Specific Growth Inhibition of Primitive Hematopoietic Progenitor Cells Mediated Through Monoclonal Antibody Binding to Major Histocompatibility Class II Molecules

By H.T. Greinix, R. Storb, and S.H. Bartelmez

In a previous study using a canine model, we reported that a certain anti-class II monoclonal antibody (MoAb H81.9), which recognizes an epitope formed by the α and β subunits of HLA-DR, prevented long-term engraftment of autologous marrow cells if administered intravenously during the first 4 days after 9.2 Gy of total body irradiation. Another MoAb (B1F6), reactive with only the β subunit of HLA-DR and -DP, had no adverse effect on engraftment, although both MoAbs detect antigens on hematopoietic long-term repopulating cells as determined from complement-mediated lysis experiments. In the present study, continuous exposure of unfractionated human marrow to MoAb H81.9 specifically inhibited the growth of primitive progenitor cells that require multiple hematopoietic growth factors for proliferation (high proliferative potential colony forming cells [HPP-CFC] and burst-forming units-erythroid [BFU-e]), but had no effect on more mature, single factor responsive (CFU-GM), progenitor cells. In contrast, MoAb B1F6 did not impair primitive progenitor cell growth cultured as unfractionated marrow. However, when cell dose-response experiments were performed using CD34-positive cells plated at low cell densities, the marked inhibitory effects of MoAb H81.9 on HPP-CFC and BFU-e colony formation were not seen. These findings suggest that MoAb H81.9 may not inhibit primitive hematopoietic cells directly, but rather indirectly through the action of potent mediators derived from other HLA-DR-positive marrow cells.

The current general model of hematopoiesis assumes that differentiated blood cells arise from a small number of pluripotent hematopoietic stem cells capable of both self-renewal and differentiation. Increasing maturation of the progenitor cells results in a diminishing proliferative and differentiative capacity that eventually culminates in a restriction to a particular cell lineage. Studies from this laboratory and others have shown that long-term hematopoietic repopulating cells (LTRC) in dogs and nonhuman primates express major histocompatibility complex (MHC) class II antigens. Committed hematopoietic progenitor cells assayed as colony-forming units granulocyte-macrophage (CFU-GM), CFU-granulocyte-erythroid-monocyte-macrophage (CFU-GEMM), and burst-forming units-erythroid (BFU-e) are also known to express HLA-DR and -DP antigens. Further maturation in the granulocyte series results in the loss of cell surface expression of class II antigens while functionally mature macrophages retain class II expression.

In a previous study using a canine model, we reported that a certain anti-class II monoclonal antibody (MoAb) prevented long-term engraftment of autologous marrow cells if the MoAb was administered intravenously during the first 4 days after 9.2 Gy of total body irradiation (TBI). Specifically, injection of 0.6 mg/kg/d MoAb H81.9, specific for an epitope formed by the α and β subunits of HLA-DR, did not affect initial marrow engraftment that proceeded normally for the first 15 days; however, profound late secondary marrow failure was consistently observed. In contrast, another isotype-matched MoAb, B1F6, reactive with only the β subunit of HLA-DR + DP, had no effect on initial or late engraftment, even though both MoAbs detect antigens on hematopoietic repopulating cells as determined from complement-mediated lysis experiments. Several mechanisms, including nonspecific removal of antibody-coated repopulating cells by the reticuloendothelial system, antibody and canine complement-mediated cytotoxic effects on repopulating cells, and inactivation of antibody-coated marrow cells by canine antimouse antibody, have been ruled out. In addition, a second infusion of autologous marrow cells depleted of “accessory cells” by L-leucine methylester after completion of MoAb infusion resulted in long-term repopulation, indicating that the supportive function of the marrow microenvironment was not permanently impaired by MoAb injections.

These findings are consistent with the possibility that MoAb H81.9 modifies a primary signal pathway between LTRC and accessory or stromal cells required for maintenance or growth of repopulating cells either directly or indirectly through secondary mediators. Thus, either inhibition of growth of primitive progenitor cells or, conversely, a potent differentiation stimulus resulting in a severely reduced proliferative potential, could be the basis for the in vivo observation. To further elucidate the mechanisms involved, in vitro studies were undertaken to test the effects of MoAbs on the growth of different classes of progenitor cells using clonal assays, purified recombinant hematopoietic growth factors, and both unfractionated and enriched progenitor cell populations. Because human hematopoietic progenitor cells are better characterized than canine marrow progenitors and both anti-class II MoAbs used in these studies cross-react with human cells, human marrow cells became the focus of this study. We found that MoAb H81.9 specifically inhibited the growth of human high
proliferative potential colony-forming cells (HPP-CFC) and partially also of BFU-e growth, but had no inhibitory effect on more mature hematopoietic progenitor cells assayed as CFU-GM in unfractonated marrow. In contrast, MoAb B1F6 did not affect HPP-CFC or BFU-e growth. However, the marked inhibitory effects of MoAb H81.9 on both HPP-CFC and BFU-e colony formation were not observed in low cell density dose-response experiments using CD34-positive marrow cells. These in vitro findings show that specific epitopes of HLA-DR expressing accessory cells may play an important role in controlling the proliferative status of primitive hematopoietic cells.

**MATERIALS AND METHODS**

**Marrow cells.** Human marrow cells were obtained from consenting normal marrow donors participating in the allogeneic transplantation program at the Fred Hutchinson Cancer Research Center (FHCRC). Samples were collected under general anesthesia from the iliac crest. Marrow cells were centrifuged over Ficoll-Hypaque (density 1.077) and the low-density cells collected, washed once in hemolytic buffer and then twice with Iscove's medium (GIBCO, Grand Island, NY) and 10% fetal calf serum (FCS) (Hyclone Laboratories, Logan, UT).

**MoAbs.** The MoAbs used were H81.98.21 (abbreviated H81.9) specific for mouse la and reactive with canine and human HLA-DR antigen,14 B1F6, produced against canine cells and reactive with canine and human HLA-DR and -DP antigen11 and 31.A, directed at the Thy 1.1 antigen in mice14 and not cross-reacting with human and canine cells. All MoAbs are of the IgG type. Also used was the anti-C3D IgM MoAb, 12.8, along with an irrelevant isotype matched MoAb, H12C12, an antimouse Thy 1.2. The latter two antibodies were kindly provided by Dr. Irv Bernstein, FHCRC. Antibodies were purified from ascites by either affinity chromatography on a Staphylococcus aureus protein A Sepharose column or by high performance liquid chromatography (HPLC) using an ABX column (IgGs) or partially purified by boric acid precipitation (IgMs) (J.T. Baker, Inc, Phillipsburg, NJ).

Cell staining and fluorescence-activated cell sorting (FACS). Low-density human marrow cells were washed and resuspended in phosphate-buffered saline (PBS) containing 2% FCS at 10^7/mL. For single-color immunofluorescence, cells were then incubated with the primary antibody for 30 minutes at 4°C and subsequently with fluorescein isothiocyanate (FITC)-conjugated F(ab')2 fragments of goat antimouse IgGs (Tago, Burlingame, CA). In the control experiments, cells were incubated in MoAb H12C12 and 31.A as irrelevant IgM and IgG controls, respectively. Flow sorting was performed on a FACSII flow cytometer (Becton Dickinson, Palo Alto, CA). For two-color immunofluorescence, isotype-specific second-stage fluorescent reagents were used. IgM MoAbs were detected with phycoerythrin (PE)-conjugated goat antibody to mouse IgM (Tago). IgG MoAbs were detected with phycoerythrin (PE)-conjugated goat antibodies to mouse IgG (Tago), respectively. Cells were analyzed as described above, except that cells in the neutrophil region of the two-dimensional scatter plot, which were uniformly negative with both anti-class II MoAbs and CD34 MoAbs, were excluded from the analysis. The positive fluorescent gate cutoff was set at 100% of the background population. Flow cytometric analysis was performed using ReProMan software (Becton Dickinson).

Hematopoietic growth factors (HGFs). Recombinant human stem cell factor (SCF), interleukin-3 (IL-3), IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF) (kindly provided by Dr. Kris Zsebo, Amgen Corp, Thousand Oaks, CA), and erythropoietin (EPO) (purchased from Amgen Corp) were produced in Escherichia coli. They were used as purified material at 100 ng/mL (SCF), 20 ng/mL (IL-1 GM-CSF), 50 ng/mL (IL-3), and 2 U/mL (EPO).

**Cytolytic treatment of bone marrow.** The reactivity of hematopoietic progenitors with anti-class II MoAbs was assessed by a complement cytotoxicity assay as described elsewhere. Briefly, low density marrow cells were incubated with a saturating concentration of antibody at 4°C for 60 minutes. After two washes in PBS supplemented with 2% FCS, marrow cells were incubated with rabbit complement (C') (Pelfreeze, Rogers, AR) at 37°C for 60 minutes. Control cells were incubated with medium alone or medium and C'. Preliminary experiments with graded concentrations of C' and MoAb were performed to establish the optimal concentrations of both reagents. For these experiments a single batch of rabbit C' was selected. Controls showed that this batch by itself was not cytotoxic for human marrow cells. Furthermore, control cultures showed that at the end of the cytotoxicity assay, marrow cells incubated with anti-class II MoAbs and C' were completely depleted of MHC class II positive cells, whereas MHC class II negative cells were unaffected by this procedure. Marrow cells were then plated in clonal agar assays to measure the specific effect of the lysis.

**Clonal agar assay for human marrow.** A double-layer nutrient agar culture consisting of a 1-mL under-layer of 0.5% agar plus hematopoietic growth factors and MoAbs and a 0.5-mL overlay of 0.5% agar plus target cells in 35-mm Petri dishes was used essentially as previously described. However, in these experiments the X2 strength medium consisted of the following: 1 part X3 o-minimal essential medium (GIBCO), 1 part FCS (Hyclone), 0.2% bovine serum album (Sigma, St Louis, MO; no. A-7906). This X2 medium was mixed 1:1 with either 1% or 0.66% DIFCO (Detroit, MI) noble agar in water. Cultures were incubated at 37°C in a 5% O2, 5% CO2, 90% N2 gas mixture, as previously described for optimal colony formation. CFU-GM and BFU-e were scored at 14 days using an inverted microscope while HPP-CFC-type colonies were enumerated at 21 days and identified as macroscopic colonies (>50,000 cells/colony) with diameters greater than 1.0 mm with a dense cell center using a dissecting microscope at x10 magnification. The morphology of cells in the colonies was determined by cytosin preparations of individually picked colonies stained with benzidine and counterstained with Wright-Giemsa (Sigma).

**RESULTS**

**MHC class II expression on hematopoietic progenitor cells: Complement-mediated cytotoxicity.** MHC class II expression on human hematopoietic progenitor cells was determined by complement-mediated cytotoxicity assays using the two described MoAbs that recognize epitopes of MHC class II antigens. One representative experiment of four performed is shown in Table I. Incubation of unfractonated, low-density marrow cells with MoAbs alone or C' alone did not significantly affect colony formation. However, incubation of marrow cells with either antibody against class II plus C' reduced subsequent colony formation by all progenitors markedly. Compared with control cultures, the numbers of progenitors surviving H81.9 + C' or B1F6 + C', respectively, were: BFU-e 7% ± 2% and 24% ± 5%, CFU-GM was 7% ± 1% and 28% ± 2%, and HPP-CFC, 0% and 0%. These findings indicate that the epitopes recognized by both our investigated anti-class II MoAbs are expressed on both early (HPP-CFC, BFU-e)
and late (CFU-GM) hematopoietic progenitor cells. The C'-mediated cytotoxicity of CFU-GM and BFU-e mediated by MoAb B1F6 was greater than with MoAb H81.9. Cytotoxicity of HPP-CFC appeared complete using either MoAb.

**Effect of continuous exposure to anti-class II MoAb on the growth of subpopulations of human hematopoietic progenitor cells using unfractionated marrow.** To investigate the effects of anti-class II MoAbs on the growth of different classes of hematopoietic progenitor cells, clonal agar assays in the presence or absence of MoAb alone were performed. In these experiments, the anti-class II MoAbs or irrelevant IgG control MoAbs were added at the start of the culture in addition to IL-1, IL-3, GM-CSF, EPO, and SCF (either pre-exposure to MoAb nor C' were used in these experiments). Results from hematopoietic progenitor cell growth in the presence or absence of anti-class II MoAb are shown in Fig 1. In three replicate experiments, colony numbers in control cultures were 120 ± 13 for CFU-GM, 40 ± 1 for BFU-e, and 5 ± 0 for HPP-CFC per 50,000 cells grown in the presence of the irrelevant IgG matched control MoAb (31.A). In Fig 1, these values are expressed as maximal growth (100%). In the presence of MoAb H81.9, CFU-GM colony formation was similar to control cultures; however, BFU-e colony growth was markedly reduced to a mean of 45% (range 24% to 66%) of control cultures (Fig 1). The isotype matched anti-class II MoAb B1F6 also had little effect on CFU-GM colony growth, and also, contrary to MoAb H81.9, did not inhibit BFU-e colony formation (Fig 1). However, most striking was the inhibitory effect of MoAb H81.9 on the development of the characteristic macroscopic colonies of HPP-CFC and greater than 1.0 mm CFC. In contrast, HPP-CFC development was not inhibited in the presence of MoAb B1F6 or the irrelevant IgG control antibody (Fig 1).

The observed inhibition of BFU-e and HPP-CFC formation grown from unfractionated marrow in the presence of MoAb H81.9 was antibody-concentration dependent. Maximal inhibitory effects on BFU-e growth were observed at concentrations greater than 25 μg/mL MoAb H81.9, with significant inhibition of colony growth still evident at antibody concentrations as low as 0.4 μg/mL (Fig 2). The dose-response curve for MoAb H81.9 inhibition of HPP-CFC development was similar to that for BFU-e, with a substantial inhibition evident at 1 μg/mL (41%) that eventually reached 5% compared with controls at 50 μg/mL (Fig 2).

**Effects of continuous exposure to anti-class II MoAbs on the growth of human unfractionated hematopoietic cells grown in the presence or absence of SCF.** In agar cultures of human marrow cells, human SCF synergizes with other hematopoietic growth factors resulting in increased progenitor cell growth of the myeloid and erythroid lineages. In the present study, CFU-GM colony formation was increased up to 1.5-fold in the presence of SCF. More strikingly, BFU-e growth was increased 7- to 10-fold in cultures containing SCF (Table 2). To investigate whether anti-class II MoAbs interfere with stimulatory signals for proliferation of early hematopoietic progenitor cells, agar assays containing hematopoietic growth factors in optimal concentrations with or without stem cell factor were performed (Table 2). In the absence of SCF, neither CFU-GM nor BFU-e colony formation was affected by either anti-class II MoAb. In contrast, a 55% inhibition of BFU-e growth was observed in cultures exposed to SCF plus MoAb H81.9. No inhibition of growth was observed in control cultures (Fig 1). These findings indicate that MoAb H81.9 selectively inhibited BFU-e that depend on SCF for proliferation. The effect of MoAb H81.9 on human HPP-CFC that can develop in the absence of SCF could not be determined in these experi-

<table>
<thead>
<tr>
<th>MoAb*</th>
<th>CFU-GM</th>
<th>BFU-e</th>
<th>HPP-CFC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute No.</td>
<td>% of Growth</td>
<td>Absolute No.</td>
</tr>
<tr>
<td>Medium + H81.9 + B1F6</td>
<td>1,800 ± 178</td>
<td>100</td>
<td>334 ± 31</td>
</tr>
<tr>
<td>Medium + C'</td>
<td>1,660 ± 203</td>
<td>92 ± 11</td>
<td>322 ± 16</td>
</tr>
<tr>
<td>H81.9 + C'</td>
<td>507 ± 34</td>
<td>28 ± 2</td>
<td>81 ± 19</td>
</tr>
<tr>
<td>B1F6 + C'</td>
<td>134 ± 19</td>
<td>7 ± 1</td>
<td>25 ± 6</td>
</tr>
</tbody>
</table>

*Where applicable, cells were first incubated with MoAb at 4°C for 1 hour, washed, then incubated at 37°C for 1 hour with complement, washed, then subsequently plated in clonal agar assays.

1Mean ± 3D expressed as absolute number or percent of values in unfractionated marrow cell suspensions, plated with IL-1, IL-3, GM-CSF, EPO, and SCF and kept in culture at 37°C. In four experiments, triplicate dishes were evaluated after 21 days in culture.

Fig 1. Unfractionated marrow. The effect of anti-HLA class II antibodies on the growth of the bone marrow progenitors. All cultures received 50,000 cells/plate in agar plus SCF, IL-3, IL-1, GM-CSF, and EPO. In addition, cultures received 50 μg/mL of one of the following MoAbs: [ ] MoAb 31.A, an irrelevant IgG control antibody; [ ] MoAb B1F6, reactive with the β subunit of HLA-DR and DP; [ ] MoAb B1F6, reactive with an epitope formed by the α and β subunits of HLA-DR. Results are expressed as percentage of colony formation ± 3D (n = 3) compared with control cultures (31.A) from three individual experiments. The average incidence of progenitors per 50,000 cells in control cultures for the three experiments was: CFU-GM, 120 ± 31; BFU-e, 40 ± 17; HPP-CFC, 4.8 ± 1.1.

Table 1. MHC Class II Expression on Human Hematopoietic Progenitor Cells Assessed by C' Mediated Lysis
MoAb H81.9 for the two experiments described was: CFU-GM, 167 of progenitors per 50,000 cells in control cultures that did not receive H81.9. Results are expressed as percentage of colony formation. In addition, cultures received either 50 pg/mL of the control monoclonal 31A (100% colony growth) or decreasing amounts of MoAb H81.9. To further clarify the cell population involved in the inhibitory effects of MoAb H81.9, enriched cell populations were cultured at low cell densities. CD34-positive marrow cells were flow sorted and either plated at 3,000 cells/plate or set up as a cell dose response in the same experiment (375 to 3,000 cells/plate) in the presence of the above-described hematopoietic growth factors, including SCF and MoAbs. In striking contrast to the results described above using unfractionated marrow plated at relatively high cell densities, no significant H81.9 MoAb-mediated growth inhibition of any progenitors was seen in low cell density, CD34-positive cultures. The results of a representative cell dose experiment are shown in Fig 3. A reanalysis of the sorted cells used in this experiment indicated that 94.5% were CD34+. When this CD34+-enriched cell population was plated at doubling cell concentrations in the presence of control MoAb, the number of hematopoietic progenitors that formed colonies was essentially linear for both BFU-e and HPP-CFC, indicating an apparent lack of cell–cell modulating effects (Fig 3). In contrast, the number of CFU-GM that formed colonies was not linear using the cell concentrations tested, regardless of the presence or absence of MoAb. That is, the frequency of CFU-GM varied from 125 ± per 1,000 CD34+ cells (n = 24) when plated at the lowest cell density and then progressively decreased to 100 ± 8 (n = 12), 63 ± 7 (n = 6), and finally 42 ± 3 (n = 3) CFU-GM per 1,000 cells as the cell density was increased by twofold increments. Thus, a cellular derived inhibitory activity in control MoAb cultures that was active on CFU-GM, but not HPP-CFC or BFU-e, could be detected. Unexpectedly, the addition of MoAb H81.9 did not result in any further significant inhibition of progenitors; however, consistently fewer HPP-CFC formed in low cell concentration cultures than contained MoAb H81.9 (Fig 3). Although these slight differences in HPP-CFC growth were not statistically significant. Thus, MoAb H81.9 was markedly inhibitory to HPP-CFC and BFU-e only when grown in the presence of unfractionated marrow cells cultured at relatively high density (40,000 cells/plate).

**Table 2. Influence of Anti-Class II MoAbs Alone on Human Marrow Progenitor Cells**

<table>
<thead>
<tr>
<th>MoAb</th>
<th>CFU-GM Absolute No.</th>
<th>%</th>
<th>BFU-e Absolute No.</th>
<th>%</th>
<th>Without SCF</th>
<th>CFU-GM Absolute No.</th>
<th>%</th>
<th>BFU-e Absolute No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>31A</td>
<td>91 ± 7</td>
<td>100 ± 8</td>
<td>42 ± 1</td>
<td>100 ± 1</td>
<td>58 ± 5</td>
<td>100 ± 8</td>
<td>5 ± 0.4</td>
<td>100 ± 8</td>
<td>8 ± 0.8</td>
</tr>
<tr>
<td>B1F6</td>
<td>86 ± 2</td>
<td>95 ± 6</td>
<td>38 ± 2</td>
<td>90 ± 4</td>
<td>64 ± 1</td>
<td>110 ± 3</td>
<td>6 ± 0.3</td>
<td>120 ± 6</td>
<td>120 ± 2</td>
</tr>
<tr>
<td>H81.9</td>
<td>82 ± 6</td>
<td>90 ± 1</td>
<td>10 ± 1</td>
<td>24 ± 2</td>
<td>55 ± 1</td>
<td>94 ± 1</td>
<td>6 ± 0.1</td>
<td>120 ± 2</td>
<td>24 ± 0.1</td>
</tr>
<tr>
<td>31A</td>
<td>113 ± 1</td>
<td>100 ± 1</td>
<td>62 ± 0</td>
<td>100 ± 1</td>
<td>107 ± 4</td>
<td>100 ± 4</td>
<td>8 ± 0.8</td>
<td>100 ± 10</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>B1F6</td>
<td>128 ± 2</td>
<td>113 ± 2</td>
<td>66 ± 1</td>
<td>105 ± 2</td>
<td>102 ± 2</td>
<td>96 ± 2</td>
<td>8 ± 0.1</td>
<td>100 ± 1</td>
<td>112 ± 2</td>
</tr>
<tr>
<td>H81.9</td>
<td>105 ± 3</td>
<td>93 ± 3</td>
<td>41 ± 1</td>
<td>66 ± 2</td>
<td>91 ± 9</td>
<td>86 ± 9</td>
<td>9 ± 1.0</td>
<td>112 ± 12</td>
<td>112 ± 12</td>
</tr>
<tr>
<td>31A</td>
<td>153 ± 12</td>
<td>100 ± 7</td>
<td>23 ± 1</td>
<td>100 ± 3</td>
<td>137 ± 7</td>
<td>100 ± 5</td>
<td>3.5 ± 0.4</td>
<td>100 ± 11</td>
<td>112 ± 11</td>
</tr>
<tr>
<td>B1F6</td>
<td>142 ± 13</td>
<td>93 ± 8</td>
<td>23 ± 0</td>
<td>100 ± 1</td>
<td>132 ± 8</td>
<td>98 ± 6</td>
<td>3.1 ± 0.2</td>
<td>89 ± 5</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>H81.9</td>
<td>173 ± 7</td>
<td>113 ± 4</td>
<td>11 ± 1</td>
<td>47 ± 2</td>
<td>159 ± 11</td>
<td>118 ± 8</td>
<td>3.4 ± 0.3</td>
<td>97 ± 8</td>
<td>3.4 ± 0.3</td>
</tr>
</tbody>
</table>

Fifty thousand unfractionated human marrow cells were plated in agar with IL-1, IL-3, GM-CSF, and EPO. Results are expressed as absolute colony numbers and percentage of growth ± SD of three individual experiments plated in triplicates. No C' was used in these experiments.

**DISCUSSION**

Major histocompatibility complex class II antigens are known to be involved in cell–cell interactions in hematopoiesis and are differentially expressed on human hematopoietic progenitors. The results of the above experiment indicate that 94.5% were CD34+ and involved in the inhibitory effects of MoAb H81.9, enriched cell population was plated at doubling cell concentrations in the presence of control MoAb, the number of hematopoietic progenitors that formed colonies was essentially linear for both BFU-e and HPP-CFC, indicating an apparent lack of cell–cell modulating effects (Fig 3). In contrast, the number of CFU-GM that formed colonies was not linear using the cell concentrations tested, regardless of the presence or absence of MoAb. That is, the frequency of CFU-GM varied from 125 ± per 1,000 CD34+ cells (n = 24) when plated at the lowest cell density and then progressively decreased to 100 ± 8 (n = 12), 63 ± 7 (n = 6), and finally 42 ± 3 (n = 3) CFU-GM per 1,000 cells as the cell density was increased by twofold increments. Thus, a cellular derived inhibitory activity in control MoAb cultures that was active on CFU-GM, but not HPP-CFC or BFU-e, could be detected. Unexpectedly, the addition of MoAb H81.9 did not result in any further significant inhibition of progenitors; however, consistently fewer HPP-CFC formed in low cell concentration cultures than contained MoAb H81.9 (Fig 3). Although these slight differences in HPP-CFC growth were not statistically significant. Thus, MoAb H81.9 was markedly inhibitory to HPP-CFC and BFU-e only when grown in the presence of unfractionated marrow cells cultured at relatively high density (40,000 cells/plate).
et al. of agar plus SCF, IL-3, IL-1, GM-CSF, and EPO. Sorted cells were enriched with a dense cell center containing greater than 50,000 cells.

CD34+ FACS-sorted cells were plated in 35-mm plates in 1.5 mL of autologous transplants in otherwise lethally irradiated dogs. CD34+ FACS-sorted cells were plated in 35-mm plates in 1.5 mL of growth medium containing SCF, IL-3, IL-1, GM-CSF, and EPO. Sorted cells were reanalyzed and were 94.5% CD34+ in the experiment shown. In addition, cultures received either 50 pg/mL MoAb 31.A (control) or MoAb H81.9. The same number of CD34+ cells were assayed for each cell concentration, ie, 9,000 cells were plated at 3,000 cells/plate (n = 3 replicate plates), 1,500 cells/plate (n = 6), 750 cells/plate (n = 12), 375 cells/plate (n = 24).

**Fig 3.** CD34-positive marrow cells. Effect of MoAb H81.9 on the growth of different progenitor populations plated at low cell densities. CD34+ FACS-sorted cells were plated in 35-mm plates in 1.5 mL of medium containing SCF, IL-3, IL-1, GM-CSF, and EPO. Sorted cells were reanalyzed and were 94.5% CD34+ in the experiment shown. In addition, cultures received either 50 pg/mL MoAb 31.A (control) or MoAb H81.9. The same number of CD34+ cells were assayed for each cell concentration, ie, 9,000 cells were plated at 3,000 cells/plate (n = 3 replicate plates), 1,500 cells/plate (n = 6), 750 cells/plate (n = 12), 375 cells/plate (n = 24).

The growth of different progenitor populations plated at low cell densities (375 to 3,000 cells/35-mm plate), although marked inhibitory effects of MoAb H81.9 on both HPP-CFC and BFU-e were markedly inhibited by MoAb H81.9, but not by the isotype-matched MoAb B1F6 or the irrelevant control MoAb. Interestingly, only SCF-dependent BFU-e growth was inhibited by MoAb H81.9 using unfractonated marrow, while BFU-e that were able to proliferate in the absence of SCF were not affected by the anti-class II MoAb. These findings suggest that MoAb H81.9 specifically inhibits more primitive progenitors, leaving more differentiated progenitors unaffected when unfractonated cells are plated at relatively high cell densities.

To further elucidate the mechanism of the effect of MoAb H81.9 on primitive progenitor cells, low-density cell dose-response experiments were performed. The marked inhibitory effects of MoAb H81.9 on both HPP-CFC and BFU-e colony formation observed with unfractonated marrow were not seen using CD34-positive cells cultured at low cell densities (375 to 3,000 cells/35-mm plate), although a consistent slight, but not statistically significant, inhibition of HPP-CFC growth occurred at all the lower cell densities tested. This is most consistent with the possibility that the inhibitory effect of MoAb H81.9 is predominantly mediated indirectly through an HLA-DR-positive cell population.

Both positive and negative regulators influence hematopoiesis and the net production of mature hematopoietic cells appears to reflect the balance between the levels and activity of inhibitors and stimulators. The target cell populations for these mediators appears to be specific for various subpopulations of progenitors ranging from relatively mature (eg, CFU-GM) to relatively "primitive" (eg, CFU-S, CFU-MIX, HPP-CFC). Whereas certain hematopoietic growth factors have positive effects on these different classes of progenitors, transforming growth factor β (TGFβ), macrophage inflammatory protein-1α (MIP-1α), tumor necrosis factor α (TNFα), and interferons have been shown to have inhibitory (as well as stimulatory) effects. The specific inhibitory effects of MoAb H81.9 on early hematopoietic progenitor cells appear to be similar to those described for certain isoforms of TGFβ that are known to inhibit SCF-dependent colony formation of both murine and human progenitors. However, a major difference between the inhibition mediated by MoAb H81.9 and TGFβ is that the TGFβ effect appears to be reversible.
on withdrawal of the TGFβ. Cessation of MoAb H81.9 after 4 days in vivo did not re-verse the impending graft failure. However, an immediate second transplant after this time resulted in long-term hematopoietic engraftment, indicating that even though the MoAb H81.9 mediated signal had subsided, the effects of such were essentially irreversible. Because MHC class II molecules are known to be involved in cell–cell interactions, binding of MoAb H81.9 to this specific epitope on MHC class II molecules may disrupt the equilibrium of stimulatory and inhibitory signals important in the proliferative pathway of long-term repopulating cells. Trede et al.38 reported transcriptional activation of TNF-α and IL-1 genes by MHC class II ligands. Their results indicated that gene expression is mediated by preformed inactive transcription factors that are modified upon signaling via class II molecules.37 However, neither TNF-α nor IL-1 have been shown to induce the in vivo effects seen with MoAb H81.9.

An alternative explanation for the observations reported in this study is that apparent inhibition may in fact be a rapid differentiation event. The failure of HPP-CFC, for example, to develop its full proliferative potential in vitro could be caused by the HPP-CFC rapidly differentiating to, eg, the low proliferative potential CFU-GM state unusually fast. Highly enriched murine repopulating cells (ie, CFU-S) have recently been shown to differentiate rapidly in vitro under the influence of specific hematopoietic growth factors. This mechanism correlates with the in vivo observations that the effect of H81.9 on LTRC appears to be irreversible.36 Most physiologic inhibitors are reversible, and thus MoAb H81.9 may be inducing the release of potent differentiative mediators.

REFERENCES

Specific growth inhibition of primitive hematopoietic progenitor cells mediated through monoclonal antibody binding to major histocompatibility class II molecules

HT Greinix, R Storb and SH Bartelmez