A number of phenotypic and functional alterations in T cells of MM patients have been reported. Some of them, such as the expansion of HLA-DR+ T cells, reflect a general state of activation induced by tumor growth. HLA-DR+ T cells are nearly independent of accessory signals for activation and produce high amounts of interleukin (IL)-2 and interferon (IFN)-γ upon in vitro multivalent crosslinking of the CD3/CD28 receptor (T cell receptor) complex.\textsuperscript{2,3} Other alterations, such as the restricted usage of Vα and Vβ segments,\textsuperscript{4,5} the presence of Idiotypic (Id)-reactive HLA-DR+ T cells,\textsuperscript{6} and the possibility of growing T cell clones after stimulation with IL-2 and Id-derived F(ab′)\textsubscript{2} fragments,\textsuperscript{7} indicate the existence of specific interactions between host and tumor cells. Despite the evidence of activation and immune recognition, T cells are unable in vivo to hold in check the disease. In particular, it is intriguing that peripheral blood counts of HLA-DR+ T cells (including Id-reactive cells) are negatively correlated with diagnosis and disease status.\textsuperscript{8}

Activated T cells have been reported to display an increased susceptibility to apoptosis,\textsuperscript{7,10} a form of programmed cell death occurring in the absence of inflammation that leads to the fragmentation of genomic DNA.\textsuperscript{11} Accumulating evidence indicates that apoptosis is involved in the regulation of cellular immune responses.\textsuperscript{9,10} A number of molecules that might regulate apoptosis have been identified in T cells, including the Fas (CD95) antigen and the bcl-2 protein.\textsuperscript{12}

Molecular cloning has shown that the former belongs to the tumor necrosis factor receptor (TNF-R)/nerve growth factor receptor (NGFR) family\textsuperscript{13}; its engagement by specific monoclonal antibodies (MoAbs) transduces apoptotic signals that result in the death of Fas+ cells.\textsuperscript{14} The protein coded by the bcl-2 protooncogene was originally discovered to be overexpressed in follicular lymphomas at the breakpoint of t(14;18) chromosomal translocation.\textsuperscript{15} It is widely distributed in lymphoid and hematopoietic cells and promotes cell survival by preventing apoptosis.\textsuperscript{16} The determination of Fas and bcl-2 levels in individual cells may thus help to predict their susceptibility to apoptosis.

A number of methods have been used to assay apoptosis, but all of them suffer from limitations, especially when apoptosis is restricted to a proportion of cells in mixed populations. Under these conditions, a reliable identification of apoptotic cells requires a combination of assays and confirmation by the qualitative analysis of DNA fragmentation by gel electrophoresis.

In this study we have investigated in purified T cells of multiple myeloma (MM) patients the expression of Fas and bcl-2 antigens and the susceptibility to spontaneous and triggered apoptosis using two independent methods and DNA gel electrophoresis. The aim of this work was to determine to what extent the activation state occurring in MM might influence the expression of Fas and bcl-2 antigens and the susceptibility to apoptosis in peripheral blood T cells.

**MATERIALS AND METHODS**

**Patients.** Forty-seven MM patients entered this study from November 1993 to December 1994. MM was diagnosed as previously reported.\textsuperscript{17} According to the Durie and Salmon MM staging system, 12 MM patients were classified as stage II, and 35 were stage III; nine were substage B. Twenty-nine were IgG, 12 IgA, and six Bence Jones myeloma. Twelve patients were evaluated at diagnosis; 35 were on first- or second-line chemotherapy according to the Italian Myeloma Study Group protocols. All patients receiving treatment were studied at least 3 weeks after the last day of chemotherapy.
Patients were not on antibiotics, did not have infections, and had not received transfusions for at least 10 days before the study. As the median age of the MM patients was above 50 years and Fas+, CD45RO+ T cells have been reported to increase with advancing age,\textsuperscript{29} the control group was age-matched and consisted of 30 normal volunteers. Experiments were designed in such a way that cells from at least one normal control were always studied simultaneously with T cells from the patients.

Cell preparation. Peripheral blood mononuclear cells were obtained by density gradient centrifugation (Ficoll-Hypaque) of heparinized venous blood.\textsuperscript{3} Monocytes were extensively removed to prevent the clearance of apoptotic cells by phagocytosis.\textsuperscript{19} The carbonyl-iron method and the lysosomotropic compound L-leucine methyl ester were used to deplete monocytes, as previously reported.\textsuperscript{3} T lymphocytes were isolated by rosetting with sheep erythrocytes at 29°C for 1 hour to exclude the majority of CD3+, CD2+ rosette-forming cells. Cells forming rosettes (T lymphocytes) were isolated from nonrosetting T cells on a Ficoll-Hypaque density gradient.

The standard medium was RPMI 1640 (GIBCO, Milan, Italy) containing 2 mmol/L glutamine, penicillin (100 U/mL), and streptomycin (100 \mu g/mL).

Antibodies and reagents. The following MoAbs were used: fluorescein isothiocyanate (FITC)-conjugated Leu4 (CD3e chain, IgG), phycoerythrin (PE)-conjugated anti-HLA-DR (IgG), FITC-conjugated goat antimouse as the second layer, and FITC-conjugated anti-Fas MoAb (quadrants 1, 2, 3, and 4). The immunocytofluorometric method identifies apoptotic cells on the basis of peculiar forward (FSC) and side light scatter (SSC) settings, as first described by Pannuti et al.\textsuperscript{29} In some experiments, normal T cells were exposed to saponin, cells were incubated with 0.5 \mu g/mL anti-bcl-2 MoAb in PBS for 30 minutes on ice. After further washing, cells were analyzed with a fluorescence-activated cell sorter (FACS; FACScan, Becton Dickinson). FITC- and PE-conjugated mouse myeloma proteins of the appropriate subclasses were used as negative controls. To evaluate triggered apoptosis, T cells were split for measurement of apoptosis. In some experiments, normal T cells were exposed to 6 Gy from a 60Co radiation source as a positive control for apoptosis.\textsuperscript{29}

Measurement of apoptosis. Apoptosis was assessed with an immunocytofluorometric method detecting changes in morphology and the decreased expression of surface molecules,\textsuperscript{19} and with the cytofluorometric analysis of hypodiploid DNA labeled with PL2* (Reg A) (see Figs 2 and 5). The immunocytofluorometric method identifies apoptotic cells on the basis of peculiar forward (FSC) and side light scatter (SSC) changes, reflecting their smaller size and their increased granularity, and on the basis of decreased expression of surface molecules such as CD45.\textsuperscript{3} This method allows the identification of apoptotic cells in mixed populations as well as their immunophenotyping. FACS settings were specifically focused on the lymphocyte region by incrementing the amplification gain of the FSC photodiode and both the amplification gain and the excitation voltage of the SSC photomultiplier, as to delimit the regions of viable (Reg V) and apoptotic cells (Reg A) (see Figs 2 and 5). In some experiments, cultured T cells were double-stained for CD45/HLA-DR and CD4/CD8 to determine simultaneously the proportion of apoptotic cells and the expression of these antigens in Reg V and Reg A.

PI staining was performed with a modification of the method by

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<th>Table 1. Fas and bcl-2 Expression in T Cells of Normal Donors and MM Patients by Percentage of Activated (HLA-DR+) T Cells</th>
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Fas+ and bcl-2+ cells were identified by cytofluorometric analysis using FITC-Leu4 (CD3), anti-Fas + FITC-goat antimouse, and FITC–bcl-2 as reported in Materials and Methods. MM patients were divided into groups by percentage of HLA-DR+ T cells in the peripheral blood. Percentages ± SD were: 6% ± 2% (group A), 18% ± 3% (group B), 25% ± 4% (group C), and 55% ± 15% (group D). HLA-DR+ T cells in the controls were 11% ± 6%. Data for means and ranges indicate percentages of positive cells. P values were calculated with the nonparametric Mann-Whitney U test.

Abbrefering: NS, not significant.
Fig 1. HLA-DR+ expression in MM T cells is associated with Fas upregulation and bcl-2 downregulation. T cells from a normal donor (A through C) and two representative MM patients with low (D through F) and high (G through I) numbers of HLA-DR+ T cells are shown. A two-color cytofluorometric analysis using FITC-Leu4 (CD3), PE-anti-HLA-DR, anti-Fas + FITC-conjugated goat antimouse, and FITC-bcl-2 was performed as reported in Materials and Methods.

Nicoletti et al. Briefly, $2 \times 10^5$ T cells were washed in PBS and resuspended in 0.8 mL of hypotonic fluochrome solution (50 $\mu$g/mL PI, 0.1% sodium citrate, 0.1% Triton X100). After gentle mixing, cells were incubated at RT for 15 minutes in the dark. The PI fluorescence of stained DNA was measured with FACScan without further washings. FACS settings were designed to identify a distinct hypodiploid DNA region (apoptotic region, Reg A) below the diploid G0/G1 DNA peak, as shown in Fig 3.

DNA fragmentation. Qualitative DNA analysis was performed by agarose gel electrophoresis to show DNA fragmentation in apoptotic cells. DNA fragmentation was evaluated in unfractionated samples at the end of the cultures or after separation of Reg A and Reg V cells by density gradient centrifugation (1,400 rpm for 15 minutes). Cells were pelleted by centrifugation and kept at $-20^\circ$C until assessed for DNA fragmentation. Cells were resuspended in 0.5 mL of 10 mmol/L Tris-HCl (pH 7.5), 1 mmol/L EDTA, 0.15 mol/L NaCl, 1% sodium dodecyl sulfate (SDS), and 0.2 mg/mL of proteinase K. After incubation for 3 hours at 37°C, high molecular weight (mol wt) DNA was extracted with phenol-chloroform, ethanol-precipitated, vacuum-dried, dissolved in 10 mmol/L Tris-HCl.
respectively, the two-tailed, nonparametric Mann-Whitney U test for unpaired samples: group A, less than 10%; group B; it increased further in group C and reached the highest value in group D. These data indicate that MM T cells are highly heterogeneous in terms of HLA-DR expression. Fas and bcl-2 expressions were evaluated in fresh MM T cells and the controls were evaluated with the two-tailed, nonparametric Mann-Whitney U test for unpaired samples: P < .05 was considered significant.

RESULTS

Dysregulated Fas and bcl-2 expression in MM. The percentage of Fas+ cells was significantly higher and that of bcl-2+ cells significantly lower in MM T cells compared with the controls (Fas+: 63% ± 27% vs. 24% ± 16%, respectively, P < .0001; bcl-2+: 67% ± 26% vs. 82% ± 12%, respectively, P = .03; Table 1).

Correlation between Fas, bcl-2, and HLA-DR expression. MM T cells are highly heterogeneous in terms of HLA-DR expression.1,3 Fas and bcl-2 expressions were evaluated in MM cells divided into four groups according to the proportion of HLA-DR+ T cells: group A, less than 10%; group B, 10% to 20%; group C, 20% to 30%; and group D, greater than 30%; (Table 1). The percentage of Fas+ cells was already significantly higher than the controls in groups A and B; it increased further in group C and reached the highest value in group D. Expression of bcl-2 was not different in groups A and B; it was significantly lower in group C and reached the lowest value in group D. These data indicate that MM cells with the highest proportion of circulating HLA-DR+ T cells also present the highest and the lowest proportion of Fas+ and bcl-2+ cells, respectively.

Two-color cytofluorometric analysis was performed on freshly isolated T cells to further characterize in individual cells the relationships between HLA-DR, Fas, and bcl-2 antigens. Samples from two representative MM patients are shown in Fig 1 to demonstrate the inversion correlation between Fas and bcl-2 expression.

Increased susceptibility to apoptosis in MM. Susceptibility to apoptosis was then investigated in MM T cells with dysregulated Fas and bcl-2 expression. In a first series of experiments, susceptibility to spontaneous apoptosis was investigated after 18-hour incubation in RPMI + 1% FCS at 37°C. Percentages of apoptosis cells were significantly higher in MM than in the controls (Table 2). Similar results were observed in 10 experiments where MM and normal T cells were incubated in RPMI + 10% FCS, and percents of apoptotic cells were determined with the immunocytometric assay based on their hypodiploid DNA content, as reported in Materials and Methods.

Data for means and ranges indicate percentages of apoptotic cells. P values were calculated with the nonparametric Mann-Whitney U test for unpaired samples.

Table 2. Spontaneous and Triggered Apoptosis in Purified T Cells of MM Patients and Normal Controls

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<td>Control</td>
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T cells were incubated for 18 hours in RPMI + 1% FCS (spontaneous apoptosis), RPMI + 10% FCS in the presence of 1.5 μmol/L methylprednisolone (MP) or 1:500 final dilution anti-Fas MoAb (triggered apoptosis). The immunocytometric assay detects apoptotic cells based on changes in morphology and the decreased expression of surface molecules, as reported in Materials and Methods. The PI staining assay detects apoptotic cells based on their hypodiploid DNA content, as reported in Materials and Methods.

In a second series of experiments, susceptibility to triggered apoptosis was investigated using MP and anti-Fas MoAb as apoptotic agents. Again, MM T cells displayed a higher susceptibility to apoptosis than normal T cells (Table 2). Results from a representative MM patient are shown in Fig 2. The increased susceptibility of MM T cells to spontaneous and triggered (1.5 μmol/L MP) apoptosis was maintained over a 4-day incubation period (data not shown).

To directly evaluate HLA-DR expression in apoptotic cells, T cells were double-stained with anti–HLA-DR and anti-CD45 MoAbs, and the expression of these antigens together with cell size and granularity were compared in Reg A and Reg V of the same sample. Results from a representative experiment are shown in Fig 3. T cells from Reg A showed the typical features of apoptotic cells, including smaller size (Fig 3A), increased granularity (Fig 3B), and decreased CD45 expression (Fig 3C). These cells also showed increased HLA-DR expression compared with T cells from Reg V (Fig 3D).

Some experiments were performed to determine whether cells gated in Reg A presented the typical DNA fragmentation of apoptotic cells. These cells were enriched by density gradient centrifugation onto a Ficoll-Hypaque gradient as previously reported.21 Dense cells recovered from the pellets and light cells recovered at the interphase were reanalyzed by FACS and fell in Reg A and Reg V of unseparated samples, respectively. In six experiments, the percentages of apoptotic and viable cells were 15% ± 3% and 78% ± 4%, respectively, in unseparated samples; 6% ± 2% and 82% ± 3%, respectively, in the light cell fraction (Reg V); and 76% ± 3% and 10% ± 1%, respectively, in the dense cell fraction (Reg A).
ENHANCED T-CELL APOPTOSIS IN HUMAN MYELOMA

Fig 2. Effect of anti-Fas and MP on purified T cells from a representative normal donor (A through D, I through N) and MM patient (E through H, O through R). T cells were incubated for 18 hours in RPMI + 1% FCS (A, C, E, G, I, M, O, Q) or in the presence of anti-Fas MoAb (1:500 final dilution) (B, D, F, H) or 1.5 μmol/L MP (L, N, P, R). The immunocytometric (A, B, E, F, I, L, O, P) and PI staining assays (C, D, G, H, M, N, Q, R) were used to identify apoptotic cells on the basis of their morphologic changes and hypodiploid DNA content. Reg A and Reg V denote regions of apoptotic and viable cells, respectively.

and gel electrophoresis. As positive controls, DNA extracted from normal T cells exposed to 6 Gy radiation and from thymocytes were included (Fig 4). The typical DNA ladder of apoptotic cells was observed in the dense but not in the light cell fraction, indicating that cells of Reg A only had undergone DNA fragmentation. Similar results were observed when DNA was extracted from MM T cells treated with anti-Fas MoAb and MP (data not shown).

**IL-2–induced recovery of spontaneous apoptosis in MM T cells.** IL-2 has been reported to prevent apoptosis.23-25
Interestingly, the IL-2/IL-2 receptor (IL-2R) system is dysregulated in MM and some of the alterations are correlated with survival. The effect of exogenous IL-2 on spontaneous apoptosis was thus evaluated in four experiments. Cells were incubated at 37°C for 24 hours in medium + 1% FCS in the presence or absence of IL-2. The proportion of apoptotic cells was decreased in the presence of IL-2 (medium, 30% ± 13%; IL-2, 7% ± 2%; P = .02; Fig S).

DISCUSSION

This study was undertaken to evaluate the role of apoptosis in the immune dysregulation of MM T cells. We have initially investigated expression of the Fas and bcl-2 antigens, which are important regulators with opposite effects on apoptosis. MM T cells showed an increase of Fas+ and a decrease of bcl-2+ cells that became progressively more evident as the percentage of HLA-DR+ T cells increased. This distribution was opposite to that observed in the controls, where Fas is expressed by a minority (20% to 40%) and bcl-2 by the majority (greater than 80%) of resting T cells. Normal T cells, however, can be induced in vitro to upregulate Fas and downregulate bcl-2, provided that they are subjected to a prolonged stimulation with mitogens such as phytohemagglutinin (PHA) or anti-CD3 MoAbs. A Fas+/bcl-2− phenotype is thus supposed to reflect a chronic activation state that is likely to occur in MM, where T cells are exposed long-term to tumor cells. A two-color cytofluorometric analysis confirmed the inverse correlation between Fas, bcl-2, and HLA-DR expression in individual MM T cells.

Fig 3. Preferential HLA-DR expression in spontaneously apoptotic MM T cells. Apoptosis was assessed with the immunocytometric assay after 18-hour incubation in medium + 1% FCS. Cells were double-stained for the HLA-DR and CD45 antigens before immunocytometric analysis. Reg A (A) and Reg V (V) were analyzed separately, and the overlayed results are shown. As expected, apoptotic cells (Reg A) were smaller (A), more granular (B), and expressed lower CD45 (C) than viable cells (Reg V). Cells from Reg A also showed higher HLA-DR expression than cells from Reg V (D). Results are from one of four experiments. No difference was observed between Reg A and Reg V when the expression of CD4 and CD8 antigens was investigated.

Fig 4. Qualitative analysis of DNA fragmentation by gel electrophoresis. DNA was extracted from (A) normal T cells, (B) viable MM T cells obtained from Reg V by density gradient centrifugation, (C) spontaneously apoptotic MM T cells obtained from Reg A from the same patient of lane B, (D) apoptotic cells from irradiated normal T cells, and (E) apoptotic thymocytes. Results are from one of four experiments.
The next step was to determine whether the Fas^{hi}$/bcl-2^{low}$ phenotype was associated with an increased susceptibility to apoptosis. The conventional understanding is that spontaneous apoptosis occurs in immature and activated T cells (also referred to as activation-induced cell death), but not in resting T cells. However, we and others have detected a small proportion of apoptotic cells in normal T cells as well,

indicating that apoptosis is not an all-or-nothing phenomenon; rather, its magnitude distinguishes MM from normal T cells. Spontaneous apoptosis may be considered as a withdrawal mode to initiate programmed cell death (i.e., apoptosis induced by deprivation of growth factors or positive cell-to-cell interactions). In a second series of experiments, susceptibility to apoptosis induced by a triggering mode was evaluated (i.e., apoptosis induced by an external agent). Examples of the triggering mode include apoptosis induced by anti-Fas MoAb and glucocorticoids. The former has little effect on normal resting T cells, and the physiologic increase of Fas$^+$ cells occurring in aged people is not paralleled by an increased susceptibility to anti-Fas treatment. Recent data indicate that the primary function of Fas in normal resting T cells may not necessarily involve apoptosis, but rather acts as an accessory molecule that promotes cell activation.

The conversion of Fas-mediated signaling from activation to apoptosis may represent a safety mechanism to downregulate a prolonged T-cell activation induced by a repetitive antigenic stimulation. Indeed, in vitro experiments have shown that normal T cells need to be extensively activated to become Fas$^+$ and susceptible to Fas-mediated apoptosis. The finding that freshly isolated MM T cells were already susceptible to Fas-mediated apoptosis confirmed that these cells had received repetitive activating signals in vivo.

Glucocorticoids induce apoptosis in immature and mature T cells through different pathways that, unlike other apoptotic agents, are independent of p53 and c-myc. When the effect of MP was tested on MM T cells, the cells showed increased susceptibility compared with normal T cells.

Enhanced apoptosis and chronic activation have also been reported in T cells from patients with Epstein-Barr virus (EBV), Varicella-Zoster virus (VZV), and human immunodeficiency virus (HIV) infections, which share many immunologic abnormalities with MM, including the expansion of HLA-DR$^+$, CD45RO$^+$ T cells, Fas upregulation, and bcl-2 downregulation. The effect of apoptosis on the final outcome of the immune response, however, is very different. In EBV and VZV infections, apoptosis is the major mechanism by which the bulk of T cells with antiviral activity are rapidly removed once the infection has been successfully cleared. In AIDS patients, where the infection is not cleared, apoptosis is believed to contribute to the progressive establishment of their immunodeficiency. MM is another disease where, despite the presence of specific interactions with the tumor cells, there is no spontaneous resolution, and T cells are subjected to a repetitive stimulation leading to chronic activation and enhanced susceptibility to apoptosis. Apoptosis is one of the mechanisms responsible for the es-
establishment of peripheral tolerance and clonal inactivation of mature functional T cells.9,10,37,38 The nonresponsive state may be maintained as long as T cells are exposed to the antigen.9,10,39 If this occurs in vivo, the immune system is depleted of effector T cells, which are among the best candidates to specifically interact with tumor cells. This may be one of the mechanisms exploited by tumor cells to weaken and escape T cell-mediated immunosurveillance. Studies are currently in progress to determine any correlation among apoptosis, tumor progression, and the clinical outcome in MM.

The molecular mechanisms by which tumor cells make T cells more susceptible to apoptosis have not yet been identified. One possibility is that tumor cells cannot provide the accessory signals required to fully activate T cells.41 In their absence, TCR occupancy may predispose to apoptosis. Exogenous cytokines have been used to provide these accessory signals. This is one of the rationales supporting the use of cytokines such as IL-2 in clinical trials. Spontaneous apoptosis was prevented in MM T cells by the addition of exogenous IL-2. It has recently been demonstrated that IL-2 can sustain bcl-2 expression even in extensively activated T cells, pointing to bcl-2 upregulation as a mechanism by which IL-2 exerts its antiapoptotic activity. Interestingly, high levels of serum IL-2 are powerful predictors of longer survival in MM.22 raising the possibility that prevention of T-cell apoptosis is a mechanism by which endogenous IL-2 positively influences the disease evolution.

In conclusion, T cells in MM are in a chronic activation state characterized by a Fas+ and bcl-2+ phenotype. This places them in a particular situation: on the one hand, they have a less stringent need of accessory signals to produce IL-2 and proliferate fully (at least in vitro); on the other hand, they reopen their death program and become more susceptible to apoptosis. Any immunotherapy-based approach should, therefore, be carefully designed to trigger antitumor activity rather than apoptosis.

ACKNOWLEDGMENT

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REFERENCES


Dysregulated Fas and Bcl-2 expression leading to enhanced apoptosis in T cells of multiple myeloma patients

M Massaia, P Borrione, C Attisano, P Barral, E Beggiato, L Montacchini, A Bianchi, M Boccadoro and A Pileri