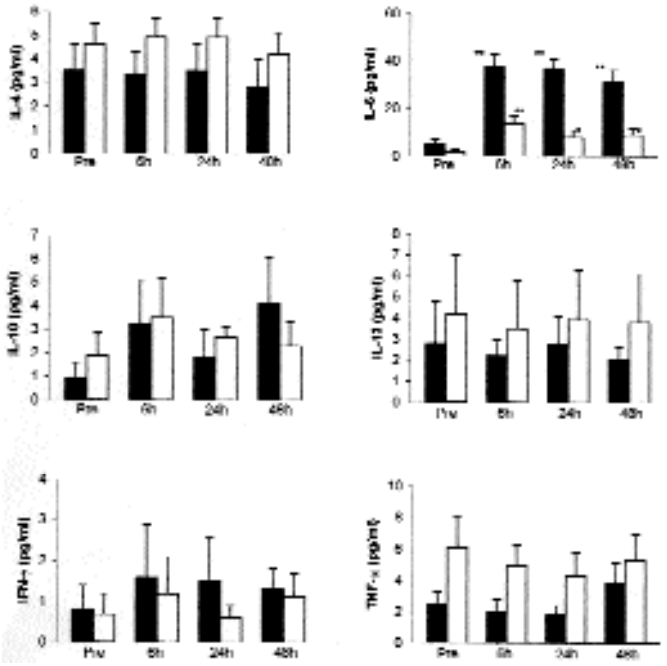

Fig. 1: Changes in serum levels of cytokines in open (black columns) versus laparoscopic (white columns) cholecystectomy before (Preop) and 6, 24, 48 h after surgery. Values are expressed as mean ± SE. Cytokines serum levels (pg/ml) in healthy control group: IL-6: 0.8 ± 0,4; IL-10: < 0.1; IL-4: 1.8 ± 0.8; IL-13: 1.4 ± 0.2; TNFα: 1.2 ± 0.2; IFN-γ: 1.8 ± 0.9. Significance versus preoperative value: * P < 0.05, **P < 0.01.

Figure 1 clearly indicates that the cytokine network is altered after cholecystectomy. IL-6 levels were significantly increased in both groups compared their baseline levels or those detected in healthy controls. In patients who underwent OC, IL-6 began to significantly increase immediately after the operation with a peak 6 hours

after operation (39±5 pg/ml). Its level remained significantly high (P >0.01) 48 hours after surgery, declining to the preoperative level 7 days afterwards (data not shown). Also in the LC group patients IL-6 levels were significantly increased overtime compared with the baseline level, but it was significantly (P <0.01) lower than that in the OC group. Furthermore, in LC patients, after the IL-6 peak at hour 6, a marked decrease was observed by day 1. Since IL-6 preoperative levels in LC and OC patients were in the range of healthy controls the increased secretion of IL-6 is strictly induced by operative procedures. IFN-γ, IL-13, IL-4 and TNFα serum levels did not vary significantly at any analysis time in comparison with those observed in both groups 24 h before surgery, or in healthy controls. IL-10 serum levels were discretely higher than before surgery and showed a similar pattern of behaviour in both OC and LC groups.

Leukocytes variation in OC and LC patients

The analysis of leukocyte counts in OC and LC groups are shown in table 2. As indicated cholecystectomy determined a significant leukocytosis 6 h, 1 and 2 days postoperatively in both groups. This increase was significantly higher in OC than in LC patients. OC was associated with a significant increase in the percentage and in the absolute number of neutrophils, with the contemporary reduction in lymphocytes. LC patients showed a significant (P <0.01) leukocytosis. Furthermore, LC subjects had a significant increase (P <0.01) in the absolute number of lymphocytes, even though their percentage values were not modified. Monocytes were not significantly altered in both groups.

Cytokine levels in the supernatants by stimulated PBMC from OC and LC

The results of the cytokine production by PBMC from cholecystectomized patients stimulated *in vitro* with the T cell mitogen, PHA, are shown in Fig. 2. Because the plateau of cytokine expression and white cells occurs within 24 h of operation, we studied this time point for the analysis of cytokines in the supernatants of all patients, even though in some others we analysed all time points. Before operation we did not observe significant differences in both groups. As clearly indicated, very significant (P <0.01) high levels of IL-4 were detected in PHA-stimulated PBMC isolated from OC patients, whereas IL-4 was reduced in not significant way in LC patients. PHA-induced IFN-γ production was reduced in PBMC from OC patients 24 hours after surgery compared with the baseline value even though in not significant way, whereas IFN-γ secretion by PBMC from LC patients was significantly increased (P <0.005) compared with the preoperative level and with that observed in OC patients. As far as other cytokines, after the operation IL-10, and TNFα activities in PHA-stimulated PBMC were significantly increased (P < 0.05

TABLE II - EVALUATION OF LEUKOCYTE MODIFICATIONS IN OC AND LC PATIENTS BEFORE SURGERY AND OVERTIME

		White cell (1 /min')	*Neutrophils %*	*Lynphocytes %*	*Monocytes %*
Time					
Preop.	**a)** OC	7255 ± 1412	65.1 ± 9	30.6 ± 8.3	2.7 ± 0.8
	b) LC	6700 ± 1862	67.9±5.8	28.6 ± 5.2	2.8 ± 0.6
+ 6h	**c)** OC	14020 ± 4830	82. ± 9	14.7 ± 10..5	2.0 ± 0.5
	d) LC	11014 ± 2809	76.6 ± 6.9	21.4 ± 6.2	2 ± 0.5
+ 24h	**e)** OC	12570 ± 3597	79.4 ± 6.7	17.1 ± 5.8	1.6 ± 0.3
	f) LC	10621 ± 2893	76.4 ± 6.9	22.1 ± 6.6	1.8 ± 0.5
+48 h	**g)** OC	10950 ± 2573	74.5 ± 6.6	22.7 ± 6.4	2.1 ± 0.4
	h) LC	9436 ± 2775	72.3 ± 6.1	23.9 ± 6.6	1.1 ± 0.6

Data are espressed as mean ± S.E.
Significance for white cells and neutrophils:
P < 0.003: a vs c, a vs e; P < 0,01: a vs g, c vs d
P < 0.002: a vs d, b vs f; P < 0.05: a vs h, e vs g
Significance for lynphocytes:
P < 0.01:b vs d, b vs f; b vs h; P < 0.05 c vs d and e vs f

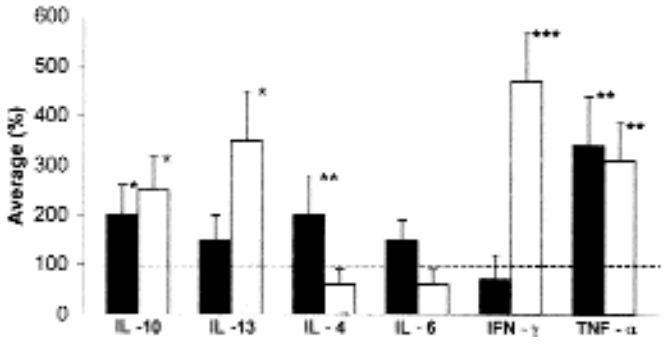

Fig. 2: Th1 and Th2 cytokine secretion profile of PHA-stimulated PBMC from patients with OC (black columns) and LC (white columns). Cytokines are expressed as percentage (mean ± SE) of baseline value. Basal (24-hour preoperative) cytokine secretion values (pg/ml): Healthy control group: IL-10: 395 ± 30; IL-13: 30 ± 18; IL-4: 20 ± 10; IL-6: 535 ± 20; IFN-γ: 740 ± 120; TNFα: 935 ± 170 ; OC patients: IL-10: 260 ± 60 < 0.1; IL-13: 12 ± 6; IL-4: 8 ± 4; IL-6: 540 ± 20; IFN-γ: 480 ± 214; TNFα: 650 ± 300; LC patients: IL-10: 350 ± 25; IL-13: 42 ± 12; IL-4: 31 ± 12; IL-6: 430 ± 90; IFN-γ: 420 ± 200; TNFα: 620 ± 250. Significance versus preoperative values: *P < 0.05; **P < 0.01; ***P < 0.005.

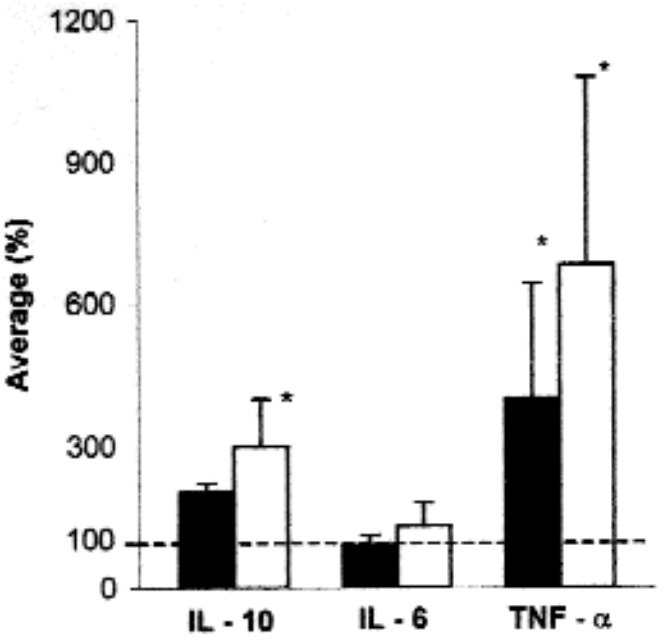

Fig. 3: PBMC from OC (black columns) and LC (white columns) patients were micubated for 24 h with LPS and supernatants tested for IL-10, IL-6 and TNFα activity. Results are expressed as percentage (mean ± SE) of baseline value. Basal (24-hour preoperative) cytokine secretion values (pg/ml): Healthy control group: IL-10: 300 ± 40; IL-6: 530 ± 20; TNFα: 1000 ± 50; OC patients: IL-10: 310 ± 70; IL-6: 550 ± 30; TNFα: 1020 ± 50; LC patients: IL-10: 360 ± 30; IL-6: 500 ± 30; TNFα: 865 ± 150. Significance versus preoperative values: *P < 0.05.

and P < 0.01 respectively) compared with preoperative values in patients who underwent OC, whereas IL-13 and IL-6 were unmodified. In the group having LC there were significant increases in IL-13, IL-10 and TNFα secretion postoperatively compared with baseline, whereas IL-6 was almost unmodified. These results indicate that in OC patients PBMC under stimulation showed in part an amplification of Th2-like cytokines associated with a decrease of Th1-like cytokines (IFN-γ). In LC patients both Th1 and Th2-like cytokines appear increased, a part from IL-4 and IL-6.

When we studied the cytokines induced in monocytes by LPS we observed that IL-10 was significantly increased compared with the baseline level (P <0.05) only in LC patients (Fig. 3). Whereas, the LPS-induced TNFα production was extremely variable in both groups. as attested by the high SE, and they were enhanced from the baseline, even though TNFα activity was higher in LC patients (Fig. 3). LPS-induced IL-6 production was not different from that observed before surgery in both groups.

Discussion

There is clinical evidence that the surgical insult experienced by patients who undergo LC differs significantly from that experienced by those undergoing OC, since LC is accompanied by less pain, better ventilatory function and almost total absence of secondary paralytic ileum (3, 4). Some of the benefits of minimal invasive surgery seem to be due to lower systemic cytokine response (IL-6, TNFα and IL-1α), infiammatory response (CRP, cortisol, monocytes and neutrophil release of 0_2) (5-7). Further possible causes of not favourable clinical outcome of OC have been found in the reduction of proliferative response (8, 9), cell-cytotoxicity (8), and of HLA-DR expression on monocytes (9, 14), associated with an increase in Th2-like activity, evaluated as *in vitro* production of IL-4 cytokine (14). Our study presents evidence that the balance between cells producing Th1 and Th2-like cytokines is certainly modified after surgical stress, but the events appear more complex than (9, 14) previously reported. In our patients the acute phase response following OC appears more significantly increased than in the LC, as attested by the relevant secretion in the blood of IL-6, according to previous report (6, 7, 9, 12, 13).

This cytokine can be secreted by various cells. At the site of incision, infiltrating T cells might be important sources of IL-6 production (16, 22, 23). On the other hand, our observation of reduction of circulating lymphocytes, as well as the already described reduction of CD4+ T cells (9) is consistent with this hypothesis, Other important sources of IL-6 could be the damaged endothelium and fibroblasts (16, 22, 23). Since there is convincing evidence that neutrophils can release IL-6 (24, 25, 26) the very high number of neutrophils could have a relevant role in causing high levels of IL-6. Altogether, these data indicate that serum IL-6 is a good indicator of response to surgical stress (27) and it could be of prognostic value.

In vitro data showing a reduction of IFN-γ and an

increase in Th2-like cytokines in OC patients compared with basal value indicate that the potential down-regulation of Th1-like cell-mediated immunity, under stimulation, makes these patients potentially more susceptible to infections with pathogens (15), because of a reduction in the macrophage antibacterial activity (28). These results indicate that the suppression of Th1-type response could be the dominant event following OC and that could explain the significant suppression of skin test response in OC patients compared with LC ones. In contrast with the report of Brune et al. (29), we detected in OC subjects a contemporary activation of Th2-type responses *in vitro*, evaluated as IL-4 and IL-10, and also an increased mitogen-induced TNFα production. In LC patients we have observed an active Thl-like response, attested by very strong mitogen-induced IFN-γ production compared with basal value and with OC. The increased Th2 response in LC patients, attested by the high production of IL-10 and IL-13, but by normal levels of IL-4 and IL-6, could be due to the fact that in LC the Th2 activation is dominated by the anti-infiammatory effects of IL-13 (28) and IL-10 (22), rather than by the inhibitory effects on macrophage activity by IL-4 (15, 22, 28) and antibody production by IL-4 and IL-6 (10, 15). Furthermore, IL-13 is able to inhibit the production of proinfiammatory cytokines (included IL-6 and TNFα) in human monocytes stimulated with LPS (10, 22, 23, 28) and the high production observed in LC patients could indicate that in these patients there is on the one hand a very sustained anti-microbial response, associated with IFN-γ and TNFα, on the other hand a potential down-regulation of the infiammatory response by IL-13. The higher IL-10 production by PBMC from LC than OC patients is a further confirmation of the ability of LC PBMC of modulating infiammation, since IL-10 acts to suppress (5, 30) production of infiammatory cytokines. In conclusion our data show than LC is associated with a reduced systemic infiammation compared with OC, associated with a potential ability to develop after stimulation a very active Th1 response, that maintains in such a way a consistent anti-microbial activity. This could be associated with the high levels of lymphocytes and, in particular, CD4+ T cells (9). In OC the strong systemic stress is associated with a normal Th1 response and an increased Th2 activity. Thus, the determination of Th1 and Th2 cell balance before operation could be of help in deciding on different surgical approaches and that a reduction of preoperative level of Th1 cells should exclude OC.

References

1) Schirmer B.D., Edge S.B., Dix J. et al.: *Laparoscopic cholecystectomy. Treatment of choice for symptomatic cholelithiasis.* Ann Surg, 213:665-677, 1991.

2) Neugebauer E., Troidl H., Spangenberger W. et al.: *Conventional versus laparoscopic cholecystectomy and the randomized controlled trial.* Br J Surg, 78:150-154, 1991.

3) Berggren U., Gordh T., Grama D., et al.: *Laparoscopic versus open cholecystectomy: hospitalisation, sick leave, analgesia and trauma responses.* Br J Surg, 81:1362-1365, 1994.

4) The Southern Surgeons Club.: *A prospective analysis of 1518 laparoscopic cholecystectomies.* N Engl J Med, 324:1073-1078, 1991.

5) Karayiannakis A.J., Makri G.G., Mantzioka A. et al.: *Systemic stress response after laparoscopic or open cholecystectomy: a randomized trial.* Br J Surg, 84:467-471, 1997.

6). Redmond H.P., Watson R.W.G., Houghton T. et al.: *Immune function in patients undergoing open vs laparoscopic cholecystectomy.* Arch Surg, 129:1240-1246, 1994.

7) Ueo H., Honda M., Adachi M. et al.: *Minimal increase in serum interleukin-6 levels during laparoscopic cholecystectomy.* Am J Surg, 168:358-360, 1994.

8) Griffith J.P., Everitt N.J., Lancaster F. et al.: *Influence of laparoscopic and conventional cholecystectomy upon cell-mediated immunity.* Br J Surg, 82:677-680, 1995.

9) Kloosterman T., von Blomberg B.M.E., Borgstein P. et al.: *Unimpaired immune functions after laparoscopic cholecystectomy.* Surgery, 115:424-428, 1994.

10) Del Prete G., Maggi E., Romagnani S.: *Human Th 1 and Th2 cells: functional properties, mechanisms of regulation, and role in disease.* Lab Invest, 70:299-306, 1994.

11) Stadnyk A.W., Gauldie J.: *The acute phase protein response during parasitic infection.* Immunol Today, 12:7-12, 1991.

12) Bellon J.M., Manzano L., Bernardos L. et al: *Cytokine levels after open and laparoscopic cholecystectorny.* Eur Surg Res, 29:27-34, 1997.

13) Schietroma M., Risetti A., Carlei F., Maggi G., Cianca G., De Santis C., Centi D., Iannucci D., Recchia C.L., Gattucci S., Simi M.: *Risposta della interleukina-6 in pazienti sottoposti a colecistectomia laparoscopica o laparotomia.* Min Chir, 53:359-62, 1998.

14) Decker D., Schondorf M., Bidlingmaier F. et al.: *Surgical stress induces a shift in the type-1/type-2 T-helper cell balance, suggesting down-regulation of cell-mediated and up-regulation of antibody-mediated immunity commensurate to the trauma.* Surgery, 119:316-325, 1996.

15) Romagnani S.: *The Th1/Th2 paradigm.* Immunol Today, 18:263-266, 1997.

16) Ivashkiv L.B.: *Cytokine expression and cell activation in infiammatory arthritis.* Adv Immunol, 63:337-376, 1996.

17) Adorini L.: *IL-12.* Chemical Immunology, 68:1-203, 1997.

18) Grace P.A., Quereshi A., Coleman J. et al.: *Reduced postoperative hospitalization after laparoscopic cholecystectomy.* Br J Surg, 78:160-162, 1991.

19) American Society Anesthesiologists: *New classification of physiology status.* Anesthesiology, 24:111, 1963.

20. Cillari E., Milano S., D'Agostino P. et al.: *Depression of CD4 T cell subsets and alteration in cytokine profile in boutonneuse fever.* J Infect Dis, 174:1051-1057, 1996.

21) Cillari E., Liew F.Y., Lo Campo P. et al.: *Suppression of IL-2*

production by cryopreserved peripheral blood mononuclear cells from patients with active visceral leishmaniasis in Sicily. J Immunol, 140:2721-2726, 1988.

22) Grau G.E.: *Implications of cytokines in immunopathology: experi - mental and clinical data.* Eur Cytokine Netw, 1:203-210, 1990.

23) Van Snck J.: *Interleukín-6:an overview.* Ann Rev Immunol, 8:253-278, 1990.

24) Chakravortty D., Kurnar K.S.: *Interaction of lipopolysaccharide with human small intestinal lamina propria fibroblasts favors neu - trophil migration and peripheral blood mononuclear cell adhesion by the production of proinfiammatory mediators and adhesion molecules.* Biochim Biophys Acta, 1453:261-72, 1999.

25) Cassatella M.A.: *The production of cytokines by polymorphonuclear neutrophils.* Immunol Today, 16:21-26, 1995.

26) Johnson J.L., Moore E.E., Taniura D.Y. et al.: *Interleukin-6 augments neutrophil cytotoxic potential via selective enhancement.* J Surg Res, 76:91-4, 1998.

27) Reith H.B., Kaman S., Mittelkotter O. et al.: *Cytokine activa - tion in patients undergoing open or laparoscopic cholecystectomy .* Int Surg, 82:389-393, 1997.

28) Zurawski G., de Vries J.E.: *Interleukin-13, an interleukin-4-like cytokine that act on monocytes and B cells, but not on T cells.* Immunol Today, 15:19-26, 1994.

29) Brune I.B., Wilke W., Hensler T., Holzmann B., Siewert J.R.: *Downregulation of T helper type 1 immune response and altered pro- infiammatory and anti-inflammatory T cell cytokine balance following conventional but not laparoscopic surgery.* Am J Surg, 177:55-60, 1999.

30) Bogdan C., Vodovotz Y., Nathan C.: *Macrophage deactivation by interleukin-10.* J Exp Med, 174:1549-55, 1991.

Commento Commentary

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Qualsiasi stimolo nocivo e/o lesione traumatica a livello tissutale evoca una risposta, la cosiddetta "reazione di fase acuta", il cui scopo è quello di minimizzare il danno e iniziare il processo di guarigione.
In corso di intervento chirurgico, nonostante che negli ultimi anni siano stati intrapresi molti studi per cercare di spiega - re i processi alla base del trauma operatorio, l'esatto meccanismo che c'è dietro alla "reazione di fase acuta" non è ancora ben chiaro. In tale condizione un ruolo molto importante è giocato dall'effetto diretto di anestetici e di farmaci sulle cel - lule immunitarie (1), dalla risposta ormonale evocata dallo stress (2), dall'attività dei linfociti T helper e suppressor (3), dalla produzione di fattori sierici soppressivi (4) e dalle citochine (5), fra l'altro, pur non riflettendo direttamente lo sta - to del sistema immunitario, sembra che svolgano un essenziale ruolo nell'"orchestrazione" della risposta immunitaria (6-9). Le citochine chiamate in causa sono soprattutto il fattore di necrosi tumorale-α (TNF-α), l'interleichina-1α (IL-1α), l'inter - leuchina-1α (IL-1β), interleuchina-6 (IL-6) e l'interleuchina-8 (IL-8), che fra l'altro modulano l'attivazione dei macrofa - gi, dei linfociti T e delle cellule "natural killer" (NK) (10). Anche l'interleuchina-10 (IL-10) sembra essere una citochina importante, in quanto ha un effetto inibitorio sull'attivazione dei monoliti e dei macrofagi (11). Attualmente si è dell'opi - nione che alcuni sintomi (dolore, febbre etc.), osservati dopo un trauma chirurgico e in situazioni patologiche, siano da ascriversi alla esagerata produzione di citochine (12).
La videolaparocolecistectomia (VLC) è una procedura chirurgica i cui vantaggi sono ormai ben conosciuti (13). Diversi autori sono del parere che il ridotto dolore postoperatorio e la minore incidenza di complicanze infettive dopo VLC siano legati ad una elevata produzione di citochine connessa al lieve trauma tissutale. A tale proposito sembra dimostrato che la IL-6 aumenta significativamente in corso di colecistectomia laparotomica (CL) a differenza di quanto accade dopo VLC (14-16). I risultati di alcuni lavori non concordano però del tutto con i suddetti dati. Roumen (17), ha sì osservato un aumento della IL-6 dopo laparotomia, ma solo nei pazienti con una età di 60 anni. Mc Mabon (18) d'altro canto non ha notato significative differenze nei livelli di IL-6 tra un gruppo operato con colecistectomia laparoscopica ed uno sotto - posto a minilaparotomia.
In questo ambito si inserisce il presente lavoro di Di Vita e coll. Che hanno valutato l'alterazione delle citochine prodot - te dalle cellule simil Th1 (INF-α) e Th2 (IL-4, IL-6, IL-10, IL-13) nei pazienti sottoposti a CL versus VLC. I loro risultati confermano i dati della maggiore parte degli studi citati per quanto riguarda il modesto incremento dopo VLC

della IL-6 che è la più indagata delle citochine della "reazione di fase acuta". Il dato nuovo e molto interessante che emerge da questo lavoro di Di Vita e coll. è però l'incremento osservato dopo VLC della IFN-γ, citochina che si comporta in modo opposto rispetto alle altre. Tale incremento per altro, considerando trattarsi di una citochina con notevole attività antimicrobica, costituirebbe un ulteriore rilievo a favore della VLC in quanto spiegherebbe in parte la minore incidenza di complicanze infettive dopo VLC. A quest'ultimo proposito, i risultati di Di Vita e coll. meritano ulteriori conferme. È da notare, tuttavia, fermo restando gli indiscutibili ben noti vantaggi della VLC che le modificazioni delle citochine dopo una colecistectomia laparoscopica o laparotomica, potrebbero avere un ruolo più dottrinario che pratico, trattandosi di un trauma chirurgico di media entità. È in un altro ambito della chirurgia mini-invasiva (chirurgia del colon-retto ad es.) che la conferma dei suddetti risultati potrebbe giocare veramente un ruolo pratico di notevole rilievo (infezioni p.o., prognosi oncologica).

All noxious stimuli and mechanical injuries to living tissue evoke a response, often called the "acute phase reaction", which aims to minimize the damage and start the healing process.
In spite of the extensive studies in recent years and growing understanding of different aspects of the processes evoked by surgical traumatic injury, the exact mechanisms behind the "acute phase reaction" are not yet fully understood.
The direct effect of anaesthetics and drugs on immune cells (1), hormonal changes evoked by stress (2), activation of suppressor activity (3), the appearance of suppressive serum factors (4), and changes in cytokine production (5), are all considered to play a very important role. In particular, attention has been turned to the role of cytokines in the "orchestration" of the immune response (6-9). The main cytokines in these conditions are tumor necrosis factor-α (TNI-α), interleukin-1α (IL-1α), interleukin-1α (IL-1α), interleukin-6 (IL-6) and interleukin-8 (IL-8), which further modulate the activation of macrophages, T lymphocytes and natural killer (NK) cells (10). Interleukin-10 also appears to be an important cytokine, as it has an inhibitory effect on the activation of monocytes and, macrophages (11). Currently several authors point out the fact that many of the symptoms (pain, fever etc.) observed after trauma and other patological situations may be evoked by exaggerated. cytokine production (12).
Laparoscopic cholecystectomy is being performed with increasing frequency. The advantages of laparoscopic surgery have been extensively discussed in recent years (13). Several investigators have suggested that that explanation of why laparoscopic surgery results in less postoperative pain and infectious complications is that it reduces the tissue trauma so implicating less local cytokine production. The acute phase response after laparoscopic surgery has been studies in several clinical trials measuring IL-6 leves after laparoscopic colecystectomy. Interleukin-6 leves have been noted to be reduced in patients undergoing laparoscopic procedures compared to traditional laparotomy (14-16).
Other studies, however, have shown contrary findings. Roumen (17) reported that IL-6 leves were only detected in patients after the age of 60 undergoing laparotomy. Mc Mahon (18) showed no significant differences between laparoscopic cholecystectomy and mini-laparotomic colecystectomy groups.
Di Vita e coll., in their study define the involvement of Th1 (INF-α) and Th2 (IL-4, IL-6, IL-10, IL-13) cytokine production in patients undergoing OC or LC.
This confirmed the results of recent studies that show only a slight increase of IL-6 levels after LC IL-6 is the most analyzed cytokine of the "acute phase reaction". An important and interesting result of the study of Di Vita e coll., was the increase in IFN-γ levels. Considering that IFN-γ is a powerful antimicrobial cytokine, its increase during LC may partially explain the low incidence of infectious complications after LC.
by the light of this last point the results of Di Vita e coll., merit ulterior confirmation by other studies. Despite, the well known advantages of LC, variation of cytokine levels after LC or OC may have a more theoretic than pratical role, since cholecystectomy is a moderate surgical procedure.
Confirmation of the above results in another sector of mini-invasive surgery (colorectal surgery, for example) may play a very important role (post-operative infections, oncologic prognosis).

Bibliografia-References

1) Salo M.: *Effect of anaesthesia and surgery on the immune response.* Acta Anaesthesiol Scand, 36:201-220, 1992.

2) Calvano S.: *Hormonal mediation of immune dysfunction following thermal and traumatic injury.* In: Davies J.M., Shires G.T. (eds): A*dvances in Host Defence Mechanism.* New York, Raven Press, 111-142, 1986.

3) Miller C.L., Baker C.C.: *Changes in lymphocyte activity after thermal injury. The role of suppressor cells.* J Clin Invest, 63:202-210, 1979.

4) Constantian M.B., Menzoian J.O., Nimberg R.B., Schmid K., Mannick J.A.: *Association of a circulating immunosuppressive polypeptide with operative and accidental trauma.* Ann Surg, 185:73-79, 1977.

5) Akira S., Kishimoto T.: *IL-6 and NF-IL-6 in acute phase response and viral infection.* Immunol Rew, 127:25-50, 1992.

6) Arai K.I., Lee F., Miyajims A., Miyalake S., Arai N., Yokata T.: Cytokines: *Coordinators of immune and infiammatory responses.* Annu Rev Biochem, 59:783-836, 1990.

7) Carfei F., Schietrorna M., Cianca G., Risetti A., Mattucci S., Enang G.N., Simi M.: *Effects of laparoscopic and conventional (open)*

cholecystectomy on human leukocyte antigen-DR expression in peripheral blood monocytes: correlations with immunologic status. World J Surg, 23:8-22, 1999.

8) Schietroma M., Risetti A., Carlei F., Angeloni R., Cianca G., Leardi S., Simi M.: *Valutazione della funzione immunitaria in pazien - ti sottoposti a colecistectomia laparoscopica o laparotomica.* Chirurgia, 11:35-40, 1998.

9) Schietroma M., Carlei F., Risetti A., Enang G.N., Tozzi D., Cianca G., Mattucci S., Simi M.: *Acute cholecystis: laparoscopic or open management? Role of the immune apparatus.* 3th World Congress of the International Hepato-Pancreato-Biliary Association (I.H.P.B.A). Proceedings, 230-232, 1999.

10) Ostensen M.E., Thiele D.L., Lipsky P.E.: *Tumor necrosis factor-alpha enhances cytolytic activity of, human natural killer cells.* J Immunol, 138:4185-4191, 1987.

11) Del Prete G., De Carli M., Almerigogna F., Guigizi M.G., Biagiotti R., Romagnani S.: *Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) cell clones and inhibits their antigen-specific proliferation and cytokine production.* J Immunol 150:353-360, 1993.

12) Marx J.L.: *Cytokines are two-edged swords in disease.* Science, 239:257-258, 1988.

13) Delaunay L., Bonnet F., Cherqui D., Rimaniol J.M., Dahan E., Atlan G.: *Laparoscopic cholecystectomy minimally impairs posto - perative cardiorespiratory and muscle performance.* Br J Surg, 82:373-376, 1995.

14) Suzuki M., Oka M., Tangoku A. et al.: *Interleukin-6 and granulocytic elastase levels following laparoscopic cholecystectomy.* Surg Endosc, 8:447, 1994 (Abstract).

15) Vander Velpen G., Penninckx F., Kerremans R., et al.: *Interleukin-6 and coagulation-fibrinolysis fluctuation after laparoscopic and conventional colecystectomy.* Surg Endosc, 8:1216-1220, 1994.

16) Schictroma M., Risetti A., Carlei F., Maggi G., Cianca G., De Santis C., Ccnti D., Iannucci D., Recchia C.L., Mattucci S., Simi M.: *Risposta della interleukina-6 in pazienti sottoposti a. colecistectomia laparoscopica o laparotomica.* Min Chir, 53:359-62, 1998.

17) Roumen R.M.H, van Meurs P.A., Kuypers H.H.C. et al.: *Serum interleukin-6 and C-reactive protein responses in patients after laparoscopic or conventional cholecystectomy.* Eur J Surg, 158:541-544, l992.

18) Mc Mahon A.J., O'Dwyer P.J., Cruikshank W. et al.: *Comparison of metabolic responses to laparoscopic and minilaparotomic cho - lecystectomy.* Br J Surg, 80:1255-1258, 1993.

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