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Expression of Antigens Associated with Small Cell Carcinoma of the Lung on Hematopoietic Progenitor Cells[1]

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ABSTRACT

We have previously described a panel of four monoclonal antibodies (MoAbs) reactive with antigens expressed on tumor cells from small cell carcinoma of the lung (SCCL). These IgM MoAbs are cytotoxic to SCCL cells in the presence of complement and thus have potential as reagents for the removal of SCCL cells contaminating bone marrow autografts. Therefore, we examined the cytotoxicity of these MoAbs against normal hematopoietic progenitor cells as a preliminary step toward their use in clinical trials. In this paper we report the results of treating normal bone marrow and peripheral blood mononuclear cells with the four IgM MoAbs, as well as an IgG2a MoAb that we have recently prepared against an SCCL cell line, DMS 406. Peripheral blood and bone marrow mononuclear cells were treated with the MoAbs alone or in combination, in the presence of rabbit complement, and then plated into colony-forming assays. Notably, only one MoAb, SCCL-1, had any demonstrable cytotoxicity against progenitor cells. This toxicity was limited to bone marrow burst-forming unit-erythroid and all classes of blood progenitor cells. A MoAb cocktail containing a combination of either four or five MoAbs + complement spared most marrow progenitor cells. These studies extend the base of information regarding the expression of SCCL-associated antigens on hematopoietic cells and indicate that selected MoAbs may be used safely for the removal of SCCL cells from autografts by complement-dependent lysis or other means.

INTRODUCTION

Small cell carcinoma of the lung (SCCL)[3] is a highly lethal form of lung cancer which spreads hematogenously early in its course (1). As many as 50% of patients have bone marrow metastases at some point in their disease (1). Chemotherapy of SCCL has resulted in significant prolongations in survival and the achievement of remissions in many patients (2). However, almost all patients die from this disease within 2 yr of diagnosis due to drug-resistant relapse (1, 2). Modern approaches to the treatment of SCCL have involved the use of higher than conventional doses of chemotherapeutic agents with or without autologous bone marrow rescue (3–7). Since the major dose-limiting toxicity of many chemotherapeutic agents is myelosuppression, autologous bone marrow transplantation offers a means of achieving higher dosage. The limitations of this approach to therapy are (*a*) the primary resistance of the tumor to even higher doses of chemotherapy and (*b*) the potential seeding of tumor cells contained in the autologous marrow graft. Thus, a means to remove tumor cells from bone marrow from these patients would at least address the latter problem and allow a more critical evaluation of the chemotherapy resistance problem.

We and others have prepared numerous MoAbs that react with antigens on SCCL cells (8–12). One or more MoAbs from our panel of four IgM MoAbs to SCCL, and an IgG2a MoAb recently prepared, react with >95% of clinical SCCL tumor samples and to all SCCL cell lines that we have studied (8). All five of these MoAbs are cytotoxic in the presence of rabbit complement (C′) and thus have the potential of being used in autologous transplantation protocols to remove residual tumor cells. A limitation to this approach could be toxicity to normal hematopoietic stem cells. This is particularly important to consider in light of recent observations that myeloid and SCCL cells have several antigens in common (13–18), which suggests the possibility that SCCL-associated antigens could be associated with hematopoietic progenitor cells. To address this question we have studied the toxicity of our panel of MoAbs against normal hematopoietic CFUs measured in methylcellulose assays. Our results indicate that at least some of these MoAbs, and possibly all of them, have potential as reagents for the removal of SCCL tumor cells from marrow autografts.

MATERIALS AND METHODS

Cells. Bone marrow aspirates were obtained from normal volunteers. Mononuclear cells were obtained by Ficoll-Hypaque gradient centrifugation (19). Mononuclear cells were isolated from the peripheral blood of normal volunteers in the same manner.

Monoclonal Antibodies. SCCL-41, SCCL-114, SCCL-124, and SCCL-175 are all IgM MoAbs prepared by immunization with an SCCL primary tumor (8). The source of MoAbs for these studies was ascites diluted 1:100 in phosphate buffered saline containing 0.01% bovine serum albumin (BSA). In some experiments, the IgM MoAbs were purified as previously described (20). SCCL-1, an IgG2a MoAb, was prepared by immunizing with cells from the SCCL cell line, DMS 406, described by Soranson *et al.* (21, 22) and was purified from ascites by protein A affinity chromatography (20). An irrelevant IgM monoclonal, sIgA-39, and an irrelevant IgG2a, Vx4, MoAb were used as negative controls. Positive controls for cytotoxicity to hematopoietic progenitor cells included W632, a MoAb to class I HLA (23), and AML-1-99, an IgM MoAb produced in this laboratory and known to be cytotoxic to hematopoietic progenitor cells (24). All MoAb preparations (purified, diluted ascites) including negative controls were tested for binding (or lack) to SCCL cell lines, and optimal binding concentrations were used in all of the cytotoxicity experiments based on these results.

Cytotoxicity and Colony-forming Assays. Peripheral blood or bone marrow mononuclear cells were suspended at 2×10^6 cells/ml in a solution of α-medium with 10% fetal calf serum containing MoAbs at a final dilution of 1:100 or at 50 μg/ml in the case of SCCL-1. These concentrations all gave saturating binding on SCCL cell lines as assessed by flow cytometry. A volume of rabbit C′ (Pel Freez, Rogers, AR) was added to achieve a final dilution of 1:6. The cells were incubated at room temperature for 1 h at which time the cells were sedimented, the supernatant was removed, and the cells were resuspended in fresh α-medium (to starting volume) and assessed for viability. Aliquots containing 2×10^5 cells (starting counts) were cultured in 1-ml cultures containing 0.9% methylcellulose, 10% bovine serum albumin (deionized), 10% PHA-LCM or GCT-conditioned medium as sources of colony-stimulating factors, erythropoietin (1 unit/ml, 2-mercaptoethanol) (2×10^{-5} M) and glutamine (2 mM). The cells were cultured for 14 days in a 5% CO_2 humidified atmosphere and scored for numbers of CFU-GM, BFU-E, and colonies comprised of both

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[3] The abbreviations used are: SCCL, small cell carcinoma of the lung; MoAb, monoclonal antibody; CFU-GM, colony-forming unit-granulocyte/monocyte; BFU-E, burst-forming unit-erythroid; CFU-GEM, colony-forming unit-granulocyte/monocyte/erythrocyte; C′, complement.

erythroid and myeloid progenitors, CFU-GEM, using an inverted microscope.

In some experiments, cells from the SCCL cell line, DMS 406, were mixed with normal bone marrow cells to a final mixture of 5% DMS 406 before performing the C′-dependent lysis and progenitor cell culture as above.

RESULTS

Effect of Single MoAbs + C′ on Bone Marrow Progenitor Cells. The viability of bone marrow mononuclear cells, as determined by dye exclusion, was not significantly reduced by treatment with any of the anti-SCCL MoAbs studied (Table 1). Treatment of bone marrow mononuclear cells with MoAbs SCCL-41, SCCL-114, SCCL-124, and SCCL-175 did not significantly decrease the numbers of any progenitor cell class from any subject studied (n = 10). MoAb SCCL-1 reduced BFU-E and CFU-GEM from the bone marrow of several but not all subjects. Results using either purified MoAb, ascites, or culture supernatant were identical.

Effect of Single MoAbs + C′ on Blood-derived Progenitor Cells. Blood-derived progenitor cells displayed a slightly different phenotype (Table 2). Blood-borne CFU-GM were more sensitive to treatment with SCCL-1 and SCCL-124 than marrow-derived CFU-GM. Both blood-derived and marrow-derived BFU-E were sensitive to SCCL-1. SCCL-41, -114, and -175 had no effect on any progenitor cell population.

Effect of Combinations of MoAbs on Bone Marrow Progenitors. In an effort to determine the potential of additive toxicity from a cocktail of several anti-SCCL MoAbs, we treated bone marrow mononuclear cells with either four or five MoAbs + C′ at the same dilutions and/or concentrations of each MoAb that were used when each was used singly. As seen in Table 3, treatment of bone marrow with all five MoAbs resulted in large decreases in marrow-derived mixed colonies and in all blood-borne CFUs. The results obtained with all five MoAbs are similar to those obtained with SCCL-1 alone although less toxicity to BFU-E was observed. These results are not surprising in light of the fact that the concentration of SCCL-1 in the cocktail was the same as in experiments with SCCL-1 alone. The effects of the cocktail and of SCCL-1 alone on mixed progenitor cells may be a reflection of selective toxicity to erythroid precursors. The MoAb cocktail containing the four IgM MoAb did not significantly deplete any class of colony-forming cell from bone marrow, consistent with the findings with each MoAb used separately.

Effect of "Seeding" DMS 406 into Normal Bone Marrow. Since DMS 406 is an SCCL cell line that expresses the antigens with which SCCL-1, SCCL-41, SCCL-114, SCCL-124, and SCCL-175 react we investigated whether different effects on hematopoietic progenitor cells might be seen if DMS 406 cells were mixed into normal marrow. As seen in Table 4, there was no effect on the numbers of any class of CFU when DMS 406 was added. These experiments were not designed to measure the efficacy of DMS 406 killing. The presence of DMS 406 cells had no effect on the numbers of CFUs observed in control cultures.

DISCUSSION

The MoAbs studied in this report all react with SCCL tumors and thus have potential for the diagnosis and treatment of this disease. For example, SCCL-175 reacts with 95% of SCCL tumors obtained from patients with this disease and thus far has shown no cross-reactions with any other types of tumor cells (25) or normal cells with the exception of renal proximal tubular cells. The other four MoAbs react with all of 15 SCCL cell lines that we have studied (8) and not normal tissues or cells. The antigens defined by these MoAbs are only partially

Table 1 *C′-dependent cytotoxicity of anti-SCCL MoAbs to normal bone marrow-derived hematopoietic progenitor cells*

Bone marrow cells were incubated with MoAb + C′ for 1 h and cultured in methylcellulose for 14 days.

MoAb	% of control[a]		
	CFU-GM[a]	BFU-E	CFU-GEM
SCCL-1	82.0 ± 8.6	32.5 ± 15.0	28.5 ± 20.0
SCCL-41	91.2 ± 5.9	98.5 ± 12.9	120.6 ± 25.8
SCCL-114	87.9 ± 8.4	125.2 ± 20.9	140.2 ± 35.0
SCCL-124	88.3 ± 10.1	115.6 ± 18.0	79.0 ± 19.8
SCCL-175	88.8 ± 8.0	111.0 ± 13.9	132.0 ± 20.9
AML-1-99	20.8 ± 5.0	18.9 ± 9.0	5.0 ± 3.0
W6-32	2.5 ± 1.5	2.5 ± 1.5	0 ± 0

[a] Numbers shown are the averages ± SE of the percentages from 10 experiments of colonies remaining after MoAb treatment compared to control values obtained by averaging colony counts from control MoAb + C′ alone. Control values used were the averages of colonies that grew after no MoAb treatment and after exposure to complement only. Colony numbers in these experiments ranged from 100 to 200 for CFU-GM and BFU-E and 5 to 20 for CFU-GEM, all per 2 × 10^5 cells.

Table 2 *C′-dependent cytotoxicity of anti-SCCL MoAbs to normal peripheral blood-derived hematopoietic progenitor cells*

Bone marrow cells were incubated with MoAb + C′ for 1 h and cultured in methylcellulose for 14 days.

MoAb	% of control[a]		
	CFU-GM[a]	BFU-E	CFU-GEM
SCCL-1	27.2 ± 6.0	10.57 ± 4.8	72.9 ± 56.2
SCCL-41	115.5 ± 26.6	118.43 ± 30.0	113.3 ± 41.5
SCCL-114	97.6 ± 24.1	122.5 ± 10.2	72.4 ± 34.7
SCCL-124	37.0 ± 8.1	92.4 ± 13.3	73.9 ± 34.0
SCCL-175	102.2 ± 42.4	99.6 ± 18.8	125.5 ± 48.3
AML-1-99	7.4 ± 3.9	2.2 ± 1.3	0 ± 0
W6-32	1.9 ± 1.3	4.4 ± 2.3	0 ± 0

[a] Numbers shown are the averages ± SE of the percentages from 7 experiments of colonies remaining after MoAb treatment compared to control values obtained by averaging colony counts from control MoAb + C′ alone. Control values used were the averages of colonies that grew after no MoAb treatment and after exposure to complement only. Colony numbers in these experiments ranged from 50 to 100 for CFU-GM and BFU-E and 5 to 10 for CFU-GEM, all per 2 × 10^5 cells.

Table 3 *Effect of MoAb combinations on bone marrow progenitor cells*

Treatment	% of control[a]		
	CFU-GM	BFU-E	CFU-GEM
4 MoAbs[b]	80.5 ± 25.8	80.0 ± 24.2	79.5 ± 12.6
5 MoAbs[c]	90.7 ± 8.9	43.3 ± 7.2	18.7 ± 17.2
W6-32	14.7 ± 10.7	5.0 ± 1.2	0

[a] Numbers shown are the mean ± SE of the percentages of colonies in each class remaining after treatment compared to control values. The data are from four experiments using different donors.

[b] Marrow cells were treated with a combination of SCCL-41, -114, -124, and -175.

[c] Marrow cells were treated with a combination of SCCL-1, -41, -114, -124, and -175.

Table 4 *Effect of MoAb combinations on bone marrow progenitor cells "spiked" with DMS 406 SCCL cells*

Treatment	% of control[a]		
	CFU-GM	BFU-E	CFU-GEM
4 MoAbs[b]	87.5 ± 10.2	86.3 ± 7.0	100.0 ± 10.0
5 MoAbs[c]	86.3 ± 9.8	43.3 ± 6.4	90.3 ± 8.9
W6-32	3.5 ± 1.2	3.5 ± 1.2	2.5 ± 1.0

[a] Numbers shown are the mean ± SE of the percentages of colonies in each class remaining after treatment compared to control values. The data are from two experiments using different donors.

[b] Marrow cells were treated with a combination of SCCL-41, -114, -124, and -175.

[c] Marrow cells were treated with a combination of SCCL-1, -41, -114, -124, and -175.

characterized to date. SCCL-175 immunoprecipitates two polypeptides, under reducing conditions, of M_r 155,000 and 115,000. SCCL-1 precipitates two proteins of M_r 100,000 and 80,000 under reducing conditions. Since these MoAbs kill SCCL cells in the presence of C′ and could potentially be used for the *in vitro* treatment of bone marrow from patients with SCCL, we have examined the question of whether hematopoietic progenitor cells are affected by treatment with these MoAbs. Notably, only one of the five MoAbs, SCCL 1, showed any toxicity to normal bone marrow progenitor cells and only from some individuals. SCCL-1 + C′ resulted in a decrease in the number of BFU-E and CFU-GEM from the bone marrow or peripheral blood of 12 of 17 subjects studied.

Recent reports have demonstrated the existence of hematopoietic cell-associated antigens on SCCL cells (13–17). These have included the C3bi receptor (defined by MoAb OKM1) (16), the Leu-7 antigen (14), AML-1-99 (24), and lacto-*N*-fucopentaose III (26). Here we have examined the expression of four SCCL-specific and one highly expressed SCCL antigen (SCCL-1) on hematopoietic progenitor cells and have found little cross-reactivity. Thus, these MoAbs selected for specificity to SCCL generally do not react with hematopoietic progenitor cells.

MoAbs SCCL-41, SCCL-114, SCCL-124, SCCL-175, and possibly SCCL-1 appear to be good immunological reagents for *ex vivo* marrow treatment in SCCL. Although SCCL-1 was toxic to erythroid precursors from some subjects, these studies do not rule out the possibility that a more primitive progenitor cell is SCCL-1 negative. For example, the antimyeloid MoAb L4F3, in the presence of C′, eliminates progenitor cells from short-term cultures, but long-term bone marrow culture has revealed that a precursor cell that is spared L4F3 toxicity exists that can give rise to progenitor cells in these cultures (27). If the same phenomenon occurs with respect to the SCCL-1 antigen, the SCCL-1 MoAb could also be included in a cocktail of MoAbs for the treatment of SCCL in marrow autografts. Regardless of the outcome of this study, it is apparent that the combination of the four IgM MoAbs is not significantly toxic to normal marrow progenitor cells and probably is safe to use to treat bone marrow. Others have also reported that selected anti-SCCL MoAbs spare normal progenitor cells (11, 12). In addition, several MoAbs to "myeloid" antigens that are expressed on SCCL cells [PM-81 (28), AML-1-99 (24), HNK-1 (13)] could also be used for this purpose of eliminating SCCL cells from bone marrow. Further study is necessary to define the optimal method for SCCL elimination. Although C′-dependent lysis is feasible and relatively uncomplicated, other possibilities such as immunomagnetic bead separation (29) or immunotoxins (30) should be considered. These studies indicate that several MoAbs are available that deserve further study for elimination of SCCL from autografts. Since antigen expression in SCCL tumors is heterogeneous, we believe that combinations of several MoAbs will be needed to remove maximum numbers of tumor cells from marrow autografts.

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