Sickle Cell Disease of Transgenic SAD Mice

By Marie Trudel, Monique E. De Paepe, Nathalie Chrétien, Nacéra Saadsne, Janik Jacmain, Martin Sorette, Trang Hoang, and Yves Beuzard

Erythrocyte sickling on deoxygenation in vitro occurs in transgenic SAD mice, hemizygous for a modified human sickle hemoglobin, HbSAD (αSAD/βSAD). β-thal/SAD mice display enhanced red cell sickling upon deoxygenation in vitro, whereas sickle cell variants in mice were only rarely observed. Therefore, SCD-like pathology in these transgenic mice was infrequent and limited to congestion in the lungs and kidneys and hypertrophy of the spleen. The mild phenotype in these mice might result from the interference of endogenous Hb with sickle Hb. For this reason, we have produced a transgenic mouse expressing Hb SAD that includes three human mutations βpA, βpΔ, and βpΔPunj. The hemizygous (βSADpA, βSADpΔ, βSADpΔPunj) transgenic mice developed a pathophysiology that strongly resembles human sickle cell disease. Moreover, this animal model allows studies on the effect of antisickling agents.© 1994 by The American Society of Hematology.

SIckle Cell disease (SCD) is a recessive disorder caused by an amino acid substitution in the β-globin chain of hemoglobin (Hb). The molecular basis of SCD has been studied extensively with respect to the mechanism of Hb polymerization and subsequent sickling of red blood cells (RBCs). However, the relationship between the RBC defects and the various clinical features of the disease is not well understood. Transgenic mouse models of SCD could contribute significantly to the understanding of the pathophysiology by providing the opportunity to study the progression of the disease, and the mechanisms of vaso-occlusions under different conditions known to trigger sickle cell crisis. Furthermore, SCD animal models could also be useful for dissecting genetic and environmental factors that may contribute to the highly variable SCD expressivity in humans. Most importantly, an animal model of SCD will allow for evaluation of specific therapies. To date, numerous attempts to produce transgenic mouse models of SCD have resulted in RBC sickling upon deoxygenation in vitro, whereas irreparable sickle cells in vivo were only rarely observed. Therefore, SCD-like pathology in these transgenic mice was infrequent and limited to congestion in the lungs and kidneys and hypertrophy of the spleen. The mild phenotype in these mice might result from the interference of endogenous Hb with sickle Hb. For this reason, we have produced a transgenic mouse expressing Hb SAD that includes three human mutations βpA, βpΔ, and βpΔPunj (βSADpA, βSADpΔ, βSADpΔPunj) in the RBC of transgenic SAD mice. To increase the Hb polymerization, SAD mice (HbSADpA/βSADpΔPunj) were crossed with mice homozygous for a β-thalassemia mutation (Hbβ−/−). Erythrocytes of compound heterozygous β-thal/SAD mice (Hbβ−/−/HbSADpA−/−) and age-matched control (Hbβ−/−/HbSADpA−/−) mice as well as the spontaneously dead SAD mice (Hbβ−/−/HbSADpA−/−) were studied with special emphasis on features of human SCD such as RBC sickling, hemosiderosis, erythropoiesis, vascular congestion, and occlusions. The iron content was semiquantitatively assessed as a percentage of fields showing iron deposits using 40× (bone marrow) or 20× (liver) high-power objectives. The level of iron content was evaluated as follows: low, less than 25%; intermediate, 25 to 75%; high, more than 75% of fields. In the spleen, the percentage of fields containing more than five iron deposits with a 40× objective was determined. Erythropoiesis of the bone marrow was defined as an increase in erythroid/myeloid ratio to at least a 1:1 proportion (normal 1:3). To quantitate the degree of extramedullary hematopoiesis (EMH) involving the strains in an attempt to produce congenic inbred strains. The SAD (HbSADpA−/−/βSADpΔ−/−) mice (age range, 19 to 88 weeks, n = 18), β-thal/SAD (Hbβ−/−/HbSADpA−/−/βSADpΔ−/−) mice (age range, 46 to 57 weeks, n = 3) and age-matched control (five Hbβ−/−/HbSADpA−/−/βSADpΔ−/−) mice as well as the spontaneously dead SAD mice (age range, 38 to 76 weeks, n = 6) were studied. Erythropoiesis of the bone marrow was defined as an increase in erythroid/myeloid ratio to at least a 1:1 proportion (normal 1:3). To quantitate the degree of extramedullary hematopoiesis (EMH) involving the...
liver, foci of EMH were counted in 20 randomly selected fields using a 10× objective. Passive congestion, microscopically characterized by engorgement of small-sized vascular structures (capillaries and venules) with RBCs, often associated with vascular distension was assessed semiquantitatively (none, mild, moderate, marked). In each kidney, 100 randomly selected glomeruli were evaluated for the presence of sclerosis (ie, 200 glomeruli per animal).

Transmission electron microscopy. To study the polymerization process in SAD and β-thal/SAD mice by electron microscopy, biopsies of the spleen of SAD (n = 3), β-thal/SAD (n = 3) and β-single/β-thal control (Hbb^ab/ab/Hbb^ab) mice (n = 2) were fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer at 4°C in which no attempts were made to deoxygenate the tissues. After postfixation in 1% buffered osmium tetroxide, the samples were embedded in epoxy resin. Ultrathin sections were stained with uranyl-acetate and lead citrate and examined with a JEOL 1200-EX transmission electron microscope at an accelerating voltage of 60 kV.

Density gradient. The stract solutions ranging in density from 1.0919 to 1.1195 (22 g/dL to 28 g/dL), were successively layered from high- to low-density in 5-mL tubes. Blood samples of 100 to 150 μL were layered on the gradient and centrifuged at 20,000 rpm for 45 minutes. Cells of all layers were harvested under oxygenated conditions and processed for light and electron microscopy. Cell smears were stained with Wright and blue cresyl. Samples of each layer were washed with bovine serum albumin solution, pelleted, fixed in 2.5% phosphate-buffered glutaraldehyde, and embedded in epon.

Erythropoietin concentration. Murine bone marrow cells were plated in methylcellulose cultures (2 × 10^5 cells/mL) in the presence of serum of SAD or control mice (1% vol/vol). Erythropoietin (IMDM; GIBCO, Burlington, Canada) with 10% fetal calf serum) served as positive and negative controls respectively. CFU-E (erythroid colonies) were quantitated by light microscopy.

Renal function. Blood urea nitrogen (BUN) and creatinine levels were determined in serum obtained from the peripheral blood of the tail vein. BUN levels were measured in SAD mice (n = 18) and control mice (n = 9) and the ratio of urine protein/creatinine was calculated for SAD (n = 29) and control (n = 18) mice.

Hypoxia. A series of hypoxic experiments were performed using a continuous flow of oxygen and nitrogen mixtures monitored by oximetry. Different oxygen levels (70 to 42 mmHg of oxygen) were produced in an enclosed environment to test tolerance of hypoxic stress in control, SAD, and β-thal/SAD mice. At the time of death of SAD or β-thal/SAD mice, the remaining animals of that experimental group were killed for comparative histopathologic studies. In all of the experiments, normal control littermates survived the various low-oxygen tension.

Antiscicking treatment with BW12C79. An antiscicking agent from Burroughs Wellcome, BW12C79 [5-(2-formyl-3-hydroxyphenoxo)pentanoic acid] was supplied by SANOFI. The BW12C79 drug was freshly dissolved in 0.1 mol/L NaOH and adjusted to pH 7.4 for the experiments. Mice were injected intraperitoneally with doses of BW12C79 at 40 mg/kg to induce 15% to 20% of modified hemoglobin. These hemoglobin values were chosen according to data reported in human studies. Ten minutes after the injection, the treated and untreated control mice were subjected to hypoxic challenge at a pO₂ of 42 or 49 mm Hg.

RESULTS

Evidence of microvascular occlusions. SAD mice manifest a unique spectrum of vasoocclusive defects. Dilated capillaries and venules filled with densely packed and deformed RBCs, reminiscent of the microvascular occlusions observed in human SCD, consistently affected the lungs, liver, kidneys, bone marrow, and spleen of all β-thal/SAD and SAD mice. In SAD mice, RBCs strongly resembling human sickled cells were readily observed in the bone marrow (90%) (Fig 1 inset, Table 1) and spleen (24%). In β-thal/SAD mice, unequivocal sickled RBCs were noted in all organs, consistent with the higher content of HbsAD in erythrocytes and a more severe phenotype. In addition to widespread microvascular occlusions due to vessel plugging by deformed RBCs, organizing microthrombi were observed in the lungs (Fig 2A, Table 1), liver, and kidneys of the transgenic mice. Microvascular occlusions also appear to underlie two characteristic clinical manifestations of the SAD disease. Skin ulcers located on the back and on the neck are abnormally frequent in SAD mice (prevalence, 16%; n = 125). Furthermore, priapism and penile hemorrhage, often followed by penile atrophy, were also repeatedly observed in transgenic mice (incidence 5% of males, Fig 2C). Histologic sections were performed in order to determine the implication of RBC sickling in penile infarction. The sections of penis taken during priapism showed congestion of the corpora cavernosa with extensive RBC sickling, followed by hemorrhagic infarction and fibrosis of the corpora in later stages. The clinical manifestations of skin ulcers and of the sequence of priapism and penile infarction in SAD mice are akin to lesions previously documented in human SCD.15-18

Visceral pathology. The transgenic mice displayed a range of degenerative organ damage. In apparently healthy SAD mice, the mean splenic weight (415.9 ± 220.6 mg) was significantly larger than that of control mice (89.8 ± 14.5 mg, P < .01, single-factor analysis of variance) with moderate to marked expansion of the red pulp related to massive sequestration of RBC and diffusely increased erythropoiesis (Fig 3, Table 1). SAD mice showed no evidence of large splenic infarction. In contrast, spontaneously dead SAD mice displayed relative atrophy of the organ (mean weight, 268.6 ± 141.3 mg, P < .05 compared with apparently healthy SAD mice), frequently associated with significant degrees of splenic fibrosis and capsular thickening. The splenic evolution from initial hypertrophy to progressive fibrosis and atrophy parallels the splenic changes that occur with age in sickle cell patients; ie, congestive splenomegaly in young children with SCD, followed by splenic atrophy later in life. In many patients with reduced severity of SCD, the spleen remains enlarged throughout their lifetime.

The lungs of the SAD mice also showed many of the alterations described in human SCD. Moderate to marked congestion of the pulmonary capillaries was noted in all β-thal/SAD and in a significant proportion of SAD mice (28%, Table 1), frequently associated with staihnable alveolar iron deposits and interstitial fibrosis. Infarcts of the lung, thrombosis and small pulmonary hemorrhages were observed in spontaneous (Table 2) or hypoxia-induced deaths of SAD mice.

An array of functional and morphologic abnormalities of the kidney and liver was observed in transgenic SAD mice, similar to that observed in humans with SCD.28-30 Focal-
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Fig 1. Bone marrow of a SAD mouse. Bone marrow section shows extensive RBCs sickling and erythroid hyperplasia. Morphology of the sickled RBCs is illustrated in the inset (hematoxylin-eosin, original magnification × 400, inset original magnification × 1,260).

Segmental and/or global glomerulosclerosis were observed in the kidneys of SAD mice (44%) and of β-thal/SAD mice (33%) (Table 1). Moreover, the severity of the glomerulosclerosis in many spontaneously dead animals, as shown in Table 2, suggested that chronic renal failure may have contributed to their death. The animals also frequently displayed glomerular hypertrophy as well as occasional renal fibrosis and papillary necrosis. Further, SAD mice showed renal dysfunction by a significant increase in the mean blood urea nitrogen and proteinuria (urine protein/creatinine ratio: SAD, 3.3 ± 1.5, control, 2.2 ± 0.7; *P < .01*). Hyperplasia of the Kupffer cells and of portal macrophages in association with stainable iron and erythrophagocytosis, as shown in human SCD, was observed in all β-thal/SAD mice and in a substantial proportion of SAD mice (33%, Table 1). In summary, SAD and β-thal/SAD mice at ambient atmosphere showed chronic organ damage as defined by the progressive morphologic lesions of various organs.

**Shortened lifespan and cause of death.** At ambient atmosphere, the mean lifespan of SAD mice is 15.4 ± 7.3 months (n = 155), which is significantly less than that of mice with the same genetic background C57BL/6J and CBA/J (23 and 28 months, respectively). Accordingly, 40% of SAD mice (63/155) experience premature death before 15 months of age. Autopsies performed on six of these SAD mice showed similar organ damage as described above, although in more severe form as shown in Table 2. Half of these animals showed evidence of an acute sickle cell vasocclusive process, as defined by sudden death caused by major tissue damage. Thrombosis of coronary arteries associated with myocardial infarct and thrombosis of the corpora cavernosa associated with penile infarcts and acute pyelonephritis correlated with a significantly shortened life span (38 to 42 weeks). Hence, SAD mice could develop acute vasocclusive defects at ambient atmosphere while longer-lived animals displayed severe renal glomerulosclerosis. Overall, the premature death of SAD mice could often be ascribed to specific chronic or acute complications of their disease.

**Polymerization and increased erythrocyte density.** The observed pathology was further correlated to the molecular defect of RBCs in vivo. Ultrastructurally, the RBCs of SAD and β-thal/SAD mice showed intracellular Hb polymerization with various patterns, ranging from scattered fibers to bundles of parallel fibers (Fig 4). The filaments had a transverse diameter ranging from 16.0 nm to 19.0 nm. Cross-sections through closely packed parallel rods often suggested an orderly paracrystalline arrangement, in which the rods showed a periodicity of approximately 28.0 nm. As in human SCD, the amount of hemoglobin polymer in SAD RBCs correlated with the degree of deoxygenation and cellular distortion. The RBCs in splenic capillaries were rounded or biconcave and contained few intracellular polymers that were randomly oriented. By contrast, the RBCs in the congested splenic sinuses and cords were more frequently distorted and elongated, containing increased numbers of polymers, usually in parallel orientation (Fig 4). Clusters of polymers per cell in RBCs of β-thal/SAD mice were more abundant than in the RBCs of SAD mice, but the average

<table>
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<tr>
<th>Table 1. Pathologic Features of Killed SAD and β-thal/SAD Mice</th>
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<tr>
<td>Mouse</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>SAD</td>
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<tr>
<td>β-thal/SAD</td>
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<tr>
<td>Control*</td>
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Abbreviations: Cong, congestion; Hyp, hyperplasia; Fe, iron deposits; all as defined in Materials and Methods.

* Control includes five (C57BL/6J x CBA/J) F2 mice with Hb of single haplotype and two C57BL/6J heterozygous for the β-thal mutation [Hbb(β−/+)].
length of the polymers was similar in both. The patterns and dimensions of the polymers observed in SAD RBCs strongly resembled those of human Hb S polymers. Therefore, the Antilles and D-Punjab mutations do not appear to affect the polymer diameter or structure, which is in agreement with the study of the polymer nucleation process in vitro of Hb SAD. The RBCs of β-thal/SAD mice did not show any of the typical ultrastructural alterations described in human β-thalassemia (Heinz bodies, remnants of organelles, or evidence of iron accumulation).

The density distribution of SAD RBCs was analyzed by discontinuous Stractan gradient. The erythrocytes of three SAD mouse littermates (nos. 10, 11, and 13) showed a distinct shift towards higher densities when compared with that of control C57BL/6J mice (Fig 5A), correlating well with the high mean corpuscular Hb concentration and the presence of irreversibly sickled cells in the transgenic animals. The high density as well as the heterogeneous distribution pattern of SAD RBCs are similar to those documented in human SCD. Although the reticulocytes of control mice were evenly distributed over all density layers, the reticulocyte fraction of SAD mice was diffusely increased with a maximum in the least-dense layer. Deformed erythrocytes with holly leaf and spiculated shapes were most abundant in the dense layers of SAD mice RBCs (maximum, 20.1%) (Fig 5B). By electron microscopy, most RBCs of high-density showed scattered Hb polymers.

Analysis of erythropoiesis. All SAD and β-thal/SAD mice showed systemic hemosiderosis, indicating the presence of a chronic hemolytic process. Extensive iron deposition was observed in 78% of SAD mice (Fig 3B, Table 1) and in all β-thal/SAD mice. In contrast, iron deposits were
Fig 2. (Cont'd) (C) SAD mouse with priapism and penile hemorrhage.

Fig 3. (A) Spleen of a nontransgenic control mouse. A normal spleen consisting of predominant white pulp with large, well-developed lymphoid follicles surrounded by a narrow rim of red pulp (arrows) with no visible iron deposits (hematoxylin-eosin, original magnification x 400). (B) Spleen of a SAD mouse. Loss of splenic architecture caused by massive expansion of red pulp showing sequestered RBCs and diffusely increased erythropoiesis. Small lymphoid aggregates constitute the residual white pulp. Numerous iron aggregates (arrows) are present (hematoxylin-eosin, original magnification x 400).
Table 2. Pathologic Studies of Deceased SAD Mice

<table>
<thead>
<tr>
<th>SAD Mouse No.</th>
<th>Time of Death (wks)</th>
<th>Bone Marrow</th>
<th>Spleen</th>
<th>Kidney</th>
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<tr>
<td></td>
<td></td>
<td>Erythroid</td>
<td>Erythroid</td>
<td>Fe</td>
</tr>
<tr>
<td>93</td>
<td>38</td>
<td>++</td>
<td>++</td>
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<td>7</td>
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<td>++</td>
<td>50%</td>
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<tr>
<td>16</td>
<td>42</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>62</td>
<td>+</td>
<td>+++</td>
<td>&lt;50%</td>
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<tr>
<td>74</td>
<td>75</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>73</td>
<td>75</td>
<td>ND</td>
<td>ND</td>
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Quantification of the morphologic features was determined as described in Materials and Methods.

Abbreviations: ND, not done; +, mild; ++, moderate; ++++, severe.

small and infrequent in control animals (28%). The main sites of RBC destruction were the spleen and bone marrow.

Despite the chronic hemolysis, transgenic SAD mice are not anemic. To understand the lack of anemia, the serum of SAD mice was analyzed for erythropoietin-like activity by measuring CFU-E stimulation in a functional assay. Erythropoietin-like activity was elevated in the serum of transgenic SAD mice as quantified by the stimulation of CFU-E (Fig SC). In the serum of half of the transgenic mice, erythropoietin-like activity was markedly elevated as compared with the range of control mice. In one set of experiments, the activity was neutralized with a monoclonal anti-mouse erythropoietin. No other colony-stimulating activity was detected in the serum.

Major sites of erythropoiesis in SAD and β-thal/SAD mice are the spleen and bone marrow, although foci of extramedullary erythropoiesis were frequent in liver and lungs as well (Table 1). Expansion of the erythroid series and increase of the erythroid/myeloid ratio to at least 1:1 was observed in the bone marrow of all SAD and β-thal/SAD mice, whereas the expected normal ratio is 1:3 (Fig 1).

Effect of acute hypoxia. Transgenic SAD mice are specifically vulnerable to hypoxia, a condition known to induce sickle cell crisis in human SCD. Preliminary studies have shown that 8% O₂ was lethal to 90% of β-thal/SAD mice but did not affect the survival of SAD mice or control littermates. Forty-eight β-thal/SAD, SAD, and nontransgenic control mice were exposed to a wide range of oxygen levels, from 70 to 42 mm Hg (Tables 3, 4, and data not shown). Results of Table 3 show the analysis of two mice per group subjected to different levels of hypoxic stress that were subsequently processed for histologic studies. All animals survived prolonged exposure to moderate hypoxia (>17 hours at 70 mm Hg oxygen). When the oxygen tension was lowered to 53 mm Hg the two β-thal/SAD mice died within 45 minutes, whereas SAD and control mice only transiently reduced their activity and survived. Other SAD and control mice were further subjected to lower oxygen tension. At 49 mmHg of oxygen, the SAD and control mice remained alive during the 3 hours of the hypoxic experiment (Table 3). In contrast, all SAD mice died within 20 minutes when the oxygen proportion was further decreased (46 and 44 mm Hg). Several SAD and β-thal/SAD mice under hypoxic conditions suffered convulsions just before death. In all experiments, control mice tolerated the low oxygen levels without obvious adverse effects. Together, Tables 3 and 4 show that all of the 10 SAD mice exposed to hypoxia between 42 and 46 mm Hg died within 19 minutes, whereas all four controls survived. Similarly, none of the eight β-thal/SAD mice survived exposure to levels ≤ pO₂ of 53 mm Hg. Although survival in hypoxic conditions might be related to a number of factors, the difference in Hb SAD levels in these mice appears to be an important determinant for their relative intolerance to hypoxia.

The pathology of SAD and β-thal/SAD mice that underwent hypoxia was similar to that described for mice at ambient oxygen levels, albeit with more extensive sickling, severe
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3195

Hb haplotype: 'ls 10 11 13 SlS SlS sls

25 1 151 - SAD - CONTROL / 0

B1.09 1.10 1.11 1.12 DENSITY

n X (D c 100 .- c 95 .- a 30 .- c- .- 0 c.- .- 25-. 0 .- c. 2. 20-. W P SAD c57 C

systemic visceral congestion, and microvascular clogging such as that seen in human sickle cell crisis as well as small pulmonary hemorrhages.

Because the time of survival and the oxygen threshold under hypoxia were strongly related with the levels of HbSAD in transgenic mice, we have used this approach to evaluate the antisickling properties in vivo of a substituted benzaldehyde, BW12C79, which specifically binds to Hb.13,14 Within 10 minutes of BW12C79 intraperitoneal injection, both 15% to 20% of the murine Hb and 16.5% of the HbSAD were modified as assessed by isoelectric focusing and the proportion decreased to 8% by 50 minutes (data not shown). Furthermore, the oxygen affinity of RBCs from SAD and β-thal/SAD mice was determined with and without BW12C79 treatment (Table 5) by P50 measurements which are inversely related to oxygen affinity of Hb. The results indicated that the BW12C79 compound increased the oxygen affinity of Hb for both SAD and β-thal/SAD mice. As a consequence, the Hb polymerization process in vivo is likely to be decreased after treatment with BW12C79. Data in Table 4 show that SAD and β-thal/SAD mice treated with BW12C79 survived exposure to 42 and 49 mmHg of oxygen respectively for at least 1 hour, i.e., for the duration of the experiment, and stayed alive, whereas untreated transgenic SAD and β-thal/SAD mice died within 14 minutes, in concordance with results in Table 3.

DISCUSSION

SAD mice show chronic organ and tissue lesions consistent with SCD as well as acute vasoocclusive events at atmospheric oxygenation. The similarity in pathogenesis between the human SCD and the SAD mouse starts at the molecular and cellular level and extends to pathologic features of SCD. The RBCs of transgenic SAD mice in vivo can display, in addition to sickling, organized Hb fibers into parallel bundles even without hypoxia, a feature typical of human SCD and specific to these transgenic mice. Our data show that HbSAD have similar polymer structure in patterns and dimensions as those of HbS. Further, the process and degree of HbSAD polymerization appear to depend on tissue oxygenation and correlate with RBC sickling. Transgenic SAD mice undergo erythrocytic sequestration, vascular congestion, and occlusions in various organs. Therefore, the transgenic SAD mice can develop all the primary characteristics of human SCD.

Human SCD is characterized by chronic hemolytic anemia. Although of clinical importance, anemia is a secondary

Fig 5. (A) Stractan density discontinuous gradient. Erythrocytes separation obtained from control mouse C57Bl/6J (S/S, single haplotype) and SAD mouse kindreds (nos. 10, 11, 13) with the single haplotype. (B) Quantification of the sickle-shaped erythrocytes in the segregated stractan density bands. Sickle-shaped RBCs with holly leaf and spiculated shapes expressed in percent of total amount of cells. Open squares represent cells from control mice and dark squares cells from SAD mice. (C) Erythropoietin-like activity in SAD mice. Data are shown for 15 SAD and 7 control (C57Bl/6J) mice within the same age range (3 to 18 months). The mean of serum values for normal control mice was taken as 1 and the stimulation indexes for individual serum samples from independent mice were calculated as a ratio over the mean.
feature observed in human SCD. SAD fetuses and neonates are anemic (manuscript in preparation) but normal hematocrit levels are restored by the time SAD mice reach adulthood. In adult SAD mice, the increased level of erythropoietin in the serum is reminiscent of the erythropoietin response in human SCD. This specific increase in erythropoietin indicates the stimulation of erythropoiesis, which is further supported by the presence of high reticulocyte counts and general erythroid hyperplasia. Hence, the combined evidence from functional and morphologic studies in SAD mice indicates the existence of a compensatory mechanism secondary to increased chronic hemolysis. The important role of rodent spleen in the adjustment of erythropoiesis throughout life may contribute to this compensatory process.

The phenotype of transgenic SAD mice consistently includes congestive splenomegaly and renal glomerulopathy whereas damage in other organs was more variable. In humans affected by SCD, genetic heterogeneity is believed to be of major importance in variable clinical manifestations. Depending on the site of occurrence of vascular occlusions, the resulting phenotype may be more or less acute, such as myocardial or skin infarcts. It is noteworthy that the pathologic severity and frequency increase with age. Histologic and functional renal defects that can be readily quantifiable (and herein) could provide for a measurement of chronic damage. Moreover, the kidney defects could serve to evaluate long-term therapy aimed at inhibiting Hb polymerization by various approaches.

Other attempts to produce a sickle cell mouse model by expressing HbS and Hb S-Antilles in transgenic mice have been undertaken. One HbS transgenic line on a homozygous β-thalassemic and heterozygous α-thalassemic background was reported to display visceral congestion and splenic enlargement. However, the RBCs even upon deoxygenation did not produce hemoglobin fascicle fibers characteristic of human SCD, in sharp contrast with SAD and β-thal/SAD mice. This difference may explain their survival to prolonged (5 days) exposure to 8% oxygen, whereas 9 of 10 β-thal/SAD mice (only heterozygous for the β-thalassemic determinant) died within 90 minutes under the same conditions.

Both transgenic SAD and β-thal/SAD mice, similar to SCD patients, are specifically sensitive to low oxygen tension. Our data indicate that the level of sensitivity to hypoxia is inversely related to HbSAD levels. Furthermore, some of the mice undergo convulsions before death, which could be reminiscent of strokes and seizures reported for SCD patients under hypoxic stress. In addition, survival of SAD mice under lethal hypoxic conditions can provide a crude but sensitive means to evaluate therapies for prevention or cure of acute sickle cell events.

To improve the conditions of SCD patients or the transgenic SAD mouse lines, two mechanisms can be considered: inhibition of hemoglobin polymerization or inhibition of RBC dehydration. The latter approach can be observed with administration of clotrimazole in transgenic SAD mice. Most pertinently, we have chosen to directly modify HbSAD by using an antisickling agent BW12C79. In humans, BW12C79 has been shown to increase the oxygen affinity ex vivo and inhibit sickling when administered intravenously to sickle cell patients. However, there is no information on the efficacy of BW12C79 in preventing sickle cell crisis in vivo. Using hypoxic stress, we showed that the administration of BW12C79 protected SAD and β-thal/SAD transgenic mice from lethal effect of hypoxia. The availability of SAD mice will allow us to determine an exhaustive dose-response study and role determination of a therapeutic index for BW12C79. Further studies to address the potential benefit of using, at the onset of sickle cell crisis, agents that interfere with polymerization through increased oxygen affinity (such as BW12C79) or by contact inhibition within the polymer are warranted.

In summary, transgenic SAD and β-thal/SAD mice can reproduce both the chronic, steady-state disease and acute, spontaneous, or hypoxia-induced vasoocclusive events of human SCD. The potential of SAD and β-thal/SAD mice to respond to a therapeutic agent provides evidence that these transgenic mice are suitable models for analysis of therapies specific to SCD and for the design of preclinical trials.

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