Up-Regulation of CD11a (LFA-1) Expression on Peripheral CD4\(^+\) T Cells in Primary Biliary Cirrhosis

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Up-regulation of CD11a expression on CD4\(^+\) T lymphocytes is considered to be one of the mechanisms involved in the initiation of the Th-1-mediated immune response. In this study, peripheral blood mononuclear cells from patients with primary biliary cirrhosis (PBC) were evaluated for CD11a\(^{\text{high}}\)CD2\(^{\text{low}}\) T cells and populations of type 1 (Th-1) and type 2 (Th-2) helper T cells. CD11a\(^{\text{high}}\)CD2\(^{\text{low}}\) T cells were found in PBC (7/15) and in active rheumatoid arthritis (4/4), but not in chronic hepatitis C (0/5) or in healthy subjects (0/6). The population of Th-1 had a positive correlation with that of CD4\(^+\)CD11a\(^{\text{high}}\)CD2\(^{\text{low}}\) cells in patients with PBC (\(P = 0.034\)). The serum levels of interferon-\(\gamma\) also had a weak correlation with the population of CD4\(^+\)CD11a\(^{\text{high}}\)CD2\(^{\text{low}}\) cells (\(P = 0.050\)). There was no statistically significant correlation of Th-2 population (\(P = 0.295\)) or serum interleukin-4 level (\(P = 0.685\)) with the population of CD4\(^+\)CD11a\(^{\text{high}}\)CD2\(^{\text{low}}\) cells. These results suggest that CD4\(^+\)CD11a\(^{\text{high}}\) cells play a role in Th-1-predominance and in the autoimmune process of PBC.

KEY WORDS: primary biliary cirrhosis; lymphocyte function-associated antigen-1; Th-1; interferon-\(\gamma\).

Primary biliary cirrhosis (PBC) comprises several autoimmune features, including the presence of serum anti-mitochondrial antibody (AMA), elevated serum levels of immunoglobulin M (IgM), infiltration of lymphocytes around the injured bile ducts, and frequent coexistence with other autoimmune diseases (1). Chronic nonsuppurative destructive cholangitis (2), a presumed histologic feature of immune-mediated cholangitis, is possibly caused by the attack of autoreactive T lymphocytes. There have been reports on the predominance of Th-1-type cytokines in liver tissue from PBC (3) in addition to the presence of autoreactive T lymphocytes in PBC, such as T-cell clones specific for the pyruvate dehydrogenase complex (PDC–E2 component (4, 5). Polarization of CD4\(^+\) T cells towards type 1 helper T cells (Th-1) has been reported to play a role in the maintenance of experimental autoimmune models, such as experimental allergic encephalomyelitis in mice (6).

Autoreactive lymphocytes are presumably eliminated when they recognize the antigen peptides derived from self-antigens by several mechanisms including immune education during development in the thymus (7). CD11a (lymphocyte function-associated antigen-1 \(\alpha\) chain; LFA1\(\alpha\)) is one of the markers for activated T lymphocytes and acts as a co-stimulatory molecule that enhances the reactivity of T cells to antigen presenting cells (8). In addition, the up-regulation of CD11a may lower the threshold of recognition of cryptic epitopes, ie, antigens ignored un-
nder steady-state conditions (9). One of the mechanisms that may be related to the autoimmune process is considered to be the up-regulation of CD11a expression on CD4+ T lymphocytes. These cells can be detected as CD11a^{high}CD2^{low} cells by flow cytometry (FACS) and can be induced by treatment with inhibitors of DNA methylation (10). CD4+ T cells with this phenotype are demonstrated in the peripheral blood mononuclear cells (PBMCs) obtained from patients with the active stage of rheumatoid arthritis (RA), in which autoreactive T lymphocytes destroy synovial tissue (11). In this study, we evaluated the prevalence of CD4^{+}CD11a^{high}CD2^{low} cells in the peripheral blood of PBC patients and compared them with the Th-1 population to determine if these immunoregulatory cells play a role in PBC.

MATERIALS AND METHODS

Patients. Fifteen patients with PBC (14 women and 1 man, age 56.1 ± 9.2, mean ± SD), 4 patients with RA (2 men and 2 women, age 55.0 ± 16.5), 5 patients with chronic hepatitis C (3 men and 2 women, age 33.0 ± 5.5), and 6 healthy control subjects (all women, age 36.8 ± 8.4) were enrolled in this study. The histologic stage of PBC was determined according to Scheuer (12): four patients were in stage 1, seven patients in stage 2, one patient in stage 3, and one patient in stage 4. This study design was approved by the Ethics Committee of Tohoku University, and informed consent was obtained from every subject.

Autoantibodies tested in patients with PBC were as follows: anti-nuclear antibody (ANA; Mesacup ANA ELISA, MBL Co., Ltd., Nagoya, Japan), anti-mitochondrial antibody (AMA; FluoroAID-1 test, MBL), anti-M2 antibody (M2; Mesacup mitochondria M2, MBL), anti-smooth muscle antibody (ASMA; FluoroAID-1 test, MBL), rheumatoid factor (RF; RA-E test, MBL), anti-double strand DNA antibody (Mesacup DNA-II test “ds” ELISA, MBL), anti-single strand DNA antibody (Mesacup DNA-II test “ss” ELISA, MBL), anti-Sm antibody (Mesacup Sm test ELISA, MBL), anti-SS-A antibody (Mesacup SS-A/Ro test ELISA, MBL), and anti-SS-B antibody (Mesacup SS-B/La test ELISA, MBL).

Reagents. Mouse monoclonal antibodies including PerCP-labeled anti-Cy3 antibody, fluorescein isothiocyanate (FITC) -labeled anti-CD11a antibody, phycoerythrin (PE) -labeled anti-CD2 antibody, FITC-labeled anti-interferon-γ monoclonal antibody, and PE-labeled anti-interleukin 4 (IL-4) monoclonal antibody together with isotype-matched control antibodies were obtained from Becton Dickinson (Mountain View, California, USA). 1,3-phorbol myristate acetate (PMA), ionomycin, brefeldin A, and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA).

Staining of Interferon-γ and Interleukin-4 in CD4+ T Cells. Heparinized blood was obtained and PBMCs were separated by Ficoll-Paque density gradient centrifugation (Pharmacia, Upsalla, Sweden). PBMCs were activated and stained following the manufacturer’s instructions with minor modifications. Briefly, PBMCs were first stimulated with PMA (25 ng/ml) and ionomycin (1 μg/ml) in the presence of brefeldin A (10 μg/ml) for 4 hr at 37°C in a CO₂ incubator. After incubation, cells were stained with PerCP-labeled anti-CD4 monoclonal antibody for 15 min at room temperature. Subsequently, cells were fixed and permeabilized with FACS lysis solution (Becton Dickinson) and FACSCalibur staining buffer (Becton Dickinson). After washing with PBS containing 0.1% bovine serum albumin, cells were stained with FITC-labeled anti-interferon-γ and PE-labeled anti-IL-4 monoclonal antibodies for 30 min at room temperature. CD4+ cells positive for interferon-γ (IFN-γ) and negative for IL-4 were considered to be Th-1 cells, and those negative for IFN-γ and positive for IL-4 were considered to be Th-2 cells.

Serum Levels of Cytokines and sICAM-1. Serum samples were simultaneously obtained and stored at −80°C until use. Serum concentrations of IFN-γ (detection limit: 16 pg/ml) and IL-4 (detection limit: 16 pg/ml) were measured using ELISA kits (Predicta human IFN-γ and Predicta human IL-4, both from Genzyme, Cambridge, Massachusetts, USA). Serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) were determined by a commercial ELISA kit (An’Alaya human soluble ICAM-1 immunoassay, Techne Co., Minneapolis, Minnesota, USA).

Staining of CD11a and CD2 on PBMCs. Separated PBMCs (1 × 10⁶ cells) in PBS containing 0.1% bovine serum albumin were placed in Falcon 6-ml culture tubes (Becton Dickinson) and incubated with PerCP-anti-CD4 for 15 min on ice. After three washes in PBS, cells were incubated with FITC-anti-CD11a and PE-anti-CD2 for 20 min on ice. Cells were washed three times in PBS and then fixed with 1% paraformaldehyde in PBS. The cells were analyzed for the expression of CD11a and CD2 by FACS-Calibur (Becton Dickinson) with CellQuest software (Becton Dickinson). The subset of CD4+CD11a^{high}CD2^{low} was defined as a relative increase in CD11a fluorescence against CD2 fluorescence (11) in the CD4+ T cells (Figure 1a–c).

Statistics. The Kruskal Wallis test was used to compare the serum titer of each autoantibody between the patients with CD11a^{high}CD2^{low} and those without CD11a^{high}CD2^{low} using SPSS for Windows ver. 9.0 software (SPSS Inc., Chicago, Illinois, USA). The correlation between the positive percent of CD11a^{high}CD2^{low} cells and the serum levels of cytokines and the Th-1, Th-2 populations in patients with PBC was evaluated with the linear regression test.

RESULTS

Prevalence of Cells with CD11a^{high}CD2^{low} Phenotype. In all the patients with active RA, a distinct fraction of CD11a^{high}CD2^{low} cells was detected by flow cytometry (11.40 ± 7.10%, mean ± SD, Figure 1a). A fraction of cells with CD11a^{high}CD2^{low} phenotype was also found in PBMCs from PBC patients (6.21 ± 5.48%, Figure 1b). On the other hand, the cells with CD11a^{high}CD2^{low} phenotype were observed...