Biochemical and Functional Characterization of MCS-2 Antigen (CD13) on Myeloid Leukemic Cells and Polymorphonuclear Leukocytes

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ABSTRACT

The antigen defined by MCS-2 monoclonal antibody (mAb) was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of surface and internally labeled cells. Surface iodination revealed that MCS-2 antigen on the surfaces of acute myelogenous leukemia cells, HL-60 cells, and polymorphonuclear leukocytes (PMN) had the same molecular weight ($M_w$, 150,000) and that their autoradiographic bands were also the same. Internal labeling of HL-60 cells with $^{35}$S-methionine followed by immunoprecipitation revealed two bands whose molecular weights were 150,000 and 130,000. HL-60 cells gave stronger bands than did PMN. The intensity of the $M_w$, 130,000 band became weaker, when internally pulse-labeled cells were cultured in the absence of labeled methionine, suggesting that $M_w$, 130,000 glycoprotein was a precursor protein of $M_w$, 150,000 glycoprotein. MCS-2 mAb precipitated two bands from tunicamycin-treated HL-60 cells whose apparent molecular weights were 100,000 and 110,000.

When cells were cultured with MCS-2 mAb, expression of the antigen decreased rapidly (within 10 min). The degree of suppression was more prominent in PMN than in acute myelogenous leukemia and in myelomonocytic cell lines. Reexpression of MCS-2 antigen by PMN after removal of the mAb from the culture medium was not observed, but it occurred rapidly in myelomonocytic cell lines, although it was blocked by pretreatment of the cells with cycloheximide. These findings suggested that the less-marked suppression of MCS-2 antigen expression by cell lines was due to its greater synthesis by these cells. These findings suggested that MCS-2 mAb reacted with identical molecules, which were recognized by other mAbs belonging to CD13. Furthermore, modulation of MCS-2 antigen was observed by MCS-2 mAb itself.

INTRODUCTION

MCS-2 mAb$^3$ which belongs to Group CD13 recognizes immature myeloid progenitor cells and mature granulocytes and monocytes (1–3). Thus, it has been used as part of a panel of mAbs useful in the differentiation of myeloid or undifferentiated leukemia from lymphoid leukemia (3, 4). It has been thought that MCS-2 mAb recognizes the same molecule as other well-characterized mAbs, such as MY7 and DU-HL60-4, which are known to react with gp150, because they react with the same cell populations (1). Recently, amplification of the genes coding for MY7 and DU-HL60-4 was observed by Look et al. (5, 6) in murine NIH-3T3 cells, which had been cotransfected with feline sarcoma virus and high-molecular-weight DNA of HL-60 cells. These investigators recently succeeded in cloning a gene which encoded gp150, and it was found to be located on human chromosome 15 at Bands q25-26 (7). This locus coincides with the regional assignment of the human c-fes protooncogene, which is also specifically expressed by myeloid cells (8, 9).

In this study we characterized the protein and its precursors recognized by MCS-2 mAb and compared our results with those previously reported studies which used mAbs belonging to the same group (CD13).

The biological roles of CD13 antigen have not been clarified. It has been reported that removal of MCS-2 antigen-positive cells from bone marrow did not reduce the efficiency of CFU-GM (10), while MY7 antigen expression appeared to recognize actively proliferating fractions of CFU-GM cells (11). In order to learn whether it plays a role in signal transduction or in cell growth, we attempted to elucidate whether MCS-2 antigen levels were regulated. Many molecules which are important in cell growth and various cell functions are known to be modulated by antibodies and growth factors. For example, the T3 antigen and T-cell receptor (12), the Tac antigen (13) of T-cells, transferrin receptors (14), and insulin receptors (15) of various cells are known to be modulated by a range of conditions. We examined whether MCS-2 antigen could be modulated by MCS-2 mAb or not. Various myelomonocytic cell lines, AML cells, and PMN were used in these studies.

MATERIALS AND METHODS

Reagents. RPMI 1640 medium and MEM-M were obtained from Nissui (Nissui, Tokyo, Japan). FCS and PHA-P were from DIFCO (Detroit, MI). $^{35}$S-Methionine, $^{3}$H-labeled and $^{3}$C-methylated protein mixture were purchased from Nissui (Tokyo, Japan). PCS and PHA-P and [3H]thymidine and [14C]methylated protein mixture were purchased from Amersham (Arlington Heights, IL). Human recombinant IFN, IL-1, G-CSF, GM-CSF, and gibbon recombinant IL-3 were kindly provided by Kyowa Hakko Pharmaceutical Co., Otsuka Pharmaceutical Co., Ltd., Kirin Beverley Co., Ltd., and Genetics Institute, respectively. DEAE-Sephadex, tressyl-activated Sepharose 4B, and dextran were from Pharma (Uppsala, Sweden). Tunicamycin, indomethacin, and CHL were from Sigma (St. Louis, MO). FITC-conjugated F(ab)’2 goat anti-mouse immunoglobulin was from Cappel Laboratories (Cochrantown, PA).

Cell Preparation. All cells were cultured in complete medium of RPMI 1640 supplemented with 10% FCS, 100 μg/ml streptomycin, and 100 units/ml penicillin G. PMN were separated from heparinized blood of five healthy donors (24 to 35 yr old) by dextran. Samples of peripheral blood or bone marrow were obtained from five AML cases who were diagnosed as having M, (3 cases), M,- (1 case), and MM, (1 case) according to the French-American-British classification (10). Leukemic cells were separated by Ficoll-Conray density gradient sedimentation and were washed 3 times with complete medium. Cell suspensions, containing more than 90% of blasts and less than 1% of PMN, were examined with cyto centrifuge preparations stained by May-Grünewald-Giemsa were used. Four myelomonocytic cell lines K562 (17), ML-1 (18), HL-60 (19), and U937 (20) were also used for experiments. HPB-ALL cells (21) were used as controls.

Immunoprecipitation of Cells. Ascites of MCS-2 (IgG1) and irrelevant antibody NU-T2 (CD1, IgG1) (22) were obtained by injecting 4 × 10$^6$...
antigen, immunoprecipitation revealed two bands which were labeled with [35S]methionine for 18 h to measure internal were negative for MCS-2 mAb. When HL-60 cells and PMN were also seen on HPB-ALL cells. Thus, these two bands must have been nonspecifically precipitated, since HPB-ALL cells have been nonspecifically precipitated, since HPB-ALL cells expressed as MFI, were determined by a laser flow cytometry system. Production of PHA-CM. PHA-CM was obtained using surgically removed spleen cells as the source. In brief, single cell suspensions (5 x 10^6) were labeled with 0.5 mCi of I25I by the lactoperoxidase method. After labeling, cells were washed and lysed in 1 ml of lysing buffer (0.5% Nonidet P-40-10 mM Tris-HCl-0.15 M NaCl-1 mM phenylmethylsulfonyl fluoride, pH 7.2) for 20 min on ice. Cell lysates were twice reacted with NU-T2-conjugated Sepharose 4B, followed by overnight incubation with MCS-2-conjugated Sepharose 4B. Following incubation with antigen, Sepharose beads were extensively washed with PBS, containing 0.5% Nonidet P-40, followed by 10 mM Tris-HCl buffer (pH 8.0) containing 0.1% SDS and 0.2% deoxycholate. Washed beads were finally suspended in a Laemmli sample buffer, and the bound antigens were eluted by boiling the beads for 5 min. Immunoprecipitated materials were analyzed by 7% SDS-PAGE and autoradiography. Regulation of MCS-2 Antigen Expression. One million cells were cultured in 2 ml of complete medium in each well of 24-well tissue culture plates (Linbro, Flow Laboratories, McLean, VA). To assess the regulation of MCS-2 antigen expression, cells were cultured in either the presence of various factors or MCS-2 mAb. After cultivation, cells were harvested by washing the wells vigorously using Pasteur pipets with PBS containing 0.5% bovine serum albumin and 0.1% sodium azide. After two washes with the same buffer, surface markers were examined. Aliquots (50 μl) of MCS-2 ascites diluted 1- to 200-fold were incubated with 5 x 10^6 cells in the presence of 50 μl of human AB serum and were then washed twice. FITC-conjugated F(ab)'2 goat antimouse immunoglobulin (50 μl, 1:160) was then added. The relative proportion of antigen-positive cells and the antigen density per cell, expressed as MFI, were determined by a laser flow cytometry system (Spectrum III; Ortho Diagnostic Systems, Westwood, MA). Proliferative Assays of Cells. Fifty thousand cells in 200 μl of medium were cultured for 24 h in the presence of various factors in flat-bottomed plates (Falcon Microtest Plates III; Falcon, Oxnard, CA). The cultured cells were pulsed with [3H]thymidine (0.5 μCi/well) for the last 6 h of culture, followed by precipitation onto glass fiber filters. Radioactivity was counted by a liquid scintillator. Production of PHA-CM. PHA-CM was obtained using surgically removed spleen cells as the source. In brief, single cell suspensions (5 x 10^6/ml) were cultured in the presence of 1% FCS, PHA-P (10 μg/ml), and indomethacin (10^-6 M) for 2 days. After centrifuging at 1000 x g for 10 min, the supernatants were collected and filtered through a Millipore filter (0.45 μm). RESULTS Characterization of MCS-2 Antigen. Leukemic cells obtained from a patient with AML (48-yr-old female) at the M1 stage were cultured with or without PMA (10^-9 M) for 2 days plus PMN, and HL-60 cells were surface iodinated, followed by immunoprecipitation with MCS-2-Sepharose 4B. Cultured AML cells were used, because expression of MCS-2 antigen increased during cultivation as described previously (2). The MCS-2 antigen of these three cell types had a similar molecular weight of 150,000 (gp150) (Fig. 1). HL-60 cell surfaces showed two additional bands corresponding to p180 and p90, but these were also seen on HPB-ALL cells. Thus, these two bands must have been nonspecifically precipitated, since HPB-ALL cells were negative for MCS-2 mAb. When HL-60 cells and PMN were labeled with [35S]methionine for 18 h to measure internal antigen, immunoprecipitation revealed two bands which corre-
of MCS-2 antigen was low in K562, and the relative proportions of MCS-2 antigen-positive cells were below 15% in K562, while over 90% of cells were positive in ML-1, HL-60, and U937. Surface marker studies with a panel of mAbs which define the myelomonocytic cell lines showed that K562 was one of the most immature cell lines (23).

Thus, reexpression of MCS-2 antigen on PMN surfaces could be due to a lower rate of synthesis by the PMN. It was speculated that the modulation of MCS-2 antigen on PMN cell surfaces could be due to a lower rate of synthesis by the PMN. This was confirmed by the findings that reexpression of MCS-2 antigen on PMN surfaces remained low, with no increase in MCS-2 antigen expression. On the other hand, the intensity of MCS-2 antigen on myelomonocytic cell lines except for K562 increased after removal of MCS-2 mAb (Table 1). However, that increase was inhibited when HL-60 cells were preincubated with CHEX (1.5 μg/ml) for 6 h prior to antibody treatment. In addition, expression of MCS-2 antigen on antibody-untreated HL-60 cells decreased by 34% when incubated in the presence of CHEX (1.5 μg/ml, 6 h) (data not shown). These findings confirmed that a prominent suppression by MCS-2 mAb of MCS-2 antigen expression on PMN surfaces could be explained by a low synthesis rate of MCS-2 antigen in PMN.

Effects of Various Factors on the Expression of MCS-2 Antigen and the Proliferation of HL-60 Cells. It is known that MCS-2 antigen expression on HL-60 cell surfaces increases after cultivation with PMA or PHA-CM (2). Our results described above suggested that synthesis of MCS-2 antigen in myelomonocytic cell lines after removal of MCS-2 mAb was studied. Cells were preincubated with CHEX (1.5 μg/ml) for 6 h prior to antibody treatment. In addition, expression of MCS-2 antigen on antibody-untreated HL-60 cells decreased by 34% when incubated in the presence of CHEX (1.5 μg/ml, 6 h) (data not shown). These findings confirmed that a prominent suppression by MCS-2 mAb of MCS-2 antigen expression on PMN surfaces could be explained by a low synthesis rate of MCS-2 antigen in PMN.

Table 1  
Reexpression of MCS-2 Ag after modulation

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Mean fluorescence intensity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium alone</td>
<td>PMN 116.9</td>
</tr>
<tr>
<td>MCS-2 mAb (1:1000)</td>
<td>22.6 ML-1 145.2</td>
</tr>
<tr>
<td>Medium alone after treatment with MCS-2 mAb</td>
<td>52.6 HL-60 127.3</td>
</tr>
<tr>
<td></td>
<td>U937 178.2</td>
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</tbody>
</table>

* Representative data of the three difference experiments with similar results are shown.
MCS-2 ANTIGEN ON MYELOID CELLS

ocytic cell lines was higher than that in PMN. Thus, it was speculated that synthesis of MCS-2 antigen and proliferation of cells might be closely associated and be affected by the actions of various biological factors. PMN and four myelomonocytic cell lines were cultured with various recombinant factors such as IFN-γ, IL-1α, G-CSF, GM-CSF, and IL-3 at a concentration of 100 units/ml. PHA-CM (10%) and PMA (10⁻⁹ m) were also used for these experiments. After culture for 24 h, expression of MCS-2 antigen and [³H]thymidine uptake were measured. However, no consistent results were obtained in biological factors or not. The effects of various factors on antigen by myelomonocytic cell lines, since treatment of HL-60 myeloid cell lines very quickly recovered. The reason for this gave only weak bands. Studies on reexpression of MCS-2 relatively resistant. It was speculated that synthesis of MCS-2 antigen or molecular cloning of the gene which codes nonglycosylated precursor molecule of CD 13 was a single band not clear. It is possible that MCS-2 mAb recognizes a different epitope from those defined by MY-7 and DU-HL-60-4 mAbs, in which the nonglycosylated precursor molecule of CD13 was a single band corresponding to p110 (5). The reasons for this discrepancy are not clear. It is possible that MCS-2 mAb recognizes a different epitope from those recognized by MY7 and DU-HL-60-4 mAbs, and recognizes another nonglycosylated precursor protein of the CD13 antigen. Alternatively, it is also possible that p100 is a cleavage product of p110 digested by cellular protease. However, the conclusion should be drawn after purification of MCS-2 antigen or molecular cloning of the gene which codes MCS-2 antigen.

In order to clarify the biological roles of MCS-2 antigen, we tested to see whether the expression of MCS-2 antigen could be regulated by various biological factors and MCS-2 mAb itself. Suppression of MCS-2 antigen expression by MCS-2 mAb was observed on PMN, AML, and myelomonocytic cell lines except for K562, the degree of suppression being most prominent on PMN, while AML and cell line cells remained relatively resistant. It was speculated that synthesis of MCS-2 antigen was low in PMN, since internal labeling of these cells followed by immunoprecipitation using MCS-2-Sepharose 4B gave only weak bands. Studies on reexpression of MCS-2 antigen on myelomonocytic cell lines and PMN showed that, as expected, MCS-2 antigen on PMN remained low even after removal of MCS-2 mAb, whereas reexpression on myelomonocytic cell lines very quickly recovered. The reason for this difference could be due to a high rate of synthesis of MCS-2 antigen by myelomonocytic cell lines, since treatment of HL-60 cells with CHEX blocked reexpression of MCS-2 antigen.

These findings prompted us to test the possibility whether the expression of MCS-2 antigen is regulated with any known biological factors or not. The effects of various factors on expression of MCS-2 antigen on myelomonocytic cell lines were studied. However, no consistent results were obtained in these studies, and there were no relationships between [³H] thymidine uptake and MFI of MCS-2 antigen of cells cultured with various factors for 24 h. These findings did not support the idea that MCS-2 antigen is a receptor for any known biological factors. In addition, MCS-2 mAb was found to have no effects on chemotaxis, phagocytosis, or superoxide production by PMN.

The close association of the CD13 gene to the fes oncogene at human chromosome 15, Bands q25-26, is interesting, because a translocation involving chromosome 15, (15;17) (q22, q21.1), is known to be frequently found in acute promyelocytic leukemia (24).

Taken together, our findings suggest that MCS-2 antigen is expressed on PMN, AML, and cell line cells is a regulatable molecule, although the factor which regulates expression of MCS-2 antigen could not be identified in this study. A comparison of nucleotide sequences of MCS-2 antigen with other well-known genes of receptors for growth factors and protooncogenes would be helpful to assess the biological role of MCS-2 antigen.

REFERENCES

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* Unpublished data.


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