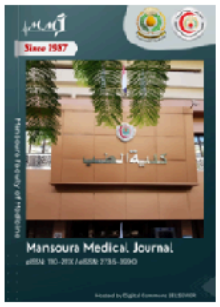
MANSOURA MEDICAL JOURNAL

ISSN - Print: 1110-211X - Online: 2735-3990

journal homepage: mmj.mans.edu.eg



Volume 31 | Issue 2 Article 3

EVALUATION OF IMMUNE RESPONSE IN PATIENTS UNDERGOING OPEN VERSUS LAPAROSCOPIC CHOLECYSTECTOMY

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EVALUATION OF IMMUNE RESPONSE IN PATIENTS UNDERGOING OPEN VERSUS LAPAROSCOPIC CHOLECYSTECTOMY

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ABSTRACT

Background / Aim : Laparoscopic cholecystectomy is a so called mini-invasive surgical procedure, and on this basis, we investigated whether and how the immune response is modified in patients after laparoscopic cholecystectomy compared to patients who underwent open cholecystectomy. Also we focused on the T cell secretion of cytokines that regulate the critical balance of either T helper type-I (Th1) - and T helper type-II (Th2) - cell mediated immune responses and the pro- inflammatory activities.

Methods : In a prospective study, immunological data of 38 patients submitted to laparoscopic cholecystectomy (LC) and 18 patients undergoing open cholecystectomy (OC) for symptomatic cholecystolithiasis were compared. Patients with acute cholecystitis and patients developing postoperative complications or receiving immunosuppressive medications were excluded. Production of interferon (IFN)-γ, interleukin(IL)-2, (IL)-4, and tumor necrosis factor (TNF)-α, by isolated T cells stimulated by cross linking of CD3 and CD28 was evaluated preoperatively as well as on postoperative days 1 and 6 or 7. Cytokines were measured by immunoenzymometric assay. Also, skin Multitest was done to share in evaluation of cell mediated immune response.

Results : IFN -γ, TNF-α and IL-2 production by T cells decreased significantly by 48.3%, 36.6%, and 36.8%, respectively, on postoperative day 1 after OC, but not after LC. These results indicate severe suppression of Th1- type and pro-

inflammatory cytokines after the open operation, while IL-4 did not show significant changes in either group suggesting that Th2 cell response remained normal. Moreover, skin tests showed a hypo-or anergic response in the majority (79.6%) of open cholecystectomy patients compared to laparoscopic cholecystectomy patients (24.3%), ($P<0.05$). In open group, we noted 2 cases (11.1%) of respiratory tract infection.

Conclusion : The present study shows that open, but not laparoscopic cholecystectomy is associated with a marked suppression of T lymphocytes functions as indicated by deregulation of both the Th1/Th2 and the pro-inflammatory cytokine balance. The results therefore suggest that down-regulation of Th1 cell-mediated immune response and pro-inflammatory activity of T cells is a hallmark of open, but not laparoscopic surgery.

Key Words : laparoscopic cholecystectomy, open cholecystectomy, immune response, skin tests.

INTRODUCTION

Laparoscopic cholecystectomy (LC) is now considered the treatment of choice for symptomatic cholelithiasis . It was first performed in Germany in1986 [1] , and rapidly spread to the United States [2], and other European countries [3] . The advantages of this method to open cholecystectomy (OC) are: absence of postoperative pain, prompt postoperative bowel activity (6-24hrs), reduced postoperative infections, shorter hospitalization (1-3 days), earlier return to normal activity as well as optimal esthetic results [4].

Postoperative alterations in host immune functions after major surgery have often been described and investigated. [5,6] In particular, a reduction of cellular proliferation and secretion of interleukin (IL)-2 and interferon (INF) -γ by mitogen-stimulated T lymphocytes has been observed after surgical trauma, burn, or injury. [5-8] Consistent with these reports, Hensler et al [5], demonstrated that major surgery results in a severe defect of CD3/CD28-stimulated T cells to proliferate and to secrete INF -γ, IL-2, and tumor necrosis factor (TNF)-a, suggesting a defect of T helper type-1 (Th1) cell functions. [5,9] Down regulation of Th2 cell-mediated humoral immune defense was also noted after major conventional surgery as demonstrated by decreased T cell secretion of IL-4 in the early postoperative

period. Interestingly, TNF-α and INF - γ deficiencies have been shown to result in impaired immune defense against protein antigens and various pathogenic microorganisms such as Listeria monocytogenes. (10-13).

Earlier studies have shown that some immunological changes such as serum IL-6 concentrations correlate with the severity of surgical trauma. (14) The clinical advantages of laparoscopic surgery including minimized pain and shorter recovery time seem to be mainly due to reduced surgical trauma. Thus, the primary objective of our study was to evaluate whether the reduction of trauma correlates with reduced suppression of host defense dependent on cell mediated (Th1) and humoral (Th2) immune responses. Effects of laparoscopic surgery on immune functions are of critical importance in patients with acute peritonitis, as postoperative immunosuppression may contribute to the development of septic complications. (5,12) Moreover, patients suffering from malignant disease may also benefit from a reduction of postoperative suppression of immune defenses since tumor recurrence and therefore prognosis of these patients are considered to be related to immune function. (11) In the present study, we compared secretion of IL-4 for evaluation of Th2 cell function (humoral immune defense) and production of IFN-γ and IL-2 to analyze Th1 cell mediated immune defense) after laparoscopic and conventional cholecystectomy. In addition, secretion of the pro- inflammatory cytokines TNF-α by T cells was measured.

PATIENTS AND METHODS

Patients

This study included 56 patients who attended Outpatient Surgery Clinic unit (8) of Mansoura University Hospital between July, (2000) and May (2002). All patients were submitted to full detailed history, clinical, laboratory, and radiological investigations. we analyzed the immune function of 38 patients submitted to laparoscopic cholecystectomy (LC) and 18 patients undergoing open cholecystectomy (OC) for symptomatic cholecystolithiasis at the General Surgery Department of the Mansoura Faculty of medicine ,Mansoura University. Mean age for laparoscopic group was 42.1 years (± 17.2), while the range varied from 28 to 55 years (29 women and 9 men). For conventional group, the mean age was 49.5 years (± 19.6) ($P < 0.1$), while the range

varied from 44 to 63 years ; this group was consisted of 10 women and 8 men .

History of upper abdominal surgery, contracted gall bladder were the main indications for conventional, otherwise laparoscopic maneuver was the preferable one. The two groups were compared for the age difference, clinical parameters including operative duration, preoperative laboratory findings, and preoperative immunological data (Tables 1&2). In both groups, patients with common bile duct stones, acquired immunodeficiencies, or acute inflammatory cholecystitis, or patients receiving medications that may interfere with immunologic response such as immuno-suppression, steroid or non-steroidal anti-inflammatory drugs were excluded from the study. Similarly, patients in whom the operation had to be converted from laparoscopy to laparotomy or who developed postoperative complications were excluded.

General anesthesia was obtained in both groups using the same procedure: pre anesthesia was performed using atropine (0.01 mg/kg) plus prométazine (0.8mg/kg), induction using sodium thiopental (5 mg/kg) plus atracurium (0.5 mg/kg), followed by tracheal intubation and assisted ventilation. Anesthesia was maintained using isofluorane (1.5%) mixed with nitrogen monoxide (NO) and oxygen (O) in a 2:1 ratio.

Laparoscopic cholecystectomy was carried out in a standardized technique using 4 trocars mini-incisions and a pneunoperitoneum of 15mmHg. Open cholecystectomy was performed through right upper paramedian incision (10 cases) , and right subcostal incision (8 cases). Mini laparotomy colecystectomy cases were excluded from this study. There were no indications for intraoperative cholangiography, neither was blood transfusion necessary pre or postoperatively.

Mean operative time was 67.8 minutes (± 23.1) for LC and 62.6 minutes (± 19.5) for OC without statistically significant difference ($P > 0.05$). Blood samples were collected preoperatively and on postoperative days 1, and 6 or 7 and tested for immunologic parameters.

Isolation Of Peripheral Blood T Cells

Human peripheral blood mononu-

clear cells(PBMC) were isolated from 25 mL heparinized blood using Ficoll metrizoate density gradient centrifugation. Isolated PBMC were washed twice with PBS, and the total cell count was determined. PBMC were plated in 6-well tissue culture plates and incubated for 1 hour at 37oC to remove adherent cells. Non adherent cells were collected , washed with phosphate-buffered saline, and resuspended in PRMI 1640 medium containing 10% fetal calf serum. Tcells were further enriched by depletion of B cells and residual monocytes from non-adherent cells using immunomagnetic beads coated with CD14 and CD19 antibodies according to the instructions of the manufacturer (Dynal, Oslo, Norway) .

Cytokine Secretion of Peripheral Blood T Cells

For cross-linking of CD3 and CD28 receptors, enriched T cells were placed into 24-well tissue culture plates(Nunc) that were precoated for 1 hour at 37oC with 250µL of a 20 µg/mL solution of CD3 antibody. CD28 antibody and goat-anti-mouse immunoglobulin were added to T cells in solution at 5 µg/mL each and T lymphocytes were stimulated for 16 hours. After stimulation, supernatant were centrifuged to remove residual cells and stored at - 20oC until analysis.

The levels of INF -γ, IL-2, IL-4, and TNF-α, in supernatants of stimulated T cells were determined by enzyme-linked immunosorbent assay (ELISA) technique. All cytokine assays were standardized by including a titration of appropriate purified recombinant cytokine of known concentration. The levels of sensitivity of the ELISAs were 0.03 IU/mL (INF -γ), 0.1 IU/mL (IL-2), 2 pg/mL (IL-4), and 3 pg/mL (TNF-α) . The absorbance of samples was determined on a MRX Microplate Reader using 450 nm as the primary and 630 nm as the reference wave length.

Immune activity was also evaluated through skin tests, using the Multitest IMC, a day before surgery and respectively, 1,3, and 6 days postoperatively. Response to seven anamnestic antigens was evaluated after intra-dermal injection on the volar side of the forearm, using the Multitest IMC (Bio- Merieux Institute, Lion, France) [4] . The Multitest IMC (conserved at temperature not higher than 25^0C), comprises a kit which evaluates skin cellular immune response. This was made up of an ap-

applicator with eight intra-dermal injectors, containing the optimal dose of a glycerinated solution of the antigens listed here below:

1 - Tetanus toxoid
2 - Diphtheria toxoid
3 - Streptococcus antigen (Group C)
4 - Tuberculin (old)
5 - Candidin
6 - Trychophyton (Metagrophytes)
7 - Proteus (Mirabilis)
8 - Control (Glycerin)

We analyzed the results after 48 hours using the Scottal technique, which assesses only the cell-mediated immune response, hence, taking into account only the cellular infiltration and not the erythema. This is a method whereby a spherical point pen is passed over the skin surface, following a line directed towards the point of inoculation, such that the pen stops when it reaches the tumefaction formed as a result of cellular infiltration. Repeating this many times, it was possible to note many points delimiting the area involved in the cell mediated response. Two reciprocally perpendicular diameters were measured. The final value, expressed in millimeters, was obtained by adding the two diameters and dividing by two.

The patient was considered normoergic when the reaction was $\geq$ 2mm (a/2 + b/2) for at least 2 of the 7 antigens, where a and b are the two diameters of the inflammatory tumefaction. The patient was considered anergic or hypoergic when the immune response was absent or $\geq$ 2mm for only 1 of the 7 antigens.

Statistical Analysis

Data were analyzed using the Mann-Whitney U test for paired samples. Results are presented as mean ±SEM. The level of significance was set at $P < 0.05$. The statistical analysis of the data from The Multitest IMC was done using the Student's t test. Significance was achieved at $P < 0.05$.

RESULTS

To evaluate the effect of minimally invasive surgery on Th1 and Th2 cell functions, secretion of the cytokines INF -γ, IL-2, and IL-4 before and after laparoscopic or open cholecystectomy was determined. In addition TNF-α was measured to compare the effects of laparoscopic and open surgery on the pro- inflammatory cyto-

kines secreted by T cells. The results in the Table 2 show the preoperative data in both groups with regard to white blood cell count and INR, as well as T cell secretion of INF -γ, IL-2, IL-4 and TNF-α.

Th1 cell function was analyzed by INF -γ, (Figure 1), and IL-2 secretion (Figure 2). Following OC, INF -γ decreased significantly from a mean value of 7.49 (± 1.6) IU/mL to 3.87 (± 0.5) IU/mL (relative decrease of 48.3%, $P < 0.05$) on postoperative day 1. At the second postoperative control (6 to 7 days after OC) INF -γ secretion had recovered to a mean of 5.12 (± 1.0) IU/mL (Figure 1). In contrast, changes of INF -g secretion observed after LC were significant (Figure 1). On the first postoperative day after OC, IL-2 production by enriched T cells dropped significantly from 19.09 (± 3.09) IU/mL to 12.06 (± 2.9) IU/mL (relative decrease of 36.8%, $P < 0.02$). After LC, however, IL-2 secretion by T cells did not undergo any significant changes (Figure 2). These data suggest that Th1 cell function was suppressed by open cholecystectomy, but not by the laparoscopic procedure.

Th2 cells are characterized by secretion of IL-4. As shown in (Figure 3), T cell production of IL-4 did not show any significant changes following either LC or OC indicating that Th2 cell function remained unimpaired by both procedures.

Pro-inflammatory (TNF-α) cytokine is secreted by human Th1 cells. TNF-α secretion was significantly impaired on the first postoperative day after OC, where its production dropped from 1,327.55 (± 123.6) pg/mL preoperatively to 841.18 (± 129.3) on postoperative day 1 (36.6%, $P < 0.05$). In contrast, TNF-α secretion was not significantly affected by LC (Figure 4). So, the secretion of pro-inflammatory cytokines was suppressed after OC, but not after LC.

Skin reaction to the most common recall antigens (Multi-test IMC) was similar in both groups when carried out a day before surgery, while a statistically difference was noted between the two groups, 1 and 3 days postoperatively. In OC patients a high degree of hypoergy was reported, and skin reaction after Mutitest returned to normal values 6 days postoperatively ($P < 0.01$) (Figure 5).

Table (1): Details of patients who underwent cholecystectomy.

	LC group	OC group	*P* value
Number of patients	38	18	
Age (years)			
mean ± SD	42.1(± 17.2)	49.5 ± (19.6)	S
Rang	28 - 55	34 - 63	
Sex M/F	9/29	8 / 10	
Operative time (min.) ± SD	67.8 (± 23.1)	62.6 (± 19.5)	NS
PO complications (bronchopneumonia)	0	2 (11.1%)	S
Hospitalization after surgery (days)	2-3 (2.3)*	7-9 (7.3)*	S

LC: Laparoscopic cholecystectomy; OC: Open cholecystectomy;
PO: Postoperative. S = Significant; NS = Not significant.
Note: ( )* represent the mean value of days.

Table (2) : Preoperative laboratory findings and immunological data in both patient groups

	LC	OC	*P* value
White blood cell count (/cmL)	6.4 (± 0.3)	6.6 (±0.4)	NS
INR	1.0 (± 0.3)	1.1 (± 0.2)	NS
IL-2 (IU/mL)	21.4 (±3.5)	18.3 (±4.3)	NS
INF-γ (IU/mL)	7.2 (±1.1)	7.4 (±1.8)	NS
IL-4(pg/mL)	30.5 (±3.4)	24.2 (±4.5)	NS
TNF-α (pg/mL)	1162 (±129)	1357 (±137)	NS

Values are given as mean and standard error of the mean.
NS = not significant as determined by unpaired Mann-Whitey U test; OC = conventional cholecystectomy; LC = laparoscopic cholecystectomy;; IL = interleukin; INF = interferone; TNF = tumor necrosis factor.

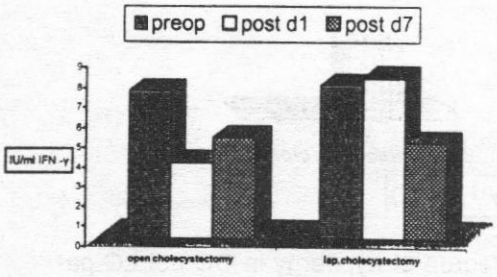

Figure. (1) : T cell interferon-γ secretion decreases significantly after OC on postoperative day 1, but not after LC.

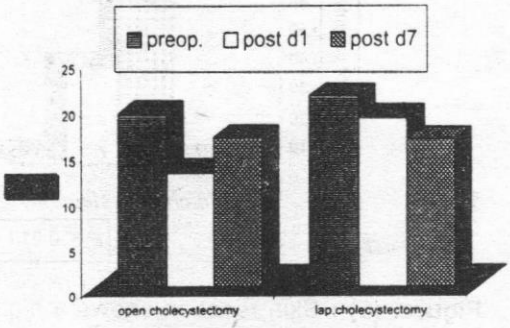

Figure. (2) : T cell interleukin-2 secretion is significantly decreased after OC on postoperative day 1, but not after LC.

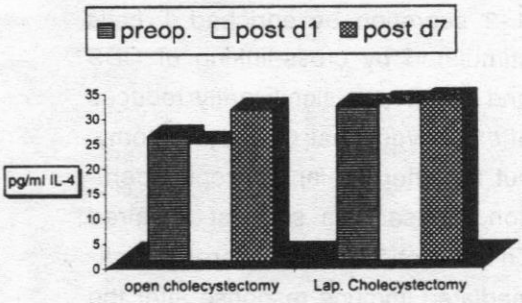

Figure. (3) : IL-4 secretion by Th2 cells did not show any significant changes after OC or LC.

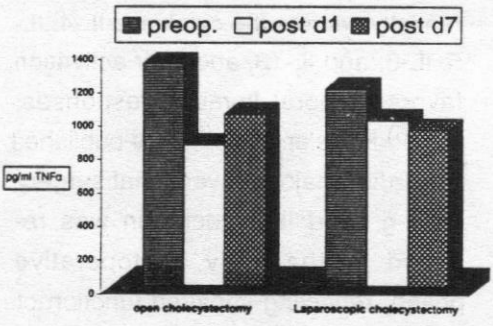

Figure. (4) : TNF-α secretion is significantly reduced on 1st P.O.D. after OC not LC.

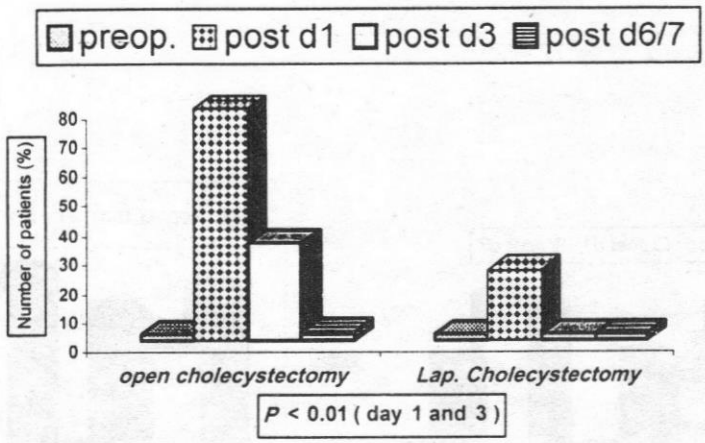

Figure. (5) : Skin reaction shows a high degree of hypoergy in OC not LC patients in 1st, & 3rd postoperative days, and return to normal values in the 6th postoperative day.

DISCUSSION

Among CD4+ T lymphocytes, Th1 and Th2 phenotypes may be distinguished. (6,9,10) Th1 cells are mainly characterized by secretion of INF -g and IL-2 and induce cell-mediated immune responses. Th2 cells preferentially secrete the cytokines IL-4, IL-5, IL-6, and IL-13, and their activation favors humoral immune responses. (15,16) Hensler et al (5) was published that, after major conventional surgery INF -g and IL-2 secretion was reduced in the early postoperative phase, reflecting impaired function of Th1 cells and therefore of cell-mediated immune responses. (5) Although to a lower extent, production of IL-4, the index cytokine for Th2 cells was also diminished suggesting that major conventional surgery results in down-regulation of both cell-mediated and humoral immunity.

In the present study, INF -γ and IL-2 secretion by enriched T cells stimulated by cross-linking of CD3 and CD28 was significantly reduced after conventional cholecystectomy, but not after the laparoscopic operation. These data suggest impaired Th1 function, and thereby cell-mediated immune response after the open operation that could not be observed after the laparoscopic procedure. These results are consistent

with by Decker et al [15], and Brune et al [16] whom reported that reduced INF -γ by mitogen-stimulated PBMC following OC, but not LC. [17] Although the pattern of these changes was similar for open cholecystectomy and for major conventional surgical procedures, the suppression of T cell IL-2 and INF -g production was not noted after the laparoscopic operation, indicating that downregulation of Th1 cell mediated immune response is a hallmark of open, but not laparoscopic surgery. Normal secretion of IL-4 by T cells of OC patients suggest dysregulated cytokine response to CD3 and CD28 delivered signals rather than reduced expression of CD3 or CD28 molecules [18] .

Previously, Hensler et al, [5] showed that after major conventional operations, IL-4 secretion of CD-3/ CD28- stimulated T cells was significantly reduced in the early postoperative period. [3] But the present study demonstrated that humoral immune responses as monitored Th2 cell secretion of IL-4 was unaltered by laparoscopic as well as open cholecystectomy. Together, these findings suggest that Th2 cell functions and humoral immune responses are only altered by major surgical trauma.

In a previous study, Decker et al [15] noted an increase of T cell IL-4 secretion following OC, whereas our results proved that IL-4 production of T cells is affected neither by OC nor by LC, which is consistent with Brune et al [17] . The possible explanation for this discrepancy is provided by differences in the experimental protocols applied by Decker et al, and proved that T cells were depleted of B cells and monocytes, thereby excluding cell-cell interactions that may affect cytokine secretion of T cell stimulation with mitogen phytohemagglutinin-P.[19] Brune et al [17] had favored cross-linking of both the T cell receptor and the coreceptor CD28. Ligation of CD28 by antibodies or natural ligand has been demonstrated to provide potent cosignals inducing T cell proliferation and IL-2 secretion. (20-23) Increased IL-4 production by PBMC as has been observed by Decker et al [15] may therefore be caused by cross-talk between different cell types and may not reflect T cell-intrinsic functional alterations. An observation showing that IL-6, which is induced by open surgery more strongly than by laparoscopic procedures (24), and may be produced by B cells and monocytes, stimulation. T cell secretion of IL-4 (25) is consistent

with this interpretation.

Our results regarding Multitest IMC skin test gave a high degree of hypoergy in open cholecystectomy cases in early postoperative period are consistent with several studies reported [4,20,21,], and proved that surgery reduces the immune responses (immunodepression) which has been clearly demonstrated through reduced skin reaction (T cell mrdiated) following open surgery but not mini-invasive surgical procedures [4].

The present study demonstrates that the pro-inflammatory cytokine TNF-a was disturbed by open cholecystectomy. These results are consistent with [11,16] who add also that these changes may result in increased susceptibility to septic complications following open surgery which were not noted after the laparoscopic procedure. Moreover, cell-mediated immune response represented by Th1 cell function remained unimpaired by the laparoscopic procedure, while secretion of Th1-type cytokines was suppressed by open cholecystectomy. Similar changes of T cell function have been observed after major conventional operations. Therefore, down-regulation of cell mediated immune response seems to correlate to a certain extent with the severity of surgical trauma [26].

In summary, we conclude that the reduction in trauma obtained by minimally invasive cholecystectomy (LC) is associated with reduced postoperative alteration of Th1 cell function and pro-inflammatory activity as compared with the open operation. So laparoscopic surgery may be an important tool for surgical management of oncology patients, avoiding immunodepression or its aggravation.

REFERENCES

1. **Muhe E; (1986) :** Die erste cholezystektomie durch das laparoskop. Langenb Arch Klin Chir; 369:804-9.

2. **Olsen DO, (1991) :** Laparoscopic cholecystectomy. Am. J. Surg; 161: 339-344.

3. **Cuschieri A; (1990) :** The laparoscopic revolution. Edinburgh: JR Coll. Surg; 34: 295-301.

4. **Schietroma M, Carlei F, Agnifili A, et al; (2001) :** laparoscopic versus open chole-

cystectomy and the immune response. Hepato-Gastroenterology; 48: 642-646.

5. **Hensler T, Hecker H, Heeg K, et al; (1997) :** Distinct mechanisms of immunosuppression as a consequence of major surgery. Infect. Immun; 65:2283-2289.

6. **Gitzelmann CA, Sagaon M, Talamini MA, et al; (2000) :** Cell-mediated immune response is better preserved by laparoscopy than laparotomy. Surgery; 127 : 65-71.

7. **Faist E, Mewes A, Baker CC, et al; (1987) :** Prostaglandin E2 (PGE2)-dependent suppression of interleukin 2 (IL-2) production in patients with major trauma. J. Trauma. 27:837-848.

8. **Wood JJ, Rodrich ML, O'Mahony BO, et al; (1984) :** Inadequate interleukin-2-production : a fundamental immunological deficiency in patients with major burns. Ann. Surg. 200:311-320

9. **O'Sullivan ST, Lederer JA, Horgan AF, et al; (1995) :** Major injury leads to predominance of the T helper-2-phenotype and diminished interleukin-12 production associated with decreased resistance to infection. Ann. Surg.; 222: 489-492.

10. **Livingston DH, Appel SH, Wellhausen R, et al; (1988) :** Depressed interferon-g production and monocyte HLA-DR expression after sever injury. Arch. Surg.; 123 : 1309-1312

11. **Eugster HP, Muller M, Karrer U, et al; (1996) :** Multiple immune abnormalities in tumor necrosis factor and lymphotoxin-a double deficient mice. Int. Immunol. ; 8:23-26.

12. **Targarona EM, Balague C, Knook MM, and Tras M; (2000) :** Laparoscopic surgery and surgical infection. British J. Surg.; 87: 536-44.

13. **Rothe J, Lesslauer W, Lotscher H, et al; (1993) :** Mice lacking the tumor necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature. 364:798-802.

14. **Cruickshank AM, Fraser WD, Burns HJG et al; (1990) :** Response of serum interleukin 6 in patients undergoing elective surgery of varying intensity. Clin. Sci.; 79:161-165.

15. **Decker D, Schondorf M, Bidlingmaier F, et al; (1996) :** 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 trauma. Surgery. 119:316-325.

16. **Romagnani S, (1994) :** Lymphokine production by human T cells in disease states. Annu. Rev. Immunol. 12:227-252.

17. **Brune IB, Wilke W, Hensler T, et al; (1999) :** Normal T Lymphocyte and monocyte function after minimally invasive surgery. Surg. Endosc. 12:1020-1024.

18. **Hajri A, Mutter D, Wacks S, Bastien C, Gury JF, et al; (2000) :** Dual effect of laparoscopy on cell-mediated immunity. Eur. Surg. Res.; 32: 261-6.

19. **Weighardt H, Heidecke CD, Emmanuilidis K, et al; (2000) :** Sepsis after major visceral surgery is associated with sustained and interferon-_ resistant defects of monocyte cytokine production. Surgery; 127: 309-15

20. **June CH, Bluestone JA, Nadler ML, and Thompson CB; (1994) :** The B7 and CD28 receptor families. Immunol. Today 15:321-331.

21. **Walker CBJ, Bruce DM, Heys SD, Gough DB, Binnie NR; (1999) :** Minimal modulation

of lymphocyte and natural killer cell subsets following minimal access surgery. Amer. J. Surg. 177: 48-54.

22. **Bluestone JA; (1995) :** New perspectives of CD28-B7-mediated T cell co stimulation. Immunity. 2:555-559.

23. **Gupta A, and Watson DI, (2001) :** Effect of laparoscopy on immune function. Brirish J. Surg.; 88: 1296-1306.

24. **Maruszynski M, and Pojda Z. (1995) :** Interleukin 6 levels in the monitoring of surgical trauma: a comparison of serum IL-6 concentrations in patients treated by cholecystectomy via laparotomy or laparoscopy. Surg. Endosc. 9:882-885

25. **Rincon M, Anguita J, Nakamura T, et al; (1997) :** Interleukin (IL)-6 directs the differentiation of IL-4 producing CD4+ T cells. J. Expe. Med.; 185:461-469.

26. **Carlei F, Schietroma M, Cianca G, et al; (1999) :** 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: 18-22.