

# Effects of Surgery on Cell-Mediated Immunity in Cancer Patients\*

KANSERLİ İLASTURDA HÜCRE ARACILI KLİ İMM ÜNİTEYE CERRAHİNİN ETKİLERİ

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## ÖZET

*In vivo ve in vitro hücre aralıklı iminin cevaplar üzerine cerrahinin etkileri, muhtelif ilerlemiş, fakat rezektabl olan karsinomali 12 hastada ve benzer kontrollerde çalışıldı. Araştırılan parametreler şunlardı: tüberkülini antijenine deri cevabı (PPD), total lökosit, lenfosit ve T-lenfosit sayıları, total ve aktif E rozet yapan hücrelerin (A-RFC) yüzdesi, T hücrelerinin alt grupları ve Fc reseptörlerince tanınan A-RFC'ler. Hastalar cerrahi tedaviden 6-8 hafta sonra yeniden incelendiler.*

*Tedavi öncesi hastalarda kontrollere göre, tüberkülin antijenine cilt cevabında azalma, lökosit ve lenfosit sayılarında artma, A-RFC'lerin yüzdesinde azalma, T hücrelerinin mutlak sayısında artma, T supressör hücrelerinin yüzdeleri ve mutlak sayılarında artma ve T helperlerin mutlak sayısında artma bulundu. T helperlerin yüzdesi ise kontrol rakamları içerisinde bulundu. Cerrahi tedaviden 6-8 hafta sonra bütün hastalarda normal immünolojik fonksiyonlar gözlemlendi.*

**Anahtar Kelimeler:** PDD. Kanserde immünite, İmmün cevap

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## SUMMARY

*The effects of surgery on in vivo and in vitro cell-mediated immune responses were studied in 12 patients with various advanced but resectable carcinoma and in appropriate controls. The parameters investigated were skin response to tuberculin antigen (PPD), total leukocyte, lymphocyte and T-lymphocyte counts, percentage of total and active E-rosette forming cells (A-RFCs) and subsets of T-Cells and A-RFCs identified by Fc receptors. Patients were retested six to eight weeks after surgery.*

*The results showed that there was a decrease in the cutaneous response to tuberculin antigen, an increase in leukocyte and lymphocyte counts, a decrease in the percentage of A-RFCs, an increase in absolute numbers of T-cells, an increase in percentages and absolute counts of T-suppressors (Tq, bearing receptor for Fc G), an increase in absolute counts of T-helpers (T\, bearing receptor for Fc M) in untreated cancer patients while the percentages of T-helpers remained within the control ranges. By six to eight weeks after surgery, all patients demonstrated normal immunological functions.*

**KeyWords:** PPD. Cancer immunity, Immun response

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**A number of studies evaluating general immunocompetence in patients with malignant disease have been carried out in recent years. These investigations have demonstrated significant impairment of delayed cutaneous hypersensitivity to recall antigens (1,2,3), decreased percentage of active E-**

rosette forming cells (4,5,6) and a fluctuation in the proportion of subsets of T-cells (7,8,9,10).

It is known that after apparently successful surgery, the depressed immunity will improve significant in most of the patients with malignencies in whom it has been previously impaired (11,12,13,14). Since cell-mediated immunity is a function of T-lymphocytes, which play a very important role in immunoregulation, we evaluated peripheral blood T-lymphocyte in patients with various carcinomas before treatment and six to eight weeks postoperatively to avoid immunodepressive effect of surgery and anesthesia (15,16,17).

## MATERIAL AND METHODS

### Patients and Controls

Twelve patients with histologically proven carcinoma of the breast (five), stomach (four) and colon (three) attending the clinics at the Black Sea University Medical School were studied. They were treated with radical mastectomy, radical subtotal or total gastrectomy and anterior resection, respectively. Patients were selected on their first attendance before any kind of therapy was given. Cancer staging was done according to TNM classification and the patients belonged to T1-3 N1-2 M0 at stages of cancer. There were eight women and four men ranging in age from 30 to 58 years with a mean age of 44 years.

Twelve healthy controls (seven women and five men) ranging from 28 to 46 years of age, with mean percent age of 38, belonging to laboratory personnel were used as controls and tested on the same days as the patients.

A count of total leukocytes and lymphocytes per cubic millimeter of blood was done using routine methods. Quantitation of T-lymphocytes was performed using spontaneous rosette formation with sheep erythrocytes.

### The Tuberculin Test

The test consisted of intradermal injection of 0.1 ml purified protein derivative (PPD) containing 0.5 T.U./0.1 ml (Refik SAYDAM INSTITUTE, Ankara, Turkey); 48 hours later the reaction was classified into three categories a) negative (-), erythema less than 5 mm in diameter; b) doubtful, erythema 5-9 mm; c) positive (+), erythema 10 mm or more with induration.

### Preparation Antisera Against Human Red Blood Cells (HRBCs)

A rabbit antiserum against HRBCs containing mainly IgM antibody was raised in rabbits by multiple intradermal injections of intact washed HRBCs (18). The antiserum was heated at 56 °C for 30 minutes and subjected to Sephadex G-200 (SIGMA) gel filtration. The antibody activity, determined by complement dependent hemolysis of HRBCs, was confined to the IgM containing exclusion peak. The mid-portion of this peak was collected and divided into small aliquots, which were stored at -20 °C. An antiserum against HRBCs containing high titres of anti-HRBC IgG antibody was raised in rabbits by similar methods and the IgG fraction was purified by DEAE-cellulose chromatography. The purity of the IgM and IgG fraction was checked by the hemagglutination procedure.

### Preparation of Lymphocytes

Peripheral blood lymphocytes were collected (19) and suspended in medium Tc-199 containing inactivated 10% fetal calf serum (SIGMA) and antibiotics. Active rosette-forming T-lymphocytes were separated using the method of Semenzato (20).

### Rosette Test

Sheep red blood cells (SRBCs) were taken under sterile conditions and stored in Alsever solution at the appropriate concentrations. Total and active E-rosette forming cells were studied by methods of Jondal et al. and Wybran and Fudenberg, respectively (21,22).

### T-Cell Purification

T-lymphocytes were purified by E-rosetting with neuraminidase (SIGMA) treated SRBCs and separated on a Ficoll-Hypaque (F-H) (SIGMA) gradient, as previously described (23). T-cells were recovered after SRBCs lysis with 0.83% Tris-ammonium chloride buffer, pH 7.2 and washed medium Tc-199 containing 10% fetal calf serum and antibiotics. Macrophage contamination was less than 1%, as at tested by alpha-naphthyl acetate esterase activity. The cells thus obtained were suspended in the medium at a concentration  $2 \times 10^6$  per ml and incubated 37 °C overnight.

**Subsets of T-Cells**

To and TM subsets (Bearing receptors for IgG and IgM respectively) were identified by of purified T-cells with HRBCs coated with subagglutinating amounts of either purified IgG or IgM antibodies to HRBCs as described earlier (24). Briefly, To and TM cells were quantitated by addition of 0.1 ml of 2% human red blood cells coated with anti-human erythrocyte antibodies either IgG or IgM respectively, to 0.1 ml of pure T-cells suspension, followed by centrifugation at 500 rpm for 5 minutes and incubation at 4 OC for 1 hour. A total of 200 cells was counted in each preparation. Subsets of A-RFCs were studied as above. The results are expressed as the mean ±SD. Student's t test was used to estimate the significance.

**RESULTS**

**Incidence Reactivity to PPD**

It can be seen from the Table 1 that the incidence of reactivity to tuberculin in the preoperative group (33%) was significantly different from the 92% in the control group ( $p < 0.01$  by  $X^2$ ). Postoperatively, the incidence of reactivity returned to normal.

**Leukocyte and Lymphocyte Counts**

The total leukocyte and lymphocyte counts from 12 cancer patients preoperatively and postoperatively are shown in Figure 1,2 in comparison with those of 12 healthy controls. It was observed that untreated cancer patients showed a significant increase in the leukocyte count ( $8550 \pm 1940$ ) and

Table 1. The Percentage of Skin Reactivity to PPD, Total and Active E-Rosettes, Subsets of T-cells and A-RFCs in Cancer Patients and Controls

	Controls	Untreated Patients	Significant	Treated Patients	Significance
PPD+ Anergic	0.00	41.70		8.00	
PPD+ Doubtful	8.00	25.00	$p < 0.01$	8.00	NS
PPD+ Normal	92.00	33.30		84.00	
Total E-Rosettes	$66.9 \pm 13.9$	$60.0 \pm 9.8$	NS	$59.5 \pm 12.5$	NS
Active E-Rosettes	$31.2 \pm 8.50$	$22.5 \pm 7.5$	$p < 0.05$	$26.7 \pm 5.80$	NS
Tc-cells	$183 \pm 5.70$	$245 \pm 4.8$	$p < 0.01$	$22.0 \pm 4.50$	NS
TG of A-RFCs	$7.00 \pm 4.00$	$10.9 \pm 4.2$	$p < 0.05$	$9.10 \pm 3.30$	NS
TM-CCHS	$54.4 \pm 12.4$	$51.3 \pm 12.3$	NS	$52.0 \pm 16.7$	NS
TM of A-RFCs	$27.9 \pm 8.90$	$29.1 \pm 7.2$	NS	$28.4 \pm 7.9$	NS
TM/TG Ratio	$3.14 \pm 0.9$	$2.11 \pm 0.47$	$p < 0.0$	$2.5 \pm 0.4$	NS

+ : Cutaneous response to PPD; NS: Not significant. Results are expressed as mean ± SD.

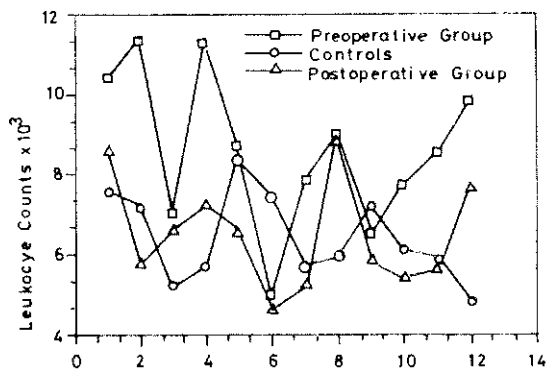


Figure 1. Leukocyte counts of cancer patients and healthy controls,

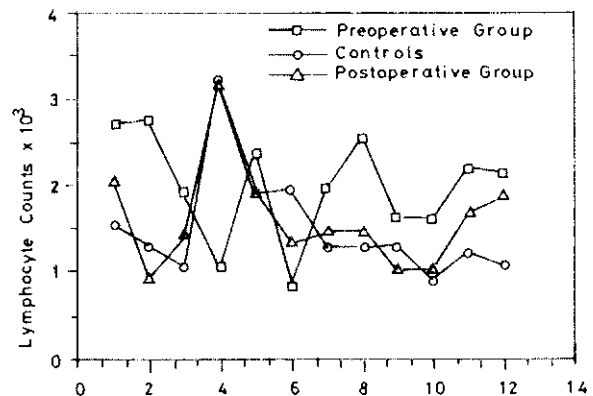


Figure 2. Lymphocyte counts of cancer patients and healthy controls.

lymphocyte count ( $2191 \pm 562$ ) in the peripheral blood, compared with normal donors ( $6291 \pm 1195$ ,  $1310 \pm 1195$  respectively). Postoperatively, the total leukocyte and lymphocyte counts were not different significantly from those of controls.

As can be seen in Figure 3, the total T-lymphocyte counts from 12 untreated cancer patients showed significant elevation ( $1331 \pm 394$ ), compared with controls ( $859 \pm 240$ ,  $p < 0.01$ ) because of differences in total lymphocyte count in circulation. Whereas the percentages of T-lymphocytes appeared to be equivalent to that of normal controls ( $60.1 \pm 9.8$  and  $66.9 \pm 13.9$ , respectively). After surgical treatment there was a significant decrease in the absolute number of T-lymphocytes in cancer patients ( $911.5 \pm 458.8$ ), when compared with untreated patients ( $p < 0.05$ ).

### Rosettes

The results of the total and active E-rosette in cancer patients and controls are summarized in Table 1. No difference could be demonstrated for the mean percentage of total E-rosette forming cells. However, the mean percentage of active E-rosette forming cells was significantly decreased in untreated patients compared to controls ( $22.58 \pm 7.5$ ,  $31.25 \pm 8.5$ , respectively,  $p < 0.05$ ). But a statistically significant increase of active E-rosette forming cells was observed postoperatively, whereas total rosette forming cells did not change.

### Subsets of T-Lymphocytes

The percentage and absolute numbers of T<sub>0</sub>-cells and T<sub>M</sub>-cells, representing suppressor and helper populations, respectively from preoperative and postoperative cancer patients and controls are

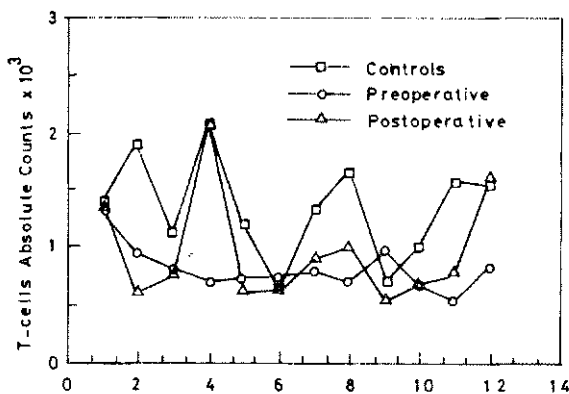


Figure 3. T-cells absolute counts from the peripheral blood of cancer patients and controls.

shown in Table 1 Figures 3,4. It appears that there was no significant difference in the percentage of T-cells in untreated cancer patients (T<sub>M</sub>, 51-0.8) when compared with controls (T<sub>M</sub>, 54±13.9). However, the total number and percentage of TG-CC11S in circulation in cancer patients ( $331.1 \pm 144$ ,  $24.5 \pm 4.8$ ) were significantly greater than in controls ( $136.1 \pm 61$ ,  $183 \pm 5.7$ ) respectively,  $p < 0.01$ ). Also the total number of TM-CC11S in the peripheral blood of untreated cancer patients ( $687.5 \pm 332.7$ ) showed significant elevation above that of normal control ( $463.9-149$ ,  $p < 0.05$ ).

The percentages and absolute of T<sub>c</sub>- and T<sub>i</sub>-cells of A-RFCs in cancer patients are shown in Table 1 and Figures 5,6, in comparison with controls. In the preoperative group, the percentage ( $10.9 \pm 4.2$ ) and absolute number ( $9.7 \pm 6.3$ ) of T<sub>c</sub>-cells increased significantly when compared with controls ( $29.1 \pm 7.2$ ) and absolute counts ( $87.8 \pm 52.8$ ) of TM-CC11S did not show significant differences from those of controls ( $27.9 \pm 8.9$ ,  $76.5 \pm 36.9$ , respectively). Postoperatively, there was no differences between patients and controls.

The T<sub>M</sub>/TG ratio in the controls was  $3.14 \pm 0.9$ , while that in the preoperative group was  $2.11 \pm 0.47$  ( $p < 0.01$ ) and in the postoperative group was  $2.5 \pm 0.4$  ( $p > 0.05$ ).

### DISCUSSION

In this study we measured in vivo and in vitro parameters of cell-mediated immune function in cancer patients and compared the result with those of controls.

The impairment of cell-mediated immunity measured in vivo and in vitro has been reported in patients with various carcinomas (3,22,25,26,28).

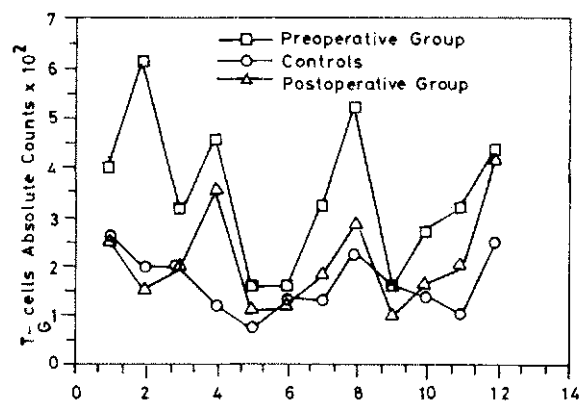


Figure 4. Tu-cells absolute counts in the peripheral blood of cancer patients and controls.

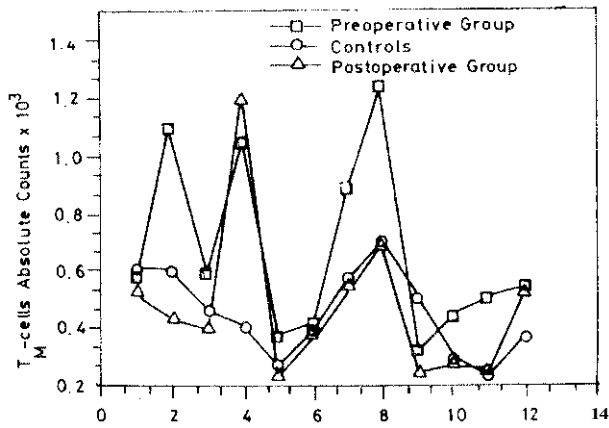


Figure 5. TM-CCIIIS absolute counts in the peripheral blood of cancer patients and controls.

These studies involve measurement of skin response to recall antigens (2,4), percentage and absolute number of lymphocyte populations and their subsets (7,8,9,29), lymphoproliferation to mitogens or alloantigens (30,31) and proportion of total E-rosette and active E-rosette (5). Among the skin test using non-specific cell-mediated immunity as a criterion, the tuberculin reaction is known to decrease in patients with malignant tumors. In our study, the decreased tuberculin reaction in untreated cancer patients returned to normal levels after complete removal of the tumor.

The total lymphocytes, T-cells and leukocytes showed significant increase in the untreated group in our study. Several workers have reported decreased percentage and total T-Lymphocyte counts in various carcinomas (8,32). Although the percentages of TM-CCIIIS in our patients were comparable to those of controls, their absolute num-

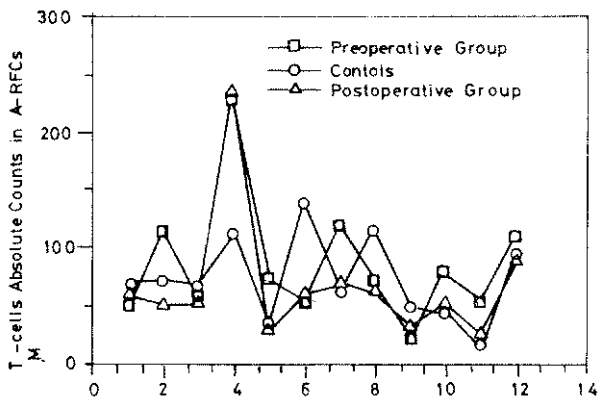


Figure 7. TM-CCIIIS absolute A-RFCs-enriched populations.

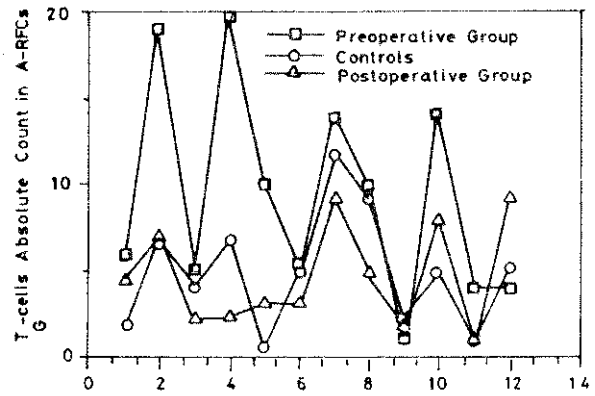


Figure 6. Tu-cells absolute counts in A-RFC-enriched populations.

bers were elevated in the untreated group because of the increased absolute lymphocyte count in the circulation. Six to eight weeks after operation, the total lymphocytes, leukocytes and T-cells showed significant reduction when compared with preoperative group.

The total rosette assay measures a surface marker on all human T-cells, while active rosettes identify a proportion of T-lymphocytes with high affinity for sheep red blood cells (33,34). Active rosette forming T-lymphocytes are thought to be a subpopulation more actively involved in cell-mediated immunity than total E-rosette forming cells and reflect cellular immunocompetence more accurately than total E-rosette forming cells (5,6,35,36). In this regard and in view of the reported impairment of delayed cutaneous hypersensitivity in various carcinomas, our data showing reduced active E-rosette forming cells and normal total E-rosette forming cells and normal total A-rosette forming cells are in agreement with these reports. Also our data indicated that functional activities are more important than the number or percentage of T-cells for reflecting immunocompetence in cancer patients. Prior to surgery, the cancer patients had significantly decreased percentage of A-RFCs (22.5 ± 7.5) when compared with the percentage in healthy controls (31.2 ± 8.5). Tumor removal resulted in increase of active E-rosette forming cell counts (26.7 ± 5.8) in our series of patients when tested six to eight weeks postoperatively.

For the analysis of subsets of T-lymphocytes we have used IgG Fc receptor (Fc R) and IgM Fc R

as markers. Since  $T_0$  and T.M-cells have been shown to conduct suppressor and helper function, respectively (37), we have used these markers to identify subsets of T-lymphocytes. Recently, monoclonal antibodies for the assessment of T-lymphocyte phenotypes have been widely used (38,39). However, as shown by Ballieux and Heijnen (40), both of these methods detect overlapping populations. Our observation on the subsets of "I"-lymphocytes indicate normal percentages of TM-CC11S and increased percentages of  $T_0$ -cells, however the absolute numbers of cells in the helper as well as suppressor population were increased in the preoperative patients group.

Elevated  $T_0$ -cells and/or decreased TM-CC11S have been reported in various malignancies (7,8,9,29) and patients with some immunodeficiency disorders (41,42,43). In our study, the elevated percentage and absolute numbers of  $T_0$ -cells returned to normal levels after successfully removing the tumor. Also our observation on the subsets of A-RFCs indicated increased percentages and absolute counts of  $T_0$ -cells ( $7 \pm 4, 5.28 \pm 3.4$ , respectively). Although the percentages and absolute counts of TM-CC11S in A-RFCs ( $29.1 \pm 7.2$ ,  $87.8 \pm 52.8$ ) were in the normal range untreated cancer patients when compared with controls. It has been shown by immunological and cytochemical studies that active rosette-forming cells include more active and immature T-cells and total E-rosette forming cells, A-RFCs and T-cell fractions differ considerably in various functional activities (20). A-RFCs were found to be consisted of cells capable to recognizing and killing allogenic cells and have immunoregulatory effect on B cell immunoglobuline production and exhibit natural killer cell activity (36). Our data showed that not only in the subsets of T-cells but also in the subsets of A-RFCs, there was significant alteration in cancer patients. We did not evaluate T suppressor/cytotoxic subsets of A-RFCs in cancer patients whether they belong to cytotoxic or suppressor subsets. Surgical removal of the tumor resulted in the significant increase of A-RFCs counts, indicating that surgery has a improving effect on impaired immune function in favor of host immune system in cancer patients.

In relation to T-helper / T-suppressor ratio, the patient group had a reduced ratio preoperatively, comparing with controls ( $2.11 \pm 0.47$ ,  $4.14 \pm 0.90$ , respectively). Low T-helper/T-suppressor ratio in patients with a wide range of malignancies was

reported by Dillman et al (10), due both to relative decrease in the percentage of T-helper cells and relative increase in the percentage of T-suppressor cells. After surgery, an increase at this ratio was also observed in cancer patients, due to significant decrease in the counts of T-suppressor cells.

Thus the major deviations in immune parameters in untreated cancer patients appear to be increased absolute number of both regulator T-cells, increased percentage of  $T_0$ -cells, decreased percentage of active E-rosette forming cells and decreased skin response to tuberculin antigen.

When we compare the pre-and postoperative parameters, the major abnormalities in regulatory cells and T-cells function disappeared after surgery. This confirms that a cancer operation may act as immunotherapy by removing the cancer cells that produce lymphocyte defects and immunodepression and allowing the patient's immune system to recover in advanced but resectable cancer patients.

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