

Preliminary Studies for an Immunotherapeutic Approach to the Treatment of Human Myeloma Using Chimeric Anti-CD38 Antibody

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Multiple myeloma is a disease in which conventional chemotherapy has only limited value, but which may be ideal for treatment with passive antibody against a suitable cell surface antigen on the neoplastic plasma cell. The CD38 antigen is known to be present on the majority of neoplastic plasma cells, and this was confirmed by detailed examination of bone marrow aspirates from three patients. Strong expression of CD38 was confined to cells which, by the criteria of light-scattering profiles and possession of cytoplasmic Ig, were plasma cells. The vast majority of neoplastic plasma cells appeared to be involved. Using a cell line as a model, it was found that the CD38 antigen acts as a target for a chimeric antibody prepared from the antibody OKT10. The chimeric antibody consists of the Fab portion of the mouse monoclonal antibody linked by a stable thioether bond to an Fc molecule derived from human IgG1, thereby forming mouse Fab-human Fc. In contrast to the parent antibody, the chimeric molecule mediates antibody-dependent cellular cy-

totoxicity (ADCC) very efficiently with human blood mononuclear effector cells, and is effective at low concentration. Also, even though the CD38 antigen is present on natural killer cells, there appears to be little deleterious action of the antibody on effector cell function. The antibody also failed to affect the growth of progenitor cells of the granulocyte/macrophage or erythroid lineages present in normal bone marrows, despite the suspicion that these cells express the antigen. Other advantages of the CD38 molecule are that it is not found in the serum of patients with myeloma, and it does not appear to modulate *in vitro*. Fourteen patients with flord myeloma and on various chemotherapeutic regimes had an undiminished capacity to mediate ADCC with the chimeric antibody, when compared with normal individuals. The maintenance of ADCC activity, coupled with the known suppression of the antibody response in these patients, augers well for treatment with chimeric antibody.

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MULTIPLE MYELOMA is a neoplasm with a poor prognosis despite modern chemotherapy. Although partial remissions of up to 60% are obtained with a variety of chemotherapeutic schedules, the median survival of about 30 months has remained constant for the last 30 years.¹ Recent protocols of intensive chemotherapy, sometimes with an autologous bone marrow transplant, have improved initial response, although it remains to be seen whether these remissions are maintained.²

Use of an immunotherapeutic approach has been limited by the fact that the plasma cell expresses few identifiable antigens at the cell surface. Monoclonal antibodies (MoAbs) that recognize plasma cells have been described, with specificity for the PCA-1, PCA-2, PC-1, and CD38 antigens,³ although only the latter has given rise to sufficient antibodies to warrant a CD designation.⁴ Another complication in selecting a therapeutic antibody for this disease is that the neoplastic clone is heterogeneous. There has been a suggestion that the plasma cell component does not have the proliferative capacity to maintain the tumor load and that a small population of less differentiated tumor cells could be involved.^{5,6} Candidates for the putative "clonogenic" cell have ranged from a pre-B cell⁷ to a lymphoplasmacytoid cell,⁸ and the situation remains confused. Hence, it is desirable to select an antibody that recognizes cells over a range of maturity: anti-CD38 is one of these, reacting with not only plasma cells, but also with mature B lymphocytes and lymphoplasmacytoid cells.⁹ However the 45,000 molecular weight antigen delineated by CD38 is not expressed throughout B-cell differentiation, and has been described as a "jumping antigen" in that it appears on pre-B cells but is then lost until the late stages of differentiation.¹⁰

One disadvantage of using antibodies against differentiation antigens on B cells is that, unlike idiotypic Ig, which is a highly restricted B-cell marker, these antigens are often expressed by other cells. The question then is whether any

of the cross-reactivity could be damaging, and although this is important, it should be remembered that for a natural effector cell to act against an antibody-coated target cell, a lethal array of Fc regions must be displayed.¹¹ This requirement may not be met by cells that express the cross-reacting antigen at low levels. For anti-CD38, there are two crucial potential cell populations that express the antigen and therefore could react: the natural killer (NK) cells, which may be involved in mediating antibody-dependent cellular cytotoxicity (ADCC),¹² and the progenitor cells, which are required for maintaining hematopoiesis.¹³ The effect of the chimeric antibody on these populations has been examined by functional assays. The other cell populations that are known to carry this antigen are thymocytes and activated T cells,¹³ and it remains to be seen if this reactivity presents a problem for treating patients with myeloma.

Several difficulties have thwarted success in the use of MoAbs to treat cancer.¹¹ In fact, the mechanism by which target cells are attacked by antibody is not fully understood, but ADCC may contribute.¹⁴ This can be mediated *in vitro* by leukocytes via receptors for the Fc region of IgG (FcR), FcRI or FcRII, or by NK cells via the FcRIII.¹⁵ However, there is evidence that NK cells are of primary importance,¹⁶

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and this presents a problem because the FcRIII carried by NK or lymphokine-activated killer effector cells in human blood does not recognize the Fc region of mouse MoAbs of the major subclasses IgG1, IgG2a, and IgG2b.¹⁷ The problem can be solved by replacing the mouse Fc by human Fc derived from IgG1 either by chemical or genetic engineering.^{18,19} Human IgG1 is in fact recognized not only by the FcRIII, but also by FcRI and FcRII.¹⁵ This replacement of the mouse Fc also removes potentially immunogenic epitopes.

A second difficulty is that many surface antigens can undergo antibody-induced modulation; this occurs more efficiently *in vivo* than *in vitro*²⁰ and varies for different antigens, but can be reduced by using univalent antibody.¹¹ Thirdly, secretion of the antigen will form a barrier to antibody attack as well as generating immune complexes.¹¹ A final problem is the immune status of the patient, and this is a double-edged sword in that an antibody response against the infused antibody is undesirable, but an active effector cell function is required for elimination of target cells. This report describes the preliminary results obtained in attempting to assess and overcome these problems to use immunotherapy as a treatment for multiple myeloma.

MATERIALS AND METHODS

Cell preparations. Patients with multiple myeloma were either inpatients or attending clinics as outpatients at Bournemouth or Poole General Hospitals. They were unselected and therefore were at various stages of disease and treatment (Table 1).²¹ Informed consent was obtained. Normal controls were from clinical and laboratory staff. Bone marrow samples were obtained from pa-

tients and normals by aspiration from either the iliac crest or sternum. Blood samples for functional assays were taken into heparin and processed within 2 hours. Both blood and bone marrow samples were separated on Ficoll-Hypaque by centrifugation at 400g for 20 minutes and cells were harvested from the interface. For investigation of cytoplasmic antigens, cytocentrifuged preparations were used. These were made by placing one to three drops of a cell suspension (10⁶/mL) in medium into a cytocentrifuge well and centrifuging at 100g for 10 minutes onto glass slides. These were air dried overnight at room temperature.

The Namalwa cell line (Flow Lab, Rickmansworth, Herts, UK) arose from an Epstein-Barr virus-transformed Burkitt's lymphoma. It is kept in continuous culture in the laboratory and grows as single cells, which express IgM λ at the cell surface.

Immunofluorescence. This was performed mainly on the FACS-SCAN (Becton Dickinson Lab Systems, Mountain View, CA), which allows selection of cell populations of varying size and granularity. For analysis of expression of the CD38 antigen, OKT10 antibody (American Type Culture Collection [ATCC] was used at 10 μ g/mL. A control mouse MoAb (see below) was also used, and detection of bound antibody was with fluorescein isothiocyanate (FITC)-sheep antimouse IgG.

In some cases, double-immunofluorescence microscopy was used to identify plasma cells that express CD38. For such cases, cell preparations were treated with anti-CD38 and detecting antibody as described above, and were then resuspended in 1% paraformaldehyde for 1 hour on ice. Cells were then centrifuged, resuspended in medium, and cytocentrifuged. After fixation for 10 minutes in 95% ethanol, they were stained for cytoplasmic IgG using polyclonal rabbit antihuman IgG, with TRITC-goat antirabbit IgG for detection. A rabbit anti-idiotypic Ig antibody specific for a patient with lymphoma was used as a control.

Immunoenzymatic analysis. This technique was applied to cyto-

Table 1. Clinical Details of Patients With Multiple Myeloma

Patient	Age	Sex	Paraprotein		Stage	Treatment	WCC		
			Type	Level (g/L)			G	M (x10 ⁹ /L)	L
1	54	M	IgG κ	40.5	IA	ABST	3.6	0.6	1.2
2	55	M	IgG κ	38.1	IIIA	Nil	2.3	0.5	1.9
3	62	M	IgG λ	10.8	IIIA	RT	8.1	1.4	0.7
4	52	F	κ Light chain		IIIA	M + P	1.3	0.2	0.3
						ABMT			
						VADx2			
5	60	F	IgG λ	31.7	IIA	HDM	0.6	0.2	0.6
						ABST			
						Relapse			
6	75	F	IgG κ	19.0	IIIA	M + P	3.4	1.1	2.3
7	52	M	IgA λ	30.3	IIB	M + P	1.5	0.3	1.1
8	72	M	IgG κ	6.6	IIA	RT	2.4	0.3	0.7
9	69	F	IgG κ	37.4	IA	Nil	2.9	0.0	0.7
10	61	F	IgG κ	32.4	IIIA	Nil	5.0	0.1	1.3
11	80	M	IgG λ	56.1	IIA	M + P	1.0	0.3	0.6
12	75	M	IgA κ	14.9	IIA	M + P			
13	75	F	IgG κ	32.0	IIA	Relapse	NA	NA	NA
						M + P			
						RT			
14	82	F	IgG κ	24.0	IA	M + P			

Abbreviations: ABST, autologous blood stem cell transplant; RT, radiotherapy; M + P, melphalan plus methylprednisolone; ABMT, autologous bone marrow transplant; VAD, vincristine, adriamycin, daunorubicin; HDM, high-dose melphalan; WCC, white blood cell count subdivided into G, granulocytes; M, monocytes; L, lymphocytes; NA, not available.

centrifuged cell preparations, fixed for 10 minutes in dry acetone, and it is able to detect cytoplasmic and some surface antigens. After exposing the cells to the mouse MoAb, a sheep antimouse IgG was added, followed, after the usual washes, by complexes of alkaline phosphatase and mouse anti-alkaline phosphatase (APAAP). Freshly made substrate, consisting of Fast Red (0.5 mg/mL; Sigma Chemical Co, Poole, Dorset, England), levamisole (0.25 mg/mL), and naphthol (2 mL), was filtered and applied to the slides for 30 minutes in the dark. Hematoxylin was used for counterstaining.

Monoclonal and chimeric antibodies. The hybridoma secreting the anti-CD38 antibody OKT10 was obtained from ATCC. It was passaged into pristane-primed Balb/c mice and ascitic fluid containing the antibody collected. A parallel control antibody of the same subclass (IgG1), with specificity for a private idiotype determinant on cells from a patient with lymphoma, was prepared in the same way. The third antibody to be made as a chimeric derivative was the mouse MoAb against human IgM heavy chain (M15/7), which was prepared in this laboratory by Dr M.J. Glennie. The mouse MoAb against the λ light chain was raised in this laboratory.

The method for preparing the FabFc has been described.¹⁸ The principle of the method is to produce a maleimide derivative of the antibody Fab fragment and couple this to human Fc, which has available reduced disulfide bonds. The two components are joined via hinge-region cysteine residues. Briefly, human Fc, prepared by papain digestion of normal IgG, was reduced with 6 mmol/L of dithiothreitol (DTT) at pH 8 and 37°C for 15 minutes. The Fc was then separated from DTT and transferred into buffer of pH 5.3 (0.5 mol/L Na acetate, 5 mmol/L in EDTA, N₂-saturated) by passage through Sephadex G-25 (Pharmacia Ltd, Milton Keynes, UK). To prepare the Fab' component, F(ab')₂ was first obtained by peptic digestion of the mouse IgG1 MoAb. The protein was then subjected to sequential reduction with DTT at pH 8, thiol-disulfide interchange with 2,2'-dipyridyldisulfide at pH 5.0, and a second reduction with DTT at pH 5.0: the resulting Fab'γ has its γ-light SS bond intact, but displays three SH groups derived from reduced inter-γ SS bonds.¹⁸ The separated Fab'γ (SH) reacted with a large molar surplus of the bismaleimide linker o-phenylenedimaleimide to yield Fab'γ (mal) with a single free maleimide group: two of the hinge-region SH cyclize with the linker, while the third reacts with one end only.¹⁸ Separation of the protein from surplus linker took place on the ion-exchanger Phospho-Ultrogel A6R (IBF, Biotechnics, Villeneuve-la-Garenne, France), from which the protein can be eluted in concentrated form. Immediately after preparation, Fab'γ (mal) was allowed to react with Fc (SH) at a molar ratio of Fab:Fc = 1.25, at pH 5.3 for 2 hours at room temperature. Finally, FabFc, with its protein moieties linked by two thioether bonds in tandem, was separated from other species by passage through Sephacryl S200HR (Pharmacia).¹⁸

Antibody-dependent cellular cytotoxicity. Target cells from the Namalwa cell line were washed in warm medium (RPMI + 10% fetal calf serum [FCS]) and then incubated with 0.4 mCi of ⁵¹Cr-sodium chromate (Amersham International plc, Amersham, UK) for 30 minutes at 37°C. The cells were washed thoroughly in medium and resuspended at 2 × 10⁵/mL for the assay. Effector cells consisting of the mononuclear cell fraction of human blood were washed and resuspended at 5 × 10⁶/mL in medium before use. The effector:target ratio for the assay was generally 50:1.

Antibody (50 μL) was placed in the wells of a 96-well U-bottomed microtiter plate and radiolabeled target cells (50 μL) were added. After standing on ice for 15 minutes, effector cells (100 μL) were also added to the wells and the plate was centrifuged gently (100g) for 5 minutes. It was then transferred to a humidified incubator, usually for 4 hours in the presence of 5% CO₂, after which the cells were pelleted and 100 μL of supernatant removed

for γ counting. The total amount of ⁵¹Cr incorporated into target cells was measured by lysing an aliquot in 1% NP40. Percent cytotoxicity was calculated from the formula: specific cytotoxicity (%) = (A - C)/(B - C) × 100, where A = cpm released in the presence of antibody, B = cpm released by NP40, and C = cpm released in the presence of medium alone.

The ADCC using target cells from the guinea pig leukemia, L₂C, has been described.¹⁷ It uses an Fab-HuFc derivative of a mouse monoclonal anti-idiotypic antibody raised against the surface IgM of the leukemia cells, with human mononuclear cells as effectors.

Progenitor cell assay. Bone marrow samples from three normal individuals were separated on Ficoll-Hypaque, and duplicate samples of the cells (6 × 10⁵) were exposed to antibody or control, at a saturating concentration, at RT for 30 minutes. For marrow 1 the antibody was then removed, but for marrows 2 and 3 it was left in the culture. To one of each duplicate was added an equal volume of sterilized complement, and the cells were incubated for 30 minutes at 37°C. For marrow 1 rabbit complement was used, and autologous human complement was used for marrows 2 and 3. Following this incubation, each aliquot of cells was cultured in Iscove's modified Dulbecco's medium containing 1.32% methyl cellulose, 5% phytohemagglutinin-stimulated leukocyte-conditioned medium, 1% bovine serum albumin (Sigma Chemical Co), 30% FCS, and 2-mercaptoethanol 10⁻⁴ mol/L. For erythroid colonies, 3 U/mL erythropoietin (Terry Fox Laboratory, Vancouver, Canada) was added.²² Quintuplicate samples (1 mL containing 10⁵ cells) of the cell suspensions were then plated into 35-mm Petri dishes and incubated in 5% CO₂ for 14 days. After incubation, the number of aggregates consisting of greater than 40 cells were counted as colonies, with burst-forming units-erythroid (BFU-Es) being recognized by their distinctive shape and color.

RESULTS

Expression of CD38 antigen by neoplastic plasma cells. Although there are extensive data available on the expression of the CD38 antigen by various cells,¹³ it was considered necessary to confirm that this target antigen was well represented on the neoplastic plasma cells, and to examine expression by other cell populations. Bone marrow samples from three patients with significant numbers of plasma cells (Table 2) were selected for detailed analysis by fluorescence activated cell sorter (FACS). A dot plot of forward scatter, a function largely of cell size, against 90° scatter, which is partially dependent on cellular granularity, was obtained (Fig 1) and was similar for all three patients. From the plot three groups of cells were delineated, with attributions as follows: the first was of granular cells of all sizes, and includes cells of myeloid origin; the second was of small cells of low granularity and includes the lymphocytes; the third was of larger cells with low and intermediate granular-

Table 2. Expression of CD38 by Bone Marrow Cells From Patients With Myeloma

Patient	Plasma Cells in Marrow (%)	CD38 ⁺ Cells (% by FACS) Cell Group			CD38 ⁺ Plasma Cells (% by APAAP)
		1	2	3	
1	8.3	8.2	—	65	79
2	12.1	30	28	95	88
3	15.4	20	22	77	97

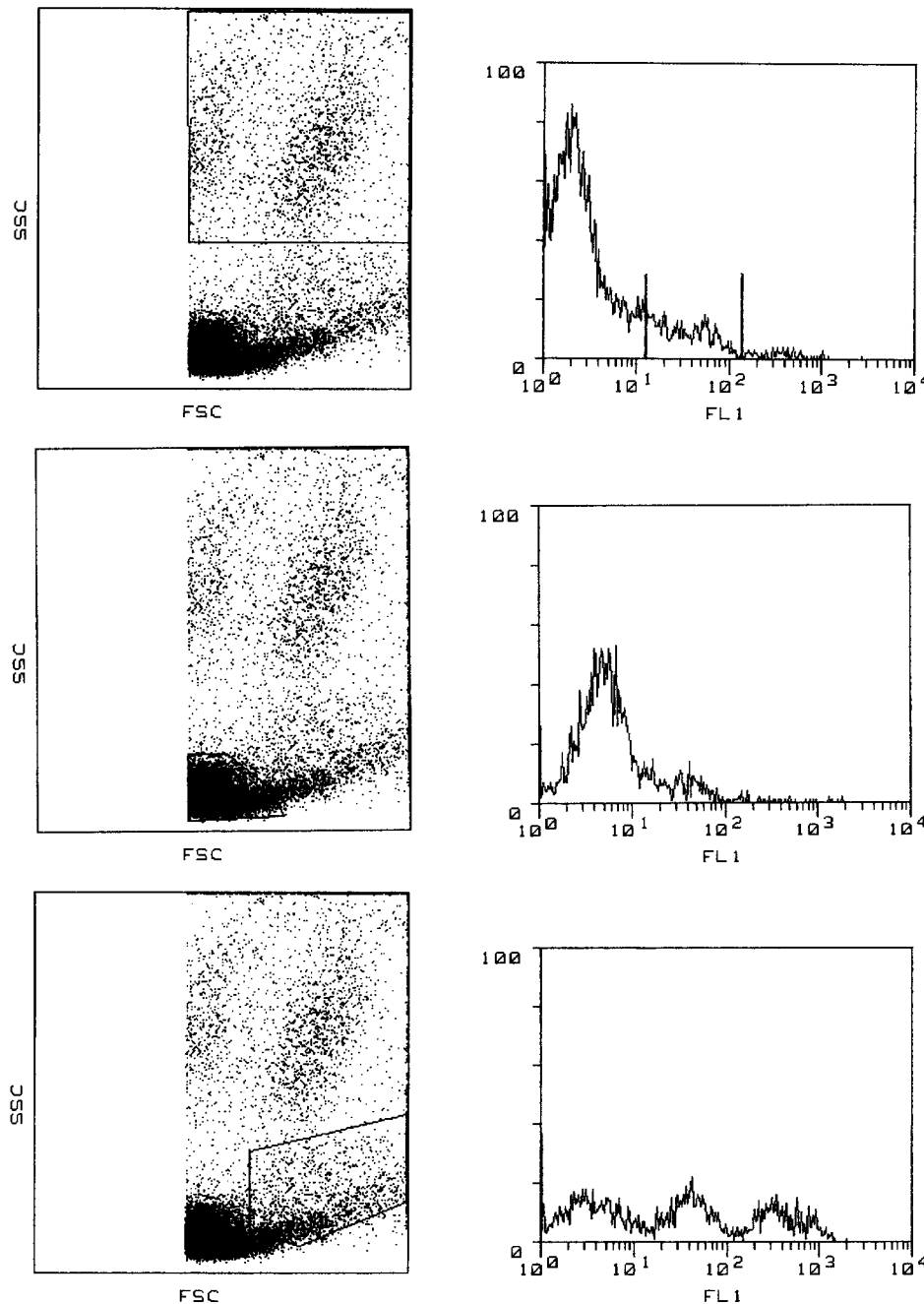


Fig 1. Expression of CD38 antigen by cells from the bone marrow of a patient (patient 3, Table 1) with myeloma. From the dot plot obtained by FACS-SCAN analysis, three cell populations (groups 1 through 3) were selected as indicated by outlining. The fluorescent profiles of each of these populations stained with OKT10 are shown. The background marker selected using a control antibody was 15 on the FL1 scale. Moderate fluorescence was in the range 15 to 150 and strong fluorescence greater than 150, as indicated in the profile for group 1.

ity and includes monocytes, plasma cells, and large granular lymphocytes (LGLs). This latter group was not further subdivided because the neoplastic clone may be heterogeneous.

The fluorescent profile of each of these populations stained with OKT10 is also shown in Fig 1. It is clear that strongly staining cells are confined to group 3. In this group there is also a cell population that expresses moderate amounts of CD38 antigen. However, in groups 1 and 2 there is only a small proportion of the total cell numbers that show this moderate reactivity, and no strongly staining cells.

The percentages of cells in group 3 that were recognized by OKT10 at both the strong and moderate levels were

calculated for each of the patients (Table 2), and clearly the majority of these cells express the antigen. To analyze specifically the plasma cell component of this population, double staining was used. For this, surface CD38 antigen was detected with OKT10 plus FITC-sheep antimouse IgG, and cytoplasmic IgG was detected with rabbit antihuman IgG plus TRITC-goat antirabbit IgG. This technique demonstrated that 80% to 90% of the plasma cells, which were readily identifiable by the presence of cytoplasmic IgG, were also strongly positive for the CD38 antigen. Other cells in the marrows appeared weak or negative for surface CD38.

The APAAP procedure was then used to examine

Table 3. Effect of Chimeric Anti-CD38 on Progenitor Cells From Normal Marrows

Incubated With:	Normal Marrows					
	1		2		3	
	Colonies/10 ⁵ MNCs					
	CFU-GM	BFU-E	CFU-GM	BFU-E	CFU-GM	BFU-E
Marrow only	19 (5.9)*		60 (4.7)	33 (9.9)	64 (10.4)	6 (5.6)
Control FabFc	35 (10.4)	8 (4.7)	41 (9.4)	23	52 (11.7)	0
Control FabFc + C'	26 (4.2)	3 (2.1)	53 (13)	26 (10)	72 (15.4)	4 (0.7)
Anti-CD38 FabFc	21 (3.8)	10 (3.3)	60 (8.5)	30	70 (4.9)	5
Anti-CD38 FabFc + C'	21 (5.0)	6 (2.1)	57 (12)	17 (0.7)	69 (8)	10

*Figures in brackets are \pm SD.

further the morphology of cells that were positive for CD38, and again the plasma cells were the most strongly reactive population in all three bone marrows, with 80% to 100% clearly stained (Table 2). Monocytes and large granular lymphocytes were weakly positive by this technique and accounted for the nonplasma cell population.

Effect of anti-CD38 on normal bone marrow progenitor cells. The effect of the chimeric antibody on the growth of colony-forming units of the granulocyte/macrophage series (CFU-GM) and on BFU-E was investigated in the presence or absence of complement, from either rabbit or autologous human serum. Results from three separate normal bone marrows (Table 3) indicate no detectable deleterious effect of the chimeric anti-CD38 antibody on the ability of these progenitor cells to form colonies. Even the addition of complement caused no measurable loss of these cells.

Mediation of ADCC by the chimeric anti-CD38 antibody. The Namalwa cell line was used as a target for investigating the ability of the chimeric antibody to mediate ADCC. These cells express CD38 and IgM λ , and the fluorescent profiles for CD38 and IgM are of similar intensity. The ability of chimeric FabFc from anti-CD38 to mediate killing of Namalwa cells by normal human blood mononuclear cells is shown in Fig 2, and it is clear that it is highly effective, causing release of $\sim 50\%$ of ^{51}Cr , down to a final antibody concentration of $\sim 3 \mu\text{g/mL}$. In contrast, in the same assay, chimeric FabFc from anti-IgM released only $\sim 20\%$ of the ^{51}Cr even at saturating concentrations, demonstrating a variable efficiency among cell surface antigens in acting as targets for the effector cells. Control FabFc from an unrelated antibody gave no significant release of radiolabel ($< 5\%$).

The parental mouse monoclonal anti-CD38 was also tested in this assay, and was found to be completely ineffective in mediating ADCC at all concentrations (Fig 2), indicating an Fc on the mouse IgG1 incapable of recruiting effector cells.

The time course of the ADCC using chimeric antibody at $5 \mu\text{g/mL}$ showed that specific lysis increased over the 5-hour period, approaching a plateau at 4 to 5 hours (Fig 3). It was also highly dependent on the effector:target ratio (Fig 4).

The role of the FcRIII receptor (CD16) in mediating

cytotoxicity by the blood mononuclear cells in this system was investigated by examining the effect of an antibody (3G8)²³ against the receptor on the ADCC assay. Results (Fig 5) indicate that the anti-CD16 antibody caused strong inhibition of ADCC, thereby implicating FcRIII in the cytotoxic mechanism.

Effect of chimeric anti-CD38 on a standardized ADCC. Because it is known that LGLs express the CD38 antigen, it is possible that the anti-CD38 could inhibit cytotoxicity mediated by these cells. This was explored using a "conventional" ADCC test involving target cells from the guinea pig leukemia, L₂C, and a chimeric Fab-human Fc antibody derivative with specificity for an idiotypic determinant on these cells.¹⁷ The effect of adding graded doses of chimeric anti-CD38 into this system was then assessed with the results shown in Fig 6, which indicate a possible minor reduction in cytotoxicity because of the anti-CD38, as compared with control chimeric antibody. However, assessment was complicated by the fact that there was some

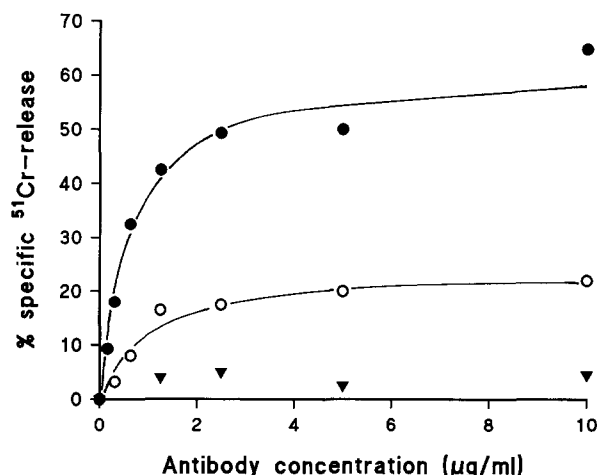


Fig 2. Mediation of ADCC against Namalwa cells by human blood mononuclear cells in the presence of MoAb or chimeric FabFc derivatives. Points represent the mean of triplicates with a range of less than 7%. (●), FabFc ex anti-CD38; (○), FabFc ex anti-IgM; (▼), parental anti-CD38.

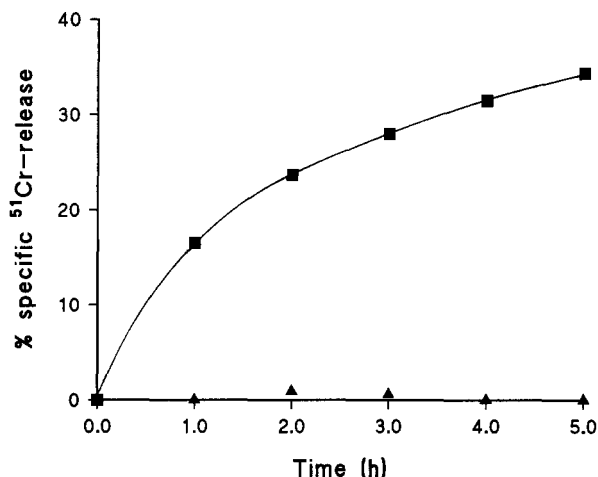


Fig 3. Time course of ADCC against Namalwa cells mediated by human blood mononuclear cells in the presence of chimeric FabFc ex anti-CD38. Radiolabeled target cells were incubated in the presence of: (■), FabFc ex anti-CD38, or (▲), control FabFc, both at 5 μ g/mL, together with effector cells at 50:1 (effector:target), for the times indicated. Results are shown for a single experiment, but have been confirmed in at least two further experiments.

inhibition at the highest concentration of IgG (25 μ g/mL) for both control antibody and anti-CD38.

Analysis of the ability of anti-CD38 or the chimeric derivative to modulate surface CD38 in vitro. The effect of incubating Namalwa cells at 37°C in the presence of either parental anti-CD38 or the univalent chimeric Fab-humanFc derivative, using FITC-sheep antimouse IgG and FITC-goat antihuman Fc, respectively, as detecting antibodies was measured over a 3-hour period. It was found that the reduction in mean fluorescence after this time was 23% for whole antibody, and 10% for chimeric antibody, consis-

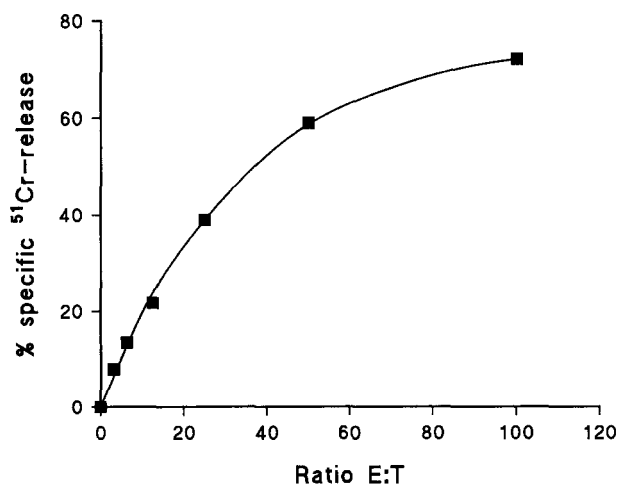


Fig 4. Variation of ADCC against Namalwa cells by using differing numbers of human blood mononuclear effector cells. Radiolabeled target cells were incubated in the presence of FabFc ex anti-CD38 at 5 μ g/mL, together with effector cells at varying effector:target ratios, for 4 hours. Radioactivity released in the presence of control FabFc (<5% for each point) has been subtracted. Results are shown for a single experiment, but have been confirmed in at least two further experiments.

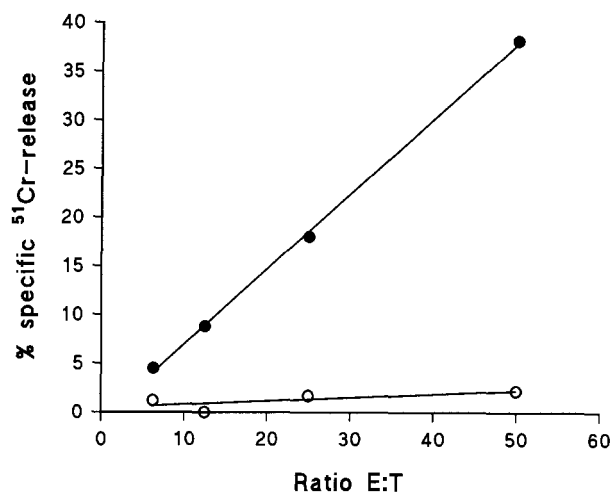


Fig 5. Effect of antibody against the FcR111 receptor (CD16) on mediation of ADCC against Namalwa cells by human blood mononuclear cells in the presence of chimeric FabFc ex anti-CD38. Radiolabeled target cells were incubated in the presence of FabFc ex anti-CD38 at 5 μ g/mL, together with effector cells in varying numbers, for 3 hours. Blocking antibody added was: (○—○), anti-CD16 (3G8, 50 μ g/mL) or (●—●), control antibody (anti-idiotypic, 50 μ g/mL). Radio-label released by the control antibody has been subtracted. Results are shown for a single experiment, but have been confirmed in at least two further experiments.

tent with a low rate of modulation for the CD38 antigen, which is decreased even further by using univalent antibody.

Investigation of potential blocking antigen in myeloma sera. For antibody therapy it is desirable to have little or no target antigen present in the patient's serum. To detect material capable of reacting with the anti-CD38 antibody, blocking studies were performed using the FACS. First, a

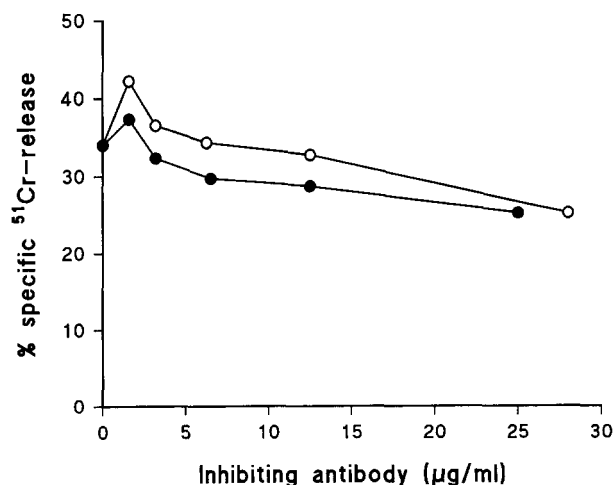


Fig 6. Effect of chimeric FabFc ex anti-CD38 on the ability of effector cells to mediate ADCC using a standardized assay. Radiolabeled target cells from the guinea pig L₂C leukemia were incubated with a chimeric FabFc ex anti-L₂C idiotype antibody at 10 μ g/mL, together with human blood mononuclear cells at 50:1 (effector:target) for 3 hours. Blocking antibody added at varying concentrations was: (●—●), FabFc ex anti-CD38, or (○—○), FabFc ex control anti-idiotypic.

saturation curve for the binding of anti-CD38 to Namalwa cells was constructed and was found to reach saturation at 10 $\mu\text{g/mL}$ antibody. Accordingly, a concentration of 5 $\mu\text{g/mL}$, which is just approaching saturation, was chosen to assess the blocking capability of six randomly chosen patients' sera, or a normal pooled human serum control, all used at 50% concentration. In all cases the presence of the serum had no effect on the amount of anti-CD38 bound to the target cells, indicating no detectable antigen in the sera.

Measurement of the ability of effector cells in patients' blood to mediate ADCC with chimeric anti-CD38. Blood samples from 14 patients with myeloma, taken at random during attendance at the clinic or from inpatients, and with disease stages as shown in Table 1²¹ were used to assess the general level of competence in such patients to act in the ADCC. A comparison was made with normal individuals, and in all cases the chimeric anti-CD38 FabFc was used. Results (Fig 7) show that all the patients were able to mediate ADCC and that the mean value for specific lysis (37.7%) was insignificantly different from that of normals (40.1%).

DISCUSSION

Attack on a tumor by an MoAb offers the patient a treatment with few, if any, side effects, but it is becoming clear that the efficacy of such an antibody is limited.¹⁴ One possible reason for this is that the primary candidate for the major mechanism of attack mediated by antibody *in vivo* is antibody-dependent cellular cytotoxicity²⁴; for this to occur, the effector cells must recognize the Fc region of the MoAb attached to the target cell. In the case of mouse IgG1, IgG2a, and IgG2b, human blood NK cells generally fail to mediate ADCC, and only the minor subclass IgG3 is active.²⁵ This has led to the development of chimeric antibodies that carry human Fc,²⁶ or where the majority of

the Ig has been replaced by human sequences, leaving only the hypervariable regions from the original antibody.²⁷ Clearly, such modified antibodies have the additional advantage of not being as immunogenic in the recipient patient.¹¹ However, in these early stages of refining immunotherapy the use of relatively simple and rapid chemical methods of attaching antibody sites to human Fc allows preparation of a wide range of antibodies in amounts required for therapeutic evaluation. It also enables other structural options to be investigated such as univalency, which reduces modulation of the target cell surface antigen,¹¹ and a double Fc, ie, FabFc2, which appears to have enhanced interaction with effector cells.¹⁸

With this technology we have been able to consider the application of antibody therapy to multiple myeloma, a disease in which chemotherapy has not been particularly rewarding. However, the problem in selecting a suitable starting antibody is particularly acute in myeloma because the plasma cell, which is a differentiated cell designed for manufacturing antibody, is rather denuded of surface antigens. There is also the question of whether less differentiated cells are maintaining the neoplastic population, and that even complete removal of plasma cells would leave a putative clonogenic cell unscathed. It has been difficult to investigate bone marrow populations for the presence of such cells,⁸ and in our experience even the use of anti-idiotypic antibodies has been thwarted by an inability to totally remove bound idiotype Ig from the surfaces of the normal cells in the marrow. At this point we consider that the CD38 antigen represents the best target for antibody attack for the reasons given in the introduction to this report, but it would be possible eventually to combine anti-CD38 with, eg, an anti-B-cell antibody, if firm evidence for a CD38⁺ B-cell precursor is found.²⁸

Expression of the CD38 antigen is quite consistent on neoplastic plasma cells as described in this study and also by others.^{9,13} The major concern was that the recorded expression of the antigen by LGLs¹² would mean that this cell population, considered to be an important component of the effector cells of ADCC, would self-destruct in the presence of anti-CD38. However, the results obtained do not indicate that this occurs, because the cells kill target Namalwa cells with high efficiency when surrounded by the antibody. Also, anti-CD38 fails to reduce significantly the ADCC mediated by human effector cells in an independent assay using anti-idiotypic antibody. The reason for this escape of LGLs from attack is not clear but could be due to the failure to bind sufficient antibody to form a lethal array²⁹ or to some mechanism inherent in the LGL. Potential problems because of the display of the CD38 antigen on other vital cells are difficult to deal with comprehensively before testing *in vivo*, but there was no detectable effect on progenitor cells of the CFU-GM or BFU-E series of normal marrow. The possibility of affecting T-cell function due to the expression of CD38 antigen by activated T cells has not yet been examined, but presumably would be transient.

The results obtained in the model system for ADCC using the CD38-expressing Namalwa cell line indicated that, rather than being diminished because of loss of LGLs,

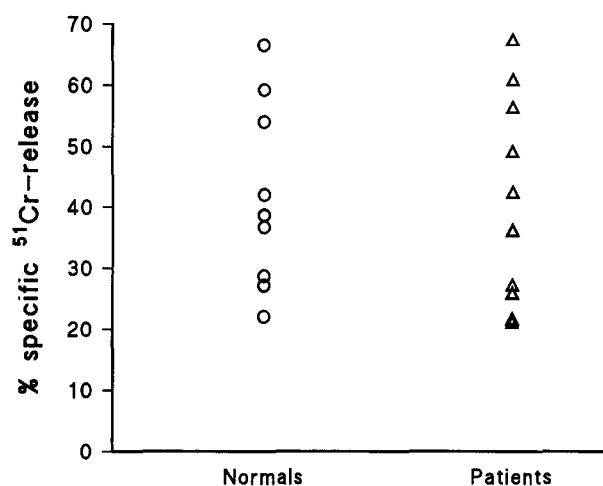


Fig 7. Measurement of the ability of effector cells in patients' blood to mediate ADCC with chimeric FabFc ex anti-CD38. Target Namalwa cells were incubated in the presence of FabFc ex anti-CD38 at 5 $\mu\text{g/mL}$, together with human blood mononuclear cells from normals, or from patients with myeloma, for 4 hours. Radiolabel released by control chimeric antibody (<5%) has been subtracted. Mean value (\pm SD) for the two groups were: normal, 40.1 ± 14.4 ; patients, 37.7 ± 15.5 .

the chimeric anti-CD38 killed target cells with high efficiency, releasing ~60% of incorporated radiolabel. In contrast, in the same experiment a chimeric anti-IgM antibody could only release ~20% radiolabel from these cells. This was not due to differences in display of the two target antigens because the immunofluorescent signals were similar for CD38 and IgM, nor is modulation likely to be a factor, because both antibodies are univalent and therefore have a much-reduced tendency to modulate.¹¹ The reason for the success of the anti-CD38 antibody is not clear and may reflect subtle steric differences. The cytotoxic capability of the chimeric antibody is in marked contrast to the parental mouse MoAb, which is of the IgG1 subclass and is completely unrecognized by effector cells. Because murine IgG1 is recognized by the human FcRI and FcRII receptors,¹⁵ this indicates that the effector cells, as obtained from human blood, are not using these receptors to any great extent. This could be an advantage because it might be expected that the FcRI would be blocked by monomeric IgG in human serum, whereas the FcRIII would not.¹⁵ However, this point requires further investigation by analyzing the effects of patients' sera or IgGs of a defined subclass on the ADCC mediated by patients' blood mononuclear cells, and these experiments are in progress. It remains to be seen if the ADCC is equally efficient in killing patients' neoplastic plasma cells, an experiment that awaits a patient with a source of cells that will take up radiolabel; a plasma cell leukemia would be ideal.

Another factor that has contributed to the irregular performance of antibody therapy is that cells can often secrete molecules normally considered to be confined to the cell surface. This has been a problem in the use of anti-idiotypic antibody for B-cell lymphoma,¹⁴ and could apply to other molecules such as the T-cell-associated antigens CD8, CD2, and the interleukin-2 (IL-2) receptor (CD25).³⁰ For CD38 this appears not to occur because serum from patients with myeloma caused no blocking of

binding of anti-CD38 when tested at the most sensitive part of the saturation curve.

Transfer of results obtained in vitro to the patient with disease is fraught with difficulty, but if ADCC has importance in antibody attack it is vital to assess the ability of the patient group to use this pathway. There is some evidence for diminution of ADCC activity by blood mononuclear cells in untreated patients with B-cell lymphoma,³¹ although often the relative contributions of disease and subsequent chemotherapy are difficult to disentangle. For patients with myeloma, who may be undergoing a variety of chemotherapeutic schedules, and who are rarely untreated, it was decided to test blood samples taken at random rather than at any particular point of therapy. The results showed that there was no apparent diminution of ADCC when compared with normals. In fact, earlier studies on NK activity against the K562 cell line in patients with myeloma had found that activity in the blood mononuclear cell population was comparable with normals, and that activity in bone marrow cells was increased above the normal range.³² Because ADCC is thought to be mainly caused by NK cells,¹⁶ these results encourage the hope that antibody attack on tumor by ADCC will occur, especially in the marrow where the disease is most manifest. Although it might be unnecessary, it would be possible to boost effector cell function by administration of IL-2.³³ With regard to immunogenicity of administrated antibody, this should not be a major problem for patients with myeloma who have a depressed ability to generate antibody responses.³⁴ Even for B-cell lymphoma the anti-antibody response has been muted¹¹ and the replacement of mouse Fc by human Fc should reduce this possibility further.

In conclusion, it appears that a chimeric FabFc anti-CD38 antibody fulfills the requirements, as far as can be analyzed in vitro, for an immunotherapeutic tool for human multiple myeloma. Scaling up of the preparative procedure for therapy is already available and the next stage will be to test the approach in selected patients.

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