Autoregulatory Circuits in Myeloma

Tumor Cell Cytotoxicity Mediated by Soluble CD16

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Abstract

Background. **Multiple myeloma remains an incurable malignancy due to marked resistance of the tumor to standard doses of chemotherapy. Treatment approaches, using chemotherapeutic dose escalation and hematopoietic stem cell support have resulted in significant augmentation of tumor mass reduction such that complete remissions are effected in ~ 50% of patients. These remissions are, however, often not durable. In the setting of minimal residual disease, therefore, adjuctive immunotherapy may be useful.**

Methods. **Peripheral blood mononuclear cells were studied from 28 untreated patients with multiple myeloma (MM). Mononuclear cell CD16 (FcRγ III) expression was determined by flow cytometry. The effect of lymphocyte-derived soluble CD16, isolated by affinity chromatography, on MM cell growth and differentiation was assessed. MM cell proliferation, viability, immunoglobulin production and gene expression was studied.**

Results. **Data are presented indicating that cells expressing CD16 are increased in untreated patients with IgG-secreting myeloma. The predominant phenotype of these cells is $CD8^+$ or $CD56^+$. These $CD16^+$ cells can produce a soluble form of the Fc receptor (sFcR, sCD16) that can bind to surface Ig on cultured human IgG-secreting myeloma cells and effect suppression of tumor cell growth and Ig secretion. This effector function is accompanied by concomitant suppression of c-*myc* as well as IgH and IgL gene transcription. Finally, prolonged exposure to sCD16 causes myeloma tumor cell cytolysis.**

Conclusions. **sCD16 and possibly other soluble FcR are candidate molecules for adjunctive immunotherapy of myeloma, once complete responses have been effected by intensive cytotoxic therapy, now possible in up to 50% of newly diagnosed patients. (*J. Clin. Invest.* 1995. 95:241–247.) Key words: Fc receptor • CD16 • suppressor T cell • tumor immunology • multiple myeloma**

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1. *Abbreviations used in this paper:* MM, multiple myeloma; sCD16, soluble CD16; sFcR, soluble forms of FcR.

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Introduction

Multiple myeloma (MM)[1] remains an incurable malignancy due to marked resistance of tumor cells to standard chemotherapy (1). Myeloablative therapies requiring hematopoietic stem cell support can effect complete remissions in up to 50% of patients, but relapses are still observed (2). Consequently, stratagies designed to sustain remissions once marked cytoreduction has been achieved need to be developed. Interferon-α has been employed to some extent in this regard, however, its use and effectiveness is controversial (3).

Many immunological perturbations have been described in patients with myeloma (4). Levels of uninvolved immunoglobulin are low, possibly as a result of suppressor macrophages that inhibit immunoglobulin production by normal B cells (5–9). In addition, increased numbers of activated T cells and CD8+ cells have been seen in the peripheral blood and bone marrow (10–14). NK cell activity is increased in blood and bone marrow, but myeloma cells are resistant to autologous NK cell-mediated lysis (15–19). Furthermore, alterations in the number of Fc receptor-bearing cells have been reported, but their function and contribution to the pathophysiology of MM remains to be ascertained (20).

FcR have been described for all Ig isotypes and the genes for most of these receptors have been cloned and extensively characterized (21–25). FcR are present on many cell types and mediate a variety of isotype-specific immunological functions such as antibody-dependent cell cytotoxicity (ADCC), opsonization and phagocytosis (26–31). Soluble forms of FcR (sFcR) mediate isotype specific regulation of B cell growth and immunoglobulin production (32–36). In a murine model of myeloma, sFcR suppress growth and immunoglobulin production of tumor cells (37–39).

In this report, we demonstrate, in untreated patients with IgG-MM, an expansion of $CD16^+$ cells in the peripheral blood. These $CD16^+$ cells are predominantly $CD8^+$ and shed soluble CD16 (sCD16) that functions as a binding factor for IgG. S-CD16 produced by patient T cells suppresses c-*myc* transcription and cell growth, as well as immunoglobulin gene transcription and the secretion of Ig in myeloma tumor lines with an IgG isotype. Prolonged treatment of cultured myeloma cell lines with sCD16 results in cell death. Thus, sCD16 is a candidate molecule for adjunctive immunotherapy of human IgG multiple myeloma.

Methods

Patients. 28 consecutive, newly diagnosed patients with multiple myeloma were studied. 19 patients secreted IgG, four IgA, one IgD, and three were light chain producers (see Table I). 14 normal volunteers were used as controls in these studies.

Immunophenotyping. After obtaining informed consent from pa-

Table I. Patient Characteristics

Patient	Age	Sex	Isotype	Percentage PC	Serum M
1	26	F	IgG-λ	15	2.8
2	65	M	IgG-κ	15	1.6
3	53	M	IgG-κ	8	1.5
4	63	F	IgG-κ	41	5.9
5	41	M	IgG-κ	36	4.4
6	38	M	IgG-κ	15	1.9
7	59	M	IgG-κ	60	1.8
8	67	M	IgG-λ	40	2.9
9	34	M	IgG-κ	45	3.5
10	42	M	IgG-κ	9	2.6
11	56	F	IgG-κ	63	4.3
12	64	M	IgG-κ	80	2.3
13	43	M	IgG-κ	60	4.0
14	49	M	IgG-λ	80	5.1
15	49	F	IgG-κ	34	4.2
16	40	M	IgG-λ	65	4.6
17	54	M	IgG-κ	63	5.9
18	58	F	IgG-κ	48	2.7
19	67	M	IgG-κ	92	6.5
20	49	M	IgA-κ	85	3.5
21	44	M	IgA-κ	61	1.8
22	71	M	IgA-κ	79	4.2
23	58	M	IgA-κ	40	1.5
24	41	M	IgD-λ	90	1.5
25	40	M	κ	23	0.3
26	27	M	κ	8	0.3
27	43	F	λ	10	0.8
28	54	F	κ	26	0.3

tients and volunteers, peripheral blood lymphocytes were isolated by Ficoll–Isopaque centrifugation (40). Cells were simultaneously stained with fluoroscein isothiocyanate-labeled anti-CD16 (Ortho Diagnostics, Raritan, N.J.) and phycoerythrin-labeled anti-CD4, CD8, CD19, CD14, and CD56 (Ortho Diagnostics) (40). Stained cells were analyzed by two-color flow cytometry using a FACStar® flow cytometer (Becton Dickinson, Mountain View, CA). Flow cytometric data were converted to absolute cells per microliter based upon measured total lymphocyte or monocyte counts.

Isolation and characterization of soluble CD16. Approximately 10^8 peripheral blood mononuclear cells (PBMC) were obtained and cultured in RPMI-1640 media supplemented with 10% fetal bovine serum and 10 mM glutamine. PBMC were stimulated with 10 μg/ml Phytohemagglutinin (PHA-P) for three days as previously described (41). Subsequent to PHA stimulation, cells were stimulated every 3 d with 10 U/ml recombinant IL2 for a total of 3 wk. At the end of the culture period ~ 10^{10} cells were harvested. Cell-free supernatants were isolated and the volume reduced to 10% of original by ultrafiltration. Soluble CD16 was isolated by affinity chromatography employing anti-CD16 monoclonal (3G8) antibody coupled to Sepharose CL4B. Bound protein was eluted with 0.1 M glycine, pH 3.0, and immediately neutralized with 1 M Tris, pH 7.4. A portion of the material was radio-iodinated by the chloramine T method (38). Radiolabeled material was subjected to analysis by standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (38). Soluble CD16 was isolated, in this fashion, from 6 separate patients with newly diagnosed IgG myeloma. The $CD16^+$ human natural killer cell clone NK3.3 was used as a source of normal sCD16 which was isolated and characterized as described above (42). Isolation of soluble, human IgA-Fc receptor has been described previously (43).

Functional studies. ARH-77 (IgG-κ), COL (IgG-κ), RAM (IgG-κ), MER (IgM-κ), ARD (IgA-κ), and LER (IgA-κ) are human myeloma cell lines producing and secreting the indicated immunoglobulins. Each cell line is grown in RPMI-1640 media supplemented with 10% fetal bovine serum (Complete Media, CM) and all cell lines were growth factor independent. Each tumor cell line was cultured in CM at 1×10^5 cells/ml. Cells were either treated for varying lengths of time with media alone, varying concentrations of affinity-purified soluble CD16, 10 μg/ml purified rat anti–human IgG or 10 μg/ml of affinity purified human IgA-Fc Receptor. Proliferation was assessed by pulsing treated cells with 0.5 μCi ^{3}H-thymidine 6 h before harvest. Harvested cells were lysed and DNA was precipitated unto glass filters with 20% ice cold trichloroacetic acid (38). The amount of radioactivity incorporated into DNA was assessed by standard liquid scintillation counting. Secretion of immunoglobulin by the tumor cells was assessed by harvesting cell free supernatants after 24 h of treatment and measuring IgG concentrations by ELISA as previously described (43). The effects of various treatments on cell viability was determined by standard trypan blue dye exclusion.

Gene expression studies. Treated ARH-77 tumor cells were harvested into guandidine isothiocyanate and total cellular RNA was isolated by cesium chloride centrifugation (40). RNA was electrophoresed on denaturing gels and subjected to standard Northern blotting (40). Blots were probed with random primer ^{32}P-labeled probes specific for IgH-γ heavy chain, IgL-κ light chain, c-*myc*, and β-actin. RNA half-life studies were performed by first treating cells with the above described reagents for 24 h. Cells were then treated with 5 μg/ml/10^6 cells actinomycin D for 2 h, harvested at various times thereafter and total RNA isolated and subjected to Northern blotting as described above (38). Specific levels of RNA were quantitated by scanning densitometry. All time points were normalized to the zero time point and data expressed as "Relative Densitometry Units."

Results

$CD8^+$ $CD16^+$ and $CD56^+$ $CD16^+$ cells are increased in patients with IgG-secreting multiple myeloma. Compared with normal controls, untreated patients with IgG-secreting multiple myeloma showed a > 5-fold increase in the number of $CD8^+$ $CD16^+$ cells and a > 2-fold increase in the number of $CD56^+$ $CD16^+$ cells in the peripheral blood (Fig. 1). Cells co-expressing CD16 with CD4, CD19 or CD14 were not increased compared to controls. Expansion of $CD16^+$ cells was not seen in patients with IgA-, IgD- or light chain only-secreting myeloma consistent with an isotype concordance between the immunoglobulin secreted by the tumor cells and the specificity of the Fc receptor.

Soluble CD16 is produced by $CD8^+$ $CD16^+$ cells. PBMC were obtained from six patients with IgG-secreting myeloma and soluble CD16 isolated. Data shown are from a single representative patient (patient JS). At initiation of culture, 63% of JS-PBMC were $CD16^+$. The isolated mononuclear cells were initially stimulated with 10 μg/ml PHA and then maintained in media containing 10 U/ml recombinant IL-2. After 3 wk of culture under these conditions, 78% of the cells co-expressed CD8 and CD16. Cell-free supernatants of these cells were harvested and sCD16 was isolated by affinity chromatography. As a control, soluble CD16 was isolated from the cell free supernatants of a human natural killer cell clone (NK3.3) which is 80% $CD16^+$. S-CD16 from both sources was radioiodinated and repurified by affinity chromatography. Analysis by standard SDS-PAGE under reducing conditions revealed two major identical components for each source of sCD16 (Fig. 2). The 67Kd band represents the glycosylated form of CD16 and the "dou-

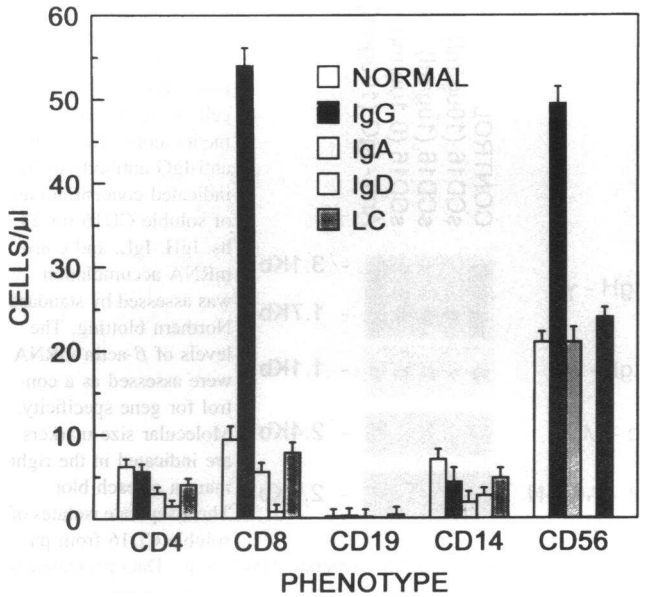

Figure 1. CD16$^+$ CD8$^+$ and CD16$^+$ CD56$^+$ cells are increased in patients with IgG myeloma. The number of peripheral blood cells simultaneously expressing CD16 with other phenotypic markers was determined by two parameter (two color) flow cytometry. Data is expressed as mean cell number/microliter ±SEM (patient N = 28, control N = 14). Note: a single patient with IgD MM was studied. The absolute number of CD19$^+$ CD16$^+$ and CD56$^+$ CD16$^+$ cells was 0 (zero).

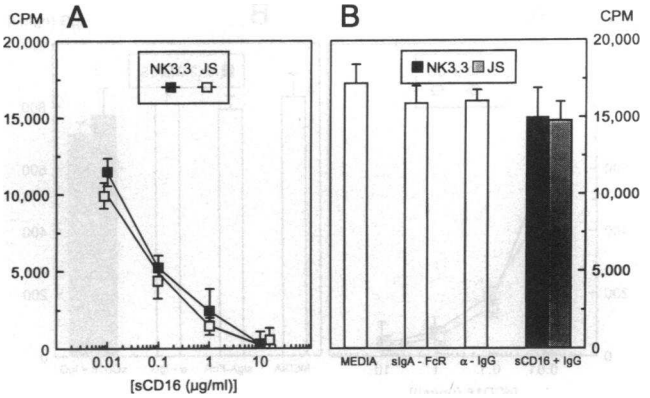

Figure 3. Soluble CD16 inhibits myeloma cell proliferation. (*A*) Myeloma tumor cells were treated with various concentrations of soluble CD16 isolated from either patient lymphocytes or normal human NK cells for 24 h. Proliferation was assessed by measuring incorporation of [^{3}H]thymidine into DNA. Three separate isolates of soluble CD16 from patient JS and NK3.3 were analyzed in three separate experiments. Data is expressed as mean counts per minute (*CPM*) ±SEM. (*B*) Controls: myeloma tumor cells were treated with media alone, 10 μg/ml soluble IgA-Fc receptor, 10 μg/ml anti-IgG antibody or 10 μg/ml soluble CD16 plus 200 μg/ml purified IgG for 24 h. Proliferation was assessed by measuring incorporation of [^{3}H]thymidine into DNA. Three separate experiments were performed on three separate isolates of sCD16 (JS), sCD16 (NK3.3), and sIgA-FcR. Three separate experiments were performed on a single lot of anti-IgG antibody. Data is expressed as mean CPM±S.E.M.

blet'' at 28–30 kD represents the nonglycosylated forms of CD16 (51). Thus, CD8$^+$ CD16$^+$ cells from a patient with IgG myeloma produce sCD16 that is identical, at the level of one dimensional SDS-PAGE resolution, to that produced by normal NK cells. Soluble CD16 isolated from five other patients showed similar results (data not shown).

Soluble CD16 suppresses human myeloma cell proliferation and immunoglobulin secretion. The human IgG-secreting myeloma cell line, ARH-77, was used as a target to study the effector function of sCD16. With increasing concentrations of purified sCD16 (isolated from patient JS), a dose dependent suppression of tumor cell proliferation was observed (Fig. 3). ARH-77 cell proliferation was not affected by treatment with media alone, 10 μg/ml affinity purified soluble human IgA-Fc receptor, or 10 μg/ml anti–human IgG antibody. Concomitant treatment of ARH-77 with 10 μg/ml sCD16 and 200 μg/ml purified IgG abrogated the suppression observed with sCD16 alone, suggesting an interaction of sCD16 with surface immunoglobulin on the tumor cells. In identically designed experiments, sCD16 also suppressed immunoglobulin secretion by ARH-77 cells in a dose-dependent manner (Fig. 4). This suppression of tumor cell proliferation and Ig secretion is isotype-specific as sCD16 failed to induce such effects in tumor cell lines producing IgM or IgA (Fig. 5). Soluble CD16 isolated from five other patients showed similar results (data not shown).

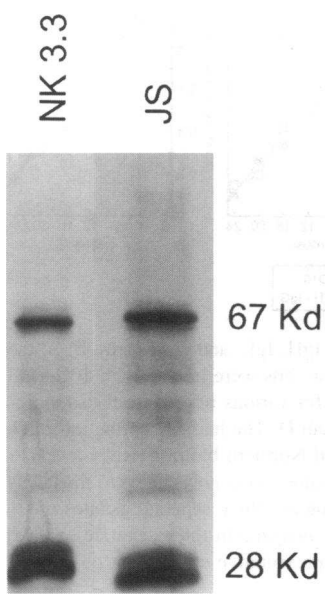

Figure 2. Soluble CD16 is produced by patient cells and normal natural killer cells. Soluble CD16 was isolated from a normal natural killer cell clone (*NK3.3*) and from a patient with IgG-secreting MM (*JS*) and subjected to analysis by SDS-PAGE. Molecular weight markers are indicated in the right margin. Data presented is one representative analysis from six separate patients and the NK3.3 cell line.

Soluble CD16 suppresses transcription of the IgH, IgL, and c-myc genes. Northern blot analysis revealed a dose-dependent suppression of the accumulation of mRNA specific for IgH, IgL, and c-*myc* when ARH-77 cells were treated with JS-sCD16, while levels of β-actin mRNA were unaffected (Fig. 6). Anti-human IgG antibody (at 10 μg/ml) had no effect on IgH, c-myc or β-actin expression. Anti-IgG antibody, appeared to cause a minor suppression of IgL gene transcription, however, this effect is < 2-fold compared to the > 10-fold suppression mediated by soluble CD16 at the same concentration. Thus, the effect of anti-IgG antibody on IgL transcription is likely not significant. sCD16 had no effect on the half-life of IgH, IgL, or c-*myc* mRNA in comparison to anti-IgG–treated cells (Fig. 7). Collectively, these results indicate that sCD16 can suppress the transcription of the IgH, IgL, and c-*myc* genes in a selective fashion. S-CD16 isolated from PBMC obtained from five other

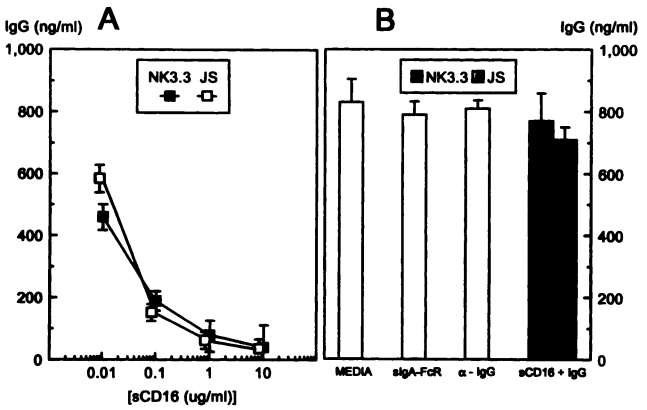

Figure 4. Soluble CD16 inhibits secretion of Ig from myeloma cells. (*A*) Myeloma tumor cells were treated with various concentrations of soluble CD16 isolated from either patient lymphocytes or normal human NK cells for 24 h. Ig secretion was assessed by ELISA. Three separate isolates of soluble CD16 from patient JS and NK3.3 were analyzed in three separate experiments. Data is expressed as mean ng/ml IgG±SEM. (*B*) Controls: Myeloma tumor cells were treated with media alone, 10 μg/ml soluble IgA-Fc receptor, 10 μg/ml anti-IgG antibody or 10 μg/ml soluble CD16 plus 200 μg/ml purified IgG for 24 h. Ig secretion was assessed by ELISA. Three separate experiments were performed on three separate isolates of sCD16 (JS), sCD16 (NK3.3), and sIgA-FcR. Three separate experiments were performed on a single lot of anti-IgG antibody. Data is expressed as mean ng/ml IgG±SEM.

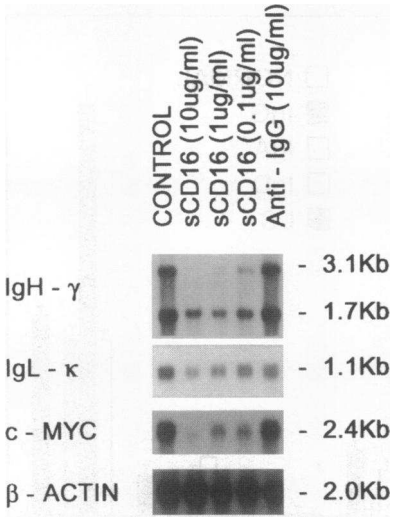

Figure 6. Soluble CD16 inhibits IgH, IgL and c-*myc* mRNA accumulation. Myeloma tumor cells were treated with media alone, 10 μg/ml anti-IgG antibody or the indicated concentrations of soluble CD16 for 24 hs. IgH, IgL, and c-*myc* mRNA accumulation was assessed by standard Northern blotting. The levels of β-actin mRNA were assessed as a control for gene specificity. Molecular size markers are indicated in the right margin of each blot. Three separate isolates of soluble CD16 from patient JS were analyzed in three separate experiments. Data presented is one representative experiment of three separate experiments.

patients with IgG MM revealed similar results (data not shown).

Prolonged exposure of human myeloma tumor cells to soluble CD16 is cytotoxic. Examination of the number of viable ARH-77 tumor cells after prolonged exposure to sCD16 revealed no change within the first 24 h of exposure, followed by progressive cell loss resulting in, at 72 h, a three log reduction (Fig. 8). Treatment with anti-IgG antibody, on the contrary, was associated with a slight increase in the number of viable cells. Thus, prolonged exposure (> 48 h) of ARH-77 cells to sCD16 is cytotoxic. Treatment of IgA- or IgM-secreting cell lines with soluble CD16 showed no effect on cell viability (data not shown).

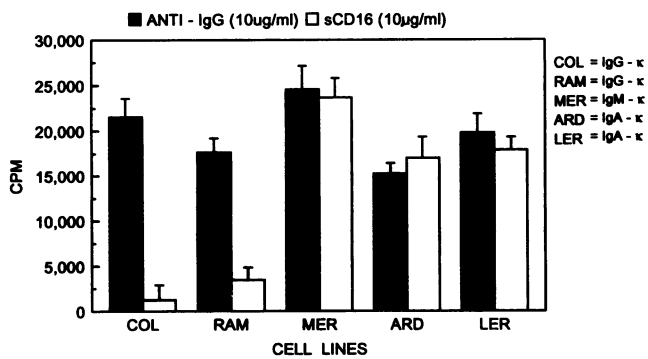

Figure 5. Soluble CD16 mediated suppression of myeloma cell proliferation is isotype-specific. Myeloma cells secreting the indicated isotypes were treated with 10 μg/ml soluble CD16 for 24 h. Proliferation was assessed by measuring the incorporation of [^{3}H]thymidine into DNA. Three separate isolates of soluble CD16 from patient JS were analyzed in three separate experiments. Data is expressed as mean CPM incorporated±SEM.

Discussion

The role of soluble Fc receptors in murine myeloma: similarities to the human disease. The results of the studies presented here resemble those conducted in the murine plasmacytoma system. Murine plasmacytoma growth is accompanied by an expansion of circulating, isotype-condordant, FcR$^+$ cells (44–47). The majority of these cells express CD8 and produce a soluble form of the FcR (44-47). The isotype concordance between the FcR specificity and the Ig secreted by the tumor cells is also observed in patients with MM. In both murine and human systems, solu-

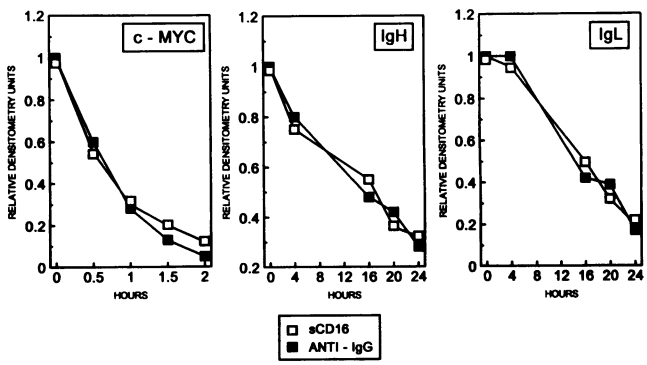

Figure 7. Soluble CD16 modulates IgH, IgL, and c-*myc* mRNA at the transcriptional level. Myeloma tumor cells were treated with 10 μg/ml soluble CD16 or anti-IgG antibody for various time periods (as indicated) in the presence of Actinomycin D. The half-life of the indicated mRNAs was determined by standard Northern blotting. RNA levels were assessed by densitometry and values were normalized to the levels observed at the start of each experiment. Three separate isolates of soluble CD16 from patient JS were analyzed in three separate experiments. The data presented is one representative experiment of three separate experiments.

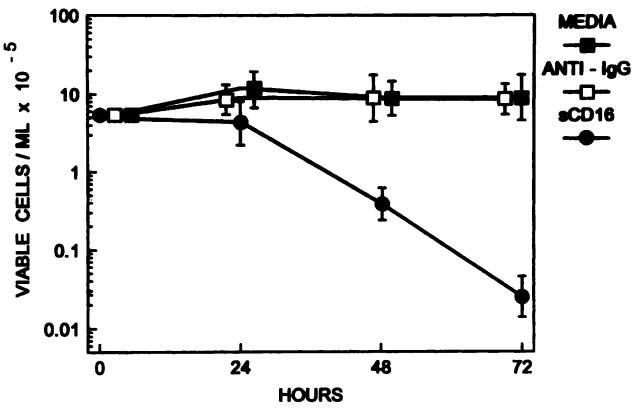

Figure 8. Prolonged exposure of myeloma cells to soluble CD16 is cytotoxic. Myeloma tumor cells were treated with media alone, 10 μg/ml anti-IgG antibody or 10 μg/ml soluble CD16 for various time periods as indicated. Tumor cell viability was determined by standard dye exclusion. Three separate isolates of soluble CD16 from patient JS were analyzed in three separate experiments. Data represents mean viable cells ($\times 10^{-5}$) ±SEM from three separate experiments.

ble FcR binds to sIg on tumor cells and suppresses growth and Ig secretion (34,38,39). Thus, our studies indicate the presence of an isotype-specific suppressor circuit that is activated in untreated patients with IgG-secreting multiple myeloma.

The biology of CD16. CD16 is a low affinity IgG Fc receptor that binds all IgG subtypes with variable affinity (48–50). Two forms of the receptor have been identified. One form, present on neutrophils, is not a transmembrane protein, but rather is linked to the membrane via a glycan phosphatidyl inositol (GPI) linkage (50). This form of the receptor can be released as a soluble molecule by breakage of the GPI linkage with phospholipase C. A second form of the CD16 receptor is found predominantly on natural killer cells. This form is a transmembrane protein linked to several accessory, transmembrane proteins (50). This transmembrane form of CD16 can be released as a soluble form through the action of a cell membrane associated metalloproteinase (51).

In humans, CD16 is not usually expressed on T cells. An exception to this is seen in Large Granular Lymphocytosis (52). In this disorder an expansion of $CD16^+$ cells expressing the phenotype of NK cells ($CD56^+$) or $CD8^+$ T cells is seen (52). Many of these patients exhibit disorders of granulopoiesis or erythropoiesis suggesting that $CD16^+$ cells play specific regulatory roles (52). Abnormalities in immunoglobulin production have also been described in these patients (52). Thus, this rare disorder suggests the presence of a population of $CD8^+$ $CD16^+$ and $CD56^+$ $CD16^+$ cells that subserve specific roles in the regulation of hematopoiesis and immune cell function. Our results suggest that $CD16^+$ cells are also expanded and play an immunoregulatory role in patients with IgG-secreting multiple myeloma.

Surface immunoglobulin and the action of soluble CD16. Our data suggests that sCD16 must interact with surface Ig on tumor cell targets in order to effect suppression. The interaction of soluble CD16 with other non-Ig determinants on tumor cells, however, cannot be excluded. Although Fc receptors interact with determinants in the CH2 region of the Ig heavy chain, substantial evidence suggests that soluble Fc receptors can interact with these determinants even when Ig is present as the membrane form (34,38,39,53). Indeed, surface membrane immunoglobulin is linked to the membrane by additional terminal amino acids, thus leaving the CH2 and CH3 determinants accessible to extracellular effector molecules (54).

All target myeloma cell lines used in our studies express surface Ig. The literature suggests, however, that the plasmacytic myeloma tumor cells in patients are either surface Ig negative or express surface Ig at low density (55). The presence of surface Ig is an important prerequisite for soluble Fc receptors to exert biological function and hence for their therapeutic use. Recent data in human myeloma indicate the presence of circulating tumor cells with a more immature phenotype including surface Ig expression (56). Although not firmly established, it has been suggested that these immature tumor cells represent the proliferating, self-renewal compartment in MM. Thus, if future research should establish these sIg^+ circulating tumor cells as the proliferative compartment in MM they would be the more appropriate target of sFcR rather than the sIg^- plasmacytic tumor cells in the bone marrow.

Suppression of myeloma cell growth by soluble CD16. It is attractive to postulate that the control of tumor cell growth by CD16 is mediated through its suppression of c-*myc* transcription. The role of inappropriate activation of the c-*myc* gene has been well established in the murine myeloma model (57). The role of c-myc in the pathogenesis of human myeloma, however, is less clear. Cytogenetic studies show that chromosome 8 abnormalities, involving the c-*myc* locus, are present in < 5% of patients with multiple myeloma (58,59). Abnormalities of c-myc gene structure, demonstrated by various molecular techniques, are also seen in a small minority of patients (60). Recent studies, however, suggest that c-*myc* may have an important role in the pathogenesis of this disease. Greil et al. (61) have used the technique of in situ hybridization in order to analyze individual myeloma tumor cells and have shown that c-myc mRNA and protein are present in tumor cells at levels comparable to normal proliferating cells in greater than 90% of patients. Interestingly, these authors did not find a correlation between c-myc mRNA/protein levels and the number of cycling cells. However, they did not examine the immature circulating tumor cells in the peripheral blood. Thus, at his point, it is unclear how the c-*myc* gene is activated, what role it plays in controlling cell proliferation or whether activation of this gene is of primary pathogenetic significance in MM. Regardless, activation of the c-*myc* gene has been shown to be of importance in the control of cell proliferation in many cell types and likely plays a similar role in MM (62). Thus, suppression of c-myc gene transcription by soluble CD16 likely plays a significant role in the ability of the soluble Fc receptor to control myeloma tumor cells growth.

Tumor cell cytotoxicity mediated by soluble CD16. Prolonged exposure of myeloma cells to sCD16 was required to induce tumor cell death. Indeed, treatment of tumor cells with sCD16 for 72 h or longer results in at least a three log reduction in cell viability. The mechanism of the tumor cell cytoxicity mediated by soluble CD16 is not clear. Preliminary evidence suggests that soluble CD16 can induce apoptotic tumor cell death. Recent studies have suggested a relationship between the expression of the c-myc gene and the control of apoptosis (63). Since soluble CD16 can regulate the expression of c-myc it is provocative to suggest that the cytoxicity induced by soluble CD16 and the regulation of c-*myc* gene expression are related.

Regulation of myeloma tumor cell growth by soluble CD16 in situ. Our studies have demonstrated an expansion $CD16^+$

cells in newly diagnosed myeloma and in vitro tumor growth suppression upon exposure to sCD16. Does sCD16 control myeloma tumor cell growth in vivo? Benign plasma cell dyscrasias are highly prevalent in the population and increase in incidence with age (64). Since a minority of these patients progress to overt multiple myeloma it is distinctly possible that expansion of tumor cell clones is prevented in these individuals by immunoregulatory cells. However, since we have not investigated the role of soluble CD16 in these disorders this supposition is entirely speculative. With regard to overt myeloma, however, independent evidence suggests a role for soluble CD16. It is generally believed that myeloma begins years prior to clinical diagnosis. It is possible that accumulated genetic damage in tumor cells coupled with the progressive immune paralysis that accompanies this disease has allowed escape of the tumor clone from immunoregulatory mechanisms. Recent studies reported by Mathiot et al. (65) have suggested a role for sCD16 in the biology of myeloma in vivo. They measured sCD16 in the serum of patients with multiple myeloma and found that the levels correlated inversely with disease stage. These findings do not permit, however, a distinction as to whether lower levels of sCD16 account for or result from tumor mass expansion. High levels of circulating Ig, present at diagnosis in most patients, could adsorb and inactivate sCD16. Thus, neither endogenous nor exogenously administered sCD16 would be effective in regulating tumor growth. However, after intensive cytoreductive therapy followed by autologous or allogeneic hematopoietic stem cell transplantion, most patients no longer demonstrate monoclonal Ig in serum or urine. Thus, in the setting of such marked tumor cytoreduction, sCD16 should be effective in eliminating residual surface Ig expressing tumor cells. Thus, soluble Fc receptors are candidate molecules for the adjunct immunotherapy of multiple myeloma or other related B cell neoplasms.

Summary. Data are presented indicating that cells expressing CD16 are increased in untreated patients with IgG-secreting multiple myeloma. The predominant phenotype of these cells is $CD8^+$ or $CD56^+$. These $CD16^+$ cells can produce a soluble form of the FcR that can bind to sIg on cultured human IgG-secreting myeloma cells and effect suppression of tumor cell growth and immunoglobulin secretion. This effector function is accompanied by concomitant suppression of c-*myc* as well as IgH and IgL gene transcription. Finally, prolonged exposure to sCD16 causes death of target myeloma tumor cells. Soluble CD16 and possibly other soluble Fc receptors are candidate molecules for the adjunct immunotherapy of multiple myeloma, once significant tumor cytoreduction has been effected by intensive cytotoxic therapy coupled with bone marrow transplantation, now possible in up to 50% of newly diagnosed patients.

Acknowledgments

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