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Increased apoptosis of circulating T cells in myelodysplastic syndromes

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Abstract

The mechanism of T cell lymphopenia in myelodysplastic syndromes (MDS) is unknown. We investigated apoptosis in freshly isolated and cultured lymphocytes; the latter were used to detect cells not yet apoptotic but destined for apoptosis. Apoptosis increased in both fresh and cultured T cells in MDS compared with those from healthy controls. Furthermore, in lymphopenic MDS patients the lymphocyte count correlated negatively with the degree of T cell apoptosis. MDS T cells showed increased Fas expression. However, in MDS but not in controls, the degree of T cell apoptosis was independent of the Fas expression level, and exogenous anti-Fas antibodies did not modulate T cell apoptosis. Mechanisms other than the Fas–Fas ligand pathway may induce T cell apoptosis in MDS.

Keywords: Apoptosis; Myelodysplastic syndromes; T cell; Fas–Fas ligand pathway

1. Introduction

Myelodysplastic syndromes (MDS) are malignant disorders of hematopoietic cells with poor prognosis that typically occur in the elderly and often transform into acute myeloid leukemia (AML) [1]. In each MDS patient, clonal hematopoietic progenitors show varying degrees of differentiation to myeloid cells. However, mainly due to apoptosis of partially or fully differentiated hematopoietic cells and insufficient differentiation capacity of the progenitors, anemia, neutropenia, and/or thrombocytopenia occur in MDS [2,3]. Although lymphocytes are not involved in a malignant clone in most MDS cases, lymphopenia is a common finding in MDS [4,5]. The immunological competence of hosts is considered to be important to protect against MDS clones, which is supported by the following observations. Immunosuppressive therapies increase mature myeloid cells in some MDS cases, however, the increased myeloid cells are thought to be clonal in origin [6]. MDS clones develop in a substantial proportion of patients with aplastic anemia who were immunosuppressed after treatment with anti-thymocyte globulin [7].

Lymphopenia in MDS is mainly due to a decrease in the number of T cells [4,5]. Few studies examined the mechanisms explaining T lymphopenia in MDS. Hamada et al. examined morphologically identifiable apoptosis of total lymphocytes in the peripheral blood (PB) [8]. They showed that, although the difference was statistically insignificant, a higher percentage of MDS lymphocytes exhibited apoptosis compared with normal lymphocytes. Amin et al. examined bone marrow (BM) samples and reported that apoptosis was increased in B cells but not in T cells in MDS [9]. In the present study, using PB samples, we examined T cell apoptosis in freshly isolated cells and cells cultured for 1–4 days; the latter system was used to detect cells preprogrammed in vivo to undergo apoptosis. We showed that T cell apoptosis

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was increased in both freshly isolated and cultured cells from MDS patients compared with cells from healthy controls. Furthermore, in lymphopenic MDS patients the lymphocyte count correlated negatively with the degree of T cell apoptosis. The increase in T cell apoptosis was more marked in MDS patients who had a high score on the International Prognostic Score System (IPSS) compared with other patients. We also investigated the mechanism of T cell apoptosis in MDS.

2. Materials and methods

2.1. Patients and controls

Twenty-three patients with MDS, diagnosed according to the French–American–British (FAB) criteria [10] and treated at our institution, were enrolled in this study. Eight had refractory anemia (RA), 1 refractory anemia with ringed sideroblasts (RARS), 7 refractory anemia with excess blasts (RAEB), and 7 RAEB in transformation (RAEB-t). Their median age was 64.5 (range 29–86) years. Patients with secondary MDS and those who had had infections within 1 month before this study were excluded. Cytogenetic analyses were performed using standard G-banding with trypsin-Giemsa staining. Karyotypes were interpreted using the International System for Cytogenetic Nomenclature criteria [11]. The IPSS of each MDS patient was determined according to the report by Greenberg et al. [12]. The control group comprised 25 healthy volunteers. We did not age-match the controls and MDS patients in this study because we confirmed that the degree of T cell apoptosis was not associated with age in preliminary experiments (upper part of Supplemental Table 1). This was also confirmed in MDS patients (see Section 3). The procedures followed were in accordance with the ethical standards of the Institutional Committee on Human Experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

2.2. Cell separation

Heparinized PB was obtained from the patients and controls after informed consent had been obtained. PB mononuclear cells (PBMCs) were prepared using Ficoll-Hypaque density-gradient centrifugation (Sigma, St. Louis, MO, USA). A portion of the PBMCs was immediately analyzed for apoptosis with flow cytometry (FCM). The remaining cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FCS), 1 mM L-glutamine, 100 μg/ml penicillin, and 100 μg/ml streptomycin (designated complete medium in this paper) at a concentration of 5×10^5 cells/ml. On days 1 and 4 of culture, the cells were analyzed for apoptosis using FCM to detect cells preprogrammed in vivo to undergo apoptosis (spontaneous apoptosis), as described previously [13]. In some experiments, $CD3^+$ lymphocytes were isolated by magnetic cell sorting as described previously [14].

2.3. Flow cytometry

Cells were treated with human immunoglobulin to block nonspecific binding before antibody staining. To detect lymphocyte apoptosis, the cells were stained with one of the PE-conjugated mouse monoclonal antibodies (mAbs) to human lymphocyte antigens, fluorescein isothiocyanate (FITC)-conjugated annexin V and propidium iodide (PI) (Trevigen, Gaithersburg, MD, USA), according to the manufacturer's instructions. To examine Fas (CD95) expression, the cells were stained with one of the FITC-conjugated mouse mAbs to human lymphocyte antigens and PE-conjugated mouse mAb to human CD95 (BD PharMingen, San Diego, CA, USA). To examine Fas ligand (FasL) expression, the cells were successively reacted with purified mouse anti-human FasL antibody (BD PharMingen), second antibody (FITC-conjugated anti-mouse IgG), and PE-conjugated mAbs to one of the human lymphocyte and monocyte antigens. The mAbs to human lymphocyte and monocyte antigens included antibodies to human CD3, CD4, CD8, CD14, CD19, CD25, and CD56 (BD PharMingen). FCM was performed with the standard method, as described in our previous report [15].

Single-labeled cells were used to compensate for the fluorescence emission overlap of each fluorochrome into inappropriate channels. Isotype-matched negative controls were used in all assays. Analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA, USA).

2.4. Induction of apoptosis via the Fas–Fas ligand pathway and by activation via CD3 receptor signaling

To induce apoptosis through the Fas–FasL pathway, an agonistic anti-Fas mAb, CH11 (MBL, Nagoya, Japan), was added to the cultures of PBMCs or Jurkat cells (Riken Cell Bank, Tsukuba, Japan) at a final concentration of 400 ng/ml. Jurkat cells have been confirmed to be sensitive to Fas-mediated apoptosis [16]. To block apoptosis through the Fas–FasL pathway, an antagonistic anti-Fas mAb, ZB4 (MBL, Nagoya, Japan), was added to the cultures at a final concentration of 2 μg/ml. Concentrations of these antibodies were determined to be optimal in preliminary experiments. To examine activation-induced T cell apoptosis, culture dishes were precoated with anti-CD3 mAb (CRIS7) (NeoMarkers, Fremont, CA, USA) according to the method previously described [17]. Then PBMCs were cultured in the dishes for 1 or 2 days.

2.5. Analysis of caspase-3 and -8 activation

PBMCs, which had been activated via CD3-mediated signaling for 1 day as described above, were subjected to caspase-3 analysis using a flow cytometric kit (BD PharMingen). In brief, the cells were stained first with FITC-conjugated anti-CD3 antibody, washed, and then stained for the active form of caspase-3 according to the manufacturer's instructions. For caspase-9 analysis, purified $CD3^+$ T cells