HIM\textsubscript{1} AND HIM\textsubscript{4}, TWO MONOCLONAL ANTIBODIES POTENTIALLY USEFUL FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION IN CHRONIC MYELOGENOUS LEUKEMIA

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We developed two complement-fixing MoAbs HIM\textsubscript{1} and HIM\textsubscript{4} (murine) that were specifically reactive with chronic myelogenous leukemia (CML) cells. They were capable of fixing human or rabbit complement and suitable for CML cells purging of remission marrow from CML patients. HIM\textsubscript{1} reacted with majority leukemic cells from 7 out of 10 CML patients by complement-mediated cytotoxicity (CMC) assay (positive cells 80\%--90\%), HIM\textsubscript{4} reacted with majority CML cells from 4 out of 5 CML by CMC assay (positive cells 80\%--90\%). Treatment with HIM\textsubscript{1} or HIM\textsubscript{4} and human C' was capable of lysing 97\% of K562, U937, HL-60 and CML cells in a 20 fold excess of unrelated cells by indirect FITC+EB stain. Using limited dilution culture, incubation with HIM\textsubscript{1} and C' produced 1.5 logs inhibition of growth in K562 cells, and 1.9 logs in U937 cells, and with HIM\textsubscript{4} and C' produced 2.9 logs inhibition in HL-60 cells and 3.0 logs in U937 cells. Both MoAbs cocktail was shown 1.8 logs in K562 cells and 3.2 logs in U937 cells. They were no suppression on the growth of CFC-GM.

The outcome of patients with chronic myelogenous leukemia (CML) has not been substantially improved by conventional therapies.\cite{1} There is general agreement that bone marrow transplantation (BMT) after high-dose chemotherapy and/or radiation therapy is probably the only curative treatment for those patients.\cite{2,3} Allogeneic BMT, though has been reported with encouraging results, was limited in its use for lacking of HLA-identical sibling donor. Consequently, considerable effort in the use of autologous BMT has been made over the past few years.

Purging autologous marrow, usually involving cytotoxic drugs or MoAbs to eradicate residual leukemic cells is a reasonable measure in the treatment of leukemias with ABMT. Recently two MoAbs, HIM\textsubscript{1} and HIM\textsubscript{4}, were developed in our institute, both were shown specifically cytotoxic to CML cells and probably offered promise of marrow purging. This paper reports the preliminary results.

MATERIALS AND METHODS

Production of MoAbs

BALB/c mice were immunized with fresh CML-cells. After the second immunization, the spleen was removed and the spleen cells were fused with mice NS-1 myeloma cells. Two hybridomas were obtained. The ascites contain high titre monoclonal Ig, named HIM\textsubscript{1} and HIM\textsubscript{4} respectively, both are mouse IgM class.

Cells

Leukemic cells were obtained from bone marrow or peripheral blood of 30 cases of leukemia patients prior to treatment (10 CML, 2 CML-BC, 5 M2, 6 M3, 4 M4, 1 M5 and 2 C-ALL). Mononuclear cells (MNC) fraction were prepared with density gradient centrifugation through Ficoll-Hypaque (F/H). The human leukemic cell lines Nalm-6, Reh, CEM, BjAb, K562, HL-60, U937 and Nawalva were also used. Normal MNC were prepared from cord blood with F/H density gradient centrifugation.

Complement

Human complement (HC') was from a serum pool of at least 3 donors with AB blood group and gave the maximal killing at a final dilution of 1:2. Baby rabbit complement (RC') obtained from
neonatal rabbit showed low nonspecific cytotoxicity and gave the maximal killing at a final dilution of 1:4. HC− and RC− were divided into aliquots and stored at −80 °C.

**Complement-mediated Cytotoxicity (C'MC)**

Cells (1×10^7/ml) 50 µl were incubated with 50 µl MoAbs (in varying dilutions) for 45 minutes at 4 °C with shaking per 15 minutes, then washed once with 5% fetal calf serum (FCS) 1640 medium. After that, 100 µl HC− or RC− (at a final dilution of 1:2 or 1:4, respectively) was added and the mixture was incubated for 60 minutes at 37 °C. Viable cells were estimated with trypan blue exclusion. The percentage of cytotoxicity was calculated by the formula:

\[
\text{C'MC\%} = \left(1 - \frac{\text{No. 1}}{\text{No. 2}}\right) \times 100\%
\]

No. 1: No. of viable cells of experiment
No. 2: No. of viable cells of control

**CFU-GM Culture**

Normal MNC (1×10^7/ml) 0.2 ml was incubated with 0.2 ml HIM₈ (1:100 and 1:300 dilution) or HIM₉ (1:50 and 1:300 dilution) for 45 minutes at 4 °C, then 0.133 ml RC− was added (at a final dilution of 1:4) and the mixture was incubated for another 60 minutes at 37 °C. Washed once with 5% horse serum 1640 medium, was used 1640 medium for negative controls instead of MoAb. 2×10⁵ cells were cultured in quadruplicate 1.0 ml aliquots in 24 flat bottom wells culture plates (Linbro, USA) containing 0.3% agarose, 20% human fetus muscles extract, 30% horse serum, 5% AB blood group human serum and 1640 medium. After incubation at 37 °C in a humidified atmosphere of 5% CO₂ for 10 days, granulocyte-monocyte colonies (CFU-GM) of greater than 50 cells/colony were counted under an inverted microscope.

**Indirect Immunofluoresence + Ethidium Bromide Stain (FITC + EB stain)**

Leukemic cells in a 20 fold excess of unrelated cells (RBC) were treated with HIM₈ or HIM₉ (both at 1:100 dilution) plus HC− (1:2 dilution) for C'MC assay. Then the cells were washed twice and incubated for another 30 minutes at 4 °C with fluorescein isothiocyanate-labeled rabbit antimouse globulin (FITC-RAMG). After that, the cells were washed once again and the cells pellet was resuspended in a minimal amount of medium, 10 µl/well of 60% buffered glycerol was added, 20 µl cells suspension plus 10 µl ethidium bromide (EB, 0.07 mg/ml). A small drop of the mixture was spread on a cover slip and scored within 2 minutes under a OPTON fluorescence microscope. At least 200 cells were examined per slide. For negative control 1640 medium was used in place of MoAb. Specific elimination was calculated by the formula:

\[
\text{Rate of specific elimination} = \left(1 - \frac{\text{No. 1}}{\text{No. 2}}\right) \times 100\%
\]

No. 1: No. of FITC + EB− cells of treated group
No. 2: No. of FITC + EB− cells of control group

**Limiting Dilution Culture (LDC)**

K562, HL-60 and U937 cells in exponential growth phase as target cells were treated with HIM₈ or HIM₉ (both at 1:50 dilution) plus HC− (1:2 dilution) for C'MC assay. After that, cells were washed twice and diluted to concentrations varying from 10⁵ to 0.5 cells per 200 µl in 10% FCS 1640 medium. Aliquots of each dilution from 8 to 16 were plated in U bottom well microculture plates (NUNC, DENMARK), cells were incubated at 37 °C with 5% CO₂ for 10 — 14 days. Cell growth at each serial dilution was assessed in an “all or none” (positive and negative) fashion under an inverted microscope. Colony formation was calculated by the formula:

\[
\text{No. of negative wells} = \text{ln} \left(\frac{\text{No. of seeded wells}}{\text{No. of seeded wells}}\right)
\]

According to a Poisson probability distribution, the dilution that yields 37% nonresponding well represents a precursor frequency of 1 cell/well (F value). Logs elimination of MoAb was calculated by the formula:

\[
\text{Logs elimination of MoAb} = \text{log}(\text{F value of treated group}) - \text{log}(\text{F value of control group})
\]

**RESULTS**

**Reactivity of MoAbs HIM₁ and HIM₄ to Various Leukemic Cells and Leukemic Cell Lines**

Figure 1 and 2 showed that HIM₁ and HIM₄ specifically reacted to various fresh leukemia cells and leukemic cell lines. HIM₁ reacted to the majority of fresh leukemia cells from 7 out of 10 CML patients in C'MC assay (positive cells 80%—92%, X ± S = 86.9% ± 5.17%), and HIM₄ reacted to 5 out of 5 fresh CML patients (positive cells 80%—93%, X ± S = 87% ± 5.48%). HIM₁ and HIM₄ cocktail was more effective than either of them (positive cells 81%—96%, X ± S = 91% ± 6.88%, as shown in Table 1). HIM₁ also reacted...