Journal of Korean Medical Science
Vol. 10, No. 3, 164-168 June 1995

Peripheral Blood Lymphocyte Subsets in Patients with Stomach Cancer

Weon-Seon Hong, M.D., Young Il Min, M.D., Young Sook Son, Ph. D.,*
Seok-Il Hong, M.D.*

Department of Internal Medicine, Asan Medical Center, University of Ulsan, Seoul, Laboratory of Cell Biology, Korea Cancer Center Hospital, Seoul, Korea*

The present study was conducted in order to investigate the immunologic alterations alongside the numerical changes in peripheral blood lymphocytes(PBL) and their subsets in stomach cancer patients. Lymphocyte surface markers were determined in 85 stomach cancer patients and 49 controls by indirect immunofluorescence technique using monoclonal antibodies. Monoclonal antibodies used were Leu 2a(CD8, suppressor / cytotoxic T cells), Leu 3a(CD4, inducer / helper T cells), Leu 4(CD3, pan T reagent), Leu 11(CD16, natural killer cells) and Leu 12(CD19, B cells). The numbers of PBL, CD3+, CD4+, CD8+, CD16+ and CD19+ cells significantly decreased and the CD4 : CD8 value increased in 85 patients with stomach cancer compared to those in controls($p<0.01$). In stage I($n=17$), neither PBL, their subsets nor the CD4 : CD8 value were significantly different from those of the controls. In stage II($n=17$), the numbers of PBL, CD3+, CD4+ and CD8+ cells decreased($p<0.01$). In stage III($n=24$) and IV($n=27$), PBL and all subsets measured decreased($p<0.01$). The CD4 : CD8 value showed significant increases in stages III and IV($p<0.01$), because the CD8+ cells decreased to a greater extent than did the CD4+ cells. The results demonstrating that the lymphocyte subsets are depressed differentially with the stage suggest that host immunity is impaired with the progression of stomach cancer.

Key Words : *Lymphocyte subsets, Differential depression, Stomach cancer, Stage*

INTRODUCTION

The studies of host response to cancer cells have demonstrated that the antitumor immune system plays a critical role in competing against the growth of cancer cells and is frequently altered in cancer patients especially in advanced stage(Wanebo, 1979 ; Braun and Harris, 1981). As antitumor immunity is known to be mediated mainly by lymphocytes, it is usually determined using the immune parameters reflecting either functional or numerical changes of lymphocytes. Both functional and numerical changes in the peripheral blood lymphocytes(PBL) have been reported in patients with various kinds of cancer, although there have been some conflicting results (Kaszubowski et al., 1980 ; Saijo et al., 1982 ; Dillman et al., 1984 ; Kim and Kim, 1989).

Address for correspondence : *Weon-Seon Hong, M.D., Department of Internal Medicine, Asan Medical Center, University of Ulsan, 388-1, Poongnap-dong, Songpa-gu, Seoul, 138-040, Korea.*
Tel : 82-02-224-3194(3180), Fax : 82-02-476-0824.

Stomach cancer is the leading cause of cancer death in Korea. Our previous study revealed that natural killer(NK) and lymphokine-activated killer(LAK) activity were suppressed in patients with unresectable stomach cancer, suggesting that PBL in advanced stomach cancer have a functional defect in their cytotoxicity to target cells(Hong et al., 1990). We have also reported that the number of lymphocytes and lymphocyte subsets in the peripheral blood decreased in patients with stomach cancer, with the lymphocyte subsets depressed differentially with the progression of stage(Hong et al., 1991). However, the number of patients was too small in each stage to draw a definitive conclusion from the latter study.

The primary concern of this study was to elucidate conclusive results by analyzing the data from a larger number of patients compared with the previous study. In this study, we have determined the lymphocyte subsets by the same monoclonal antibodies and methods as in the previous study and demonstrated that lymphocyte subsets were depressed differentially with the progression of stage.

MATERIALS AND METHODS

Patients

Eighty-five patients with stomach cancer(52 males and 33 females) and 49 age and sex-matched normal controls(22 males and 27 females) were included in this study. The controls were selected from healthy persons who had undergone routine medical examinations, including complete blood count, blood chemistry, urinalysis, chest X-ray and upper gastrointestinal series, the results of which were confirmed as being within normal limits. The age and sex distribution are summarized in Table I. Criteria for entry into the study included : histologically proven adenocarcinoma of the stomach, no previous therapy for the cancer, bilirubin$\leq$2.0 mg/dl, BUN$\leq$30 mg/dl, creatinine$\leq$2.0 mg/dl and no evidence of infection or other major illness. Cancer staging was performed according to the TNM staging groups approved by UICC and AJC(Beahrs, et al., 1988).

Measurement of lymphocyte subsets

Lymphocyte subsets were determined by using the same indirect immunofluorescence technique as previously described(Hong, et al., 1991). Briefly, mononuclear cells were isolated by centrifugation on a Ficoll-Paque(Sigma Chemical Co., St. Louis, MO, USA) density gradient from the heparinized blood diluted with Hank s balanced salt solution(HBSS) (Gibco, Grand Island, NY, USA). The cells collected were washed twice with HBSS and once with Roswell Park Memorial Institute(RPMI)-1640 medium(Gibco) supplemented with 10% heat-inactivated fetal bovine serum(Gibco) (RPMI-FBS) and adjusted to 20×10^6 cells/ml in phosphate buffered saline(PBS) containing 10% AB serum, 2% FBS and 0.02% sodium azide. The cell suspension(50 $\mu\ell$) was mixed with 50 $\mu\ell$ of PBS containing 0.02% sodium azide and monoclonal antibody in three wells of a microtest plate coated with PBS-FBS. The monoclonal antibodies used were Leu 2a(CD8, suppressor/cytotoxic T cells), Leu 3a(CD4, inducer/helper T cells), Leu 4(CD3, pan T reagent), Leu 11(CD16, NK cells) and Leu 12(CD19, B cells) (Becton Dickinson, Mountain View, CA, USA). The plates were then kept for 60 min at 4℃. After the removal of the supernatant, 150 $\mu\ell$ of PBS-FBS and 100 $\mu\ell$ of fluorescein isothiocyanate(FITC)-conjugated monoclonal antibody were added to each well. The cells were then washed twice and resuspended in 30 $\mu\ell$ of PBS containing 25% fluorescein-free glycerol. The lymphocytes having each surface marker were counted using a fluorescence microscope and the results

Table 1. Age and sex distribution in stomach cancer patients and controls

Age(yr)	Control			Stomach cancer		
	Male	Female	Total	Male	Female	Total
20-39	6	7	13	8	6	14
40-49	5	7	12	12	12	24
50-59	8	7	15	20	6	26
60-79	3	6	9	12	9	21
Total	22	27	49	52	33	85
Mean Age	47.4	47.3	47.3	52.5	49.3	51.2
SD	11.8	14.0	13.1	11.4	11.0	11.3

Table 2. Number(/ μℓ) of peripheral blood lymphocytes(PBL) and their subsets in 85 patients with stomach cancer

PBL	CD3+	CD4+	CD8+	CD16+	CD19+	CD4 : CD8
Controls (n=49)						
2728±526[a]	1965±373	1196±229	773±174	468±132	313±101	1.57±0.20
Stomach Cancer (n=85)						
1958±792*	1357±558*	853±336*	516±228*	373±173*	224±125*	1.75±0.30*
Stage I (n=17)						
2694±1239	1900±861	1169±517	736±342	492±253	313±168	1.61±0.18
Stage II (n=17)						
1808±599*	1230±398*	775±249*	473±165*	403±176	262±132	1.67±0.29
Stage III (n=24)						
1941±428*	1343±322*	831±189*	522±138*	358±110*	203±82*	1.80±0.27*
Stage IV (n=27)						
1604±370*	1108±248*	724±178*	400±108*	293±88*	163±66*	1.85±0.34*

a, mean ± SD

*, $P < 0.01$ compared with control value

expressed as percentages. The significance of difference of the results between experimental groups and controls was determined using Student's *t*-test.

RESULTS

The numbers of lymphocytes, CD3+, CD4+, CD8+, CD16+ and CD19+ cells in the peripheral blood of 85 patients with stomach cancer and 49 controls are summarized in Table 2. The PBL and all lymphocyte subsets in the controls were not different between age groups and sex(Fig. 1) (data on sex not shown).

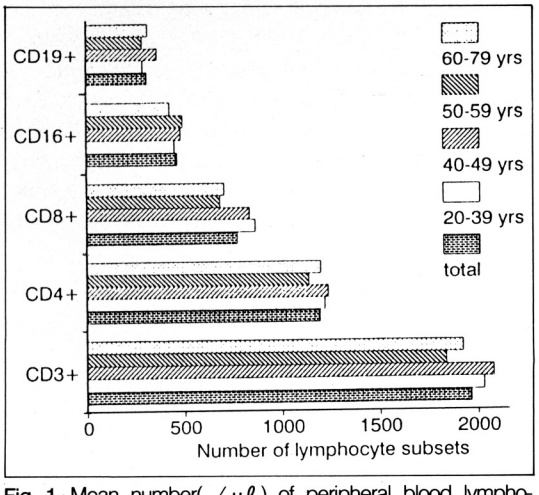

Fig. 1. Mean number(/ μℓ) of peripheral blood lymphocyte subsets in different age groups of 49 controls. No difference in lymphocyte subsets was observed between the age groups.

As shown in Table 2, the numbers of PBL and all lymphocyte subsets measured significantly decreased in 85 patients with stomach cancer. The CD4 : CD8 value increased in stomach cancer, however, because the CD8+ cells decreased to a greater extent than did the CD4+ cells($p<0.01$). Lymphocyte subsets were analyzed with regard to stage. In stage I(n=17), neither the PBL, their subsets nor the CD4 : CD8 value were significantly different from those of the controls. In stage II(n=17), the numbers of PBL, CD3+, CD4+ and CD8+ cells decreased($p<0.01$), while CD16+ and CD19+ cells were not depressed. The CD4 : CD8 value showed an increase in stage III and IV because the extent of decrease in CD8+ cells was greater than that of CD4+ cells($P<0.01$). In stage III(n=24) and IV(n=27), PBL and all the subsets measured decreased($P<0.01$).

DISCUSSION

Increased understanding of antitumor immune parameters in patients with cancer has provided insight into the role of the immune system on the development and progression of cancer. Although we do not know the mechanism that underlies the immunologic alterations completely, it is clear that both functional and quantitative defects in immunity develop with cancer especially in advanced stage(Kaszubowski et al., 1980; Saijo, et al., 1982; Hong, et al., 1990; Hong et al., 1991). To investigate the immunologic alterations from cancer, both functional and quantitative parameters for antitumor immunity have been

measured in patients with a variety of cancers. As a functional parameter, the proliferative activity of PBL in response to certain antigens or mitogens and the cytotoxicity of PBL or PBL activated with a certain compound against target cells have been frequently used. In the previous study, we have demonstrated that NK and LAK activity were suppressed in patients with unresectable stomach cancer, suggesting that PBL in advanced stomach cancer have a functional defect in both their cytotoxic activity against target cells and their responsiveness to interleukin-2 for inducing LAK activity(Hong et al., 1990).

We have also reported that the number of lymphocytes and lymphocyte subsets in the peripheral blood decreased in patients with stomach cancer, with the depression of lymphocyte subsets being related to stage(Hong et al., 1991) : No depression of lymphocyte subsets was observed in stage I. T cells, T and B cells and T, B and NK cells were depressed in stage II, III and IV, respectively, indicating that quantitative change in PBL develops in the advanced stage of stomach cancer. As mentioned in the discussion of the previous paper, one of the problems in interpreting the data was the small number of patients in each stage ; 4, 6, 5 and 16 patients in stages I, II, III and IV, respectively. To get conclusive results, we have to analyze lymphocyte subsets in a large number of patients. In the present study, we have studied a large number of patients and confirmed that PBL and lymphocyte subsets were depressed differentially according to stage : no depression in stage I, T cell depression in stage II, and T, B and NK cell depression in stage III and IV. In the previous study, NK cells were depressed only in stage IV, whereas in the present study, all subsets tested were depressed in stage III and IV. The difference in stages of NK cell depression between the previous and present studies was attributed to the different numbers of patients.

In the present study, lymphocyte subsets were determined by the surface markers expressed on the surface of PBL using the specific monoclonal antibodies. Although significant advances have been made in determining lymphocyte subsets by the identification of lymphocyte surface markers, this method has some limitations in the interpretation of the results(Moretta et al., 1982). For instance, lymphocyte subsets classified by surface markers are not always identical to those functionally classified, such as T cells, B cells and K cells, etc. Moreover, one lymphocyte may have several surface markers and may change as a result of activation. Classification of lymphocyte subsets by surface marker is, in our opinion however, one of the most practical and precise methods currently available for determining lymphocyte subsets.

Two fundamental questions have been present in the field of research for antitumor immunity. The first question is about the relation between immune status and cancer development or progression. The second one is the relation between immune status and prognosis of cancer patients. Numerous previous investigators have reported that general immunity is impaired in patients with advanced cancer(Wanebo, 1979 ; Braun and Harris, 1981). It is, however, not yet clear whether the impairment of the antitumor immunity is a cause of cancer development or a result of cancer progression. In order to obtain information on this point, we have analyzed the results with regard to stage, and demonstrated that the lymphocyte subsets were not depressed in stage I, but depressed differently with progression of the stage. In order to investigate host immune response to cancer cells more distinctly, studies on tumor infiltrating lymphocytes and regional lymph node lymphocytes should be conducted concurrently, since it is not yet clear whether the decrease in subsets is due to an actual depression or is a consequence of lymphocyte subset deposits in the cancer tissue with a corresponding deficit in the subsets in the peripheral blood(Ginns et al., 1982 ; Vose and Moore, 1985).

The alterations in the numerical balance of helper T cells to suppressor T cells, CD4 : CD8 value, is generally accepted as reflecting the balance of immune regulation. The increased CD4 : CD8 value in stage III and IV was due to a decrease in CD8 + cells greater than that in CD4 + cells. Tsuyuguchi et al.(1987) have reported that the CD4 : CD8 value decreased in the early stage of lung cancer and speculated the reduced value to be a causative factor in the development of cancer. Our data, however, support the different hypothesis that the alterations in CD4 : CD8 value is in consequence of cancer progression, because no change of CD4 : CD8 value was observed in stage I and II. Further studies are necessary to find out the role of CD4 : CD8 value and each lymphocyte subset in cancer development and progression.

REFERENCES

Beahrs OH, Henson DE, Hutter RVP, Myers MH. *Manual for staging of cancer, 3rd ed. Sydney : JB Lippincott Co., 1988 ; 69-73.*

Braun DP, Harris JE. *Relationship of leukocyte numbers, immunoregulatory cell function, and phytohemagglutinin responsiveness in cancer patients. J Natl Cancer Inst 1981 ; 67 : 809-14.*

Dillman RO, Koziol JA, Zavanelli MI, Beauregard JC, Halliburton BL, Glassy MC, Royston I. *Immunoincompetence in cancer patients. Assessment by in vitro stimulation tests and quantification of lymphocyte subpopulations. Cancer 1984 : 53 : 1484-91.*

Ginns LC, Goldenheim PD, Miller LG, Burton RC, Gillick L, Colvin RB, Goldstein G, Kung PC, Hurwitz C, Kazemi H. *T-lymphocyte subsets in smoking and lung cancer. Analysis by monoclonal antibodies and flow cytometry. Am Rev Resir Dis 1982 ; 126 : 265-9.*

Hong W-S, Kim C-M, Lee J-O, Kang T-W, Yun T-K, Kim C-Y. *Natural killer and lymphokine-activated killer activities in stomach cancer patients with special emphasis on the effect of 5-fluorouracil, adriamycin and mitomycin-C chemotherapy. Jpn J Clin Oncol 1990 ; 20 : 87-93.*

Hong W-S, Hong S-I, Kim C-M, Kang Y-K, Song J-K, Lee M-S, Lee J-O, Kang T-W. *Differential depression of lymphocyte subsets according to stage in stomach cancer. Jpn J Clin Oncol 1991 ; 21 : 87-93.*

Kaszubowski PA, Husby G, Tung KSK, Williams Jr RC. *T-lymphocyte subpopulations in peripheral blood and tissues of cancer patients. Cancer Res 1980 ; 40 : 4648-57.*

Kim J-O, Kim S-Y. *Changes of peripheral T lymphocyte subsets following treatment in patients with bronchogenic carcinoma. Korean J Int Med 1989 ; 4 : 101-7.*

Moretta L, Mingari MC, Moretta A, Fauci AS. *Human lymphocyte surface markers. Semin Hematol 1982 ; 19 : 273-84.*

Saijo N, Shimizu E, Irimajiri N, Ozaki A, Kimura K, Takazawa T, Niitani H. *Analysis of natural killer activity and antibody-dependent cellular cytotoxicity in healthy volunteers and in patients with primary lung cancer and metastatic pulmonary tumors. J Cancer Res Clin Oncol 1982 ; 102 : 195-214.*

Tsuyuguchi I, Shiratsuchi H, Fukuoka M. *T-lymphocyte subsets in primary lung cancer. Jpn J Clin Oncol 1987 ; 17 : 13-7.*

Vose BM, Moore M. *Human tumor-infiltrating lymphocytes : a marker of host response. Semin Hematol 1985 ; 22 : 27-40.*

Wanebo HJ. *Prognostic significance of immunologic tests in cancer patients. A review. Surg Clin North Am 1979 ; 59 : 323-47.*