MINIREVIEW

Lessons from Transgenic Mouse Lines Expressing Sickle Hemoglobin (43708)

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Sickle-cell anemia was discovered over 80 years ago, and is known as a molecular disease (homozygous state for the β^S gene) consisting in a single amino acid substitution (β6 Glu → Val) for more than 40 years (1, 2). The origin of the β^S gene and some of the molecular basis for its phenotypic variability has been elucidated (3), there is a considerable body of knowledge about its interaction with malaria (4), and the mechanism involved in the polymerization reaction of deoxy hemoglobin S (HbS) are now essentially solved (5). This account attests to a considerable amount of progress in the basic understanding of the genetics and pathophysiology of HbS.

Nevertheless, our comprehension of the disease, beyond the molecular events surrounding the β^S product, has been much slower in coming. For example, we are only beginning to understand the process of vaso-occlusion that underlies the acute events of painful crises (marrow and bone infarct), and chronic and acute organ damage (vaso-occlusion in kidneys, liver, brain, and lungs).

In retrospect, the dramatic advances on the molecular basis of the disease actually stymied the advances on the pathogenic physiologic events. Early on, the notion prevailed that if we understood the basis of SS red cell sickling, we could understand the disease in its entirety. Editorials (6) and reviews (7) were written in this vein. In these reviews, the microcirculation, for example, was depicted as rigid tubes, seen as passive bystanders contemplating the pirouettes of the sickling process. The pathophysiology of sickle-cell anemia began and ended with the sickle cell. Some challenged the notion espoused by Eaton (who pioneered the understanding of the deoxy HbS polymerization reaction) that delay times of the sickling of red cells determine the variability of sickle syndromes, by proposing that the sickling of SS cell begins in the arterial side and that the overall tendency to polymerize of the SS cell is central (7). In effect, the idea of arterial sickling was not new, since it had been well known for some time that the blood of sickle-cell anemia patients is desaturated as a consequence of the right shift of the oxygen equilibrium and chronic lung damage (8), and, hence, it was clear that some dense cells do polymerize in the arterial side. Of course, this challenge to Eaton did not mean that the variation in delay time of polymerization that he proposed does not play some role in organ and marrow vaso-occlusion. However, what both of these analyses unfortunately missed was that many other pathogenic functions are involved in the generation of the phenotype of the disease, specifically determining vaso-occlusion, beyond the capacity of the sickle cell to polymerize—which explains why patients with very similar tendencies to polymerize at the level of their red cells, have very different incidence of painful crises (9).

Three examples of these pathogenic phenomena, important in the phenotypic expression of sickle-cell anemia, but beyond the polymerization of HbS, come to mind. The proposal of Hebbel *et al.* (10) that sickle cells have a higher tendency to adhere to the vascular endothelium and its validation by Kaul *et al.* (11) in an *ex vivo* microcirculatory preparation (and the added notion that the SS red cell adhesion is limited to small venules) and by Fabry *et al.* in an intact animal model (12), have opened a new avenue of understanding of vaso-occlusion. Von Willebrand factor seems to be involved in the binding of red cells to endothelium (13, 14). Moreover, Kaul *et al.* (14) have proposed that the

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sickle vaso-occlusion is the consequence of a first stage in which young SS red cells adhere to the venular vascular endothelium, followed by the trapping of denser SS cells.

Another area of recent development has been our increased understanding of the volume regulation in young erythrocytes, which the blood of SS patients have in abundance. In addition to the well known deoxy-dependent K^+ efflux, which is specific to SS cells, Brugnara *et al.* (15) described a K^+ efflux triggered by red cell swelling, and Canessa *et al.* (16) defined this K^+ efflux as having K:Cl cotransport properties, that is partially inhibited during deoxygenation (17). Since these transport systems are likely to determine dense cell formation (18, 19, 20), it follows that their interindividual variability will also modulate the SS phenotype.

Finally, the copresence of α-thalassemia ($-\alpha/\alpha\alpha$ or $-\alpha/-\alpha$) with SS disease (present in about 50% of adults with sickle-cell anemia in New York), decreases hemolysis, decreases the generation of dense cells, and increases the survival of the carriers (21, 22).

In conclusion, while the presence of HbS is a single gene abnormality, the phenotype of sickle-cell anemia is the product of multiple genes and should be considered a multigene disease (23).

Based on this conceptual framework of complicated and multifactorial pathogenesis, the study of sickle-cell anemia requires animal models. Specifically, transgenic animal models of different intensities and characteristics will allow for biochemical, pharmacological, and genetic intervention. The present review examines the progress so far which has centered on the generation and characterization of β^S expressing transgenic lines. Despite some shortcomings, significant progress has already occurred.

The Genotype and Phenotype of Existing Transgenic Lines Expressing the β^S and α Human Gene

Transgenic mice expressing the human β^S or variants of the sickle genes, in combination with the human α-globin genes, have been developed and characterized with different degrees of thoroughness by several groups. The constructs utilized in the generation of these transgenic lines are illustrated in Figure 1. The use of the locus control region (LCR), associated with globin genes, has achieved site independence and high-level expression of the α- and/or β-globin gene being integrated into the mouse genome. In all cases in which this was tested, the construct/s integrated in the same site in a concatenation of several copies.

Greaves *et al.* (24) obtained five transgenic mice, including one that expressed high levels of human α- and β^S-chains, and contained eight to 10 copies. The

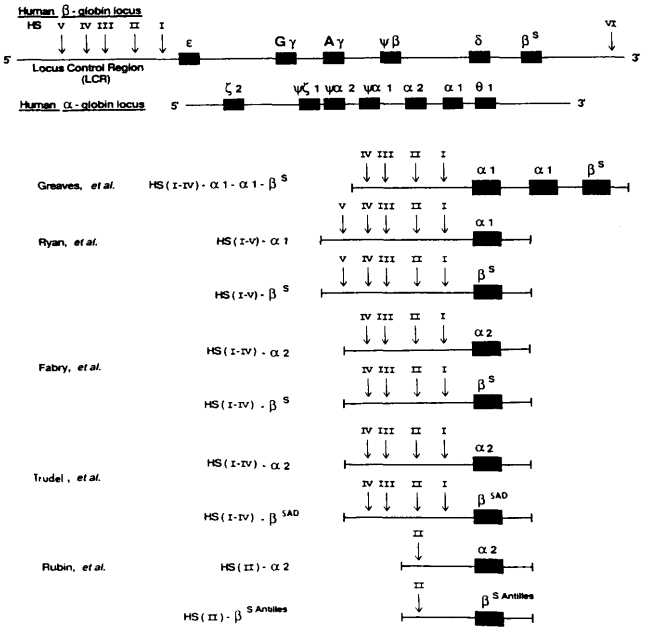

Figure 1. Constructs used to create the five transgenic mouse lines. In all cases, with the exception of Greaves *et al.*, the α- and β-globin constructs were made separately and were coinjected and cointegrated into the mouse genome. The SAD line was generated by recombinant construction of a β-chains containing the mutations found in the sickle mutation, the second mutation of the $S^{Antilles}$ and the mutation found in Hb D Punjab. Modified from Reference 53 by M. E. Fabry.

mouse with the highest expression was not anemic, but the red cells sickled in a conventional sickle test. Unfortunately, it was not propagated.

Ryan *et al.* (25) generated a transgenic line expressing 40% human HbS (based on IEF) on a heterozygous β^{Major} deletional background. They observed a mildly elevated reticulocyte count and sickling of most cells *in vitro*. When introduced into a homozygous β^{Major} deletional background, the percent of β^S increased to 77% of all β-chains, the mice had no anemia, and the MCHC was elevated, had increased levels of reticulocytes, and had a 4-fold increase of membrane-associated denatured hemoglobin, regardless of the level of β^S expression (26). Exposure of transgenic mice to a low oxygen environment (7% O_2 for 14 days) resulted in increased hematocrit and a further increase in red-cell density.

Rubin *et al.* (27) have described a transgenic line which expresses the low O_2 affinity hemoglobin S double-site mutant, $Hb^{S\text{-}Antilles}$ (β6 glu → val; β23 val → ile). This line was also bred into a homozygous background for mouse β^{Major} deletion and expressed about 51% of their β-globin as $\beta^{S\text{-}Antilles}$; but 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 tetramers containing mouse α-globin, which is known to be inhibitory to the polymerization of HbS (39). The mice had slightly reduced hematocrits, but as hemoglobin levels were not determined, the animals were not necessarily anemic. Hy-

poxic conditions (10 days at 8.4% O_2) generated an increase in dense cells and increased numbers of irreversibly sickle cells (ISCs).

Trudel, working in Costantini's laboratory, reported the development of a mouse strain bearing α^H and β^{SAD} ($\beta^{S\text{-Antilles-D Punjab}}$; β6glu → val; β23 val → ile; β121Glu → Gln) genes that, when bred into a heterozygous β^{Major}-deletional background, express 26% hemoglobin SAD (28). Hemoglobin SAD, a recombinant product, has a low oxygen affinity and an enhanced polymerization potential compared with HbS. The characterization of this transgenic line, completed mostly in Beuzard's laboratory, demonstrated that completely deoxygenated hemolysate from the SAD founder, which contains 20% β^{SAD}, polymerized in 8 min; hemolysate from β^{SAD} mouse heterozygous for the β^{Major} deletion that expresses 26% β^{SAD}, polymerized in 25 sec (a control solution of 19% HbS and 81% mouse hemoglobin polymerized in more than 15 hr). The later transgenic mice had increased fetal and neonatal wastage, and attempts to breed to homozygosity for the β^{Major} deletion were unsuccessful. When SAD mice were mated to nontransgenic mice, only 32% (instead of the expected 50%) of the progeny expressed HbSAD, and the neonatal mice had 32% lower hematocrits than their litter mates. All SAD mice had enlarged spleens. Beuzard, in the Cyprus meeting on hemoglobinopathies in 1993, reported that the SAD mice exhibited priapism. Interestingly, adult SAD mice were not anemic, but the SAD mice also heterozygous for the β^{Major} deletion were extremely susceptible to hypoxia, and nine out of 10 animals died after 90 min of exposure to 8% oxygen.

The most thoroughly studied transgenic line expressing the sickle hemoglobin has been the one generated by F. Costantini and characterized by Fabry *et al.* (29–32). The founders progeny, while displaying a moderately high expression of human β^S-globin and human α-globin (α^H) (30%), had red cells that did not sickle on deoxygenation. To increase expression of human globins and reduce the level of mouse globins, they were bred into a background homozygous for a β-globin deletion (β^{MDD}, MD = mouse deletion; $\alpha^H\beta^S[\beta^{MDD}]$ mice). The percent of β^S expressed was 73% (SD 2.4) and 45% for α^H (SD of 2.4%). Isoelectric focusing revealed 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. Experiments under deoxy conditions are in progress to render the full picture: the extent of formation of both symmetrical (as in $\alpha_2{}^M\beta_2{}^S$) or asymmetrical (as in $\alpha^M\alpha^M\beta^M\beta^S$) tetramers in these red cells.

The phenotype exhibits no anemia, normal MCH, elevated MCHC, a moderate but statistically significant reticulocytosis, and a small number of early ISCs. These findings, coupled with increased spleen red pulp and iron deposits, suggest a compensated hemolysis. Density gradient separation revealed a narrow range of densities, but a fraction of cells with elevated MCHC. Mice without the transgene, but homozygous for the β^{Major} deletion, have a dramatically different density distribution which is characteristic of β thalassemia. The normal density distribution and normal MCH found in transgenic mice establishes that the expression of the transgenes ($\beta^S + \alpha^H$) cures the mouse thalassemia (β^{Major} deletion). In both lines of transgenic mice, the oxygen affinity, in conditions in which polymerization was retarded, was elevated in proportion to the percent of β^S present.

Slow deoxygenation of the transgenic red cells resulted in 95% of the cells sickling, which demonstrates that essentially all cells contain sickle hemoglobin (Fig. 2). Electron microscopy detects intraerythrocytic HbS-type polymer in the form of fibers. The rate of sickling, under fast deoxygenation, was variable from animal to animal and was related to the percent β^S and α^H found in their red cells. Between 25% and 75% of all cells sickled in less than 5 min, slower than the rate for human red cells. In collaboration with Hofrichter and Christoph (32), we found that the delay time of polymerization was sensitive to both pH and osmolarity of the suspending media: many cells sickled in 300 msec at pH 7.1. In density-defined human sickle red cells (MCHC 32–34 g/dl), the most prevalent delay time was 300 msec; therefore, at least some of the cells of the transgenic mice had delay times comparable to those of human cells. In contrast, the C_{Sat} of the hemolysate from the transgenic mice was closer to the hemolysates of sickle trait individuals (AS) than SS patients (30). This finding suggests a low probability of pathological findings. The basis for the apparent discrepancy will be discussed below.

Several indications of organ damage occurred in

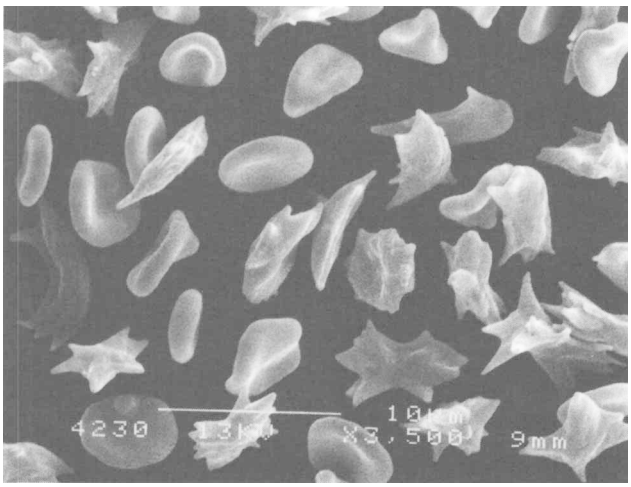

Figure 2. Mouse sickled cells generated by slow deoxygenation from $\alpha^H\beta^S[\beta^{MDD}]$ blood.

the transgenic mice described by Fabry *et al.* (31) under *ambient conditions:* (i) the lung, spleen, and kidney were all heavier than the controls (C57BL/6J strain) (31); (ii) the spleen showed expanded red pulp (suggestive of elevated erythropoiesis), the presence of iron (suggestive of red cell destruction), and scarring (suggestive of infarct); (iii) in collaboration with N. Bank and H. Aynedjian, renal function was examined in these animals (31), and the glomerular filtration rate or GFR was found to be elevated (13.5 ± 0.3 ml/min-kg vs controls 9.7 ± 0.4 ml/min-kg; $P < 0.01$) (elevated GFR is seen in patients with sickle cell disease); (iv) in collaboration with G. Lutty and D. S. McLeod, the retinas of transgenic animals were examined (33). Retinal neovascularization and choroidal neovascularization were first detected in mice at a year old and was found in most, but not all, animals by 18 months; (v) magnetic resonance imaging revealed enlarged kidneys with elevated water content in some of the animals; (vi) thickened alveolar septa were noted in the lungs of older transgenic animals. In brief, under ambient conditions these transgenic mice had red cell properties and a degree of organ pathology intermediate between AS individuals and SS patients (analogous to S/β^+ thalassemia).

Moreover, exposure to *hypoxia* (10% oxygen, 0.5% CO_2 for 5–7 days) resulted in a significantly decreased hematocrit, increased abnormally shaped and ISC formation, and increased red cell density (31). During hypoxia, a renal concentrating defect, which is common in sickle-cell anemia, became apparent. Transgenic mice, but not control mice, lost about one-third of their renal concentrating ability, as determined after overnight deprivation of water. The renal papilla of the hypoxic animals sacrificed showed distended capillaries packed with cells, suggesting that blockage had occurred involving a greater number of capillaries than that in transgenic animals not made hypoxic. In addition, in some of the animals under hypoxic conditions, priapism was observed. In sixteen hypoxia experiments involving transgenic animals, four animals died between Days 4 and 7.

Mice have also been bred into a heterozygous background for an α-globin deletion (α^{MD}), referred to as $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$, expressing 65% of β^S (SD 8.5%), while the percent of α^H was 53 (SD 6.2%). In other words, the β^S expression decreased, and the α^H increased, compared with $\alpha^H\beta^S[\beta^{MDD}]$. Nevertheless, the phenotype is quite similar, except that the mice with α deletion have higher reticulocyte counts and a more prominent increase in the red pulp in the spleen. Since these mice have not been examined extensively, other differences might yet emerge.

Finally, in an attempt to increase the severity of the phenotype, we have recently bred the $\beta^{S\text{-Antilles}}$ line of Rubin *et al.* (34) with our $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mice (30), creating a second generation of transgenic mice. Offsprings were obtained which express both transgenes and are either heterozygous (β^{MD}) or homozygous (β^{MDD}) for deletion of mouse β^{Major}. Expression of human α was 50% ± 10% of all α-globin chains for all animals, while the expression of β^S and $\beta^{S\text{-Antilles}}$ was 38% and 27% of all β-globin chains for β^{MD} mice, and 43% and 35% for β^{MDD} mice. Interestingly, during breeding it was apparent that the $\alpha^H\beta^S\beta^{S\text{-Antilles}}[\beta^{DD}]$ mice were under-represented, suggesting increased fetal death. The hematocrit, hemoglobin, and mean corpuscular hemoglobin were normal for all mice, but reticulocytes were elevated for the doubly transgenic mice to 7.9% ± 2.0% versus 4.2% ± 0.9% for $\alpha^H\beta^S[\beta^{MDD}]$ mice and 2.7% ± 0.4% for control mice. Strikingly, urine concentrating ability was reduced 30% in all mice expressing both transgenes when compared with control mice under the same conditions, a significant difference from $\alpha^H\beta^S[\beta^{MDD}]$ mice. In addition, the sickling rate was clearly accelerated: at 2 omm O_2 after 10 min the $\beta^S/\beta^{S\text{-Antilles}}$ mice red cells were 45% sickled, while the $\alpha^H/\beta^S[\beta^{MDD}]$ mice red cells were 18% sickled. At 2 min, the difference was even more dramatic: 15.1% and 1%, respectively. There is no doubt that this new model appears to be more severe than either of the two parent lines.

Raymond Popp (35) has bred the Rubin's transgenic mice to the MHOAH mouse, which has a P_{50} of 24.5 mm Hg, which is lower than that of C57BL mice (P_{50} = 40 mm Hg), and is comparable to that of sickle hemoglobin. The rationale behind this model is based on the notion that the low oxygen affinity of normal mouse hemoglobin prevents the easy deoxygenation of HbS in mouse red cells, since the mouse hemoglobin will be preferentially deoxygenated. The mice expressing both the MHOAH hemoglobins and the $\beta^{S\text{-Antilles}}$ were found to have quite elevated reticulocytes (−15%) and misshapen and dense erythrocytes, but were not anemic. Pathology consistent with intravascular destruction of red cells was also observed. It will be interesting to see if these animals develop sickle type organ damage.

Can a "Perfect" Model for Sickle-Cell Anemia be Generated?

While the generation of the sickle transgenic mouse models outlined above represent a significant advance in the study of this disease, we have to keep in mind that there will never be a "perfect" animal model of the disease. The reason is simple: animals will contribute their own red cell characteristics, their own vascular system, and their own differences in the myriad of other gene products that contribute to the phenotype of sickle-cell anemia. To make this point clear, we will review the areas in which we know or suspect that mice might be different from humans:

1. Mouse red cells are significantly smaller with an average diameter of about 4μ (45–55 μm^3) than human red cells (average diameter 8μ, 75–90 μm^3), but the diameter of vessels in the microcirculation of these two mammals is comparable (36). Nevertheless, Kaul *et al.* (37) have demonstrated that mouse red cells can cause vaso-occlusion in the *ex vivo* rat mesoappendix and the *in vivo* cremaster preparation.

2. Mouse red cells do not have the same cation transport systems and/or they may not be expressed to the same degree. In collaboration with Canessa (38), we have found that transgenic mouse red cells (from $\alpha^H\beta^S[\beta^{MDD}]$ mice), but not those of control mice, exhibit a deoxygenated induced K^+ efflux, which is analogous to that observed by Tosteson *et al.* (39) in human sickle cells. Nevertheless, mouse red cells differ from human red cells in that they do not express K:Cl cotransport activity, which is elevated in human SS cells. This condition could affect the generation of dense and irreversibly sickled cells, as we will discuss below.

3. Mouse α-chains inhibit this polymerization of HbS as shown by Rhoda *et al.* (40). The tetramer formed from the heterodimer $\alpha^M\beta^S$, and found in most models, did not polymerize under the conditions used by the author. Although it is clear that the pure tetramer $(\alpha^M\beta^S)_2$ polymerizes very slowly, Roy *et al.* (41) have recently shown that it is capable of polymer formation, and studies of how effectively it can be incorporated into an existing polymer need to be done.

4. The oxygen equilibrium curve of adult mouse red cells is considerably to the right of that of normal human red cells and close to that of human SS red cells (42), due to the high level of 2,3-Diphosphoglycerate (DPG) in mouse red cells (43). The mouse hemoglobin response to DPG is close to that of human hemoglobin (43). As stated before, this means that the HbS in the mouse does not have an advantage of deoxygenating selectively. Hence, this was the rationale of Popp to create a sickle mice model with a mouse carrying a high ligand affinity hemoglobin.

5. There is no exact equivalent to HbF in the mouse. In the "diffuse" mouse Hb β-minor responds to thalassemia or anemia by increasing its expression. The details of the interaction of β-minor with HbS are unknown. The potential absence of a fetal-like hemoglobin (capable of the inhibition of the HbS polymerization) will leave the fetal and neonatal periods of the β^S mouse without the protection accorded by HbF to their human counterparts. Some investigators have reported that the β-minor mouse hemoglobin is down-regulated during the neonatal period (44). The absence of the protection of HbF could be the reason for the severe fetal wastage observed by Trudel *et al.* (28) for SAD mouse.

6. Reticulocyte counts are higher in mice (average about 3%, range 1%–6%) (36), than in humans, and the life span of the red cells is shorter (40–43 days) (45). In addition, there are circulating stress reticulocytes in normal mice (unlike normal humans). This feature could increase the severity of the model because K:Cl cotransport is expressed primarily in young red cells. Unfortunately, the mice red cells do not express K:Cl cotransport at all (see above). On the other hand, young red cells are also more prone to adhering to the vascular endothelium (see above). Of course, mouse red cells may not express the same cell surface receptors to "sticky" proteins; hence, adhesion of sickle cells to endothelium might be altered.

7. During the neonatal period, the average size of mouse red cells are a little higher than human neonatal red cells (110 μm^3) and the size decreases to 50 μm^3 by 14 days (36). DPG is low during the neonatal period and increases to higher than human levels in the third week. High DPG favors sickling and low DPG inhibits sickling (46). Judging from the neonatal mortality of SAD mice, this phenomena does not compensate for the pro-obstruction features of prenatal and neonatal mouse blood, the ability of HbS to deoxygenate preferentially, and the large size of the red cells, if indeed the SAD mice are dying because of increased vaso-occlusion.

8. The microscopic, and sometimes the gross, anatomy of organs is different between mice and humans. We have already detected that the vasa recta anatomical arrangement is different in mice (bundles of partially fused vasa recta) compared with the individual vasa recta characteristic of the human kidney medulla (47). This could modify the sensitivity to the concentration defect.

9. In the retina, Lutty (48) has noted that the capillaries of the choroid are smaller and presumably easier to obstruct than the same vessels in the human choroid, which could explain the higher incidence of choroidal neovascularization in the mouse.

10. Plasma of mice has an osmolarity around 330 mOsm, which is higher than that of human plasma (290–300 mOsm) (32) and should be considered a pro-sickling condition (5).

Apparent Shortcomings of the Transgenic Mouse Models Expressing HbS in Reproducing the Human Disease

Lack of Anemia. The most striking, and for some discouraging, result is the lack of anemia of the order seen in the human disease in any of the transgenic mice lines. In the neonatal period for the SAD mouse, there is some anemia, but it is only mild. Most models, nevertheless, have evidence of compensated hemolytic anemia (high reticulocyte count, increase in stress reticulocytes, and increased red pulp in the spleen, which in the mouse is a normal source of erythropoi-

esis). The lack of anemia has been blamed on the inhibitory effect of the α mouse chains and the reduction in the propensity to sickle of many transgenic lines compared with humans. Nevertheless, in the SAD model in the neonatal period when the sickling is close to the human rate, no anemia comparable to humans is found.

Another interesting feature of these models, and related to the anemia, is the absence of significant levels of very dense cells [SS4 type (22)] and/or ISCs. When found, they are in low number (<3%) and they appear as early ISC, i.e., in the first stages of dehydration. According to Serjeant *et al.* (50), very SS dense cells (ISCs) determine the level of anemia, since they correspond to a fraction of red cells predetermined to become dense and endowed with a very short life span (49). Our group (19) and others (18, 20) consider that the K:Cl cotransport mechanism, expressed in young SS red cells, is indispensable for the generation of very dense cells, as those seen in the bottom of the gradient and in ISC cells. The lack of anemia might be due to the absence of this transporter in the mouse red cell as demonstrated by Romero *et al.* (38). When this transporter is cloned in the future, a transgenic line could be generated with the corresponding construct and bred into the existing transgenic sickle lines to resolve this issue. Whatever the result, this represents an instance in which a shortcoming of the mouse model can be turned into an advantage: defining the role of K:Cl in the generation of hyperdense cells and anemia in sickle-cell anemia.

Of course, most laboratories working in this area are attempting to create mouse lines devoided of their α and β mouse genes, by "knock out" techniques based on homologous crossing over.

Slower Sickling. The present models can be made to increase their rate of sickling by developing second generation transgenic animals. The cross between the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse and the $\beta^{S\text{-Antilles}}$ mouse, in which there are already encouraging results, and the cross between the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse and the SAD mouse, seem attractive possibilities. It should be remembered, nevertheless, that too severe a phenotype (in the absence of HbF protection) will render transgenic lines with overwhelming fetal wastage and few animals to study. The ones who survive might be genetically biased.

On the other hand, by defining the pathology in transgenic mice with comparatively lower sickling rates, we can define the most sensitive systems in the economy to sickle obstruction. In addition, by having models that only develop pathology under hypoxic conditions, we can time and manipulate the pathogenic event more easily.

Increase of the Choroidal Neovascularization in the Retina. That the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse has the tendency with age to present significant choroidal neovascularization (most likely the product of obstruction) and consequent death of the neuroretina that have not been described in sickle-cell anemia, was at first intriguing. The fact that the vessels in mouse choroid are smaller (48) might be the underlying reason, but what is interesting is that recent examination of human retina with the new techniques developed by Lutty and associates (51) has revealed the presence of choroid neovascularization to a greater extent than previously known (after all, the choroid is not apparent in a fluorescein retinogram). This sequence of events we can call reverse pathology.

Early Death of Severe Phenotypes. While this is a setback, particularly, for the SAD mice line, genetic and pharmacological experiments trying to rescue these animals will be very informative.

The potential shortcomings which transgenic mice may unavoidably exhibit should not discourage investigators, since they may be turned to our advantage. Nevertheless, investigators planning to develop transgenic models should make themselves familiar with the differences between mice and humans as these distinctions pertain to the system of interest. In the development of a sickle mouse model, two shortcoming could be turned into advantages. One was mentioned above: the lack of K:Cl cotransport in the mouse. Another is that, if β^{S} mouse cells do not adhere to the vascular endothelium (because they lack the appropriate receptors), this contribution to vaso-occlusion can be dissected from the other determinants (such as increased rigidity) of SS red cells.

Specific Contribution So Far of the Transgenic Mouse Model to the Understanding of Sickle Cell Anemia

In brief, three important findings have occurred in the study of the transgenic lines reviewed here that would have been difficult to obtain in the absence of this animal model: (i) The GFR increase has been classically interpreted to be the consequence of high output cardiac regime imposed by the perversive anemia characteristic of the disease. In the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse, GFR is elevated in the absence of anemia. Now we have to find out what mechanism underlies this phenomena; (ii) choroidal abnormalities were not considered an important part of the retina pathology in sickle cell anemia. Stimulated by the transgenic mouse findings, Lutty (48) has been able to document choroidal neoformation of vessels; and (iii) simple C_{Sat} determinations have done a bad job of predicting organ damage and other abnormalities in the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse, in spite of the supposed close correlation of these parameters (7). The reason is not mysterious and can be derived from first principles (5) in combination with sound physiological insight. The mouse red cells,

as humans, encountered a variety of conditions in the body that favor sickling, such as low pH and hyperosmolarity. These conditions can be encountered normally (as in the kidney) or any place at times of circulatory stasis. So even red cells with high C_{Sat} can sickle (a generate vaso-occlusion) in certain circumstances. In addition, the mouse has a normal plasma osmolarity of 330 mOsm, so, since the red cells will have an increased MCHC as a consequence, the raw C_{Sat} has to be corrected.

Conclusions and Future Work

The transgenic mice lines already developed have a significant potential to increase our understanding of the pathogenesis of kidney, lung, and retina damage in sickle-cell anemia. Second generation transgenic mice, interbreeding of existing lines, promise to improve this score even more. The capacity of eliciting sickle complications by hypoxic treatment renders these animals particularly useful in the study of the renal concentration defect and priapism.

While the shortcomings of the transgenic models (i.e., when mice do not behave as humans) have discouraged those who naïvely expected a "perfect" model, they also have the potential of furthering our comprehension of the disease, by allowing replacement experiments and by exposing the relative importance of pathogenic determinants.

Even the less severe phenotypes among the transgenic mouse model will demonstrate their usefulness. A panoply of different levels of severity will assist in the study of specific pathogenic mechanisms, since solid predictions can be in a graded field of subjects.

Finally, the transgenic models are in the early stage of their use as test sites for pharmacological intervention (52), and this is an area likely to expand rapidly and be fruitful.

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