Surface Membrane Antigens of Hemopoietic Tumor Cell Lines and Normal Marrow Progenitor Cells

Suzanne Hasthorpe, Jean Rogerson, and Ian F. C. McKenzie

Cancer Institute, 481 Little Lonsdale St., Melbourne, 3000, Australia [S. H., J. R.], and Department of Pathology, University of Melbourne, Parkville 3052, Australia [I. F. C. M.]

ABSTRACT

A xenoantiseraum raised against a mast cell tumor line (FMP1.1) was found to have cross-reactivity with surface antigens on primitive hemopoietic precursor cells in normal mouse bone marrow: erythroid burst-forming units; granulocyte-macrophage colony-forming cells; and high-proliferative-potential granulocyte-macrophage colony-forming cells. In the presence of anti-FMP1.1 serum and complement, only 15 ± 2% (S.E.) (n = 5) of normal nucleated marrow cells were lysed, demonstrating that the majority of mature hemopoietic cells did not express the antigens detected on their primitive counterparts. A variety of hemopoietic and other tumor cell lines were examined with anti-FMP1.1 serum, and all B- and pre-B-lymphomas, one plasmacytoma, one mastocytoma, and a monocytic-macrophage line exhibited significant lysis. Direct typing of the FMP1.1 tumor demonstrated that it did not express the B-cell surface antigens such as la, surface immunoglobulin, Fc, and complement (C3) receptors. Although the Ly.6 alloantigen was present on FMP1.1 cells, this antigen was not found on marrow erythroid burst-forming units and granulocyte-macrophage colony-forming units. Absorption of anti-FMP1.1 serum with cross-reacting (WEHI-3 and W-279.1) and a nonreacting (P-815) cell line confirmed the specificity of the antisera reaction with these cells and marrow progenitors. These experiments indicated that more than one antibody is contained in anti-FMP1.1 serum. Thus, the mast cell tumor (FMP1.1) carries an unusual array of antigens, which are found on bone marrow progenitors and are not expressed on the majority of differentiated cells. It has been demonstrated that tumor cell lines provide an important basis for the analysis of surface membrane antigens expressed on normal hemopoietic progenitors.

INTRODUCTION

Studies of the function and properties of primitive hemopoietic cells have been limited by the lack of specific antisera to isolate them from the very heterogeneous bone marrow population which is the richest source of these cells. Further knowledge of the surface antigens of bone marrow cells should provide a versatile means of isolating and characterizing hemopoietic progenitor and stem cells, but at present a composite picture of these cell surface antigens is not available. It has been reported that pluripotent spleen colony-forming units (27) have antigens in common with brain (10, 28) and testis (2, 19) and do not express la antigens (1). Spleen colony-forming units continuously give rise to progenitor CFC which can be assayed by colony formation in vitro when stimulated by specific growth factors. These colonies contain either mature erythroid cells, granulocytes-macrophages, megakaryocytes, or mixtures of these cell types. GM-CFC (5) appear to be selectively enriched by treatment with anti-testis cell serum (2) but do not appear to have brain-associated antigens (28). Other studies with fluorescent lectins (23, 24) have shown enrichment of progenitors, but in all there are few reproducible methods to isolate bone marrow precursors.

To produce antiserum against surface antigens restricted to primitive hemopoietic cells, we have used a mast cell tumor line (FMP1.1) as immunogen. The rationale for this choice was that progenitor CFC and FMP1.1 share a particular requirement for a growth factor, found in PWCM, which is necessary to stimulate their proliferation in vitro. PWCM stimulates erythroid (BFU-E) (14, 15, 26), megakaryocytic (21), pluripotent CFC (17), and GM-CFC (5) and acts synergistically with other factors to stimulate more primitive HPP-GM-CFC (4) progenitors to form colonies containing the respective mature cells in semisolid medium. The reactivity of HPP-GM-CFC with anti-FMP1.1 serum has also been studied since they represent a new, relatively primitive subpopulation of GM-CFC. They require a combination of specific growth factors and are revealed at high frequency in marrow only after treatment of mice with 5-FU, whereas spleen colony-forming units and other GM-CFC are eliminated by the drug (4).

A small proportion of nucleated bone marrow cells were lysed in the presence of anti-FMP1.1 serum and complement, and all progenitor cell types tested (GM-CFC, HPP-GM-CFC, and BFU-E) expressed surface antigens in common with FMP1.1 as did some other tumor cell types. Selective absorption experiments have shown that this antiserum contains more than one antibody. However, it provides us with a tool to enrich for progenitors and to explore surface antigen expression during differentiation and maturation of hemopoietic cells.

MATERIALS AND METHODS

Tumor Cell Lines. The cell lines described in Table 1 were cultured in DMEM (Grand Island Biological Co., Grand Island, N. Y.) with 10% FCS (Flow Laboratories, Stanmore, New South Wales, Australia). The culture medium for FMP1.1 contained DMEM, 20% FCS, a nonessential amino acid and vitamin supplement (11), and 20% PWCM (12). Cultures were incubated at 37° in a humidified 10% CO₂ atmosphere.

Received January 9, 1981; accepted June 3, 1981. 1 Supported by the National Health and Medical Research Council (Australia) and Grant CA-22080 from the National Cancer Institute, NIH, Bethesda, Md. 2 The abbreviations used are: CFC, colony-forming cells; GM-CFC, granulocyte-macrophage colony-forming cells; PWCM, pokeweed mitogen-stimulated spleen cell-conditioned medium; BFU-E, erythroid burst-forming unit; HPP-GM-CFC, high-proliferative-potential granulocyte-macrophage colony-forming cells; 5-FU, 5-fluorouracil; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; PBS, phosphate-buffered saline; IMDM, Iscove's modified Dulbecco's medium; BSA, bovine serum albumin; PMUE, pregnant mouse uterus embryo extract; HUS, human spleen-conditioned medium; WEHI-3CMB, WEHI-3-conditioned medium concanavalin-A-Sepharose-bound fraction; i.d., intradermal; NRS, normal rabbit serum; FLSP cells, Friend virus-infected erythroleukemia cells; SRC, sheep red blood cells.
Cells were tested when in log phase of growth. Adherent cells were removed from culture vessels by incubation with 0.01% Pronase in PBS for 3 to 5 min. Before testing, all cells were washed in IMDM (Grand Island Biological Co.) containing 1% BSA (Fraction V; Sigma Chemical Co., St. Louis, Mo.).

In Vitro Clonogenic Assay. Cells were cultured in 0.8% methylcellulose containing DMEM with an α-medium supplement. Transferrin (0.6 mg/ml; Beringwerke, Marberg, W. Germany), sodium selenite (0.1 μM; Merck AG, Darmstadt, W. Germany), egg lecithin (30 μM; Merck), 2-mercaptoethanol (100 μM; Sigma), 1% BSA (Miles Laboratories, Elkhart, Ind.), and 20% FCS (heat inactivated at 56° for 30 min) were added to cultures. The stimulus for growth of GM-CFC was either 2.5% heat-inactivated FCS (20). The antisera were made as anti-Ly.6.2 (C57 x A) F, anti-thymus, but has been detected on the P-815 mast cell tumor and the test and complement control group. The complement background lysis was less than 10% in almost all cases. Cytotoxic assays were also performed to detect Ly.6.2 and La antigens. In brief, Ly.6.2 has been detected on T-lymphocytes and some B-cells, is absent from thymus, but has been detected on the P-815 mast cell tumor and several other mast cell tumors (20). In all the assays present on B-cells, are absent from most T-lymphocytes, and occur on other cell types but have not been detected on mast cells or mast cell tumors (20). The antisera were made as anti-Ly.6.2 (CXBG x A) F, anti-B10.D2 and anti-La.8 made as (B10.A x A) F, anti-C57BL/6. Their cytotoxic titers were 1/128 and 1/256, respectively (20), on spleen cells.

Surface Immunoglobulin. Surface membrane detection of immunoglobulin was done on viable cells using fluorescein-conjugated anti-mouse immunoglobulin. Cells (1 x 10^6) were washed with 1% BSA in IMDM and incubated with 1/4 dilution of antiserum for 30 min on ice. Cells were washed twice with 1% BSA and PBS and examined immediately on a Leitz Orthoplan microscope with a Ploem Opak 2 incident light illuminator. Positively stained cells had uniform "ring staining" around the cell periphery.

Fc and C3 Receptors. SRC were washed and coated with rat anti-SRC IgG for the Fc assay or with rat anti-SRC IgM for the C3 receptor assay. For the C3 assay, cells were subsequently coated with fresh mouse serum as a source of complement. The formation of C3b, all the assays performed as described (25).

PS: PBS contained 0.14 mM NaCl, 2.7 mM KCl, 81.6 mM Na_2HPO_4, 1.3 mM KH_2PO_4, 0.5 mM MgCl_2·6H_2O, and 0.9 mM CaCl_2·2H_2O in distilled water.

RESULTS

Cytotoxicity with Anti-FMP1.1 Serum. Dilution curves of FLSP-absorbed premun and anti-FMP1.1 sera are shown in Chart 1 using FMP1.1 cells and an optimal concentration (final dilution, 1/9) of NRS as a source of complement. NRS had no demonstrable cytotoxic activity for FMP1.1 cells, whereas anti-FMP1.1 serum was cytotoxic to a dilution of 1/160 reaching 50% kill at about 1/100. Of the 3 mast cell lines

Table 1

<table>
<thead>
<tr>
<th>Cell line-Cell type</th>
<th>Mouse strain</th>
<th>% of tumor cells killed at following anti-FMP1.1 serum dilutions</th>
<th>Complement control</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMP1.1 Mastocytoma</td>
<td>DBA/2</td>
<td>99.5 ± 0.5*</td>
<td>98.5 ± 0.9</td>
<td>98 ± 0.62 ± 0.7</td>
</tr>
<tr>
<td>HC-3 Mastocytoma</td>
<td>C57L/J</td>
<td>7 ± 1</td>
<td>10 ± 1</td>
<td>7 ± 1 ± 4</td>
</tr>
<tr>
<td>WEHI-3 Monocyte-macrophage</td>
<td>BALB/c</td>
<td>38 ± 5</td>
<td>12 ± 2</td>
<td>9 ± 2 ± 7</td>
</tr>
<tr>
<td>PU-51 Macrophage</td>
<td>BALB/c</td>
<td>28 ± 14</td>
<td>24 ± 17</td>
<td>11 ± 2 ± 4</td>
</tr>
<tr>
<td>W-265 Pre-macrophage</td>
<td>BALB/c</td>
<td>24 ± 14</td>
<td>11 ± 7</td>
<td>10 ± 6 ± 4</td>
</tr>
<tr>
<td>W-231 B-lymphoma</td>
<td>BALB/c</td>
<td>72 ± 8</td>
<td>8 ± 30</td>
<td>15 ± 15 ± 6</td>
</tr>
<tr>
<td>W-279**.c B-lymphoma</td>
<td>NCB</td>
<td>60 ± 3</td>
<td>71 ± 6</td>
<td>48 ± 1 ± 29</td>
</tr>
<tr>
<td>ABL5-8.1** Pre-B-lymphoma</td>
<td>BALB/c</td>
<td>52 ± 2</td>
<td>47 ± 16</td>
<td>35 ± 11 ± 25</td>
</tr>
<tr>
<td>HPC-108** Plasmacytoma</td>
<td>BALB/c</td>
<td>91 ± 8</td>
<td>83 ± 5</td>
<td>71 ± 9 ± 10</td>
</tr>
<tr>
<td>MPC-11 Plasmacytoma</td>
<td>BALB/c</td>
<td>20 ± 1</td>
<td>20 ± 5</td>
<td>21 ± 3 ± 20</td>
</tr>
<tr>
<td>FLSP Erythroleukemia</td>
<td>DBA/2</td>
<td>8 ± 4</td>
<td>7 ± 3</td>
<td>7 ± 3 ± 7</td>
</tr>
<tr>
<td>EMT-6 Mammary tumor</td>
<td>BALB/c</td>
<td>33 ± 3</td>
<td>38 ± 7</td>
<td>37 ± 4 ± 40.5</td>
</tr>
<tr>
<td>L-cell Sarcoma</td>
<td>C3H</td>
<td>4.5</td>
<td>9</td>
<td>6.5 ± 5.5</td>
</tr>
</tbody>
</table>

Addition of 5% BSA in IMDM to cells followed by NRS as a source of complement. Mean ± S.E. Student's t-test was used to determine reaction significance.

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tested, HC-3 reacted with the antiserum while P-815 cells were not lysed (Table 1); thus, the reactivity was not "mast cell" specific. It should be noted that P-815 and FMP1.1 have the same H-2 haplotype and that the results observed excluded an anti-H-2 reaction, particularly because the antiserum was absorbed with FLSP, also an H-2d line. Cell lines with macrophage characteristics were positive but generally showed a low degree of antiserum-mediated lysis (14 to 18% above controls), although 38% of WEHI-3 cells were reproducibly killed at a 1/10 dilution of antiserum. By contrast, the B-lymphomas (W-231, W-279.1) and a pre-B-lymphoma line (ABLS-8.1) had high levels of reactivity (50 to 70%) with anti-FMP1.1 serum. One plasmacytoma (HPC-108) exhibited 90% cytology with anti-FMP1.1 serum, whereas another (MPC-11) was negative. Mammary tumor and sarcoma cells were not reactive in the cytotoxic test with anti-FMP1.1 serum, although the background was high with EMT-6 cells. Thus, the antiserum recognizes cells of several different lineages, but not uniformly so.

By contrast to these findings with a range of tumor cells, normal bone marrow cells showed restricted activity, with a maximal lysis of only 15 ± 2% (mean ± S.E. of 5 experiments) with a 1/10 dilution of anti-FMP1.1 serum. Although repeated experiments have been done, results from one experiment only are shown in Tables 2 and 3. Normal and 5-FU-treated (predominantly B-lymphocytes and depleted of other mature cells) marrow exhibited similar cytology (Table 2). This dilution of antiserum was optimal for lysis of normal marrow GM-CFC and BFU-E, inasmuch as there was a 98 to 100% reduction of these colonies after treatment with anti-FMP1.1 serum and complement (Table 3). 5-FU-treated marrow contained few of the PMUE-responsive GM-CFC, which were not reduced in the presence of anti-FMP1.1 serum and complement (Table 3). However, the primate 5-FU-treated marrow HPP-GM-CFC which form colonies only when stimulated by a combination of either PMUE plus HUS or PMUE plus WEHI-3CMB were also totally eliminated (91 to 96% reduction) by the cytotoxicity assay. Results for BFU-E are not presented for 5-FU-treated marrow due to their absence 4 days after 5-FU treatment.

With 5-FU-treated marrow, PMUE plus HUS stimulated a higher number of CFC to form colonies, but the colonies were small in comparison to those of PMUE plus WEHI-3CMB-stimulated cultures. Normal marrow GM-CFC numbers were slightly enhanced by combination of PMUE with either HUS or WEHI-3CMB (Table 3), although colony morphology differed in that small colonies resulted with the former combination (comparable to those with PMUE only), and very large (>0.5-mm-diameter) ones resulted with the addition of the latter stimulus. The significance of colony number and morphology with respect to the relative maturity of the GM-CFC has not been resolved, and although different populations of GM-CFC appeared to be stimulated all were susceptible to cytolyis with anti-FMP1.1 serum and complement. The only obvious exception was GM-CFC from 5-FU-treated marrow stimulated by PMUE alone (Table 3).

**Effects of Absorption of Anti-FMP1.1 Serum with Various Cell Lines.** Two cell lines, WEHI-3 and W-279.1, which reacted with anti-FMP1.1 serum (Table 1) and one which was not reactive (P-815) were used for absorbing the antiserum to determine whether cross-reactivity with marrow progenitor cells could be removed. Although consistent, the reactivity of WEHI-3 cells with anti-FMP1.1 serum was not high, but these cells very effectively absorbed antibodies from the serum. Lysis of WEHI-3 cells was reduced from 32% (Table 1) to 1%, above the complement control level, by WEHI-3 absorption (Table 4). Absorption with WEHI-3 removed almost all activity for nucleated marrow, GM-CFC, and BFU-E as opposed to 92 to 99% lysis of CFC with non-WEHI-3-absorbed antiserum (Table 5). There was also a considerable effect on the titer of anti-FMP1.1 serum, with the 50% lysis of FMP1.1 cells being reduced from a dilution of 1/100 to one of about 1/10 (Chart 1; Table 4). Successive absorptions were done with W-279.1, and only after the third were PMUE-responsive GM-CFC colony numbers not significantly different (p < 0.05) from both the complement and antiserum plus 5% BSA control numbers (Table 6). However, GM-CFC responsive to PMUE plus WEHI-3CMB factors showed no further decrease in percentage of colony reduction after the second absorption. Complete recovery of PMUE-responsive GM-CFC but not of PMUE plus WEHI-3CMB-responsive GM-CFC suggests that 2 antibodies are responsible for GM-CFC cytotoxicity, one of which is not absorbed by W-279.1 cells. The lysis of nucleated marrow cells was reduced by W-279.1 absorption, although it still exceeded that of controls (Table 6). Triple-W-279.1-absorbed anti-FMP1.1 serum showed no reaction with W-279.1 cells (Table 4), which was also the case after only one absorption (results not shown), whereas normal bone marrow cells

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3 S. Hasthorpe, personal observation.
Table 3
Reduction of GM-CFC-, BFU-E-, and HPP-GM-CFC-derived colonies following anti-FMP1.1 and complement cytotoxicity

This experiment is referred to in Table 2. The percentage of reduction was calculated by comparing the colony reduction between test and 5% BSA plus complement control. Anti-FMP1.1 serum was used at a dilution of 1/10 in all cases shown.

<table>
<thead>
<tr>
<th>No. of colonies/5 x 10⁶ cells plated</th>
<th>Anti-FMP1.1 + complement</th>
<th>Anti-FMP1.1 + 5% BSA + complement</th>
<th>5% BSA + complement</th>
<th>% of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal marrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CFC</td>
<td></td>
<td>2.2 ± 1</td>
<td>760 ± 5</td>
<td>130 ± 8</td>
</tr>
<tr>
<td>PMUE</td>
<td></td>
<td>0.4 ± 0.4</td>
<td>150 ± 9</td>
<td>183 ± 16</td>
</tr>
<tr>
<td>PMUE + WEHI-3CMB</td>
<td></td>
<td>2.4 ± 0.7</td>
<td>154 ± 8</td>
<td>179 ± 5</td>
</tr>
<tr>
<td>BFU-E</td>
<td></td>
<td>0</td>
<td>11 ± 3</td>
<td>13 ± 5</td>
</tr>
</tbody>
</table>

For 5-FU-treated bone marrow GM-CFC, PMUE, and PMUE + WEHI-3CMB:

<table>
<thead>
<tr>
<th>No. of colonies/5 x 10⁶ cells plated</th>
<th>Anti-FMP1.1 + complement</th>
<th>Anti-FMP1.1 + 5% BSA + complement</th>
<th>5% BSA + complement</th>
<th>% of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMUE</td>
<td></td>
<td>9 ± 3</td>
<td>5 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>PMUE + HUS</td>
<td></td>
<td>12 ± 1</td>
<td>173 ± 3</td>
<td>195 ± 16</td>
</tr>
<tr>
<td>PMUE + WEHI-3CMB</td>
<td></td>
<td>5 ± 1</td>
<td>59 ± 5</td>
<td>56 ± 8</td>
</tr>
</tbody>
</table>

*Mean ± S.E.

Table 4
Effects of absorption of anti-FMP1.1 serum with various cell lines

Anti-FMP1.1 serum (FLSP absorbed) was further absorbed once with WEHI-3, 3 times with W-279.1, and twice with P-815 cells.

<table>
<thead>
<tr>
<th>Absorbed anti-FMP1.1 serum dilutions</th>
<th>% of cells killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target cell</td>
<td>Further absorption</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>FMP1.1</td>
<td>WEHI-3</td>
</tr>
<tr>
<td>W-279.1</td>
<td></td>
</tr>
<tr>
<td>P-815</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± S.E.

50 to 60% of W-279.1 cells (Table 1). In contrast to this, no reduction in the titer of antisera was observed for FMP1.1 cell lysis after W-279.1 absorption as compared with non-W-279.1-absorbed anti-FMP1.1 serum (Table 4; Chart 1). These observations demonstrate that multiple antibodies are present in anti-FMP1.1 serum. Absorption of anti-FMP1.1 serum with P-815 cells reduced the titer of the antiserum cytotoxicity to FMP1.1 cells somewhat (50% lysis at dilution of 1/70) (Table 4). However, reactivity on bone marrow and GM-CFC was not altered by P-815 absorption; i.e., marrow lyses were 14.5 and 15.5%; GM-CFC colony numbers were reduced by 94 and 99% with unabsorbed and P-815 absorbed antisera, respectively.

In conclusion, absorption experiments have shown that GM-CFC, BFU-E, and WEHI-3 cells share common surface antigens which are also in part found on W-279.1. However, the residual kill of a subpopulation of marrow GM-CFC (responsive to PMUE + WEHI-3CMB) with W-279.1-absorbed anti-FMP1.1 serum suggests that W-279.1 cells lack an antigenic component found on WEHI-3 and FMP1.1 cells. When anti-FMP1.1 serum was absorbed with P-815 cells, which did not cross-react with anti-FMP1.1 serum, very little activity was removed with respect to FMP1.1, nucleated marrow and CFC cytolysis.

Detection of Known Cell Surface Antigens on FMP1.1 Cells. Antiserum specificities peculiar to the mast cell line (FMP1.1) and marrow colony precursors indicated that it was necessary to further characterize this important cell line with known antisera. Both FMP1.1 and FLSP lines were derived from the DBA/2 mouse which express antigens coded for by the Ly-6α allele and the H-2d haplotype. Anti-Ly-6.2 serum elicited maximal lysis (100%) of FMP1.1 cells, in the presence of complement, over a plateau range from 1/2 to 1/16 dilution, whereas FLSP cells were not significantly killed (8 ± 4%). By contrast, lysis of FMP1.1 cells was not elevated above background levels (2 ± 1%) with anti-laα serum in the cytotoxicity assay.

Surface immunoglobulin was also shown to be absent from FMP1.1 cells and a pre-B-lymphoma line (ABLS 8.1), whereas the B-lymphoma W-231 showed a strong positive immunofluorescent reaction with fluorescein-labeled anti-mouse immunoglobulin. Receptors for Fc and complement (C3) were also not detected on FMP1.1 cells. P-815 cells were positive for Fc receptors with approximately 61% of cells forming rosettes, and these were negative for the C3 receptor. The FMP1.1 line
Table 5
Effect of WEHI-3 cell absorption on anti-FMP1.1 serum cytotoxicity for marrow GM-CFC and BFU-E
Anti-FMP1.1 serum was used at a dilution of 1/10 in all cases shown. The percentages of kill of GM-CFC and BFU-E are calculated by reduction in colony number relative to the 5% BSA plus complement control.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Anti-FMP1.1 WEHI-3 absorption</th>
<th>Anti-FMP1.1 WEHI-3 5% BSA + complement</th>
<th>Anti-FMP1.1 WEHI-3 absorption + complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (normal marrow)</td>
<td>Anti-FMP1.1 + complement</td>
<td>Anti-FMP1.1 + 5% BSA + complement</td>
<td>Anti-FMP1.1 + complement</td>
</tr>
<tr>
<td>GM-CFC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMUE</td>
<td>3.5 ± 1.3</td>
<td>73 ± 4</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>PMUE + HUS</td>
<td>0.6 ± 0.6</td>
<td>76 ± 3</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>PMUE + WEHI-3CMB</td>
<td>5 ± 0.6</td>
<td>51 ± 6</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>BFU-E</td>
<td>Erythropoietin + WEHI-3CMB</td>
<td>0.2</td>
<td>10.3</td>
</tr>
</tbody>
</table>

% kill of nucleated marrow cells

29 9 3.5 3 5 5

* Mean ± S.E.

Table 6
Effect of W-279.1 cell absorption on anti-FMP1.1 serum cytotoxicity for nucleated marrow cells and marrow GM-CFC
Anti-FMP1.1 serum was used at a dilution of 1/10 in all cases shown. The numbers of GM colonies and the percentage of reduction of colony numbers with 3 successive W-279.1 absorptions of anti-FMP1.1 serum are shown. The reductions in colony numbers are relative to results in the 5% BSA plus complement control group.

<table>
<thead>
<tr>
<th>Anti-FMP1.1 with W-279.1 absorptions</th>
<th>Anti-FMP1.1 with W-279.1 absorptions + complement</th>
<th>Anti-FMP1.1 with W-279.1 absorptions + complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal marrow (GM-CFC)</td>
<td>Anti-FMP1.1 + complement</td>
<td>Anti-FMP1.1 + complement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMUE</td>
<td>16 ± 1 *</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>PMUE + WEHI-3CMB</td>
<td>39 ± 4</td>
<td>78 ± 9</td>
</tr>
</tbody>
</table>

% of colony reduction

8.88 14.5 11.5 10 2 4

* Mean ± S.E.

is therefore Ia<sup>-</sup>, Ly-6<sup>-</sup>, Fc<sup>-</sup>, C3<sup>-</sup> and Ig<sup>-</sup>.

Cytotoxicity Assay for Ly.6 Antigen on Marrow GM-CFC and BFU-E. Cytotoxicity tests for the presence of Ly.6 on marrow cells, GM-CFC, and BFU-E were done to determine whether the cytotoxicity of anti-FMP1.1 serum was due, in part, to the Ly.6 determinant. DBA/2 mice express the Ly.6.2 specificity but not Ly.6.1 which was included as a control (Table 7). BFU-E colony numbers were not reduced following cytotoxic treatment of marrow cells, and GM-CFC similarly did not appear to be preferentially killed by anti-Ly.6 in the presence of complement. There was a small reduction in GM-CFC colony number with anti-Ly.6.2 in Experiment A, but this appeared to be nonspecific since an equivalent reduction also occurred with anti-Ly.6.1 serum. A repeat experiment confirmed that GM-CFC do not have detectable Ly.6 on their cell surfaces (Table 7).

DISCUSSION
 Serum from rabbits immunized with FMP1.1 cells has been shown, after absorption, to contain antibody to FMP1.1 and some other tumors. A finding of interest with this broadly reacting antiserum was the cross-reactivity with normal marrow progenitor cells. The technique of cytotoxicity was used to demonstrate this, with cytolysis by anti-FMP1.1 serum being dependent upon classical complement-mediated lysis. Although this obviously confirms specificity of the results at this time, it cannot necessarily be extrapolated to other techniques. The anti-FMP1.1 serum was produced with the aim of obtaining an anti-stem cell serum based quite arbitrarily on the observation that FMP1.1 and hemopoietic progenitor CFC share a requirement for a growth factor, present in PWCM, for proliferation in vitro. The rabbit antiserum could obviously have
that other types of progenitors have these antigens. The very
with W-279.1 still lysed at least 10% of nucleated marrow cells.
though these have not been characterized. The cytotoxicity
were not detectable on FMP1.1 cells.
myeloid (GM-CFC) and erythroid (BFU-E), and it is possible
primitive progenitor, HPP-GM-CFC expresses these antigens
mature cell types are reacting with anti-FMP1.1 serum, al
tumors, and absorption experiments with WEHI-3 and W-279.1
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markers on FMP1.1, certain tumor lines, a minority of
ance of 2 antibody specificities within anti-FMP1.1 serum
reactions has been shown and can be distinguished by their selec

tions indicate that anti-FMP1.1 serum when used for
GM-CFC and lesser enrichment of BFU-E and CFU-

tations of GM-CFC and lesser enrichment of BFU-E and CFU-

many different antibodies such as anti-H-2 and antivirus, but
these have been removed by absorption with FLSP cells to
produce an antisera which reacted with primitive marrow
precursor cells. However, the serum at this point is not mono-
specific, inasmuch as it has a broad reactivity with other
tumors, and absorption experiments with WEHI-3 and W-279.1
cells confirm this. At present, it is difficult to reconcile these
findings to specificity (for GM-CFC and BFU-E) on one hand
and broad reactivity (with tumors) on the other.

To further characterize the FMP1.1 antiserum, an investiga-
tion of known lymphocyte cell surface markers on FMP1.1
cells was performed. A previous report has shown the absence
of Thy.1 antigen, but the presence of IgE receptors (12) which
are characteristic of mast cells and basophilic leukocytes (16).
FMP1.1 cells also express the Ly.6 antigen which is common
to T- and B-lymphocytes and mast cells (20); however, la,
immunoglobulin, and receptors for Fc and complement (C3)
were not detectable on FMP1.1 cells.

Although anti-FMP1.1 serum had reacted strongly with BFU-
E, GM-CFC, and HPP-GM-CFC, about 15 ± 2% (average of 5
experiments) of normal marrow cells were lysed. Bone marrow
consists of 40 to 45% mature granulocytes, 25 to 30% eryth-
ropoietic cells, and about 20% lymphocytes (T and B) (9), with
CFC comprising between 0.1 to 1%. It is obvious that some
mature cell types are reacting with anti-FMP1.1 serum, al-
though these have not been characterized. The cytotoxicity
screening of tumor cell lines suggests that B-lymphocytes
would be predominantly involved, although antiserum absorbed
with W-279.1 still lysed at least 10% of nucleated marrow cells.
Progenitor cells were studied in only 2 differentiation lineages,
myeloid (GM-CFC) and erythroid (BFU-E), and it is possible
that other types of progenitors have these antigens. The very
primitive progenitor, HPP-GM-CFC expresses these antigens
as do the CFU-S. Our studies about the distribution of FMP1.1-
associated antigens in the marrow population suggest they

occurs on comparatively primitive cells but are not expressed
on most mature types.

The presence of la-like antigen on human marrow cells has
been shown on GM-CFC and BFU-E (13, 29). Related to this
are the human B-lymphocyte antigens (8, 18) which occur on
GM-CFC but not on mature granulocytes. Anti-la antibodies
are not responsible for the present findings, since FMP1.1 cells
are la− which is also the case for CFU-S (1). The Ly.6 antigen
is detectable on FMP1.1 cells; thus, antibodies to this may be
contained in anti-FMP1.1 serum and may be responsible for
killing of GM-CFC and BFU-E. These progenitors were tested
directly for Ly.6 expression, and no significant reaction with
anti-Ly.6.2 serum was found (Table 6), indicating that this
determinant was not the active component in anti-FMP1.1
serum.

Selective absorption of anti-FMP1.1 serum with WEHI-3 cells
has reinforced the observations (Table 1) that WEHI-3 cells do
have specific antigens in common with GM-CFC and BFU-E (Table 5). WEHI-3 cells undergo colony formation in vitro and
possess other properties comparable to those of normal GM-
CFC (22), although common surface antigens, between these
2 cell types, have not been reported previously. Absorption
experiments with the B-lymphoma W-279.1 clearly showed the
presence of multiple antibodies in anti-FMP1.1 serum. With
complete removal of activity against W-279.1 cells, the anti-
serum titer for FMP1.1 cells was still 50% at a 1/100 dilution.
In addition, these absorption experiments suggest the pres-
ence of 2 antibody specificities within anti-FMP1.1 serum
which react on the GM-CFC population: (a) the antibody spec-
ifity which detects an antigen on W-279.1, WEHI-3, PMUE-
responsive GM-CFC, and more than one-half of the PMUE plus
WEHI-3CMB-responsive GM-CFC; (b) one which is not present
on W-279.1 and detects antigens on a minor population of GM-
CFC (30 to 40%) which are responsive to the combined PMUE
plus WEHI-3CMB stimuli. Also, by deduction, this antigen
would be present on WEHI-3, since these cells absorbed out
all GM-CFC reactivity. The existence of GM-CFC subpopu-
lations has been shown and can be distinguished by their selec-
tive responsiveness to combinations of specific growth stimuli
and differences in their buoyant density characteristics (3, 7).
It would be of great interest to investigate the GM-CFC detected
by unabsorbed and W-279.1-absorbed anti-FMP1.1 serum
relative to these defined GM-CFC subpopulations.

Findings presented here have shown that particular tumor
cell lines provide a useful model system for identification of
surface antigens on primitive hemopoietic progenitor cells.
Membrane antigens have been identified which are expressed
on the surface of FMP1.1, certain tumor lines, a minority of
nucleated marrow cells, and marrow BFU-E, GM-CFC, and
HPP-GM-CFC. Although not presented here, preliminary ex-
periments indicate that anti-FMP1.1 serum when used for
fluorescent-activated cell sorting yields highly enriched popu-
lations of GM-CFC and lesser enrichment of BFU-E and CFU-
S. Furthermore, the presence of the antigens on primitive cells
and only a minority of mature types suggests that their expres-
sion has an important association with hemopoietic differen-
tiation.

ACKNOWLEDGMENTS

Thanks go to Dr. R. Kemler (Pasteur Institute, Paris, France) for his advice on
raising the anti-FMP1.1 serum and to Dr. A. W. Harris (Walter and Eliza Hall
Institute, Melbourne, Australia) for providing many of the tumor cell lines tested
in this study.

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Table 7
Tests for Ly.6 alloantigen on marrow GM-CFC and BFU-E
The percentage of DBA/2 marrow cells killed by cytotoxicity using anti-Ly.6.1
and anti-Ly.6.2 sera is shown. GM-CFC and BFU-E numbers are assayed by the
number of colonies formed.

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<th>Anti-Ly.6.2</th>
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<th>Colony no. per 5 x 10^6 cells plated</th>
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<td>PMUE + WEHI-3CMB</td>
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<td>% kill of marrow cells</td>
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<td>Experiment B</td>
<td>GM-CFC PMUE</td>
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* S. Hasthorpe and J. Rogerson, manuscript in preparation.
here. The production and testing of sera for detection of lymphoid antigens and receptors were carried out in the laboratory of Dr. I. F. C. McKenzie.

REFERENCES

Surface Membrane Antigens of Hemopoietic Tumor Cell Lines and Normal Marrow Progenitor Cells

Suzanne Hasthorpe, Jean Rogerson and Ian F. C. McKenzie