# Lymphocyte Subpopulations in Peripheral Blood and the Spleen from Gastric Cancer Patients

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## ABSTRACT

Lymphocyte subpopulations in peripheral blood and the spleen from gastric cancer patients were identified by either single or two color analysis with fluorescence activated cell sorter-IV (FACS-IV) using monoclonal antibodies. The absolute and percentages of $CD3^+$ (mature T) cells and $CD4^+$ (helper/inducer T) cells in peripheral blood from the advanced cancer patients were significantly lower than those from normal healthy controls. In peripheral blood, a significant decrease of $CD4^+$ cells was due to the decrease of $CD4^+$ $CD45RA^+$ (suppressor inducer T) cells, while $CD8^+$ $CD11b^+$ (suppressor T) cells tended to increase with progress of the disease. $CD4^+$ $CD45RA^+$ cells located more predominantly in peripheral blood and spleen than in the splenic vein, while $CD8^+$ $CD11b^+$ cells were more predominant in the splenic vein.

**Key words:** *Gastric cancer, Lymphocyte subpopulations, Peripheral blood, Spleen, Monoclonal antibodies*

Recent studies have revealed that cell-mediated immune responses play a central role in tumor immunology of cancer patients[3,9]. The regulation of these immune responses is mediated by T cells[17]. These are mainly classified into two lymphocyte subpopulations; "helper/inducer" and "suppressor/cytotoxic" T cells using monoclonal antibodies based on expression of CD4 and CD8 antigens, respectively[14]. Immune responses may be regulated by the interactions among these lymphocyte subpopulations[15,17]. It is now appreciated that there are functional heterogeneities in these lymphocyte subpopulations identified by either single or two color analysis using monoclonal antibodies[4,5,11-15].

In this study, lymphocyte subpopulations in peripheral blood and spleen are identified by either single or two color analysis with fluorescence activated cell sorter-IV (FACS-IV) using monoclonal antibodies in different stages of gastric cancer.

## MATERIALS AND METHODS

*Patients*

Fourty-two patients with primary gastric cancer underwent examination prior to surgical treatment and 10 patients with recurrent gastric cancer prior to the immunochemotherapy were also studied. The determination of the stage was made according to the general rules adopted by the Japanese Society for Gastric Cancer Research[7]. Twelve healthy donors matched for age and sex served as controls.

*Preparation of peripheral blood lymphocytes (PBLs), splenic vein lymphocytes (SVLs) and spleen cells (SCs)*

PBLs and SVLs were prepared as described previously[10]. Splenic venous samples from patients were obtained during the laparotomy and SCs were collected by mincing specimens and passing them through the stainless steel wire mesh.

*Monoclonal antibodies*

The monoclonal antibodies used in this study were OKT3 (CD3), OKT4 (CD4) and OKT8 (CD8) (Ortho Diag., USA), anti-Leu7 (CD57) and anti-Leu15 (CD11b) (Becton Dickinson, USA) and 2H4 (CD45RA), Mo-1 (CD11b) (Coulter, USA). PBLs, SVLs and SCs were stained by indirect immunofluorescence method for single color analysis and direct immunofluorescence method for two color analysis as described previously[10]. In brief, for single color analysis, $1 \times 10^6$ cells were incubated with 5 $\mu$l of each purified monoclonal antibody. After incubation for 45 min at 4°C, the cells were washed twice, and then 20 $\mu$l of 1/10 dilution of fluorescein isothianate (FITC) labeled goat F(ab')$_2$ anti-mouse IgG or IgM antibody (Tago, USA) added to the cells. After the additional incubation for 45 min at 4°C, the cells were washed twice and resuspended in 1 ml of RPMI-1640 medium (GIBCO, USA). For two color analysis, $1 \times 10^6$ cells were incubated with FITC conjugated monoclonal antibody against one surface antigen and pycoerythrin (PE) conjugated monoclonal antibody against the second antigen for 45 min at 4°C.

After incubation, the cells were washed twice and resuspended in 1 ml of RPMI-1640 medium.

*Flowcytometric analysis of lymphocyte subpopulations*

Flowcytometric analysis was performed on FACS-IV (Becton Dickinson, USA) equipped with an argon laser 164-15 (Spectra-Physics, USA) turned to emit a 488 nm line (260 mW) capable of simultaneously exciting both FITC and PE, as described previously[10]. In single color analysis, linear amplifier was used, and the data from 10,000 cells were collected. In two color analysis, logarithmic amplification was used, and the data from 30,000 cells were collected. The percentage of $CD8^+$ $CD11b^+$ cells was calculated as that of bright $CD8^+$ $CD11b^+$ cells[5].

*Lymphocyte counts*

Lymphocyte counts were determined from white blood cell (WBC) counts and differential counts using Coulter counter Model S-plus (Coulter, USA),

and the absolute number of each lymphocyte subpopulation was calculated by multiplying the percentage of lymphocyte subpopulation with lymphocyte count.

The results were evaluated for statistical significance by Student's $t$-test.

## RESULTS

*Lymphocyte subpopulations in peripheral blood*

Lymphocyte counts in PBLs from Stage IV and recurrent patients were significantly lower than those from normal healthy controls (p < 0.05)(Table 1). In single color analysis, the absolute numbers and percentages of $CD3^+$ and $CD4^+$ cells in PBLs from Stage III, IV and recurrent patients were significantly lower than those from normal healthy controls (p < 0.05), while those of $CD8^+$ cells were not. In the other hand, the absolute number and percentage of $CD57^+$ cells in PBLs tended to increase as the disease advanced.

**Table 1.** Single Color Analysis of Subpopulations in Peripheral Blood Lymphocytes from Gastric Cancer Patients.

| | Number | WBC counts ($/mm^3$) | Lymphocyte counts ($/mm^3$) | Lymphocyte Subpopulations ($/mm^3$) | | | |
|---|---|---|---|---|---|---|---|
| | | | | $CD3^+$ | $CD4^+$ | $CD8^+$ | $CD57^+$ |
| Control | 10 | 5618.2 ± 1173.7[a] | 1900.0 ± 458.2 | 1283.3 ± 160.8 (63.4)[b] | 768.7 ± 131.7 (43.6) | 326.9 ± 39.9 (21.5) | 226.9 ± 43.5 (13.8) |
| Gastric Cancer | | | | | | | |
| Stage I,II | 10 | 5633.3 ± 1006.6 | 1840.0 ± 495.4 | 1136.5 ± 297.3 (61.4) | 734.2 ± 175.8 (40.2) | 332.6 ± 101.6 (18.6) | 193.2 ± 46.5 (11.4) |
| Stage III | 10 | 6182.2 ± 1736.4 | 1658.3 ± 414.4 | 975.8 ± 165.2 (53.5) | 640.1 ± 122.4* (38.1) | 295.9 ± 96.2 (20.1) | 218.3 ± 79.3 (15.4) |
| Stage IV | 12 | 5547.8 ± 1111.0 | 1368.8 ± 448.3* | 715.6 ± 238.9** (51.6) | 494.9 ± 169.4** (36.4) | 293.1 ± 72.5 (18.4) | 235.2 ± 98.0 (16.5) |
| Rec | 10 | 6500.2 ± 1541.4 | 1416.7 ± 496.5* | 812.9 ± 224.2** (40.8) | 361.8 ± 103.2** (27.5) | 310.5 ± 108.7 (18.3) | 238.7 ± 64.9 (17.8) |

a) Mean ± SD.
b) Mean percentage of each lymphocuyte subpopulation.
Significant differences from the value of control, * p < 0.05, ** p < 0.01.

**Table 2.** Two Color Analysis of Subpopulations in Peripheral Blood Lymphocytes from Gastric Cancer Patients.

| | Number | Lymphocyte Subpopulations ($/mm^3$) | | | |
|---|---|---|---|---|---|
| | | $CD4^+$ $CD45RA^+$ | $CD4^+$ $CD45RA^-$ | $CD8^+$ $CD11b^+$ | $CD8^+$ $CD11b^-$ |
| Control | 7 | 209.7 ± 51.1[a] (12.3) | 520.2 ± 98.5 (30.5) | 83.0 ± 17.1 (5.1) | 218.2 ± 52.4 (14.2) |
| Gastric cancer | | | | | |
| Stage I,II | 10 | 202.4 ± 53.7 (11.8) | 579.9 ± 96.9 (32.6) | 59.3 ± 16.1 (3.3) | 170.8 ± 45.1 (9.1) |
| Stage III | 6 | 126.9 ± 71.9* (7.0) | 498.7 ± 90.6 (29.2) | 60.8 ± 46.4 (3.6) | 145.8 ± 49.9* (7.5*) |
| Stage IV | 6 | 80.8 ± 38.4** (6.3**) | 430.7 ± 86.3 (29.5) | 75.7 ± 30.5 (5.5) | 137.1 ± 43.1* (8.9*) |
| Rec | 7 | 78.3 ± 26.7** (5.3**) | 320.5 ± 64.9** (25.5*) | 74.5 ± 40.5 (5.2) | 124.4 ± 42.1** (8.2*) |

a) Mean ± SD.
b) Mean percentage of each lymphocyte subpopulation.
Significant differences from the value of control, * p < 0.05, ** p < 0.01.

**Table 3.** Subpopulations in Spleen Cells, Splenic Vein Lymphocytes and Peripehral Blood Lymphocytes from Stage III, IV Gastric Cancer Patients.

| Cells | Subpopulations (%) | | | CD4$^+$/CD8$^+$ ratio |
|---|---|---|---|---|
| | CD3$^+$ | CD4$^+$ | CD8$^+$ | |
| Spleen cells (n = 18) | 34.5 ± 8.9$^{a)**}$ | 20.5 ± 5.5$^{**}$ | 17.2 ± 5.2 | 1.29 ± 0.37$^{b)**}$ |
| Splenic v. ly. (n = 11) | 47.5 ± 10.9 | 31.4 ± 8.9 | 17.6 ± 9.4 | 1.79 ± 0.61 |
| Peripheral blood lymphocytes (n = 22) | 52.5 ± 7.9 | 37.2 ± 9.1 | 19.2 ± 5.2 | 1.88 ± 0.56 |

Spleen cells, splenic v. ly. and peripheral blood lymphocytes were obtained from Stage III, IV gastric cancer patients.
a) Mean ± SD. b) Mean ratio ± SD.
Significant differences from the value of peripheral blood lymphocytes, ** $p < 0.01$.

**Table 4.** Ratios of Subpopulations between Spleen Cells, Splenic Vein Lymphocytes and Perpheral Blood Lymphocytes from Stage III, IV Gastric Cancer Patients.

| Cells | CD4$^+$ CD45RA$^+$/CD3$^+$ ratio | CD8$^+$ CD11b$^+$/CD3$^+$ ratio | CD4$^+$ CD45RA$^+$/CD8$^+$ CD11b$^+$ ratio |
|---|---|---|---|
| Spleen cells (n = 10) | 0.27 ± 0.07$^{a)*}$ | 0.10 ± 0.09 | 2.31 ± 0.26$^{**}$ |
| Splenic v. ly. (n = 5) | 0.15 ± 0.04$^{*}$ | 0.15 ± 0.04$^{*}$ | 1.35 ± 0.22$^{**}$ |
| Peripheral blood lymphocytes (n = 10) | 0.20 ± 0.03 | 0.09 ± 0.04 | 1.88 ± 0.36 |

Ratios of each subpopulations were examined in Stage III, IV gastric cancer patients.
a) Mean ratio ± SE.
Significant differences from the value of peripheral blood lymphocytes, * $p < 0.05$, ** $p < 0.01$.

In two color analysis, the absolute number and percentage of CD4$^+$ CD45RA$^+$ cells in PBLs from Stage III, IV and recurrent patients was significantly lower than those from normal healthy controls ($p < 0.05$), while that of CD4$^+$ CD45RA$^-$ cells was not (Table 2). On the other hand, significant decrease of the absolute number and percentage of CD4$^+$ CD45RA$^-$ cells was found in patients with recurrent gastric cancer ($p < 0.05$). Both absolute number and percentage of CD8$^+$ CD11b$^+$ cells in PBLs tended to increase with progression of the disease, while that of CD8$^+$ CD11b$^-$ cells in the advanced cancer patients was significantly lower than those from normal healthy controls ($p < 0.05$).

*Lymphocyte subpopulations between peripheral blood, splenic vein and spleen*

The percentage of CD3$^+$ and CD4$^+$ cells was decreased gradually from PBLs to SVLs, and those in SCs were significantly lower than those in PBLs ($p < 0.01$) (Table 3). The lowest CD4$^+$/CD8$^+$ ratio was observed in the spleen ($p < 0.01$).

*Rations of CD4$^+$ CD45RA$^+$ cells, CD8$^+$ CD11b$^+$ cells to CD3$^+$ cells and CD4$^+$ CD45RA$^+$ cells to CD8$^+$ CD11b$^+$ cells in SCs, SVLs and PBLs*

Because of the differences in the distribution of CD3$^+$ cells among SCs, SPVs and PBLs, CD4$^+$ CD45RA$^+$/CD3$^+$, CD8$^+$ CD11b$^+$/CD3$^+$ and CD4$^+$ CD45RA$^+$/CD8$^+$ CD11b$^+$ ratios were investigated

in the advanced gastric cancer (Table 4). The highest CD4$^+$ CD45RA$^+$/CD3$^+$ ratio was observed in the SCs ($p < 0.05$), while the lowest was in SVLs ($p < 0.05$), and the CD8$^+$ CD11b$^+$/CD3$^+$ ratio was highest in SVLs ($p < 0.05$). SCs showed the highest CD4$^+$ CD45RA$^+$/CD8$^+$ CD11b$^+$ ratio, while SVLs did the lowest ($p < 0.01$).

## DISCUSSION

The impairment of immune responses seemed to be related to changes in the distribution of lymphocyte subpopulations[2,10,15]. It has been shown earlier that the distribution of lymphocyte subpopulations is abnormal in a number of human immunological diseases[2,15] and cancer[6,8,10], as compared with healthy controls. Our present data showed that CD4$^+$ cells in PBLs from advanced gastric cancer patients decreased as the disease progressed, suggesting that the impairment of cell-mediated immune responses may be due to the decrease of CD4$^+$ cells in advanced gastric cancer patients.

CD4$^+$ CD45RA$^-$ cells, which contain helper T cells[12], was comparable to that in normal healthy control in gastric cancer patients at any stages except recurrent patients. Helper functions of PBLs seemed to be maintained even in Stage III, IV patients although there was apparent diminution in

recurrent patients. On the other hand, CD4$^+$ CD45RA$^+$ cells were found to decrease with disease advance. Accordingly, the decrease of CD4$^+$ cells was considered to be due to the decrease of CD4$^+$ CD45RA$^+$ cells. Furthermore, CD4$^+$ CD45RA$^+$ cells located more predominantly in peripheral blood and spleen than in the splenic vein. Herrman et al[6] also reported that accumulation of helper/inducer T cells in spleen resulted in a decrease of such cells in peripheral blood from Hodgkin disease. CD4$^+$ CD45RA$^+$ cells are considered to be most important cells act as suppressor inducer cells which activate the suppressor T cells[12]. From these results, it is postulated that CD4$^+$ CD45RA$^+$ cells might migrate into spleen from peripheral blood and act on suppressor precursors to become mature suppressor cells in spleen. On the other hand, CD8$^+$ CD11b$^+$ cells were more predominantly found in the splenic vein. CD8$^+$ CD11b$^+$ cells, which contain mature suppressor T cells[11], tended to increase in peripheral blood with progress of the disease. Toge et al[18] reported that spleen contained a much higher proportion of suppressor precursors which might be activated to become suppressor cells with advance of the disease. An increasing presence of CD8$^+$ CD11b$^+$ cells with advance of the disease might indicate the increase of mature suppressor cells in peripheral blood.

CD8$^+$ CD11b$^-$ cells have been shown to contain precursor and effector cells of cytotoxic T cells[4]. Spina et al[16] reported that mixed lymphocyte culture-induced cytotoxity was depressed in bladder cancer patients; the decrease of these cells might cause the depression of activity of cytotoxic T cells.

CD57$^+$ cells, which contain natural killer cells[1], tended to increase with advance of the disease. However, Yanagawa et al[19] reported that NK cell activities of PBLs from gastric cancer patients were depressed as compared with those from normal healthy controls. An increasing presence of CD57$^+$ cells may not be directly attributed to the increase of NK cell activities in gastric cancer patients due to the presence of suppressor cells.

Thus it may be postulated that the decrease of CD4$^+$ CD45RA$^+$ cells and CD8$^+$ CD11b$^-$ cells and the increase of CD8$^+$ CD11b$^+$ cells in PBLs is attributable to the impairment of cell-mediated immune responses.

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