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Ferritin-Bearing Lymphocytes in Patients with Malignant Disease

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Abstract The proportion of ferritin-bearing lymphocytes (FBL) in peripheral blood has been proposed as a useful parameter in evaluating the clinical status of patients with breast carcinoma. In the present report, we have determined whether the proportion of FBL may be useful in additional malignant disorders including gastrointestinal malignancies. The proportion of FBL determined by a flow cytometric method was assessed in 20 patients with malignancy (8 with colorectal carcinoma, 6 with gastric carcinoma, 3 with breast carcinoma, and 3 with miscellaneous tumors), 5 patients with benign disease, and 21 healthy controls. A test result was considered positive if the percentage of FBL was greater than 2%. The levels of serum ferritin, carcinoembrionic antigen (CEA), and alpha-fetoprotein (AFP) were also measured in patients with malignancy. Our results show that the FBL test was positive in 10 of 20 patients (50%) with malignancy, 1 of 5 patients (20%) with benign disease, and 4 of 21 (19%) controls. The positive rate in patients with malignancy was significantly greater than that in controls ($p<0.05$). In addition, the percentage of FBL in 8 of 10 patients with malignancy decreased when measured one month after surgery. There was no significant correlation between the percentage of FBL and the levels of serum ferritin, CEA, and AFP.

It is concluded that the percentage of FBL could be useful independent new parameter for malignant disorders including gastrointestinal malignancies.

Key Words : Ferritin-bearing lymphocytes, Parameter for malignant disease, Flow cytometry

Introduction

Moroz et al. have demonstrated that subset of lymphocytes from the peripheral blood of patients with breast carcinoma or Hodgkin's disease bear ferritin on their cell surface membranes (1, 2). The propotion of ferritin-bearing lymphocytes (FBL) was proposed as a useful clinical parameter with breast carcinoma since the more advanced the mal ignant process, the greater the proportion of FBL (3, 4). Moreover, it has been reported that the FBL assay is useful in screening and diagnosis of breast carcinoma (5). It has been suggested that ferritin, a heat-stable, high-molecular-weight, intracellular iron storage protein, may be considered an oncofetal antigen. In clinical studies, the levels of ferritin in the serum of patients with variety of tumors have been shown to be elevated (6, 7). Ferritin has also been linked to immunosuppressive states that involve cellular immunity (8). The immunologic function of FBL remains uncertain, although they have been reported to possess a suppressor cell function (9).

The aim of the present study was to evaluate whether FBL may be useful parameter for additional malignant disorders including gastrointestinal carcinoma.

Subjects and Methods

Subjects

From 1989 to 1990, we studied 21 normal subjects (14 males and 6 females; mean age 30.1 y), 5 patients with benign disease(1 male and females; mean age 54.8 y), and 20 patients with malignant disease (8 males and 12 females; mean age 60.7 y) (Table 1).

Patients with malignancies had not received any anti-cancer therapy before surgery. All 5 individuals with benign disease had cholelithiasis without acute inflammation. Patients with malignant disease underwent surgical therapy in the Department of Surgery II, Yamaguchi University School of Medicine. They included 8 patients with colorectal carcinoma (Dukes A; 2, Dukes C; 5, recurrence; 1), 6 with gastric carcinoma (TNM, Stage 1; 2, Stage III; 4), 3 with breast carcinoma (TNM Stage II; 2, Stage IIIb; 1), and 3 others (retroperitoneal tumor; 1, hepatocellular carcinoma; 1, gall bladder carcinoma; 1), Informed consent was obtained from all patients and controls.

Antibody

Monospecific rabbit antispleen ferritin antibody was purchased from Calbiochem-Behring, La Jolla, CA.

Flow cytometry

Heparinized blood was obtained from patients and controls. An aliquot was incubated in 1 μl of anti-ferritin antibody for 30 minutes at 4℃. After incubation, samples were washed twice in phosphate buffer Solution (FBS), incubated in 20 μl of fluorescein-conjugated goat anti-rabbit immunoglobulin (Zymed Laboratories, Inc. CA) for 30 minutes at 4℃, and lysed by Orthomine (Coulter). After lysis, samples were washed three times and pellets were resuspended in 200 μl of PBS and analyzed on EPICS flow cytometer (Coulter Electronics, Inc., Hialeath, FL), using fluorescence excitation of 200 to 500 mW at nm. For each sample we analyzed 5,000 lymphocytes, which were gated on the basis of forward and side scatter. A test was considered positive if the percentage of FBL was greater than 2%.

Table 1. Patients list.

Subjects	Number of patients
Malignant diseases	20
Colorectal carcinoma	8
Gastric carcinoma	6
Breast carcinoma	3
Others	3
Benign diseases	5
Cholelithiasis	3
Inguinal hernia	1
Chrohn's disease	1
Healthy control	21

Ferritin, carcinoembrionic antigen (CEA) and alpha-fetoprotein (AFP)

Serum ferritin and AFP levels were measured by latex photomnetric immunoassay, and CEA by enzyme immunoassay. The normal range of ferritin, CEA and AFP was less than 104.8ng/ml, 5.0ng/ml, and 10ng/ml, respectively.

Statistical analysis

Student's t-test and Fisher's exact test were used for statistical analysis. A p value less than 0.05 was considered statistically significant.

Results

Percentage of FBL in patients with malignant or benign disease and controls

Ten of 20 patients with a malignancy had more than 2% of FBL as did one of 5 patients with benign disease, and 4 of 21 controls. There was a statistically significant difference between the patients with malignancy when compared to controls ($p<0.05$) (Fig. 1). Of the patients with a malignancy who had more than 2% of FBL there were 5 of 8 with colorectal carcinoma, 4 of 6 with gastric carcinoma, and one of 3 with breast carcinoma.

Relationship between FBL, and serum ferritin, CEA and AFP levels

Patients with malignancy having an abnormal level of ferritin, CEA, or AFP were 5, 5, and 5, respectively. There was no correlation between the percentage of FBL and the serum ferritin, CEA, or AFP levels (Fig. 2).

Change of tbe percentage of FBL following surgery

Ten of 20 patients with a malignancy, including 5 with gastric carcinoma, 3 with colorectal carcinoma, and 2 with breast carcinoma, who did not have complications after curative surgery, were assessed postoperatively for the proportion of FBL. In all patients, except two both with breast carcinoma, the percentage of FBL decreased after surgery (Fig. 3).

Discussion

Moroz et al. reported a subset of ferritin-bearing T-cells present in the blood of patients with breast carcinoma and Hodgkin's disease (1, 2). They also reported that in patients with breast carcinoma those positive for ferritin-bearing lymphocytes (FBL) was 89% in Stage I or II carcinoma while only 37% in benign breast disease (3). Moreover,

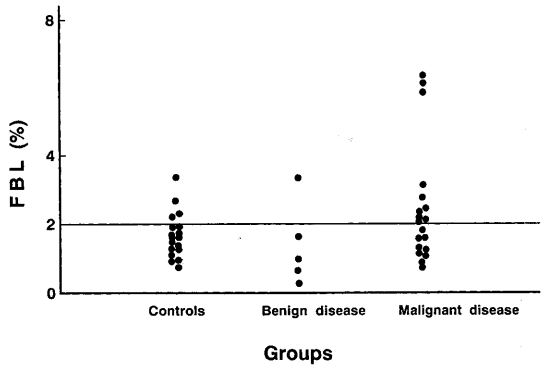

Fig 1. The proportion of FBL in patients with malignant disease and controls. Ten of 20 patients with malignancy had more than 2% FBL, as did one of 5 with benign disease, and 4 of 21 controls. The difference between patients with a malignancy and controls was statistically significant ($p<0.05$).

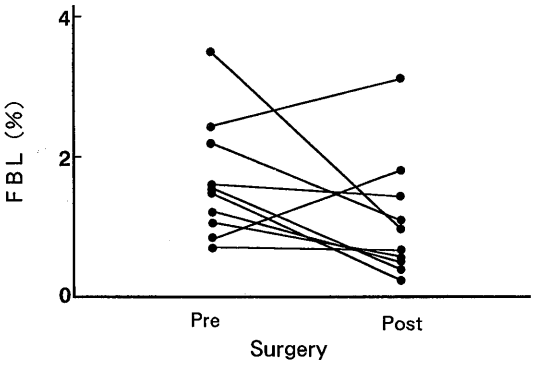

Fig 3. Change in the proportion of FBL postoperatively. Ten of 20 patients with malignancy were assessed. In all patients, except two with breast carcinoma, the percentage of FBL decreased after surgery.

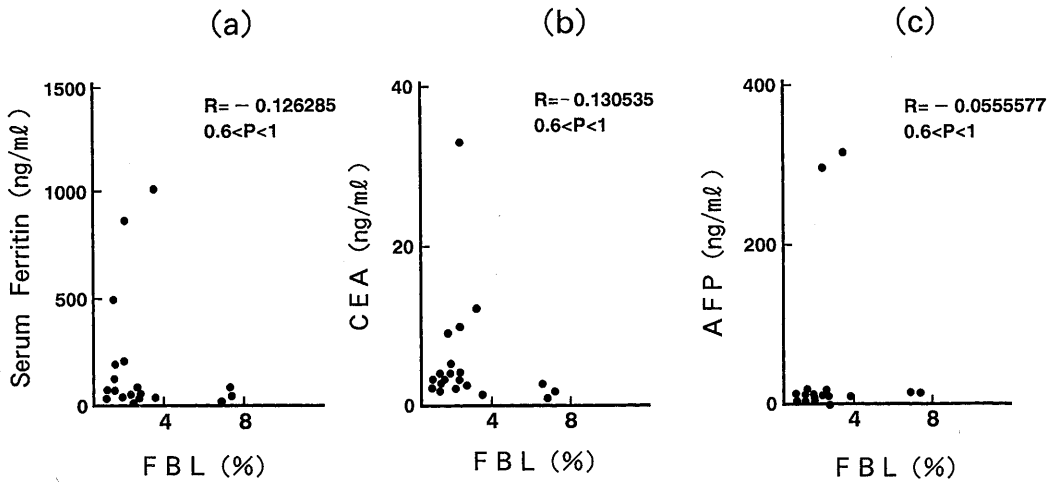

Fig 2. Relationship between FBL and serum ferritin (A), CEA (B) and AFP (C) levels. There was no significant correlation between the percentage of FBL and these other tumor markers.

they demonstrated the usefulness of FBL measurement for the screening, diagnosis, and follow-up of patients with early breast malignancy (5). The percentage of women with a positive FBL and carcinoma in situ, Stage I, or Stage II breast carcinoma, was 100, 77.8, and 66.7% respectively. In our cases, the number was too small to analyse the relationship between FBL index and tumor stages.

There have been few reports of FBL in patients with gastrointestinal malignancies. Papenhausen et al. (10) roported that in 44 patients with carcinomas of either the head and neck (n=26), colon (n=14), or lung (n=4) the mean percentage of FBLs in their peripheral blood was 10% as compared with a value of 3.1% in controls. In this study, eleven of 14 patients with colon carcinoma had more than 5% of FBLs. We report here a value of more than 2% of FBL in 5 of 8 patients with colorectal carcinoma, and 4 of 6 with gastric carcinoma. Thus, the proportion of FBL may be a useful parameter for gastrointestinal malignancies.

It has been reported that the FBL test may be useful for the postoperative follow-up of patients with breast carcinoma (5). After excision of either premalignant or malignant tumors, the positive FBL test became negative in the majority of patients and became positive again upon tumor recurrence and the appearance of metastases indicating an association between the FBL and tumor status (3). In the present report we also demonstrate that in eight of 10 patients, all with gastric carcinoma, the percentage of FBL decreased following surgery.

The function of FBL remains uncertain. Moroz et al. (9) have shown that FBL in breast carcinoma patients may represent a lymphocyte suppressor cell subset because the addition of FBLs to a mixed lymphocyte culture resulted in a decrease in the proliferative capacity of the patients' T lymphocytes. In addition, in vitro treatment of the patients' lymphocytes with levamisole, which removes cell surface ferritin, increased the reactivity of the lymphocytes in mixed lymphocyte culture. Pattanapanyasat et al. (11) observed an increase in HLA-DR positive T-cells, suppressor T-cells, as well as FBLs in patients. On the other hand, Papenhausen et al. (10) found correlation between levels of FBL and rosette-forming T-cells active rosette-forming T-cells in patients with breast cancer. We have not, as yet, identified the precise subset of lymphoid cells to which FBL belong; however, a variety of monoclonal antibodies against T-cells should make this identification possible. Such studies are currently in progress.

The significant elevation of serum ferritin levels in patients with malignancies has been reported (6, 7, 12, 13). However, in a previous study, no significant correlation between FBL and serum ferritin concentration was observed (14). We therefore determined the relationship between the percentage of FBL and other tumor markers such as ferritin, CEA, and AFP. We found there was no correlation between the proportion of FBL and these other tumor markers. This finding suggests that the increased number of ferritin bearing lymphocytes is not secondary to elevated levels of these tumor markers and that proportion of FBLs represents an independent marker of malignant tumorigenesis.

Finally, although the function characteristics of ferritin-bearing lymphocytes remains unresolved, their appearance in peripheral blood seems to be associated with malignant disease and we conclude that the determine of FBL may be useful for gastrointestinal malignancies.

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