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Transgenic animal models of sickle cell disease

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Abstract. An animal model which allows study of chronic processes occurring in sickle cell disease has finally been realized with the development of several lines of transgenic mice which express high levels of β^S or β^S-variants in their red cells. The red cells of all mouse lines exhibit characteristic sickle shapes on deoxygenation and most lines have enlarged spleens and mildly elevated reticulocytes in adult mice; both of these properties are suggestive of enhanced red cell destruction and erythropoiesis. However, all lines examined to date have normal hemoglobin levels in adult mice. In one mouse line under ambient conditions, retinopathy and abnormal renal function have been observed and in the same line under hypoxic conditions, anemia, irreversibly sickled cell formation, and urine concentrating defect have been observed. The current mouse lines will allow some aspects of sickle cell disease to be studied, but significant improvements can still be made in the transgenic mouse model.
Key words. Transgenic mouse; animal model; sickle cell; polymerization; vasoocclusion; renal; sickling; retinopathy.

Introduction

Sickle cell anemia (SCA) is the result of a single amino acid mutation which results in the polymerization of deoxygenated hemoglobin S. Sickle cell disease has long been notable for its myriad pathological manifestations and the variability of its severity from patient to patient. The consequences of anemia are superimposed on those of chromic ischemia and periodic vasoocclusion. It is both a disease of acute events such as sickle cell painful crisis and splenic sequestration and of chronic effects such as anemia, loss of renal function, retinopathy, and femoral head necrosis. The young red cell population, which is due to rapid cell destruction, has properties which are not found in normal human blood such as adhesiveness and the ability to regulate red cell volume. In addition, modulating factors exist which contribute to the variability of severity, such as the concomitant presence of α-thalassemia and the percent and distribution of hemoglobin F which interferes with polymer formation. Because of the wide range of pathology and the variety of factors which modulate sickle cell disease, it is unlikely that a single animal model will adequately represent all aspects of the disease.

The primary animal models used to date for studying sickle cell anemia have been the intact rat transfused with varying quantities of human sickle blood[6, 7, 13, 5, 22] or ex vivo tissue preparations[1, 21]. Smaller series of studies have been conducted with baboons[8, 16] and other animals. Transfused animal models and ex vivo preparations have allowed only acute studies. Despite this limitation, significant conclusions have been drawn about sequestration, vasoocclusion and adhesion of SS cells. Chronic effects associated with sickle cell disease such as the role of various factors leading to and modulating anemia and organ damage have not been accessible using these models.

The development of transgenic mice expressing high levels of β^S-chains promises to allow the chronic aspects of sickle cell disease to be studied. Three areas of study seem most suited to the transgenic mouse: 1) anemia, 2) vasoocclusion and painful crisis, and 3) end organ damage. Different animal models may be appropriate for the different aspects of the disease. For example, to study anemia and potential treatments to prevent or ameliorate it, an animal model which is spontaneously anemic would be desirable. However, for the study of renal damage, early onset and multi-organ pathology would complicate experimental design and an animal in which renal disease of varying degrees of severity is inducible may be a more suitable experimental model.

A brief review of factors affecting polymerization, sickling and vasoocclusion

Hemoglobin S polymerizes when deoxygenated, but polymer formation does not occur immediately. The time between complete deoxygenation and polymer formation is called the delay time. The delay time may be physiologically important because, if it is longer than the transit time through the circulation, in vivo sickling will be reduced[20]. Both the rate and extent of polymer formation are extremely sensitive to the intracellular hemoglobin concentration (MCHC which is directly proportional to red cell density and inversely proportional to red hydration and red cell volume)[9]. For example, increased red cell MCHC is thought to account for the unexpected severity of SC disease[14]. It has

been appreciated for a long time that the potassium efflux which occurs when cells containing HbS are deoxygenated may result in red cell dehydration[27]. More recently it has been shown that sickle cells and all young red cells have elevated levels of K:Cl co-transport activity which may also contribute to red cell dehydration and a concomitant increase in the intracellular hemoglobin concentration[2–5].

The amount of deoxy hemoglobin S available for polymer formation will be affected by the intracellular hemoglobin concentration: the degree of deoxygenation, the oxygen affinity (p50) of the hemoglobin, and the percent hemoglobin S present. Tetramers which are not fully deoxygenated and tetramers which contain only one β^S-chain can be incorporated into the polymer, but with reduced efficiency[9]. Reduced intracellular pH is also a pro-polymerization, pro-sickling factor.

Vasoocclusion occurs when polymer-filled, non-deformable cells attempt to pass through the microcirculation. Adhesion of red cells to the endothelium may further narrow the capillary circulation and adhesion of cells to the venular endothelium may allow cells with long delay times to polymerize and obstruct[18,21,29]. Other factors which may favor occlusion are vasoconstriction, local hypoxia which may also result in low tissue pH, and microcirculatory beds in which local stasis can occur.

Characteristics of five lines of transgenic mice

Transgenic mice expressing the human β^S (or variants thereof) and human α-globin genes have been developed and studied by several groups. The constructs used in producing these animals are summarized in figure 1 and the hematological properties of these mice are summarized in table 1. The presence of the locus control region (LCR) associated with the beta gene cluster, or some of the hypersensitive sites located within it, allow high level expression of the alpha and/or beta gene after integration into the mouse genome.

Greaves et al.[17] produced a construct that contained an LCR in tandem with two human $\alpha 1$ genes and a single human β^S gene. They reported the production of one transgenic mouse expressing high levels of human β^S and human α chains, most of whose red cells were able to sickle in vitro. The founder was not anemic; however, it was not propagated.

Ryan et al.[26] co-introduced two constructs consisting of the β^S gene or the human $\alpha 1$ gene, each in tandem with a LCR. They described a transgenic line expressing 40%

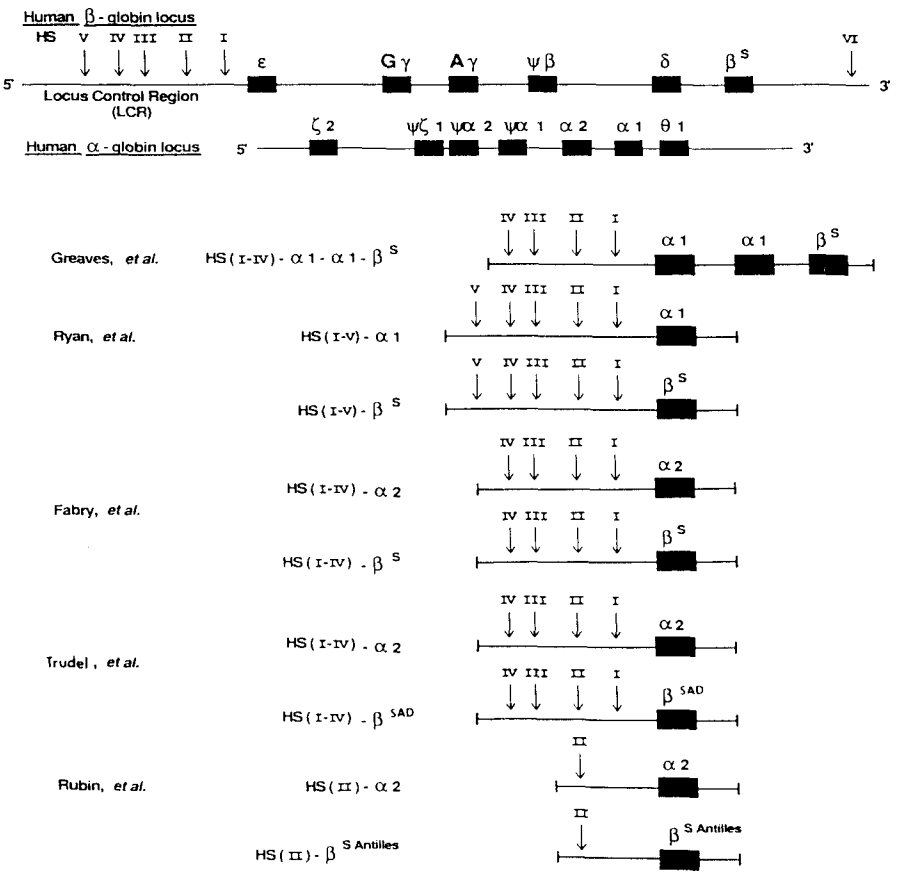

Figure 1. Constructs used to create the five transgenic mouse lines. In all cases with the exception of Greaves et al., the α- and β-globin constructs were made separately and were co-injected and co-integrated into the mouse genome.

Table 1. Characteristics of transgenic mice produced by five different research groups

Research group	Control	Ryan[26]		Rubin[25]		Fabry[10,11,12]		Greaves[17]	Trudell[28]	
%β^S Founder	C57BL/6J	33.2% β^S		13.7% $\beta^{S\text{-Antilles}}$		37.3% β^S		83% β^S	20.6% β^{SAD}	
Thalassemia	-	β^{MD}	β^{MDD}	-	β^{MDD}	β^{MDD}	$\alpha^{MD}\beta^{MDD}$	-	-	β^{MD}
% β^S	-	52	77.4	13.7	49.3	72.7	65.1	83	20.6	26.6
% α^H	-	46.7	44.4	9.0	17.3	44.7	53.1		52.0	53.2
% $\alpha^M\beta^S$	-			7.8	40.4	29.4	18.4		2	3
% $\alpha^H\beta^M$	-			7.5	5.9	11.9	20.4		39.5	
% HbS	-	40	40	5.5	8.3	41.9	40.6		19	26
Hct	45–47	45.0			45.9	47	45.9	49	44.0	39.4
Hb g/dl	14–16	13.5				17.4	17.3	14.4	16.5	15.2
MCV	33–34				44.4	43.0	41.9	47	40.7	35.4
MCH	14–15					14.1	14.3	13.8	15.1	13.6
MCHC	33–34				33.5	35.1	36.3	29.4	37.3	38.6
Reticulocytes %	2–4	10			2.9	6.7	10.3		3.6	6.2
ISC %	-	2.5				2.4	1.4			5–15
P50 mm Hg	41–42					33.5	37.4		44.6	44.8
Sickling	-	>90			>95	>95	>95	>95	>95	>95
Enlarged spleen	-	+				+	+		+	+
Other pathology#	-					R, GFR	R, GFR			
Hypoxia@	-				H	M(d), H, K	M(d), H, K			M(m)

#R retinopathy (G. Lutty), GFR-elevated glomerular filtration rate (N. Bank); @M(m, d) death following exposure to hypoxia in minutes or days; H increase in RDW or red cell density following hypoxia; K decrease in renal concentrating ability following hypoxia. β^{MD}, heterozygous β-deletion; β^{MDD}, homozygous β-deletion; α^{MD}, heterozygous α-deletion.

human HbS (based on IEF), on a heterozygous β^{major} deletional background, a mildly elevated reticulocyte count, and sickling of most cells in vitro. HbS polymer was detected inside deoxygenated cells by transmission electron microscopy. These mice had elevated levels of membrane associated denatured hemoglobin when compared to control mice. When introduced into a homozygous β^{major} deletional background the percent β^S increased to 77% of all β-chains.

Rubin et al.[25] have described a transgenic line which expressed the low oxygen affinity hemoglobin S mutant, $HbS^{Antilles}$. In contrast to sickle trait patients (AS), patients with $HbS^{Antilles}$ trait exhibit symptoms of vaso-occlusion due to a combination of the low solubility and low oxygen affinity of this mutant Hb. The animals studied, which were homozygous for mouse β^{major} deletion, expressed about 51% of their β-globin as $\beta^{S\text{-Antilles}}$; however, the ratio of $\alpha^H/\beta^{S\text{-Antilles}}$ was much less than 1 and most of the $\beta^{S\text{-Antilles}}$ was found in dimers which contained mouse α-globin. These mice had a slightly reduced hematocrit; however, hemoglobin levels were not determined and, although the reduction in hematocrit was statistically significant, it probably does not mean that the animals were anemic. In most other transgenic lines hemoglobin was found to be normal or above normal even in the presence of a slightly decreased hematocrit. Hypoxic conditions were created by placing the mice in a hypobaric chamber for ten days at 0.42 atm which resulted in an equivalent oxygen content of 8.4%. After exposure to hypoxia the distribution curve of red cell volume was increased in transgenic but not control animals and increased numbers of cells identified as ISCs (irreversibly sickled cells) were observed.

Trudel et al.[28] reported the development of a mouse strain bearing α^H and β^{SAD} ($\beta^{S\text{-Antilles D-Punjab}}$) genes, which on a heterozygous β^{major}-deletional background express 26% hemoglobin SAD. Hemoglobin SAD has a low oxygen affinity and an enhanced polymeriztion potential. A completely deoxygenated hemolysate from the SAD founder, which contains 20% β^{SAD}, polymerized in eight minutes; hemolysate from the β^{SAD} mouse which is heterozygous for the β^{major} deletion and expresses 26% β^{SAD} polymerized in 25 seconds; while a hemolysate containing 19% HbS and 81% mouse hemoglobin did not polymerize in 15 hours. These mice displayed increased fetal and neonatal wastage and attempts to breed a mouse which was homozygous for the β^{major} deletion were unsuccessful. When SAD mice were mated to non-transgenic mice, only 32% of the progeny expressed HbSAD instead of the expected 50%; in addition, the neonatal mice had a hematocrit 32% lower than their litter mates. Both SAD mice and SAD mice heterozygous for the β^{major} deletion had enlarged spleens. Adult SAD mice were not anemic, but the SAD mice which were also heterozygous for the β^{major} deletion were extremely susceptible to hypoxia and nine out of ten animals died after 90 minutes exposure to 8% oxygen while eight non-transgenic homozygous β^{major} thalassemic mice and eight SAD mice survived under the same conditions.

Fabry et al.[10–12] have reported on a strain of transgenic mice carrying human LCR-β^S and human LCR-α2 constructs co-integrated in the genome. The original transgenic animals, with a normal genetic background, displayed moderately high expression of human β^S-globin and human α-globin (α^H) in their red cells. These

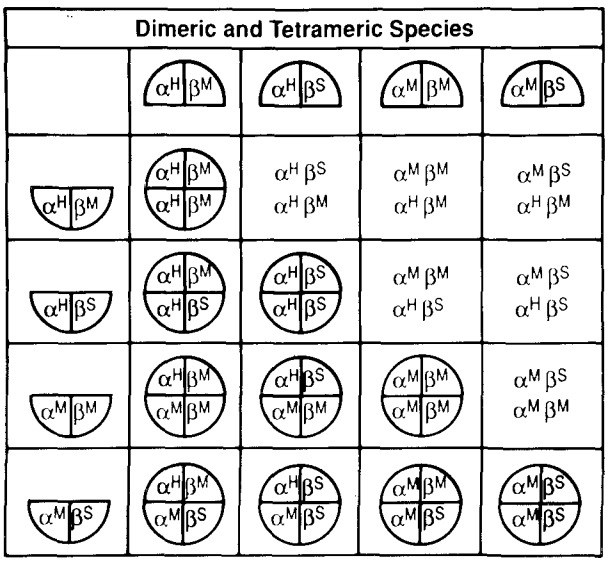

Figure 2. Ten tetramers formed from the four dimers present in the hemolysate of red cells from transgenic mice. Dimers do not disassociate into monomers under physiological conditions, but the dimers do freely recombine into all possible tetramers in the red cell. Tetramers with at least one β^S can be incorporated into the polymer formed from human hemolysates; however, since the mouse α-chains differ from the human by 17 or more amino acids, the situation may be more complex.

mice were bred with mice expressing deletions of mouse β^{major} or α-genes to increase expression of human globins and reduce the level of mouse globins. Two types of transgenic mice expressing high levels of β^S and α^H chains were extensively characterized: mice bred into a background homozygous for a β-globin deletion (β^{MDD}, MD = mouse deletion) which are referred to as $\alpha^H\beta^S[\beta^{MDD}]$ mice; and mice which were also heterozygous for an α-globin deletion (α^{MD}) which are referred to as $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mice. The percent β^S expressed in $\alpha^H\beta^S[\beta^{MDD}]$ and $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mice was found by HPLC to be 72.7 ± 2.4 and 65.1 ± 8.5 respectively while the percent α^H was 44.7 ± 2.4 and 53.1 ± 6.2 respectively. Isoelectric focusing demonstrated the presence of heterodimers containing both human and mouse chains (for example, $\alpha^M\beta^S$ and $\alpha^H\beta^M$) and confirmed the presence of more than 40% HbS. The actual solution composition is more complex since all of the dimers can recombine to form a total of ten tetramers, most of which can potentially be incorporated into the polymer (fig. 2).

Both types of transgenic mice demonstrated normal mean corpuscular hemoglobin (MCH), elevated mean corpuscular hemoglobin concentration (MCHC), no anemia, a moderate but statistically significant reticulocytosis, a small number of irreversibly sickled cells, and detection of dense cells by gradient separation. The oxygen affinity of both lines of transgenic mice was elevated in proportion to the percent of β^S present.

When whole blood from either type of transgenic mouse was deoxygenated slowly, more than 95% of the cells sickled (fig. 3). Hemoglobin S polymer can be detected inside deoxygenated mouse red cells, but the fascicles (polymer bundles) commonly seen in human SS red cells are difficult to observe (fig. 4). The rate of sickling was variable from animal to animal and was related to both the type of animal ($\alpha^H\beta^S[\beta^{MDD}]$ or $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$) and the percent β^S and α^H found in their red cells. Between 25 and 75% of all cells sickled in less than five minutes, which is slower than the rate for human red cells. The delay time is the time interval between the

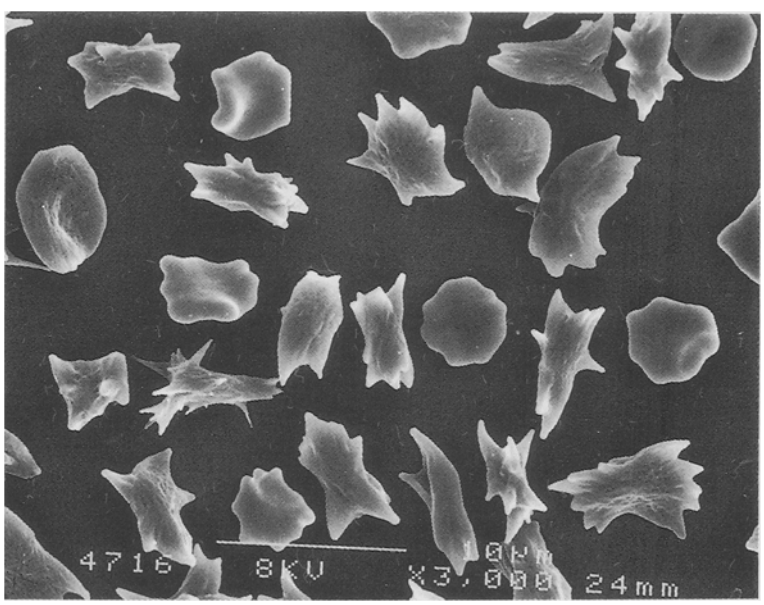

Figure 3. Sickled cells from a $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mouse following 24 h of deoxygenation. Note the small size of the cells when compared to the ten micron bar. Also note that one cell has not sickled and that one cell (in the center) appears to have numerous small domains which are characteristic of cells with high MCHC.

Figure 4. Polymer inside deoxygenated red cells from a $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse. Polymer strands run parallel to the walls of the spicules, which is similar to the case for human cells; however, no fascicles are observed.

time when complete deoxygenation is achieved and the time when polymerization begins. In collaboration with J. Hofrichter and G. Christoph, we found that the delay time was sensitivie to both pH and the osmolarity of the suspending media. At pH 7.1 many cells sickled in less than 30 seconds in both the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ and the $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ mice.

Several indications of organ damage occur in both lines of transgenic mice described by Fabry et al. under ambient conditions: 1) The lung, spleen, and kidney are all enlarged when compared to the C57BL/6J strain which is the background for these mice (fig. 5). 2) The spleen shows expanded red pulp (particularly in $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ mice which is suggestive of elevated erythropoiesis); the presence of iron (which is suggestive of red cell destruction); and scarring which is suggestive of infarct. 3) In collaboration with N. Bank and H. Aynedjian[11], renal function was examined in these animals. The glomerular filtration rate or GFR is elevated in the kidneys of transgenic animals under ambient conditions (fig. 6). Elevated GFR is seen in children with sickle cell disease. 4) Magnetic resonance imaging reveals enlarged kidneys with elevated water content. 5) Thickened septa are noted in the lungs of older transgenic animals. 6) In collaboration with G. Lutty the retinas of transgenic animals were examined. Retinal neovascularization and choroidal neovascularization were first detected at one year of age in $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice and were found in most but not all animals by 18 months. We conclude that, under ambient conditions transgenic mice with high β^{S} expression have red cell properties and a degree of organ pathology intermediate between AS individuals and SS patients (analogous to S/β^{+} thalassemia).

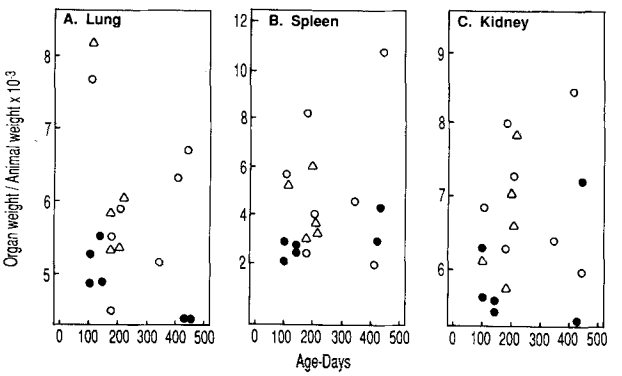

Figure 5. Organ weights in control (filled circles) and transgenic animals (open circles, $\alpha^{H}\beta^{S}[\beta^{MDD}]$; open triangles, $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$) versus the age of the animal in days. Note that there is more variation between transgenic animals than between control animals.

Exposure to hypoxia (10% oxygen, 0.5% CO_2 for 5–7 days) resulted in a significantly decreased hematocrit[11] (fig. 7), increased ISC formation, and increased red cell density. In collaboration with M. Canessa, we have demonstrated that mouse red cells exhibit a potassium efflux when deoxygenated which is analogous to that observed when human sickle cells are deoxygenated[24]. The increased red cell density suggests that the deoxy potassium efflux which may lead to red cell dehydration occurs in vivo in hypoxic mice. If these newly formed dense cells are isolated by density gradient centrifugation and examined by scanning electron microscopy,

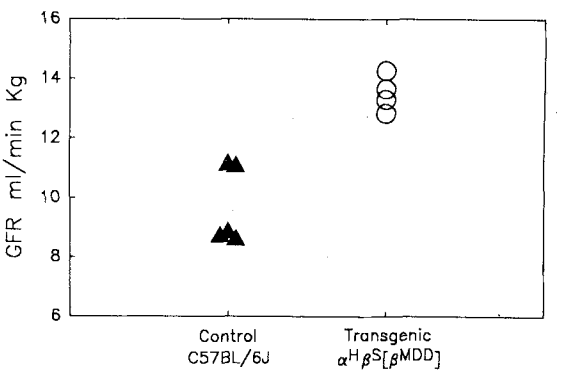

Figure 6. The glomerular filtration rate for control (filled triangles) and transgenic (open circles) mice under ambient conditions. The result is statistically significant with $p > 0.01$.

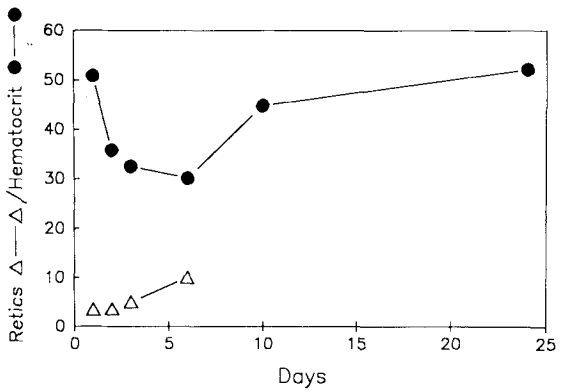

Figure 7. The effect of hypoxia on the hematocrit of a transgenic mouse. Note the increase in reticulocytes by the 6th day of hypoxia. After the mouse was returned to ambient air on day 7, the hematocrit returned to normal.

some are found to be new irreversibly sickled cells (ISCs, cells with a length to width ratio of more than 2) and small dense cells which appear to be the result of membrane loss (fig. 8). During hypoxia a renal concentrating defect was also elicited[11] (table 2). Transgenic mice, but not control mice, lost about one third of their renal concentrating ability as measured when challenged with overnight deprivation of water. Renal concentrating defect is universally found in adults with sickle cell disease and is even a characteristic of sickle trait individuals. This defect is developed after infancy and progresses more rapidly in homozygous sickle cell patients than in those with sickle trait. The kidney is expected to be one of the most sensitive organs to sickle cell vasoocclusion due to its poor oxygenation, high osmolarity and low pH. During hypoxia experiments involving 16 transgenic animals, there were four deaths, all between

Table 2. Effect of hypoxia on urine concentrating ability in control and transgenic mice

Days of hypoxia	Day 0	Day 3	Day 7
Control #1	2938	2975	3100
Control #2	2900	2912	3200
$\alpha^{H}\beta^{S}[\beta^{MDD}]$ #1	2863	2892	2175
$\alpha^{H}\beta^{S}[\beta^{MDD}]$ #2	2850	2800	2075
$\alpha^{H}\beta^{S}[\beta^{MDD}]$ #3	2850	2812	1925

day 4 and 7. At this point during hypoxia, the red cell density is increasing and the hematocrit and renal concentrating ability are falling. It seems likely that there is an increasing rate of polymerization during hypoxia which is due to the increase in MCHC. In some animals reticulocyte production also increases and, if mouse reticulocytes also exhibit increased adhesion, it may contribute to vasoocclusion as it does in the rat model[21].

Areas of difference between current transgenic mice and human sickle cell disease

All current models have features which may lead to differences between the model and sickle cell disease:

1) All models express some mouse hemoglobins which have been shown by Beuzard et al.[23] to interfere with polymerization. In particular, the tetramer formed from the heterodimer $\alpha^{M}\beta^{S}$ has been shown to polymerize very poorly[23] and seems to be readily formed in most models. Although it is clear that the pure tetramer $(\alpha^{M}\beta^{S})_2$ polymerizes very poorly, more studies will be needed to determine whether it can be incorporated into an existing polymer.

2) The p50 of mouse hemoglobin is substantially higher than that of human hemoglobin. The result of this is

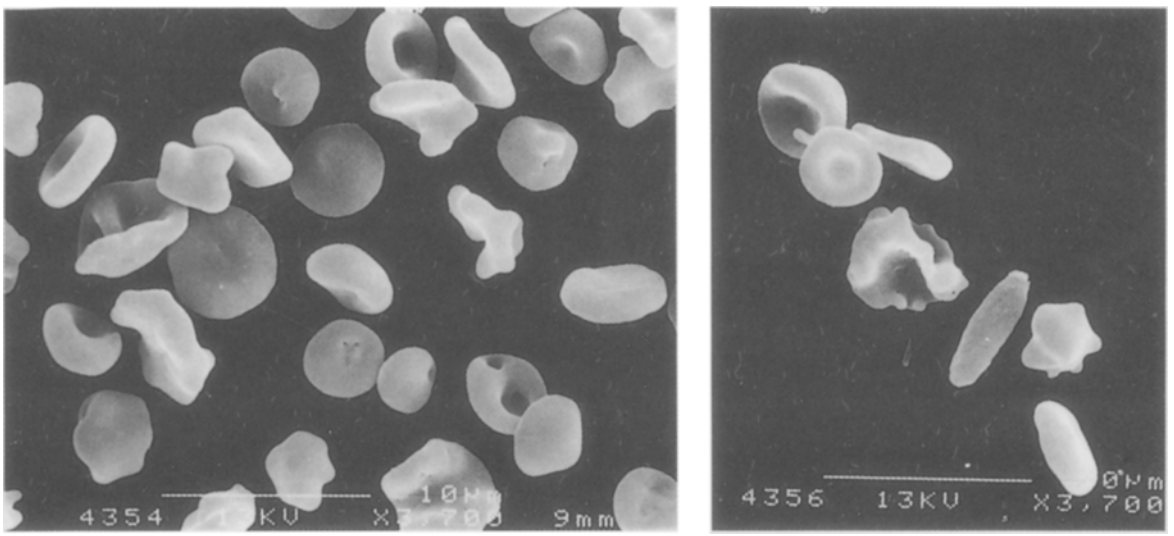

Figure 8. Dense cells isolated from mouse whole blood following exposure to hypoxia. The panel on the right has three ISCs which are similar to those observed in human sickle blood. The panel on the left has several small cells which were very dense and may be the result of membrane loss or fragmentation; some of these are also pitted.

that mouse tetramers will be preferentially deoxygenated and HbS tetramers in the mouse red cell will be partially protected from deoxygenation. This defect is overcome in the SAD mouse and the mouse with $\beta^{S\text{-Antilles}}$ both of which have low oxygen affinity.

3) All transgenic mouse lines have been bred into thalassemic mouse backgrounds to enhance expression of the transgene. Some investigators have suggested that this implies that these mice have thalassemic red cells and that this accounts for some of the abnormalities observed. However, in the line established in our laboratory, we have demonstrated that expression of the transgene completely cures the mouse thalassemia and the resulting animals have the same mean corpuscular hemoglobin (MCH) as control mice; a similar finding has been reported by other laboratories (table 1). Furthermore, the density distribution is not characteristic of thalassemic mice and in the $\alpha^{H}\beta^{S}[\alpha^{D}\beta^{MDD}]$ mice α- and β-chain synthesis is completely balanced. In fact, the presence of the human transgene cures mouse thalassemia in all lines.

4) The mouse red cell is much smaller than the human red cell while the mouse capillary diameter is comparable to that of the human. Kaul et al.[21] have demonstrated that despite this liability mouse red cells can cause vascoocclusion in the isolated rat mesoappendix just as human red cells can. However, it is interesting to note that SAD mice are anemic during the neonatal period when mouse red cells have a volume of 90 femtoliters; whereas adult SAD mice have a normal hemoglobin level. Mice older than 14 days have a red cell volume which is only 45 femtoliters. Since the mouse has no fetal hemoglobin and the hemoglobin composition immediately after birth is the same as the adult, neonatal anemia may be due to more rapid destruction of the larger red cells.

5) The human hemoglobin is encased in a mouse red cell membrane which may differ in its adhesion and cation transport properties. Romero et al. have demonstrated that mouse red cells differ from human red cells in the activity of the transport proteins present[24]. This could affect the generation of dense and irreversibly sickled cells. The potential role of adhesion in sickle cell disease has received a great deal of recent attention[19,21,29] and it is unclear whether receptors similar to those necessary for the adhesion of human reticulocytes are present in mouse reticulocytes. However, this apparent problem may allow the role of adhesion in inducing pathology to be clarified.

6) Finally, the structure and physiology of mouse organs differ from those of humans. However, as the pathology of both the human and the mouse are better understood, these differences may lead to improved understanding.

To date the site of integration of the human transgene has not been elucidated for any of the sickle cell mouse lines. None of the lines (with the exception of a line producing only 6% β^{S} produced by F. Costantini) have been bred to homozygosity. This raises the question of whether this failure is due to the vasoocclusive effects of high levels of human β^{S} in utero (which is relatively hypoxic environment) or whether the transgenes in some or most of the mouse lines have integrated in a site which then produces a lethal defect in the homozygous condition.

Can current transgenic mouse models contribute to our understanding of sickle cell disease?

All current mouse lines share a number of similar features listed in table 3. All models show extensive sickling when subjected to long periods of deoxygenation; however, only in the SAD mouse with concomitant β-thalassemia[28] does the delay time approach that of human SS patients. In vivo, a short delay time (the interval between deoxygenation and polymer formation) should contribute to intravascular sickling; however, other mechanisms such as stasis due to localized vasoconstriction, localized hypoxia, adhesion, or high osmolarity and low pH such as that found in the kidney may either detain cells with longer delay times or shorten the effective delay time to allow for in vivo sickling to occur. Three of the models exhibit a mildly elevated reticulocyte count and enlarged spleen. Both of these observations imply an increased rate of in vivo red cell destruction and erythropoiesis which is compatible with the occurrence of in vivo sickling. In addition, three of the models exhibit increased MCHC which indicates that the red cell dehydration which is characteristic of human sickle cell disease also occurs in the transgenic mice. The mechanism for the dehydration may be abnormal cation transport and permeability. Romero et al.[24] have demonstrated that the deoxy potassium efflux which is characteristic of human sickle cell disease also occurs in transgenic mice. In two of the models hypoxia, which increases both the rate and extent of sickling, led to death in minutes or days. Finally, in the model of Fabry et al., abnormal renal function and retinopathy have been demonstrated under ambient conditions and hypoxia has been shown to lead to significant anemia and loss of renal concentrating ability.

From these observations we can conclude that the transgenic mouse lines reported have some characteristics of sickle cell disease under ambient conditions and that more severe pathology can be elicited by exposure to hypoxia. These results suggest that each of these lines may contribute to our understanding of sickle cell disease.

Conclusion and future directions

The interference of mouse hemoglobin with polymerization can be dealt with by making a mouse in which

Table 3. Common properties of transgenic mouse lines

Properties related to sickle cell disease	(refs.)
Extensive sickling on deoxygenation	11, 12, 17, 25, 26, 28
Mildly elevated reticulocytes (6–10%)	11, 12, 26, 28
Enlarged spleen	10, 26, 28
Elevated MCHC	11, 12, 26, 28
Properties dissimilar to sickle cell disease	
Expression of mouse hemoglobin	11, 12, 17, 25, 26, 28
Presence of human/mouse heterodimers	11, 12, 17, 25, 26, 28
Use of murine thalassemia to enhance production of human hemoglobin	11, 12, 25, 26, 28
Normal hemoglobin levels in adult mice	11, 12, 17, 25, 26, 28
Small red cell volume	11, 12, 17, 25, 26, 28

the human Hb has a lower oxygen affinity and with better polymer contacts (mice with either $\beta^{S\text{-Antilles}}$ or $\beta^{S\text{-Antilles D-Punjab}}$ are examples of this approach). Alternatively, a mouse with only α^{human} and β^{S} could be created by a 'knock-out' experiment. However, since the mouse has no naturally occurring HbF to afford protection to the fetus in the low oxygen environment during gestation, it may be necessary to also introduce human HbF to allow the birth of healthy pups who can than be challenged in a more controlled manner by exposure to a low oxygen environment after birth. The differences between mouse and human red cell membranes and between the structure and physiology of mouse organs needs to be elucidated and accounted for in any interpretation of the data; however, the effect of these differences may themselves by informative.

Since the lines of transgenic mice currently available differ significantly, one would anticipate that they would have different pathological manifestations. This can best be documented by testing these lines under uniform conditions and comparing the results. At a minimum, comparison will allow the possibility that some of the pathology is due to the site of integration and not to vasoocclusive effects to be eliminated. At best, comparison of different lines under the same conditions may lead to conclusions about the relative importance of delay times, oxygen affinity, and MCHC in the production of pathology.

A surprising obstacle to application of transgenic mouse models to understanding human sickle cell disease is a lack of definitive, modern pathology on human sickle cell patients. For example, the functional pathology of renal disease in SCA is relatively well studied, but there are few electron microscopy studies of the human sickle cell kidney. A similar situation exists for the retinopathy of sickle cell disease. Acute chest is another life threatening illness affecting sickle cell patients which could be potentially studied with animal models, but once again the pathology is so poorly defined that it would be difficult to validate an animal model. It seems likely that the most productive approach will be parallel studies of human and mouse pathology.

In conclusion, current transgenic mouse models can be substantially improved and other transgenic animal models (such as the transgenic rat proposed by T. Townes) will also eventually contribute to our understanding of sickle cell disease. However, the currently available lines of transgenic mice exhibit organ damage under ambient conditions and more severe pathology when exposed to low oxygen. We therefore conclude that currently available mice can yield considerable new information about the evolution and prevention of pathology in sickle cell disease.

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