Canine Pluripotent Hematopoietic Stem Cells and CFU-GM Express Ia-like Antigens as Recognized by Two Different Class II-Specific Monoclonal Antibodies

By Friedrich Schuening, Rainer Storb, Sondra Goehle, Joey Meyer, Theodore Graham, H. Joachim Deeg, and John Pesando

A previous study showed failure of autologous engraftment in lethally irradiated dogs when marrow was treated before infusion with anti-class II antibody 7.2 and complement. The current study extended this observation to a second monoclonal antibody (HB10a) that identifies a different determinant on Ia-like molecules. These results suggest the presence of Ia-like antigens on pluripotent hematopoietic stem cells or on "accessory cells" needed for sustained engraftment to occur. To distinguish between these two possibilities, stem cell-depleted Ia-positive peripheral blood leukocytes obtained by discontinuous albumin density gradient were added as probable source of accessory cells to the marrow inoculum that was depleted of Ia-positive cells by treatment with antibody 7.2 and complement. Eight of ten dogs failed to show engraftment, providing further support for the hypothesis that pluripotent stem cells and not accessory cells were affected by cytolytic treatment. To provide direct evidence for the presence of Ia-like antigens on canine pluripotent hematopoietic stem cells, autologous transplants were performed using 0.7 to 13 x 10^6 Ia (7.2)-positive marrow cells per kg obtained with the help of fluorescence-activated cell sorter. Of three evaluable dogs, two showed sustained and complete engraftment, indicating that Ia-like antigens, as recognized by anti-class II antibody 7.2, are expressed at least on part of canine pluripotent hematopoietic stem cells. Concurrent in vitro studies revealed that canine CFU-GM also express Ia-like antigens as recognized by the class II-specific monoclonal antibodies 7.2 and HB10a.

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cells were eliminated by treating the marrow with mAb and complement (C') as follows:

transplantation. Autopsies with histological examinations were performed on all dogs that died.

Cytolytic treatment of bone marrow. Ia-positive bone marrow cells were eliminated by treating the marrow with mAb and complement (C') as follows:

Bone marrow was diluted 1:3 with Waymouth's medium and separated by density gradient centrifugation over Ficoll-Hypaque (density 1.077) at 1,000 g for 30 minutes. Cells at the interface were washed three times, resuspended to 1 x 10^6 cells/mL in Waymouth's medium, and incubated with Protein A-Sepharose-purified mAb 7.2 or HB10a (1 mg/10^6 cells) at room temperature for 60 minutes. The cells were then pelleted and the supernatant was stored at -20 °C for later testing for residual antibody activity (see below). After three further washes, the cells were incubated with undiluted rabbit C' of prescreened batches (absorbed for DR typing, Pelfreeze, Rogers, AR; 1 x 10^6 cells/50 mL) at room temperature for 60 minutes. The cells were then washed three times and the incubation with C' was repeated. After three washes, the cell pellet was resuspended to 30 mL and infused into the irradiated dog.

Two dogs received marrow cells obtained by density-gradient centrifugation over Ficoll-Hypaque treated twice with C' only as described above but without prior incubation with mAb.

Immunofluorescence studies. In order to determine the efficiency of the cytolytic treatment, bone marrow cells were examined for Ia+ cells before and after incubation with mAb and C' using indirect immunofluorescence and flow microfluorometry. The staining procedure has been described. Briefly, samples of marrow were incubated with mAbs 9.6, 7.2, or HB10a at saturation for 30 minutes at 4 °C. The cells were then washed and incubated with a 1:20 dilution of fluorescein isothiocyanate-conjugated F(ab')2, fragments of goat antimouse IgG (Tago, Burlingame, Calif). After further washing, cells were analyzed with a fluorescence-activated cell sorter FACSCALIBER (Becton Dickinson FACS Systems, Sunnyvale, Calif). Positively labeled cells were defined as having fluorescence greater than background fluorescence using mAb 9.6.

Cell sorting. To investigate the characteristics of Ia-positive marrow cells, we collected those cells using a FACS 440 cell sorter. Bone marrow cells obtained by density gradient centrifugation over Ficoll-Hypaque were stained with mAb 9.6, 7.2, or HB10a and fluorescein isothiocyanate-conjugated F(ab')2, fragments of goat antimouse IgG as described above. Cells were then suspended in Waymouth's medium at a concentration of 5 x 10^6 cells/mL and sorted into labeled and unlabeled populations on the FACS 440 at a flow rate of 3,000 cells/sec. Fluorescence intensity of the positively stained cells was greater than that of 99% of cells stained with irrelevant control mAb 9.6. One dog (C108) receiving 7.2 positive autologous marrow cells could not be evaluated because it died in the first week after marrow transplantation from intercurrent infection.

Cell separation by discontinuous albumin density gradient centrifugation. We used a discontinuous albumin density gradient to
deplete or enrich for hematopoietic precursor cells. Peripheral blood lymphocytes and bone marrow cells were separated by density gradient centrifugation over Ficoll-Hypaque. Interface cells were treated with hemolytic buffer (0.155 mol/L ammonium chloride) and separated by a discontinuous albumin density gradient as described by Dicke. The stock solution of bovine serum albumin (BSA; 3%) was made using Sigma Fraction V Powder (Sigma Chemical Co., St Louis, Mo). Eighteen mL of a 27% (w/v) BSA solution in Tris buffer were placed in a glass tube (17 x 2 cm) and followed sequentially by 3.75 mL aliquots of solutions containing 25%, 23%, 21%, and 19% BSA. The cell suspension was layered in 9 mL of 17% BSA on top of the gradient. Total cell load per gradient was 0.1 to 1.3 x 10^6. The osmolality of the Tris buffer and the albumin stock solution was 350 mosmol. The gradient was then centrifuged at 1,000 g for 30 minutes at 10 °C.

**CFU-GM.** CFU-GM were carried out as described. Briefly, bone marrow cells were cultured at a concentration of 2 x 10^6 cells/0.1 mL plasma clot together with PBL at a concentration of 2 x 10^6 cells/clot. Cells were incubated for 72 hours in the presence of 1.0 U of erythropoietin (Step III, sheep plasma. Connaught Lab, Grand Island, NY) containing 40% (v/v) heat-inactivated human AB plasma, 10% FCS, and double-strength Dulbecco's Modified Eagle's Medium (GIBCO, Grand Island, NY) containing 40% (v/v) heat-inactivated human AB plasma. PHA-stimulated lymphocyte-conditioned medium was added to the Petri dishes (0.1 mL/plate) prior to the incubation.

**-autografts with marrow treated by mAb 7.2 and C' and added stem cell–depleted Ia-positive peripheral blood leukocytes.** We first showed that treatment of marrow with C' alone does not interfere with engraftment (Table 1, dogs C46 and C142). Then, in confirmation of results by others, we determined that peripheral blood leukocytes from fraction 5 of albumin density gradient were depleted of hematopoietic stem cells insofar as they failed to achieve hematopoietic reconstitution after lethal irradiation (Table 1, dog C86). By further characterizing peripheral leukocytes in fraction 5 using differential counts and flow microfluorometry, we showed that 63 ± 11% of the cells are lymphocytes, 4 ± 2% monocytes, 34 ± 11% polymorphonuclear granulocytes, and that 72 ± 18% of the cells reacted with anti-class II mAb 7.2, 78 ± 15% with anti-class II mAb HB10a, and 66 ± 11% with anti-Thy1 mAb F3-20-7 (data represent mean value ± 1 SD of three independent experiments).

Next, 10 dogs received marrow cells treated in vitro with mAb 7.2 and C' before infusion. The number of mononuclear marrow cells infused after in vitro treatment ranged from 0.2 to 2.4 x 10^6/kg (Table 1). In addition, eight of the 10 dogs received between 0.04 to 0.9 x 10^9/kg of Ia-positive peripheral blood leukocytes from fraction 5 of albumin density gradient. To increase the number of stem cell–depleted Ia-positive peripheral blood leukocytes, two of the 10 dogs received cells from albumin density gradient fractions 4 and 5 (Table 1, dogs C122 and C115).

Table 1. Results in Dogs Given 9.2 Gy Total Body Irradiation and Infusion of Autologous Bone Marrow Treated With Complement Only (Group 1), Stem Cell–Depleted Ia+ Peripheral Blood Leukocytes Only (Group 2), Autologous Bone Marrow Treated With mAb 7.2 and Complement, and Stem Cell–Depleted Ia+ Peripheral Blood Leukocytes (Group 3)

<table>
<thead>
<tr>
<th>Group/ Dog No.</th>
<th>Incubation Temp</th>
<th>Marrow Cell # infused (x 10^6/kg)</th>
<th>Marrow Cell # infused and Fraction of Engraftment at Autopsy</th>
<th>Grafted</th>
<th>Histological Signs of Engraftment at Autopsy</th>
<th>Survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C46</td>
<td>Room temp</td>
<td>1.3</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>&gt;60</td>
</tr>
<tr>
<td>C142</td>
<td>Room temp</td>
<td>0.8</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>&gt;60</td>
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<tr>
<td>Group 2</td>
<td>C86</td>
<td>None</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C106</td>
<td>5 °C</td>
<td>2.4</td>
<td>15 4 4 0 Frac 5: 0.9</td>
<td>Yes</td>
<td>9</td>
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<tr>
<td>C107</td>
<td>5 °C</td>
<td>0.6</td>
<td>11 14 14 14 Frac 5: 0.1</td>
<td>Yes</td>
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</tr>
<tr>
<td>C110</td>
<td>Room temp</td>
<td>0.5</td>
<td>11 0 15 0 Frac 5: 0.06</td>
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<td>No</td>
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<tr>
<td>C114</td>
<td>Room temp</td>
<td>0.16</td>
<td>7 0 6 0 Frac 5: 0.07</td>
<td>No</td>
<td>No</td>
<td>18</td>
</tr>
<tr>
<td>C126</td>
<td>Room temp</td>
<td>0.4</td>
<td>16 0 17 0 Frac 5: 0.12</td>
<td>No</td>
<td>No</td>
<td>11</td>
</tr>
<tr>
<td>C111</td>
<td>Room temp</td>
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<td>28 2 43 0 Frac 5: 0.10</td>
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<td>No</td>
<td>12</td>
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<tr>
<td>C46</td>
<td>Room temp</td>
<td>0.4</td>
<td>9 0 13 0 Frac 5: 0.04</td>
<td>No</td>
<td>No</td>
<td>21</td>
</tr>
<tr>
<td>C58</td>
<td>Room temp</td>
<td>0.4</td>
<td>35 0 12 0 Frac 5: 0.14</td>
<td>Yes</td>
<td>21</td>
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<tr>
<td>C122</td>
<td>Room temp</td>
<td>0.3</td>
<td>25 2 19 0 Frac 5: 0.2</td>
<td>No</td>
<td>No</td>
<td>14</td>
</tr>
<tr>
<td>C115</td>
<td>Room temp</td>
<td>0.8</td>
<td>21 1 5 0 Frac 5: 0.2</td>
<td>Yes</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as percentage of a total of 4,000 cells counted by flow microfluorometry whose fluorescence exceeded a gate defining the negative control population (stained with irrelevant mAb 9.6), corrected for the percentage of the negative control.
Density gradient. A marked enrichment of committed hematopoietic precursor cells, CFU-GM and CFU-E, was seen and then split into fractions using the discontinuous albumin gradient. Cells obtained by fluorescence-activated cell sorting. Colony numbers represent mean value ± SD. All supernatants were tested for residual antibody by incubating 1 x 10^6 normal canine peripheral blood mononuclear cells. Supernatants from marrow treated with mAb 7.2 and C were tested for residual antibody by incubating 1 x 10^6 normal canine peripheral blood mononuclear cells with 100 μL of the supernatants and then fluorescein isothiocyanate-conjugated F(ab')2 fragments of goat antimouse IgG. All supernatants reacted with the target cells, confirming that conditions of antibody excess were met.

Autologous transplants with 7.2-positive marrow cells obtained by fluorescence-activated cell sorting. Additional studies were designed to provide direct evidence for the presence of 7.2-like antigens on canine pluripotent stem cells. Marrow cells were separated by Ficol-Hypaque gradient and then split into fractions using the discontinuous albumin density gradient. A marked enrichment of committed hematopoietic precursor cells, CFU-GM and CFU-E, was seen in fraction 3 of the gradient (Table 2). Autologous transplants of cells from fraction 3 resulted in prompt, sustained, and complete hematopoietic engraftment (Table 3, dogs C116 and C141). To exclude the possibility that coating of marrow cells with mAb 7.2 interferes with engraftment, we performed autologous transplants using marrow cells incubated with mAb 7.2 in the absence of C. Both dogs so treated showed sustained engraftment (Table 3, dogs C124 and C117).

Marrow cells obtained in fraction 3 of the albumin density gradient were then stained at saturation with antibody 7.2 and fluorescein isothiocyanate–conjugated F(ab')2 fragments of goat antimouse IgG. 7.2-positive cells were selected on the fluorescence-activated cell sorter and infused into lethally irradiated autologous recipient. FACS analysis after cell sort demonstrated that 99% of the positively sorted cells had fluorescence intensity greater than that of cells stained with irrelevant control mAb 9.6. Transplantation results are shown in Table 3, group 3. Dog C134 received 0.007 x 10^8 cells/kg body weight. This dog showed a rise in white blood cell count on day 9 after marrow transplant, with 60% of the cells being granulocytes. The dog died on day 11 after marrow transplant due to septicemia. Marrow histology at autopsy showed 2% of normal cellularity identifying myeloid as well as erythroid precursor cells. In order to increase the number of 7.2-positive marrow cells transplanted into the dogs, we increased the duration of the cell sort and used two cell sorters simultaneously. As a result dogs C228 and C281 received 0.03 and 0.13 x 10^8 marrow cells/kg body weight, respectively. Both dogs showed sustained hematopoietic engraftment and are now surviving longer than 210 days after marrow transplant with complete recovery of marrow function. Speed of neutrophil and platelet recovery was prolonged compared to control dogs and varied depending on number of marrow cells transplanted. Dog C228 receiving 0.03 x 10^8 marrow cells/kg body weight had granulocyte counts above 1,000 granulocytes/μL on day 26, compared to a median of 12 days (range 8 to 14 days) in controls. Platelet counts were above 20,000 platelets per μL on day 52 (median in control dogs day 15, range 11 to 23 days). Dog C281 receiving 0.13 x 10^8 marrow cells/kg body weight had

![Table 2](https://example.com/Table2.png)

<table>
<thead>
<tr>
<th>Group/ Dog #</th>
<th>Marrow Cell # Infused (x 10^8/kg)</th>
<th>Method to Preenrich for Stem Cells Before Cell Sort</th>
<th>Sustained Graft</th>
<th>Histological Signs of Engraftment at Autopsy</th>
<th>Survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C116</td>
<td>frac 3: 0.3</td>
<td>—</td>
<td>Yes</td>
<td>—</td>
<td>&gt;60</td>
</tr>
<tr>
<td>C141</td>
<td>frac 3: 0.25</td>
<td>—</td>
<td>Yes</td>
<td>—</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C124</td>
<td>7.2 coated: 1.0</td>
<td>—</td>
<td>Yes</td>
<td>—</td>
<td>&gt;60</td>
</tr>
<tr>
<td>C117</td>
<td>7.2 coated: 1.0</td>
<td>—</td>
<td>Yes</td>
<td>—</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Group 3*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C134</td>
<td>7.2 +: 0.007</td>
<td>frac 3</td>
<td>No</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>C228</td>
<td>7.2 +: 0.03</td>
<td>frac 3</td>
<td>Yes</td>
<td>—</td>
<td>&gt;210</td>
</tr>
<tr>
<td>C281</td>
<td>7.2 +: 0.13</td>
<td>frac 3</td>
<td>Yes</td>
<td>—</td>
<td>&gt;210</td>
</tr>
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</table>

*Fluorescence intensity of the positively sorted cells was greater than that of 99% of cells stained with irrelevant control mAb 9.6.
circumstantial evidence favoring the presence of Ia-like antigens on canine pluripotent stem cells.

Another explanation for the failure of engraftment seen with antibody-treated autologous marrow is that Ia-like antigens are not expressed on pluripotent hematopoietic stem cells but rather on "accessory" cells required for stem cells to engraft. We tried to distinguish between these two possibilities by adding stem cell-depleted Ia-positive peripheral blood leukocytes to a marrow inoculum that was depleted of Ia-positive cells, thereby presumably providing accessory cells. As it is possible to achieve complete marrow repopulation after otherwise lethal total body irradiation with peripheral blood cells only, this cell population contains stem cells as well as accessory cells and therefore can be used as a source for accessory cells. However, eight of ten dogs so treated failed to show sustained hematopoietic engraftment. This result provided further support for our hypothesis that Ia-like antigens as detected by anti-class II mAb 7.2 are expressed on canine pluripotent stem cells. The sustained engraftment seen in two of the ten dogs was most likely due to the fact that incubation with antibody and C' was carried out at 5°C rather than at room temperature, resulting in incomplete elimination of Ia-positive cells from the marrow.

It is possible, of course, that if those dogs receiving Ia-depleted marrow had survived longer, reconstitution of hematopoiesis might have occurred. With existing supportive measures, this possibility cannot presently be investigated. We further tried to test the hypothesis of expression of Ia-like antigens on pluripotent canine stem cells directly by carrying out grafts with marrow cells positively selected for Ia-like antigens as detected by anti-class II mAb 7.2 using the fluorescence-activated cell sorter. Of the evaluable three dogs, one had only transient hematopoietic engraftment, most likely due to very low marrow cell numbers infused (7 x 10^6/kg). With an increase in the duration of the cell sort, we increased the number of Ia-positive marrow cells transplanted. Two subsequent dogs given 3 and 13 x 10^6 Ia-positive marrow cells/kg showed sustained and complete hematopoietic engraftment. This result is in keeping with our hypothesis, and provides direct evidence for the expression of Ia-like antigens as detected by anti-class II mAb 7.2 on at least part of pluripotent canine hematopoietic stem cells.

Prendergast et al recently described complete although slow marrow recovery in a total of 7 out of 10 dogs after lethal TBI and infusion of autologous Ia-depleted marrow using a different anti-class II mAb (WM-2). These results are in contrast to what we have seen using two different

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### Table 4. Results in Dogs Given 9.2 Gy Total Body Irradiation and Infusion of Autologous Marrow Treated With Monoclonal Antibody HB10a and Complement

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Marrow Cell # (x 10^7/kg)</th>
<th>HB10A+ Cells (%)* Pretreatment</th>
<th>Posttreatment</th>
<th>7.2+ Cells (%)* Pretreatment</th>
<th>Posttreatment</th>
<th>Sustained Graft</th>
<th>Histological Signs of Engraftment at Autopsy</th>
<th>Survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C173</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>No</td>
<td>6</td>
</tr>
<tr>
<td>B935</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>No</td>
<td>13</td>
</tr>
<tr>
<td>C120</td>
<td>1.7</td>
<td>20</td>
<td>3</td>
<td>13</td>
<td>1</td>
<td>Yes</td>
<td>—</td>
<td>&gt;60</td>
</tr>
<tr>
<td>BC 5</td>
<td>0.2</td>
<td>43</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>17</td>
</tr>
<tr>
<td>C146</td>
<td>0.5</td>
<td>28</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>No</td>
<td>No</td>
<td>14</td>
</tr>
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*Expressed as percentage of a total of 4,000 cells counted by flow microfluorometry whose fluorescence exceeded a gate defining the negative control population (stained with irrelevant mAb 9.6), corrected for the percentage of the negative control.
Table 5. Effect of Anti-Ia-antibody Plus Complement Treatment on the Growth of CFU-GM of Dog Marrow Cells (Experiment 1); In Vitro Culture Results of Ia+ and Ia −Canine Marrow Cells as Detected by Monoclonal Antibodies 7.2 and HB10a

<table>
<thead>
<tr>
<th>Colony Number After Treatment With*</th>
<th>7.2 + C′</th>
<th>HB10a + C′</th>
<th>7.2 + C′</th>
<th>HB10a + C′</th>
</tr>
</thead>
<tbody>
<tr>
<td>C′ Only</td>
<td>116 ± 17</td>
<td>0.4 ± 0.7</td>
<td>0.4 ± 0.7</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>CFU-GM (per 10^6 BMC)</td>
<td>278 ± 114</td>
<td>28 ± 5</td>
<td>5 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55 ± 6</td>
<td>0.9 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.2 + BMC*</td>
<td></td>
<td>34 ± 12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CFU-GM (per 10^5 BMC)</td>
<td>135 ± 19</td>
<td>0</td>
<td>34 ± 12</td>
<td>0</td>
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<td></td>
<td>96 ± 14</td>
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<tr>
<td></td>
<td>56 ± 7</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>7.2 − BMC*</td>
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<td></td>
</tr>
<tr>
<td>HB10a + BMC*</td>
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<tr>
<td>HB10a − BMC*</td>
<td></td>
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</tr>
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</table>

* Cultures were plated in triplicate. Cell number plated after treatment with mAb + C′ was adjusted to cell count after treatment with C′ only. Colony numbers represent mean value ± 1 standard deviation.

† Data represent % inhibition of antibody plus complement-treated cells compared to cells treated with complement only.

Data represent % inhibition of antibody plus complement-treated cells compared to cells treated with complement only.

The detection of Ia-like antigens on most of human committed hematopoietic precursor cells, and the demonstration of Ia-like antigens on human B lymphocytes and monocytes as well as on human lymphocytic and myelocytic leukemia cells, have prompted the suggestion that human pluripotent hematopoietic stem cells also express Ia-like antigens. For lack of an assay for human pluripotent hematopoietic stem cells, it is not presently possible to prove this hypothesis directly. An in vitro assay for multipotential progenitor cells (CFU-GEMM, colony-forming units: granulocyte, erythroid, monocyte, megakaryocyte) has been developed. These human multipotential progenitors may be closely related to murine CFU-S in that they represent primitive precursors of multiple lines of hematopoietic differentiation. Several groups of investigators have shown that Ia-like antigens are expressed on these very immature hematopoietic precursor cells. Controversial results have been published concerning the expression of Ia-like antigens on those immature precursor cells responsible for proliferation of committed progenitor cells in liquid long-term hematopoietic cell cultures. Moore et al could not identify Ia-like antigens on those progenitor cells in long-term cultures. However, Andrews et al found inhibition of hematopoietic precursor cells in a similar long-term culture system after treatment with anti–HLA-DR antibody and C'. Keating et al could not detect Ia-like antigens on precursor cells responsible for the proliferation of CFU-GM in a different (one stage) long-term culture system. In all these studies, only depletion techniques were used to identify Ia-like antigens on precursor cells, giving rise to long-term culture systems. Recently, Falkenburg et al showed the expression of HLA-DR determinants on precursor cells responsible for sustained proliferation of CFU-GEMM, BFU-E, and CFU-GM in liquid long-term cultures, using both fluorescence-activated cell sorting and C'-dependent cytotoxicity assays.

Three genetic loci for coding distinct Ia-like molecules have been identified in man, DR, DP, and DQ locus. While HLA-DR and DP antigens have been detected on human CFU-GM, BFU-E, CFU-meg, CFU-GEMM, and, by some investigators, HLA-DR antigens on progenitor cells in long-term cultures, HLA-DQ antigens appeared not to be expressed on these hematopoietic progenitor cells. However, a recent publication by Sparrow et al describes expression of HLA-DQ antigens on three subpopulations of CFU-GM.

Using the CFU-S assay in mice, Basch et al have reported that a heteroantiserum against Ia antigens was not reactive with murine CFU-S in cytotoxicity and fluorescence-activated cell sorting studies. They concluded that CFU-S lack Ia antigens. Recent evidence, however, suggests that the situation is more complex. The murine I region is composed of at least five subregions.48 Murine Ia antigens encoded by the I-E subregion have amino acid homology with human DR antigens.49 Fitchen et al found no effect of monoclonal antibodies against I-A subregion gene products on CFU-S, but potent inhibition of these cells by anti–I-E subregion heteroantibodies and C'. These observations suggest that murine pluripotent hematopoietic stem cells express I-E subregion antigens (equivalent to human HLA-DR) but not I-A subregion antigens (equivalent to human HLA-DQ). Performing autologous marrow transplants in rhesus monkeys, Gerritsen et al observed graft failure after incubating the marrow with a combination of anti–RhLA-DR7 antibodies and C', suggesting that the RhLA-DR7 antigen is present on the hematopoietic stem cells of the rhesus monkey. Our previous and present results in dogs, showing that Ia-like antigens as detected by anti-class II mAb 7.2 are expressed at least on part of canine pluripotent hematopoietic stem cells, are therefore in agreement with observations reported for human multipotential progenitor cells and for hematopoietic stem cells.
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poeitic stem cells of mice and rhesus monkeys. Most importantly, we showed this to be true not only by methods of negative selection but also by demonstrating complete hematopoietic reconstitution with transplants of cells positively selected for expression of Ia-like antigens as detected by anti-class II mAb 7.2.

Concurrent in vitro studies revealed that canine CFU-GM also express Ia-like antigens as recognized by two mAbs (7.2 and HB10a) that identify different determinants on canine Ia-like molecules. We showed this by employing positive and negative selection for the appropriate cells.

Results on canine CFU-GM are in agreement with those reported for man. Both with C'-dependent cytotoxicity assays and fluorescence-activated cell sorting, Ia-like antigens were demonstrated on human CFU-GM using polyclonal and monoclonal antibodies.

Our findings indicate that Ia-like antigens, recognized by anti-class II antibody 7.2, are expressed on most true pluripotent hematopoietic stem cells in the dog. This seems to be relevant for clinical bone marrow transplantation. Both human and canine lymphoma cells express Ia-like antigens, and transplantation of autologous marrow purged of Ia-positive tumor cells seems an attractive treatment modality. Present results suggest that this may not be safe. At the present time, it is uncertain whether true pluripotent hematopoietic stem cells in man are Ia positive or negative and, if Ia positive, whether they express only HLA-DR but not HLA-DQ, thus allowing selective removal of HLA-DQ-positive neoplastic cells.

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