S-100 Beta Positive Human T Lymphocytes: Their Characteristics and Behavior Under Normal and Pathologic Conditions

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The characteristics of human S-100 beta-positive T lymphocytes (S-100 beta T cells) and their fluctuation in peripheral blood under normal and various pathologic conditions were investigated. S-100 beta T cells are small lymphocytes with no particular subcellular structures and showed a proliferative response to mitogens. They were present mainly in peripheral blood under normal conditions but accumulated in T zones of lymph nodes with nonspecific T-zone hyperplasia, where numerous interdigitating reticulum cells existed. In healthy adults approximately 1% to 4% (mean 3.4%) of peripheral blood mononuclear cells were S-100 beta T cells. The proportion of S-100 beta T cells in peripheral blood tended to significantly decrease (<0.5%) in patients with neoplastic diseases; this tendency was apparently related to tumor progression.

MATERIALS AND METHODS

Analysis on the Characteristics of Circulating S-100 Beta T Cells

Preparation of Cells

Peripheral blood mononuclear cells (PBM) were obtained from heparinized peripheral venous blood of healthy adults by Ficoll-Hypaque (FH) density gradient centrifugation, as described previously.11 T cells were enriched by E rosetting method as described previously.12 Non-E rosetted-cells were used as a B cell-enriched fraction. T gamma cells (IgG-Fc receptor-positive T cells) were isolated by rosetting technique using IgG-conjugated bovine erythrocytes. Briefly, 1 x 10^6 cells of T cell fraction were incubated with 5 x 10^6 bovine erythrocytes conjugated with rabbit IgG (IBL, Takasaki, Japan) for 30 minutes at 37°C. The rosetted cells were separated from nonrosetted cells by FH centrifugation. The erythrocytes in the rosette positive pellet were lysed with 0.85% Tris-NH4Cl. Complement C3 receptor-positive cells (C3-R cells) were isolated from PBM by rosetting technique with the use of C3b-conjugated bovine erythrocytes (IBL, Takasaki, Japan) in the same manner. Enriched T cells, enriched B cells, T gamma cells (IgG-Fc-R T cells), IgG-Fc-R T cells, C3-R cells, and C3-R cells were examined.

Mitogen Stimulation

PBM were cultured in 10% fetal calf serum-supplemented RPMI 1640 containing 10 μg/mL of concanavalin A (con A, Pharmacia, Uppsala, Sweden) or 20 μg/mL of phytohemagglutinin (PHA-M, Gibco, NY) for three days in a CO2 incubator. The stimulated cells were examined immunohistochemically. The percentage of S-100 beta T cells was compared with that of nontreated PBM cultured for three days without mitogen.

Preparation of Antibodies

Affinity purified rabbit antibodies specific for S-100 alpha or beta subunits were used. The preparation and specificities of these antibodies were described previously.10,11 For T cell staining we used monoclonal antibodies (MoAbs), Leu-1 (Becton Dickinson Monoclonal Center Inc, Mountain View, CA), and MT-1 (Bioscience, Switzerland), which recognize all T cells and monocytes but not B cells. To eliminate monocytes that were also positive for MT-1, we used rabbit antilysozyme antisera (DAKO, Denmark), which could specifically detect monocytes. We used MT-1 so that MT-1 could clearly recognize T cells even after formaldehyde fixation, which was essential for detection of the water-soluble S-100 beta subunit. For S-100 and lysozyme staining, we used peroxidase-antiperoxidase (PAP) method using goat antirabbit IgG (DAKO, Denmark) and peroxidase antiperoxidase complex (DAKO, Denmark). For Leu-1 and MT-1 staining we used a Vectastain ABC kit (Vector Laboratory Inc, Burlingame, CA).

Immunoperoxidase Staining at the Light Microscopic Level

Cells were fixed in 2% paraformaldehyde solution for five minutes at room temperature, washed with phosphate-buffered saline (PBS), and air dried on glass slides. The cells were immersed in -20°C acetone for ten minutes and incubated in methanol containing 0.3%
H$_2$O$_2$ for 15 minutes to block endogenous peroxidase activity. After washing with PBS the cells were incubated successively with 10% normal goat serum and rabbit antibodies against either 5-100 alpha or S-100 beta subunit (15 μg/mL) for 60 minutes. After washing with PBS the cells were stained by PAP method, as described previously. For Leu-I or MT-I staining, cells treated with normal goat serum were incubated with MoAb Leu-I (1:20) or MT-I (1:10) and stained by the ABC method according to the staining procedure of a Vectastain ABC kit.

**Immunoperoxidase Staining at the Electron Microscopic Level**

Affinity-purified antibody to S-100 beta subunit was conjugated with horseradish peroxidase (HRPO) by the method of Nakane and Kawai. Enriched T cells were fixed, stained with the HRPO-conjugated anti-S-100 beta, embedded in Epon 812, and examined as described previously.

**Analysis of Morphology and Distribution of S-100 Beta+ T Cells in Human Lymphoid Tissues**

**Preparation of Lymphoid Tissues**

Each of the five formalin-fixed specimens of normal lymph nodes, thymus, and spleen was obtained at autopsy. Fifteen lymph nodes with nonspecific lymphadenitis showing marked T-zone hyperplasia were obtained by surgical biopsy. Tissues were routinely fixed in formalin and embedded in paraffin.

**Immunoperoxidase Staining of Tissues at Light Microscopic Level**

De-paraffinized sections were stained with rabbit anti-S-100 beta antibody by PAP method as described previously.

**Immunoelectron Microscopic Analysis**

Slices 60-μm thick were cut from formalin-fixed specimens by an Oxford Vibratome. The slices were stained with anti-S-100 beta by indirect immunoperoxidase method using HRPO-conjugated goat antirabbit IgG (DAKO, Denmark), as described in our previous paper.

**Analysis of the Fluctuation of Circulating S-100 Beta+ T Cells**

**S-100 Beta+ T Cells Under Normal and Pathologic Conditions**

**Selection of Patients and Controls**

**Controls.** Healthy adult donors consisted of 38 individuals 20 to 92 years of age, 24 males and 14 females. Of 38 healthy individuals, 24 persons were under 60 years of age (mean = 30 years, younger age group) and 14 persons were over 60 years of age (mean = 76 years, older age group).

**Patients with advanced neoplastic diseases.** Patients of this group had a disseminating or systemic metastasis of the neoplasm. They consisted of 49 individuals 22 to 88 years of age, 25 males and 14 females. Of 38 healthy individuals, 24 persons were under 60 years of age (mean age, 24 to 81 years of age, three males and four females.

**Patients with a postoperative status of gastric cancer.** Patients in this group had received surgical therapy over 2 years before lymphocyte analysis and had a curative status without evidence of metastasis or recurrence. The patients consisted of 16 individuals 30 to 80 years of age, nine males and seven females.

**Patients with non-neoplastic diseases.** Patients consisted of 39 individuals 24 to 81 years of age, 25 males and 14 females, including eight patients with diabetes mellitus; four patients each with chronic active hepatitis, liver cirrhosis, and nephrotic syndrome; three patients each with lower respiratory disease and hypertension; two patients each with systemic lupus erythematosus (SLE) and chronic thyroiditis; and individual patients with chronic pancreatitis, gastroenteritis, polymyositis, progressive systemic sclerosis (PSS), sialoadenitis, splenic infarction, primary myocardiopathy, necroizing lymphadenitis, and cerebrovascular disease. None of the patients received chemotherapy or radiotherapy during the month before lymphocyte analysis. The patients with SLE, nephrotic syndrome, polymyositis, and PSS received extensive steroid therapy.

**Analysis of the Percentages of Circulating S-100 Beta+ T Cells and Total T Cells**

PBM were obtained from 5 mL of heparinized peripheral venous blood of the healthy controls and patients as described above. PBM of all persons were stained with anti-S-100 beta as described above. Besides S-100 beta staining, PBM from patients with gastric cancer, including 13 advanced cases, seven early cases, and 16 cases with postoperative stages, were stained with MT-I and lysozyme to analyze the proportions of total T cells. Both S-100 beta-positive and negative cells were precisely counted by tracing these cells in pictures of nonselected fields until they reached 1,000 in number, and the percentage of the positive cells was calculated. After four counts the average percentage was used as the result. The percentages of total T cells were calculated by counting MT-I positive cells after eliminating lysozyme-positive monocytes.

**RESULTS**

**Characteristics of Circulating S-100 Beta+ T Cells**

**S-100 Subunit Immunoreactivities**

S-100 beta+ cells were detected exclusively in the T cell-enriched fraction. Approximately 4% of T cells were positive for the S-100 beta subunit (Fig 1A). Almost all cells of this fraction were positive for Leu-I, but none was positive for S-100 alpha subunit. No S-100 beta+ cells were detected in the B cell-enriched fraction. Monocytes in the B-enriched fraction were clearly positive for S-100 alpha. These findings indicated that approximately 4% of the human peripheral T cells contained S-100b protein, a dimer of S-100 beta subunit. Therefore we named them S-100 beta+ T cells.

**Response to Mitogens**

The majority of PBM from healthy donors treated with con A or PHA-M for three days displayed a marked blastic change, and some of the blastic cells were clearly positive for S-100 beta. Occasionally S-100 beta immunostaining was found in mitotic cells (Fig 1B). The percentages of S-100 beta positive cells in con A- or PHA-treated PBM were 3.5 or 3.4 respectively and were almost equivalent to that in non-treated PBM (3.3%). These findings indicated that S-100 beta+ T cells had an ability to display a proliferative response.
devoid of the reaction products.

Fig 1. (A) S-100 beta immunoperoxidase staining of T cell-enriched fraction of healthy donors. Approximately 4.0% of T cells of peripheral blood are S-100 beta⁺ T cells (original magnification ×80; current magnification ×60). (B) S-100 beta-immunoperoxidase staining of peripheral blood mononuclear cells stimulated by con A for three days. Note a mitotic cell showing intense S-100 beta immunoreactivity (arrow) (original magnification ×1,000; current magnification ×750). (C, D) Immunoelectron micrographs of S-100 beta⁺ T cells in peripheral blood of healthy donor. S-100 beta⁺ T cells possess narrow cytoplasm with scanty cellular organelles and round (A) or singly indented (B) nuclei (original magnification ×9,300; current magnification ×6,975).

to con A and PHA, and the S-100b protein in the cytoplasm was stable during the proliferative reactions.

IgG-Fc Receptor

S-100 beta immunoreactivity was detected in approximately 5% of T gamma cells. S-100 beta immunoreactivity was also detected in about 3% of IgG-Fc-R⁺ cells. Of 1 × 10⁷ T cells, approximately 20% were IgG-Fc-R positive (T gamma cells), and 80% were IgG-Fc-R negative. From these findings it was estimated that about 30% of S-100 beta⁺ T cells expressed IgG-Fc receptor, but the rest did not.

Complement C3 Receptor

No S-100 beta⁺ T cells were detected in C3-R⁺ fraction. S-100 beta⁺ T cells were found exclusively in C3-R⁻ fraction. These findings indicated that S-100 beta⁺ T cells did not express complement C3 receptor.

Fine Structure of Circulating S-100 Beta⁺ T Cells

Ultrastructurally S-100 beta⁺ T cells in peripheral blood were small, smooth-surfaced lymphocytes containing round or singly indented nuclei and had narrow cytoplasm with scanty cellular organelles (Figs 1C, 1D). Immunoreaction products were evenly distributed throughout the cytoplasm except for mitochondria and vacuoles. Usually nuclei were devoid of the reaction products.

Morphology and Distribution of S-100 Beta⁺ T Cells in Human Lymphoid Tissues

Immunohistochemistry

In each case of normal lymph node, thymus, and spleen, only a small number of S-100 beta⁺ T cells were detected in T zones. Thus under normal conditions S-100 beta⁺ T cells circulated mainly in the peripheral blood. However, relatively large numbers of S-100 beta⁺ T cells were found in lymph nodes with reactive T-zone hyperplasia, and these cells were found exclusively in T zones where numerous S-100 beta⁺ IDC were present (Fig 2A). In addition, it was of great interest that a variety of S-100 beta⁺ cells showing morphological transition from lymphocytes to IDC were frequently observed in these areas (Fig 2B).

Immunoelectron Microscopy

S-100 beta immunoreactivity was detected in IDC and small lymphocytes (Figs 3A, 3C). As shown in our previous study, S-100 beta⁺ IDC possessed considerable cytoplasm with interdigitating projections and irregularly shaped nuclei with evenly distributed nuclear chromatin (Fig 3C). S-100 beta⁺ T cells in lymph nodes were small lymphocytes with singly indented nuclei and narrow cytoplasm without characteristic subcellular structure (Fig 3A). Occasionally small lymphocyte-sized or intermediate-sized cells that possessed
round or irregular nuclei and interdigitating cytoplasmic projections were found to be positive for S-100 beta subunit (Fig 3B).

The Fluctuation of S-100 Beta⁺ T Cells Under Normal or Pathologic Conditions

Healthy Controls

The percentages of circulating S-100 beta⁺ T cells in the peripheral blood of 38 healthy controls ranged from 0.5 to 10.0, and the mean was 3.4% (Fig 4). No sexual difference was detected. As for ages, the mean of the older age group (over 60 years of age) was 2.9%, and that of the younger age group (under 60 years of age) was 3.6%. The former was statistically lower than the latter (t test, P < 0.05). Of 38 healthy controls, only four persons showed less than 1.0%, but none of them showed less than 0.5%. Thus we concluded that a percentage lower than 0.5 indicated an abnormally low proportion of S-100 beta⁺ T cells in peripheral blood.

Patients With Advanced Stages of Neoplastic Diseases

In almost half of the patients (24 of 49), the ranges for S-100 beta⁺ T cells were less than 0.5% (Fig 4). Of these 24 patients, 18 subjects showed less than 0.2%. About two thirds of subjects showed less than 1.0%. The mean was 0.9%, and the lowest percentage was 0.035, which was observed in a patient with gastric cancer. A marked decrease in S-100 beta⁺ T cells was observed in both young and old individuals with advanced cancer but was not correlated with the ages as shown in Fig 4. As for types of the diseases, a percentage of S-100 beta⁺ T cells lower than 0.5 was observed in eight of 13 patients with gastric cancer, in seven of 15 patients with lung cancer (four of six cases with adenocarcinoma, two of four...
patients with squamous cell carcinoma, and one of four patients with small cell carcinoma), in three of seven patients with non-Hodgkin lymphoma, in two of three patients with mammary carcinoma, in one of two patients with hepatocellular carcinoma, and in one of three patients with gallbladder carcinoma, and in single patients each with esophageal carcinoma and laryngeal carcinoma.

Patients With Early or Postoperative Status of Gastric Cancer

In contrast to the patients with advanced gastric cancer, only one of seven patients with early gastric cancer and none of 16 patients with postoperative status of gastric cancer ranged less than 0.5%. No statistical difference in the percentage of S-100 beta T cells was noticed between these groups and healthy controls (Fig 5).

Patients With Non-neoplastic Diseases

Of 39 individuals, seven persons showed less than 0.5%, including two of eight patients with diabetes mellitus, two of four patients with chronic active hepatitis, two of two patients with SLE, and one of three patients with hypertension. In contrast to patients with advanced neoplastic diseases, the majority of this group (29 out of 39) showed more than 1.0% (mean = 2.3%). The highest percentage was 19.0, observed in patients with nephrotic syndrome (Fig 5A).

The Proportions of Total T Cells in Patients With Gastric Cancer

The percentages of total T cells recognized by MT-1 in peripheral blood of these groups ranged from 60 to 85, and no statistical difference was detected among these three groups.

DISCUSSION

Previously we reported that the S-100 positive T lymphocytes expressed T-3, T-8, and T-11 antigens but not T-4, HLA-DR, Leu-7, or OKM1 antigens. In the present study we further characterized the S-100 positive human T lymphocytes. They showed solely S-100 beta subunit immunoreactivity and ultrastructurally were small lymphocytes with no characteristic subcellular structure; they displayed a proliferative response to con A and PHA. These findings further indicated the distinct T cell nature of S-100 beta T cells. The S-100 beta T cells were present mainly in peripheral blood under normal conditions. Approximately 3% to 4% of peripheral blood mononuclear cells were S-100 beta T cells in healthy persons. We also found that the proportion of circulating S-100 beta T cells of healthy persons displayed a tendency to decrease with age. In contrast, a markedly decreased percentage of S-100 beta T cells (less than 0.5%) was observed in nearly 50% of patients.
with advanced neoplastic diseases. The marked decrease in the percentage of S-100 beta+ T cells was not significantly related to age or histologic types of tumors. The present analysis of the patients with various stages of gastric cancer also revealed that such a decrease was apparently related to the advanced stage of the disease. As for T cell profiles, no significant difference in total T cell proportion was observed among the patients with various stages of gastric cancer. Although S-100 beta+ T cells have been shown to belong to the T-8+ cell subpopulation,12 there has, as far as we know, been no report that indicates the proportional decrease of T-8+ cells in cancer patients accompanied by tumor progression, which could account for such a marked proportional decrease of S-100 beta+ T cells. Ginn et al14 reported that in the patients with primary lung cancer, no significant difference in the proportion of total T cells (T-3+), helper/inducer T cells (T-4+), or suppressor/cytotoxic T cells (T-8+) was detected as compared with healthy controls, although their absolute numbers differed according to the histologic types of the tumors. It has been shown that the proportion of T gamma cells may increase in the patients with advanced cancer.15,16 We found, however, that the vast majority of T gamma cells was negative for S-100 beta. Thus it was likely that the proportional fluctuation of S-100 beta+ T cells was independent of those of other types of T cells. Proportional independence of S-100 beta+ T cells from other T cell subsets suggests that their roles are distinct from those of other T cell subsets. It is also possible that such a marked proportional decrease of S-100 beta+ T cells resulted in or from certain immunologic abnormalities in patients with advanced neoplasms. It has been well documented that cancer patients may display various immunodeficiency disorders correlating with stages of disease.15,21 which have generally been considered to be important for tumor cells in escaping from the host's immunologic surveillance system. Therefore it is possible that a marked proportional decrease in S-100 beta+ T cells may be linked to known or unknown immunodeficiency disorders in the patients with advanced cancer.

In this study we found that S-100 beta+ T cells circulated mainly in peripheral blood under normal conditions, but in certain pathologic conditions they accumulated in T zones of lymphoid tissue where numerous IDC were usually present. Moreover a considerable number of S-100 beta-positive cells of various intermediate sizes between lymphocytes and IDC were detected in these areas. They seemed to be in the course of conversion from lymphocytes into IDC. Such coexistence of S-100 positive, small, lymphocyte-like cells and intermediate cells with IDC in T zones was also noticed and reported by Watanabe et al.22 They regarded the S-100 positive, small, lymphocyte-like cells and intermediate cells as precursors of IDC but did not recognize S-100 positive, small, lymphocyte-like cells as T lymphocytes. However, the present immunoelectron microscopic findings clearly indicated that they possessed the distinct morphological features of small lymphocytes. We also detected IL2 receptor on con A-stimulated S-100 beta+ T cells (unpublished finding). As shown in our previous12 and present studies, the S-100 positive, lymphocyte-like cells definitely are T cells, and they form a novel human T cell subset, namely, the S-100 beta-positive T cell subset. Thus the question as to whether S-100 beta+ T cells are precursors of IDC and their related cells should be settled because the origin of IDC still remains undetermined. There are, however, extensive differences between S-100 beta+ T cells and IDC. Namely, IDC and its related cells have been shown to possess HLA-DR, T-6 antigens, complement receptor, ATPase activity, none of which has been proved in S-100 beta+ T cells.12,23 IDC have generally been considered and accepted to be antigen-presenting cells for T cells but not to be cells originating from T lymphocytes.24 At present we have no evidence to support the view that S-100 beta+ T cells can convert into IDC, except for the immunomorphological findings. Further studies should be made to resolve these questions.

Recently Ruco et al25 reported an unusual case of lymph node neoplasm in which tumor cells were positive for S-100 protein and expressed sheep erythrocyte receptor, T-3, T-8, and T-11 antigens. According to their observations, the neoplastic cells with S-100 immunoreactivity undoubtedly were T cells. Thus their particular case of lymph node neoplasm implies the presence in a neoplastic disorder of S-100 beta+ T cells. In fact, we found a patient with a neoplastic proliferation of S-100 beta+ T cells in peripheral blood (unpublished data). Therefore we are considering proposing a new entity of T cell malignancy, namely S-100 beta+ T cell leukemia/lymphoma.

Recently, Nomori et al26 suggested the important role of S-100 positive T-zione histiocytes, IDC, and Langerhans cells in antitumor immunity of humans. They found that the degree of the density of S-100 positive T-zone histiocytes, but not that of ordinary macrophages, in the primary site of nasopharyngeal carcinoma had a significant relationship to prognosis. Together with our findings it is more likely that S-100 beta-positive cells in the human immune system may play important roles in antitumor immunity.

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