

Expansion of Fc Receptor-Bearing T Lymphocytes in Patients with Immunoglobulin G and Immunoglobulin A Myeloma

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ABSTRACT Lymphocytes obtained from the blood of normal individuals and six patients with newly diagnosed multiple myeloma were separated into T and non-T cell populations by rosette-formation with sheep erythrocytes, and were then assayed for the presence of surface membrane Fc receptors. When compared with normal individuals, four patients with IgG myeloma had a three- to fourfold increase in T cells with IgG receptors (T γ cells) and two patients with IgA myeloma had a two- to threefold increase in T cells with IgA receptors (T α cells). Patients with IgG or IgA myeloma had normal numbers of non-T lymphocytes with surface receptors for IgG and IgA, respectively. The finding that human myeloma is accompanied by elevated numbers of T cells with Fc receptors for the heavy chain class of the myeloma protein: (a) may account for the apparent "monoclonal" lymphocyte population in patients with myeloma; (b) extends to humans similar observations made in mice with secretory plasmacytomas; and (c) is of interest because T cells with Fc receptors are immunoregulatory lymphocytes.

INTRODUCTION

Several laboratories (1-4) have reported that many patients with multiple myeloma have a population of blood lymphocytes that appear to be monoclonal because they express the idiotypic antigens of the myeloma protein on their surface membranes. Similar observations were originally described in mice with subcutaneous plasmacytomas (5). The origin and sig-

nificance of idiotype-bearing lymphocytes in myeloma have been matters of considerable disagreement (6).

In recent studies (6, 7) we observed that several murine myelomas were accompanied by an extraordinary increase in the number of circulating T lymphocytes that expressed surface membrane receptors specific for the heavy chain class of the myeloma protein. Those studies established that the idiotype-bearing lymphocytes in mice with those IgA myelomas resulted from adsorption of the IgA myeloma proteins to T lymphocytes with IgA-Fc receptors (T α cells). In subsequent studies¹ we observed that mice with IgG myeloma developed large numbers of circulating T cells with surface membrane receptors for IgG (T γ cells). In the mouse, therefore, myeloma can be accompanied by an elevation in Fc receptor-bearing (FcR⁺)² T cells that can mimic a monoclonal population because their Fc receptors are occupied by myeloma protein.

In the present studies we examined blood lymphocytes from six newly diagnosed patients with multiple myeloma and observed that each patient had elevated numbers of T lymphocytes with receptors for immunoglobulin of the same heavy chain class as their myeloma protein.

METHODS

Preparation of lymphocytes. Heparinized blood was obtained by venipuncture from normal volunteers or from patients with myeloma seen at Washington University Medical Center. Blood lymphocytes were quantitated by Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.) and microscopic examination of Wright-stained peripheral blood

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² Abbreviations used in this paper: FcR, receptor-bearing cells; RFC, rosette-forming cells.

smears. Mononuclear cells were isolated from the blood by the Ficoll-isopaque procedure of Boyum (8), washed in media, and stored at -70°C in sterile Cryotubes (1076-2; Vangard International, Neptune, N. J.) at a density of 5×10^6 cells/ml in RPMI 1640 media containing 13% dimethylsulfoxide (DMSO) and 50% human AB serum.

Detection of surface membrane immunoglobulin receptors on lymphocytes. 5×10^6 blood mononuclear cells per ml (Freshly obtained or recovered from freezer storage) were incubated for 18 h at 37°C in 93% air and 7% CO_2 . Cells recovered from freezer storage were $>90\%$ viable as determined by trypan blue exclusion. Cell cultures were carried out in Falcon 3002 culture plates (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) containing RPMI 1640 media with 20% fetal calf serum. These cultures were performed to reverse any receptor occupancy by immunoglobulin adsorbed in vivo, and to remove adherent cells. Although in the present studies we did not assay for receptor occupancy by immunoglobulin adsorbed in vivo, an incubation step was nonetheless included because of previous studies (7) in murine myeloma where receptor occupancy and its reversal with incubation have been consistently observed. Nonadherent cells were rosetted with washed neuraminidase-treated sheep erythrocytes and separated into T cell and non-T cell fractions by Ficoll-isopaque centrifugation as described by Ferrarini et al. (9). When re-rosetted 95–97% of the T cell fraction formed rosettes with neuraminidase-treated sheep erythrocytes. Cells in the T cell and non-T cell fractions were assayed for surface membrane IgG receptors by rosette formation with IgG-coated ox erythrocytes as described by Ferrarini et al. (9). T cell and non-T cell fractions were assayed for IgA receptors by the method described by Lum et al. (10). Briefly, the cells were incubated for 15 min at 37°C with an equal volume of serum that contained a high titer of M315 (an $\text{IgA}\lambda_2$, anti-2,4,6-trinitrophenyl antibody secreted by the murine plasmacytoma MOPC 315). The cells were washed five times in RPMI 1640 medium containing 20% fetal calf serum, and then rosetted with 2,4,6-trinitrophenylated sheep erythrocytes according to the procedure of Hannestad et al. (11).

Statistical analysis. The comparison of individual patient vs. control were carried out using the Satterthwaite form of Student's *t* test (12).

RESULTS

The presenting clinical, laboratory, and radiographic findings established the diagnosis of multiple myeloma (Table I). The four patients with IgG myeloma had a three- to fourfold increase in the number of erythrocyte-rosette-forming cells (E-RFC) with IgG receptors and the two patients with IgA myeloma had a two to threefold increase in the number of E-RFC with IgA receptors (Table II). The four patients with IgG myeloma had normal levels of E-RFC with IgA receptors. One patient with IgA myeloma had normal numbers of E-RFC with IgG receptors while the other patient with IgA myeloma had a modest, but statistically insignificant, increase in T γ cells (Table II). Non-E-RFC with IgG or IgA receptors were present in normal numbers in all six patients. Excellent reproducibility was observed between the results obtained when the cells were recovered from the freezer and analyzed on different days.

DISCUSSION

These studies show that human myeloma is accompanied by increased numbers of circulating T lymphocytes that have surface membrane receptors for the immunoglobulin heavy chain class of the myeloma protein. In four patients with IgG and two patients with IgA myeloma, we found increased numbers of T γ and T α cells, respectively. Although many more

TABLE I
Patient Data

Patients	Paraprotein	Paraprotein	Marrow*	Bone
		Concentration	Plasmacytosis	Roentgenograms
		<i>g/100 ml</i>		
47 yr old male	IgA-kappa	4.2	+	Multiple rib lytic lesions.
57 yr old female	IgG-kappa	6.3	+	Compression fractures of 2 thoracic vertebrae, multiple lytic lesions of skull.
77 yr old male	IgG-kappa	2.6	- ‡	Lytic lesions in right scapula, right parietal-occipital area, and left clavicle.
87 yr old male	IgG-kappa	5.5	+	Pathologic fractures of ribs 4–9 on right side.
75 yr old female	IgA-lambda	3.4	+	Pathologic fracture of right 7th rib.
75 yr old male	IgG-kappa	1.15	+	Compression fractures of three thoracic vertebrae and multiple lytic skull lesions.

* Marrow plasmacytosis defined as $>10\%$ plasma cells.

‡ Patient 3 had 3% plasma cells in an iliac crest marrow sample.

TABLE II
Receptors for Immunoglobulin on Lymphocytes in Human Myeloma

Patient	Myeloma protein class	E-RFC/mm ³ *	E-RFC/mm ³ * with IgA receptors	Non-E-RFC/mm ³ * with IgA receptors	E-RFC/mm ³ * with IgG receptors	Non-E-RFC/mm ³ * with IgG receptors
		<i>g/100 ml</i>				
1	IgA (4.2)	741±32	642±64 (P = 0.01)†	162±14	74±11	145±51
2	IgG (6.3)	681±10	168±20	184±30	323±31 (P = 0.007)	204±11
3	IgG (2.6)	1,373±95	262±27	289±46	488±79 (P = 0.004)	485±30
4	IgG (5.5)	843±62	191±27	168±11	344±68 (P = 0.03)	268±21
5	IgA (3.4)	846±23	400±40 (P = 0.08)	211±7	158±26	262±7
6	IgG (1.15)	552±12	150±22	121±13	250±18 (P = 0.02)	193±6
Normal adults‡	None	1,111±26	265±30	242±17	128±9	317±7

* Data expressed is mean±SE, three determinations per patient.

† Satterthwaite modified Student *t* test used to determine *P* values.

‡ Normal control represents three separate controls done three times each.

patients need to be studied before the invariance of these associations can be determined, these observations were made in the first six patients with newly diagnosed myeloma that we have examined. The immunoglobulin-binding cells have been identified as T lymphocytes because >95% of them formed spontaneous rosettes with sheep erythrocytes. This degree of E-rosette formation ruled out the possibility that the immunoglobulin-binding cells were B cells or blood monocytes that had not been removed by adsorption to the plastic culture dish during the 18-h incubation period.

The mechanism of increased FcR⁺ T cells in human myeloma is unknown but in murine myeloma an association between high frequencies of secretory myeloma cells and increased FcR⁺ T cells has been observed (6, 7). In the murine model we observed that IgA myeloma was accompanied by increased numbers of T α cells (7), whereas IgG myeloma was accompanied by increased numbers of T γ cells.¹ In those studies T α cells were not increased in mice with variant IgA myelomas that produced only a heavy or a light chain, or secreted only small quantities of an intact IgA protein (6, 7). Those findings implied that high circulating levels of myeloma protein might play an important role in the mechanism whereby immunoglobulin-binding T cells were increased. It may be relevant to our findings that other investigators have identified an association between elevated serum levels of a class of immunoglobulin and increased numbers of T cells with receptors for that immunoglobulin class. Spiegelberg et al. (13) have observed that individuals with atopic disorders and elevated serum IgE levels have increased numbers of T cells with IgE-Fc receptors. Yodoi and Ishizaka (14) have made similar observations in rats with elevated serum IgE, and have reported *in vitro* induction of Fc ϵ

receptors on rat lymphocytes by purified rat IgE (15). An association between the serum level of a monoclonal immunoglobulin and the frequency of lymphocytes with Fc receptors for that class of immunoglobulin might be relevant to the observation of Lindstrom et al. (1) that patients with benign monoclonal gammopathies developed small numbers of monoclonal blood lymphocytes while patients with multiple myeloma developed large numbers of these cells. In the single patient with benign monoclonal gammopathy that we have examined thus far we did not observe an alteration in the number of T cells with Fc receptors (unpublished data).

The significance of increased numbers of FcR⁺ T cells in multiple myeloma remains to be established. One possibility is that the expansion of FcR⁺ T cells represents an exaggerated, but otherwise, normal immunoregulatory response. A major rationale for this view is the increasing body of evidence that FcR⁺ T cells are immunoregulatory cells that can influence the growth and differentiation of normal B cells (16).

Although it is still unproven that the FcR⁺ T cells in myeloma actually influence the malignant B cells, this is not an unreasonable possibility since myeloma cells are responsive to other immunoregulatory signals (17). If FcR⁺ T cells were found to influence myeloma cells they might offer a new therapeutic strategy for the clinical manipulation of myeloma.

Finally, our findings provide an explanation for the occurrence in human myeloma of large numbers of circulating lymphocytes that express the myeloma idiotype on their surface membranes (18). It is interesting that myeloma, a disease in which a single B cell clone expands in an autonomous fashion while other B cell clones are suppressed (19, 20), is also accompanied by increased numbers of a set of T cells that are thought to regulate B cells (16).

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