A Transgenic Mouse Model of Hemoglobin S Antilles Disease


Hemoglobin (Hb) S Antilles is a naturally occurring form of sickling human Hb but causes a more severe phenotype than Hb S. Two homozygous viable Hb S Antilles transgene insertions from Tg58Ru and Tg98Ru mice were bred into MHOAH mice that express high oxygen affinity (P50 ~ 24.5 mm Hg) rather than normal (P50 ~ 40 mm Hg) mouse Hbs. The rationale was that the high oxygen affinity HMOAH Hb, the lower oxygen affinity of Hb S Antilles than Hb S (P50 ~ 40 v 26.5 mm Hg), and the lower solubility of deoxygenated Hb S Antilles than Hb S (~11 v 18 g/dL) would favor deoxygenation and polymerization of human Hb S Antilles in MHOAH mouse red blood cells (RBCs). The Tg58 × Tg98 mice produced have a high and balanced expression (~50% each) of hα and hβ° Antilles globins, 25% to 35% of their RBCs are misshapen in vivo, and in vitro deoxygenation of their blood induces 30% to 50% of the RBCs to form classical looking, elongated sickle cells with pointed ends. Tg58 × Tg98 mice exhibit reticulocytosis, an elevated white blood cell count and lung and kidney pathology commonly found in sickle cell patients, which would make these mice useful for experimental studies on possible therapeutic intervention of sickle cell disease. © 1997 by The American Society of Hematology.

From the Biology Division, Oak Ridge National Laboratory, Oak Ridge; the University of Tennessee—Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge; the Comprehensive Sickle Cell Center, Meharry Medical College, Nashville, TN; and the Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, Berkeley, CA.

Submitted September 19, 1996; accepted January 21, 1997.

Supported by Grants No. HL-43375 and DE-AC05-96OR22464 with Lockheed Martin Research; Grants No. P60 HL-38737 and K14 HL-03141 with Meharry Medical College; and Grants No. HL-20985 and DE-AC03-76SF00098 with the University of California.

Address reprint requests to R.A. Popp, PhD, Biology Division, PO Box 2009, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1997 by The American Society of Hematology.

Blood, Vol 89, No 11 (June 1), 1997: pp 4204-4212

AID Blood 0044 / 5H36$$S861 05-01-97 18:01:38 blda WBS: Blood
adult α-globin gene is normal. The P50 of a mixture of 55% m\textsubscript{s2}\alpha and 45% m\textsubscript{a}\alpha Hbs in RBCs of Hba\textsubscript{g2}/Hba\textsubscript{g2}; Hbb\textsubscript{a}/Hbb\textsubscript{a} mice is 31.5 ± 0.2 mm Hg. Another Hb mutation, Hbb\textsubscript{b}, was found in a progeny of a female C57BL/6 mouse treated with ENU. In mice that are homozygous for Hbb\textsubscript{b}, 70% of the β\textsubscript{b} globin encoded by the 5’ adult β-globin gene contains a β\textsubscript{98,99-}Bu substitution and 30% of the β\textsubscript{b} globin encoded by the 3’ adult β-globin gene is normal. The P50 of a mixture of 70% m\textsubscript{o}α,m\textsubscript{b}β; 30% m\textsubscript{o}α,m\textsubscript{b}β Hbs in RBCs of Hba\textsubscript{g2}/Hba\textsubscript{g2}; Hbb\textsubscript{a}/Hbb\textsubscript{b} mice is 31.2 ± 0.2 mm Hg. Mice that carried each of these mutations were mated to produce a line of mice that were homozygous for both mutations. The line was called MHOAH because RBCs of these mice contain high oxygen affinity Hbs. Overall, the mixture of ~39% m\textsubscript{o}α,m\textsubscript{b}β; (P50 ~17 mm Hg), and ~16% m\textsubscript{o}α,m\textsubscript{b}β; (P50 ~24.5 mm Hg), ~32% m\textsubscript{o}α,m\textsubscript{b}β; (P50 ~27.5 mm Hg), and ~13% m\textsubscript{o}α,m\textsubscript{b}β; (P50 ~40 mm Hg) Hbs in RBCs of MHOAH mice (Hba\textsubscript{g2}/Hba\textsubscript{g2}; Hbb\textsubscript{a}/Hbb\textsubscript{b}) has a significantly higher affinity for oxygen (P50 ~24.5 mm Hg) than normal mouse Hb (P50 ~40 mm Hg). In MHOAH mice, ~87% of the Hb has an affinity for oxygen comparable with or greater than that of human Hb A.

Constructs of the ha and hG\textsubscript{S}ANTILLES\textsubscript{G} genes used to produce Tg58/β-thal and Tg98/β-thal mice have been described. The Hb S Antilles transgene insertions from Tg58/β-thal and Tg98/β-thal mice were bred into the genome of MHOAH mice; these transgenic mice will be called M-Tg58Ru and M-Tg98Ru, respectively. Finally, the transgene insertion from M-Tg98Ru mice was bred into the genome of M-Tg58Ru mice to produce the doubly homozygous Tg58 × Tg98 line of Hb S Antilles transgenic mice.

Hematology and erythrocyte characteristics. Hematologic data on peripheral blood were obtained by an Ortho Diagnostics Systems, Inc. ELT-15 Hematometric Analyzer (Westwood, MA) and by manual methods. Lysates of blood from M-Tg58Ru and Tg58 × Tg98 transgenic sickle cell mice are turbid. After adding blood to Drabkin’s reagent, the samples were clarified by centrifugation at 10,000 g for 10 minutes to obtain an accurate optical density reading of the Hb concentration. The incidence of reticulocytes was determined from blood films stained with new methylene blue and also from blood stained with thiazole orange (Retic Count; Becton Dickinson, San Jose, CA) and analyzed by flow cytometry. Blood films of peripheral blood were made on glass coverslips and stained with Wright’s stain, and the morphology of 500 or more erythrocytes per animal was examined and classified as normal or misshaped by light microscopy.

Stepwise Percoll gradients were used to examine the density distribution of erythrocytes from 6 MHOAH, 9 M-Tg58Ru, 6 M-Tg98Ru, and 7 Tg58 × Tg98 transgenic sickle cell mice. A 100-μL aliquot of freshly drawn blood was washed and suspended in 0.4 mL of phosphate-buffered saline (PBS), layered on top of a stepwise gradient of Percoll diluted with PBS at the specific gravities shown, and centrifuged at 400g for 20 minutes.

Sodium metabisulfite-induced RBC sickling. To examine the morphology of erythrocytes in vivo, blood was collected from the orbital sinus and transferred directly into 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.3. To induce deoxygenation and RBC sickling, blood was transferred directly into 1% phosphate-buffered Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5} or was allowed to stand in sealed microhematocrit tubes. Aliquots of induced sickle cells were removed from buffered 1% Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5} after 1 to 6 hours or from sealed hematocrit tubes after 24 hours, fixed in buffered 2.5% glutaraldehyde, and stained with Wright’s stain for examination by light microscopy. Deoxygenated blood was also fixed in 2.5% glutaraldehyde, coated with OsO\textsubscript{4}, dehydrated in a series of increasing concentration of ethanol, gold shadowed, and photographed by scanning electron microscopy.

Hb P50 values. Hb oxygen association-dissociation analyses were performed by a TCS HemoAnalyzer (TCS Medical Products Division, Southampton, PA). The P50 values were read from oxygen association curves as deoxygenated blood samples were slowly and fully oxygenated at 37°C over a period of 10 to 15 minutes.

Analysis of Hb and globin. High performance liquid chromatography (HPLC) was used to separate and quantify the multiple mouse, mouse-human hybrid, and human Hb tetramers. The globin chains were separated by electrophoresis in 12% polyacrylamide gels (PAGE) containing 6 mol/L urea, 2% Triton X-100, and 5% acetic acid. The relative amount of each globin was determined by quantitative scanning transmission densitometry of Coomassie blue stained gels. Two or more concentrations of each sample were separated on the same gel to establish that densitometric readings of all bands were on a linear scale.

Necropsy and histology. Age- and sex-matched MHOAH controls and transgenic sickle cell mice were weighed and necropsied. Tissues from 10 controls, 10 M-Tg98Ru, 42 M-Tg58Ru, and 23 Tg58 × Tg98 mice were fixed in 10% buffered formalin. Histological sections were stained with hematoxylin and eosin for routine histological examination or stained with Pearl’s Prussian blue to identify deposits of stainable iron.

RESULTS

MHOAH-transgenic sickle cell mice. The ha and hG\textsubscript{S}ANTILLES\textsubscript{G} transgenes from Tg58/β-thal and Tg98/β-thal mice were bred into the genetic background of MHOAH mice. Transgenic mice homozygous for either the Tg58Ru or Tg98Ru insertion (M-Tg58Ru and M-Tg98Ru, respectively) and mice doubly homozygous for both transgene insertions (Tg58 × Tg98) were viable and most of them were fertile. The MHOAH controls and the three lines of transgenic sickle cell mice have the same major histocompatibility genotype (H-2\textsuperscript{b}) and by pedigree they are alike at ~90% of the genome. Studies in progress indicate that the mean age at death for M-Tg58Ru and Tg58 × Tg98 mice will be ~16 months compared with ~24 months for MHOAH and M-Tg98Ru mice.

Hematology and erythrocyte characteristics. The peripheral blood of MHOAH mice has been shown to have normal hematologic values so the hematologic indices of blood from transgenic sickle cell mice were compared with MHOAH controls in Table 1. Except for the small increase in the reticulocyte count, the hematology values for M-Tg98Ru mice were not significantly different from the control. However, blood from the M-Tg58Ru and Tg58 × Tg98 transgenic sickle cell mice had significantly elevated white blood cells (WBC) and reticulocyte counts. The serum from MHOAH and M-Tg98Ru mice had a reddish tinge, indicating that hemolysis was occurring in vivo in M-Tg58Ru and Tg58 × Tg98 mice. Although the RBCs, blood Hb concentration (HGB), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC) values for M-Tg58Ru and Tg58 × Tg98 mice were significantly lower than for MHOAH controls, these transgenic mice were not anemic. RBCs from MHOAH and M-Tg98Ru mice were normal in size and shape, but anisocytosis, poikilocytosis, polychromatic RBCs, and intercellular debris (fragments of RBCs and microvesicles) were observed in Wright’s-stained films of freshly drawn blood from M-Tg58Ru and Tg58 × Tg98 mice. The RBC fragments and microvesicles imparted...
from Tg58, but 30% to 50% of the RBCs in deoxygenated blood Tg98Ru (0% to 1%) and M-Tg58Ru (1% to 5%) mice (Table 1 and Fig 2A). Very few elongated sickle cells P50 value 24.5 RBCs, equivalent amounts of h HbS Ant (%) 0 13.4 MCHC (g/dL) 31.6 MCV (fL) 43.4 HCT (%) 49.7 HGB (g/dL) 15.7 Reticulocytes (%) 0-1 1-3 10-15 25-35 Misshapen RBC (%) 0-1 1-3 10-15 25-35 Induced SC (%) 0 0-1 1-5 30-50 P50 value 24.5 ± 0.2 36.0 ± 0.5 31.6 ± 1.6 33.3 ± 0.8 * Values statistically different from the MHOAH control (<.05) using the Student’s t-test. Values are the mean ± standard error of mean of 12 to 23 mice per group.

The Hb tetramers and globin chains in RBCs of Tg58 were observed in the peripheral blood from M-Tg58Ru and Tg581 Tg98 mice, respectively. The globin chains were separated by PAGE (Fig 5), and the relative amount of each globin was quantified by scanning densitometry. The average percentage of each α globin/total α globins and of each β globin/total β globins were comparable for PAGE and HPLC analyses and are shown in Table 3. Analysis of the PAGE electrophoreograms of five samples of M-Tg98Ru and seven samples each of M-Tg58Ru and Tg58 × Tg98 RBCs showed that the relative amounts of hβ globin/total globins and of hββ Antillem/total globins were 19.64% ± 0.66% and 11.93% ± 0.75% in M-Tg98Ru RBCs, 13.85% ± 0.90% and 15.96% ± 1.44% in M-Tg58Ru RBCs, and 24.06% ± 0.76% and 23.10% ± 0.94% in Tg58 × Tg98 RBCs. Moreover, the total α globin/total β globin ratios were 1.00, 0.98, and 0.97 in M-Tg98Ru, M-Tg58Ru, and Tg58 × Tg98 RBCs, respectively. Although there was significantly more hβ globin in M-Tg98Ru RBCs and slightly but not significantly more hβ Antillem globin than hβ globin in M-Tg58Ru RBCs, equivalent amounts of hα and hβ Antillem globins were present in the RBCs of Tg58 × Tg98 mice.

In each line of transgenic sickle cell mice, the quantities of mα1hβ2 and hαhβ2 Antillem tetramers were significantly more, and the quantities of mα2hβ Antillem and hα2hβ were significantly less than would be expected if assembly of the Hb tetramers was random and proportional to the concentration of the globin chains in the RBCs (Table 4). Analysis of the Hb tetramers and globin chains in RBCs of Tg58 × Tg98 mice had denser than normal RBCs that sedimented to the interface of the 1.107 and 1.111 specific gravity Percoll.

Table 1. Hematological Data for Blood From 4-Month-Old MHOAH Control and Transgenic Sickle Cell Mice

<table>
<thead>
<tr>
<th>Indices</th>
<th>MHOAH</th>
<th>M-Tg98Ru</th>
<th>M-Tg58Ru</th>
<th>Tg58 × Tg98</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×105/mL)</td>
<td>6.9 ± 0.6</td>
<td>9.7 ± 3.2</td>
<td>18.9 ± 1.6</td>
<td>16.8 ± 1.9*</td>
</tr>
<tr>
<td>RBC (×106/mL)</td>
<td>11.4 ± 0.2</td>
<td>11.5 ± 0.4</td>
<td>9.9 ± 0.3*</td>
<td>10.8 ± 0.4*</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>2.5 ± 0.3</td>
<td>5.8 ± 0.5*</td>
<td>14.8 ± 0.5*</td>
<td>13.3 ± 0.5*</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>15.7 ± 0.2</td>
<td>14.9 ± 0.8</td>
<td>12.6 ± 0.4*</td>
<td>14.1 ± 0.3*</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>49.7 ± 0.7</td>
<td>50.7 ± 0.9</td>
<td>45.4 ± 1.4*</td>
<td>48.8 ± 1.1</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>43.4 ± 0.7</td>
<td>40.6 ± 0.8</td>
<td>46.3 ± 0.7</td>
<td>45.3 ± 1.3</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>13.8 ± 0.2</td>
<td>13.0 ± 0.4*</td>
<td>12.8 ± 0.2*</td>
<td>13.1 ± 0.4*</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>31.6 ± 0.3</td>
<td>29.4 ± 1.0*</td>
<td>27.8 ± 0.4*</td>
<td>29.0 ± 0.3</td>
</tr>
<tr>
<td>HbS Ant (%)</td>
<td>0</td>
<td>13.4 ± 0.3</td>
<td>15.7 ± 0.6</td>
<td>37.9 ± 0.9</td>
</tr>
</tbody>
</table>

Fig 1. Percoll density-gradient centrifugation of blood. (A) MHOAH, (B) M-Tg98Ru, (C) M-Tg58Ru, and (D) Tg58 × Tg98. Blood from M-Tg98Ru and Tg58 × Tg98 mice contained a large number of dense RBCs. A few (1% to 3%) abnormally shaped erythrocytes were seen in freshly drawn, glutaraldehyde-fixed peripheral blood from M-Tg58Ru mice (Table 1). However, many more misshapen erythrocytes were observed in the peripheral blood from M-Tg58Ru (10% to 15%) and Tg58 × Tg98 (25% to 35%) mice (Table 1 and Fig 2A). Very few elongated sickle cells were induced in samples of deoxygenated blood from M-Tg98Ru (0% to 1%) and M-Tg58Ru (1% to 5%) mice (Table 1), but 30% to 50% of the RBCs in deoxygenated blood from Tg58 × Tg98 mice formed elongated sickle cells with pointed ends (Table 1, Fig 2B, and Fig 3).

Hb P50 values. The oxygen association P50 values of Hbs from transgenic sickle cell mice were intermediate between those of MHOAH Hb and Hb S Antilles (Table 1). The values recorded represent the P50 values for the mixtures of Hb tetramers (Table 2) in RBCs of these mice.

Hb and globin analyses. The HPLC profiles of Hb from M-Tg58Ru and Tg58 × Tg98 mice are shown in Fig 4. The nine kinds and quantities of symmetrical tetramers of Hb produced in these transgenic sickle mice are shown in Table 2. Increased amounts of Hb S Antilles (~13%, 16%, and 38%) were found in M-Tg98Ru, M-Tg58Ru, and Tg58 × Tg98 mice, respectively. The globin chains were separated by PAGE (Fig 5), and the relative amount of each globin was quantified by scanning densitometry. The average percentage of each α globin/total α globins and of each β globin/total β globins were comparable for PAGE and HPLC analyses and are shown in Table 3. Analysis of the PAGE electrophoreograms of five samples of M-Tg98Ru and seven samples each of M-Tg58Ru and Tg58 × Tg98 RBCs showed that the relative amounts of hβ globin/total globins and of hββ Antillem/total globins were 19.64% ± 0.66% and 11.93% ± 0.75% in M-Tg98Ru RBCs, 13.85% ± 0.90% and 15.96% ± 1.44% in M-Tg58Ru RBCs, and 24.06% ± 0.76% and 23.10% ± 0.94% in Tg58 × Tg98 RBCs. Moreover, the total α globin/total β globin ratios were 1.00, 0.98, and 0.97 in M-Tg98Ru, M-Tg58Ru, and Tg58 × Tg98 RBCs, respectively. Although there was significantly more hβ globin in M-Tg98Ru RBCs and slightly but not significantly more hββ Antillem than hβ globin in M-Tg58Ru RBCs, equivalent amounts of hα and hββ Antillem globins were present in the RBCs of Tg58 × Tg98 mice.

In each line of transgenic sickle cell mice, the quantities of mα1hβ2 and hαhβ2 Antillem tetramers were significantly more, and the quantities of mα2hβ Antillem and hα2hβ were significantly less than would be expected if assembly of the Hb tetramers was random and proportional to the concentration of the globin chains in the RBCs (Table 4). Analysis of the Hb tetramers and globin chains in RBCs of Tg58 × Tg98 mice had denser than normal RBCs that sedimented to the interface of the 1.107 and 1.111 specific gravity Percoll.
some lungs and kidneys, some lungs had a reddish color indicative of interstitial hemorrhage, the kidneys had a dark brownish color indicative of hemosiderin deposits, and extensive sequestration of blood was observed in the liver of two M-Tg58Ru mice.

Necropsy and histology. No pathology was observed in MHOAH mice, and no pathology was observed in M-Tg98Ru mice. In contrast, gross and histologic pathology was observed in M-Tg58Ru and Tg58 × Tg98 mice. The spleens of 10 MHOAH mice averaged 80 ± 5 mg, and the spleens of 42 M-Tg58Ru and 23 Tg58 × Tg98 mice averaged 168 ± 22 and 177 ± 28 mg, respectively. The lungs of M-Tg58Ru and Tg58 × Tg98 mice were often retracted in the pleural cavity, petechiae were observed on the surfaces of some lungs and kidneys, some lungs had a reddish color indicative of interstitial hemorrhage, the kidneys had a dark brownish color indicative of hemosiderin deposits, and extensive sequestration of blood was observed in the liver of two M-Tg58Ru mice.
In histologic sections, marked congestion was observed in the spleens, kidneys, lungs, and livers of M-Tg58Ru and Tg58 × Tg98 mice. Their spleens exhibited expanded erythropoiesis, capsular and trabecular fibrosis, and large deposits of stainable iron. The alveolar septa in the lungs of M-Tg58Ru and Tg58 × Tg98 mice were thickened (Fig 6A, C, and E). Their lungs also showed fibrosis of interstitial tissue, hemorrhage, and an accumulation of numerous iron-laden macrophages. In the kidneys of M-Tg58Ru and Tg58 × Tg98 mice, the glomerular tufts were often shrunken and many of the capillary loops were filled with erythrocytes (Fig 6B, D, and F). Some glomeruli had completely atrophied. Proteinaceous deposits were observed along the collecting tubules in a few kidneys of M-Tg58Ru and Tg58 × Tg98 mice. The epithelial cells of Bowman’s capsule and of the proximal tubules contained large amounts of stainable iron (Fig 6G and H), indicating that plasma Hb had leaked from the glomerulus into Bowman’s capsule. The liver of M-Tg58Ru and Tg58 × Tg98 mice contained foci of extramedullary hematopoiesis and areas of ischemic necrosis. Sickled RBCs and fibrin thrombi were also observed in small venules of the liver. Liver macrophages and Kupffer cells were laden with stainable iron but iron deposits were not observed in liver parenchymal cells.

**DISCUSSION**

The principal aim of these studies was to produce transgenic mice that would exhibit symptoms of sickle cell disease similar to those found in humans who are heterozygous for Hb S Antilles. To accomplish this, the hα and hβ^S_Antilles transgene insertions from Tg58/β-thal and Tg98/β-thal mice were bred into the genome of MHOAH mice that produce mouse Hbs having an overall oxygen affinity property similar to that of human Hb A. Three lines of Hb S Antilles transgenic mice were produced. The M-Tg98Ru, M-Tg58Ru, and Tg58 × Tg98 lines of transgenic sickle cell mice expressed ~13%, 16%, and 38% Hb S Antilles, respectively (Table 1). M-Tg98Ru mice that produced the lowest amount of Hb S Antilles did not exhibit signs of sickle cell disease. M-Tg58Ru and Tg58 × Tg98 mice that produced larger amounts of Hb S Antilles had reduced RBC, HGB, MCV, and MCHC values but were not anemic; however, they did exhibit reticulocytosis, elevated WBC, and kidney and lung pathology (Table 1 and Fig 6). These clinical and pathological symptoms are similar to those commonly found in patients with sickle cell disease.

The number of misshapen RBCs found in vivo and the number of elongated sickle cells induced by deoxygenation of blood in vitro correlated well with the quantities of hα and hβ^S_Antilles globins synthesized and the quantities of Hb S Antilles assembled in the RBCs (Tables 1 through 4). RBCs of M-Tg98Ru mice synthesized significantly more hα than hβ^S_Antilles, and RBCs of M-Tg58Ru mice synthesized slightly more hβ^S_Antilles than hα (Table 3). High and balanced amounts of hα and hβ^S_Antilles were synthesized in RBCs of Tg58 × Tg98 mice that express both transgene insertions.

In all lines of Hb S Antilles sickle cell mice, some of the Hb precipitated and adhered to the inner surface of the RBC membrane and many of the RBCs became denser than normal (Fig 1). This was particularly evident in the Percoll gradients of all six samples of RBCs from M-Tg98Ru mice that express more hα than hβ^S_Antilles globin. In M-Tg98Ru mice the concentration of Hb S Antilles (3.94 g/dL) was too low to form polymers when the blood was deoxygenated in vitro. Although anisocytosis, poikilocytosis, and polychromatic RBCs were commonly observed (10% to 15%) in peripheral blood of M-Tg58Ru mice, only 1% to 5% of the RBCs formed elongated sickle cells, although...
25% to 30% of the RBCs became multispiculated when blood from M-Tg58Ru mice (~16% Hb S Antilles) was deoxygenated in vitro (Table 1). The concentration of Hb S Antilles (4.36 g/dL) plus other Hbs that contain hβS Antilles (5.27 g/dL) was ~9.6 g/dL in RBCs of M-Tg58Ru mice, which was also too low to form long polymers of deoxygenated Hb S Antilles. However, in erythrocytes of Tg58 × Tg98 mice the concentration of Hb S Antilles (10.99 g/dL) plus other Hbs that contain hβS Antilles (4.41 g/dL) was high enough (~15.4 g/dL) to exceed the Csat of ~11 g/dL for deoxygenated Hb S Antilles. In Tg58 × Tg98 mice, 25% to 35% of the erythrocytes became deformed in vivo (Table 1 and Fig 2); furthermore, when blood from Tg58 × Tg98 mice was deoxygenated in vitro, 30% to 50% of the RBCs formed classical looking, elongated sickle cells with pointed ends (Table 1 and Figs 2 and 3).

How does our Tg58 × Tg98 mouse model of Hb S Antilles compare with other transgenic sickle cell mouse models? Greaves et al.² reported on a transgenic mouse that expressed 83% Hb S. The mouse had a sightly elevated reticulocyte count (4.4%) but the mouse was not anemic. RBCs in the peripheral blood had normal morphology, although most of the RBCs were sickleshaped when the mice were deoxygenated in vitro.
Fig 6. Histologic sections. (A) Lung of an MHOAH mouse showing thin alveolar septa. (B) Kidney of an MHOAH mouse. (C) Lung of an M-Tg58Ru mouse showing congestion and thickening of the alveolar septa. (D) Kidney of an M-Tg58Ru mouse showing RBC congestion in the glomerular tufts and swelling of the capsular epithelium. (E) Lung of a Tg58 × Tg98 mouse showing congestion and thickening of the alveolar septa. (F) Kidney of a Tg58 × Tg98 mouse showing RBC congestion in the glomerular tufts and swelling of the capsular epithelium. (G) Kidney of an MHOAH mouse stained with Pearl’s Prussian blue and counterstained with eosin showing no deposit of stainable iron. (H) Kidney of a Tg58 × Tg98 mouse stained with Pearl’s Prussian blue and counterstained with eosin showing that deposits of stainable iron are present in epithelial cells of Bowman’s capsule and proximal tubules. (Original magnification × 100 for lung and × 200 for kidney.)
the blood RBCs sickled when the blood was deoxygenated in vitro. In another transgenic mouse that expressed 35% Hb S, the RBCs did not sickle when deoxygenated in vitro.

Ryan et al. develop two lines of transgenic Hb S mice that expressed ~50% Hb S in mice of normal genotype and also in mice heterozygous for murine β-thalassemia. The latter group of mice had enlarged spleens, an elevated reticulocyte count (7.4%), and slightly lower than normal RBC, HGB, and hematocrit (HCT) values. Less than 1% of the RBCs from the former group of mice sickled, but more than 90% of the RBCs from the latter group of mice sickled when their blood was deoxygenated in vitro. In mice homozygous for murine β-thalassemia, 77% of the β-globin was hβS and large amounts of denatured Hb accumulated on the inner surface of the RBC membrane. 18

In the Tg58/β-thal line of mice developed by Rubin et al.4 the hemizygous insertion of hαa and hββAntilles transgenes expressed 17.3% hαa and 49.3% hββAntilles globins that assembled to form 8.3% Hb S Antilles plus 40.4% of another Hb (mαa, hββAntilles). About 30% of these RBCs exhibited varying degrees of abnormal shapes and sickling when deoxygenated in vitro, and similar abnormal RBC shapes and sickling were induced in vivo when these mice were exposed to hypoxia (8.4% oxygen) for 10 days. Rubin et al.4 did not describe the characteristics of Tg98/β-thal mice; however, we found that Tg98/β-thal mice, which were bred homozygous for both murine β-thalassemia and the Tg98Ru transgene insertion, expressed 27% Hb S Antilles. Their hematological values were normal and their RBCs did not sickle when the blood was deoxygenated in vitro.

Trudel et al.19,20 used a recombinant hβ-globin gene construct, hβSAD, to produce Hb SAD mice that exhibit a very severe form of sickle cell disease. The hβSAD globin chain has a third, δ121Glu-Gln, amino acid substitution in addition to the two amino acid substitutions in the hβS Antilles globin chain compared with the amino acids in the hββ globin chain. Coexpression of the hαa and hβSAD transgenes produced 19% Hb SAD in normal mice and 26% Hb SAD in heterozygous β-thalassemic mice. Hb SAD has a low oxygen affinity similar to that of Hb S Antilles; in addition, deoxygenated Hb SAD has a lower solubility than Hb S Antilles. 19 Neonatal Hb SAD mice were anemic but adults had normal hematocrits, although they exhibited chronic hemolysis and elevated (6.2%) reticulocyte counts. 19 Sickled RBCs were visible in fixed sections of bone marrow and spleen, and microvascular occlusions and secondary end-organ pathology were observed in the spleen, lung, kidney, and liver of Hb SAD mice. 20 The reported pathological findings were congestive splenomegaly, pulmonary congestion, hemorrhage, thrombosis and fibrosis, renal congestion, glomerulopathy and fibrosis, extramedullary hematopoesis in the liver and lung, systemic hemosiderosis, priapism, and penile hemorrhage.

The Tg58/β-thal line of transgenic Hb S Antilles mice from Rubin et al. 4 was bred to a line of transgenic Hb S mice developed by F. Costantini 7 to produce a second generation of transgenic mice that expressed both Hb S and Hb S Antilles. 5 The latter line of mice showed a more severe phenotype than the former. The doubly transgenic mice expressed 58% hαa, 34% hββ, and 28% hββAntilles globins in heterozygous β-thalassemic mice, and 58% hαa, 42% hββ, and 36% hββAntilles globins in homozygous β-thalassemic mice. The quantities of Hb S and Hb S Antilles were not reported. Blood from these mice contained 10% reticulocytes and a large number of high-density RBCs, and most of the RBCs sickled in a conventional sickle test or when allowed to deoxygenate slowly in sealed microhematocrit tubes. The mice had a reduced ability to concentrate urine, indicative of a reduced renal function. In addition, the mice had elevated levels of aspartate amino transferase and alanine amino transferase, indicative of a reduced liver function. The mice also exhibited hepatosplenomegaly, ischemic infarcts in the liver, splenic congestion and fibrosis, glomerular and peritubular vessel congestion, septal thickening in the lung, and pyknotic neurons in the brain.

From these published reports, we conclude that the symptoms of sickle cell disease are more severe in Hb SAD mice than in our Tg58 × Tg98 mouse model of Hb S Antilles disease described in this report. The pathobiology of sickle cell disease in Tg58 × Tg98 mice is comparable with that in doubly transgenic Hb S and Hb S Antilles mice 4 and is more severe than in other transgenic sickle cell mice. 21,22 Although Hb SAD mice express lower amounts of a sickling Hb than Tg58 × Tg98 mice, the low oxygen affinity of Hb SAD and the lower solubility of deoxygenated Hb SAD compared with Hb S Antilles favors the deoxygenation and polymerization of Hb SAD even in the presence of large amounts (~75%) of mouse and mouse-human hybrid Hbs. Similarly, although Tg58 × Tg98 mice express lower amounts of a sickling Hb than some transgenic Hb S mice 19,23 and doubly transgenic Hb S and Hb S Antilles mice, 6 the lower oxygen affinity and lower solubility of deoxygenated Hb S Antilles compared with Hb S favors deoxygenation and polymerization of Hb S Antilles in the presence of appreciable amounts (~60%) of mouse and mouse-human hybrid Hbs, which contain mutant globins and have a higher affinity for oxygen than normal mouse and mouse-human hybrid Hbs. 8

Other factors also contributed to the expression of sickle cell disease in Tg58 × Tg98 mice. The combined expression of the Tg58Ru and Tg98Ru transgene insertions in Tg58 × Tg98 mice resulted in a high and balanced synthesis of hαa and hββAntilles globins (Table 3). Furthermore, the hαa and hββAntilles globins assembled preferentially so that more than the expected amount of Hb S Antilles was present (Table 4). The presence of 38% Hb S Antilles in RBCs of Tg58 × Tg98 mice is comparable with the 40% Hb S Antilles present in RBCs of humans who are heterozygous for Hb S Antilles. 1 Because these Tg58 × Tg98 mice exhibit erythrocyte sickling, reticulocytosis, elevated WBC, and the secondary end-organ pathology commonly found in the lung, liver, kidney, and spleen of patients with sickle cell disease, these mice should be an excellent experimental animal model to study the long-term effects of current drug therapies, including hydroxyurea, 21 for the treatment of sickle cell disease, and to evaluate future experimental methods, including gene therapy 22 and bone marrow transplantation, 23 for possible intervention of sickle cell disease.

REFERENCES

A variant with lower solubility than hemoglobin S and producing sickle cell disease in heterozygotes. Proc Natl Acad Sci USA 83:9363, 1986


7. Popp RA, Marsh CL, Skow LC: Expression of embryonic hemoglobin genes in mice heterozygous for α-thalassemia or β-duplication traits and in mice heterozygous for both traits. Develop Biol 85:123, 1981


A Transgenic Mouse Model of Hemoglobin S Antilles Disease


Updated information and services can be found at:
http://www.bloodjournal.org/content/89/11/4204.full.html

Articles on similar topics can be found in the following Blood collections
Red Cells (1159 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml