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Will Developments in Allogeneic Transplantation Influence Treatment of Adult Patients with Sickle Cell Disease?

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ABSTRACT

With improvements in the treatment of children with sickle cell disease (SCD), there has been a significant increase in the number of patients with SCD in adult hematology practice. Quality of life and life expectancy continue to be severely compromised in adult patients; hydroxyurea is the only treatment currently available that could reduce the severity and frequency of painful episodes. Allogeneic stem cell transplantation (SCT) has been offered to children with SCD as a curative option. We discuss the implications of new developments in the field of allogeneic SCT in the treatment of adult SCD patients in light of the experience derived from pediatric transplantation. These developments include innovations in the conditioning regimens, GVHD prophylaxis, and alternative donor SCT and their possible effect on adult SCD patients. Finally, we discuss a nonmyeloablative conditioning protocol for adult SCD patients and the eligibility criteria for adult SCD patients undergoing allogeneic transplantation.

KEY WORDS

Sickle cell disease • Alemtuzumab • Nonmyeloablative transplantation • Haploidentical • Mixed chimerism

SICKLE CELL DISEASE IN ADULTS

The last few decades have witnessed a significant improvement in the management of sickle cell disease (SCD) in early childhood; 85% of the SCD patients survived beyond 20 years of age in the Cooperative Study of Sickle Cell Disease (CSSCD) cohort of North America [1]. Improved survival of children with SCD has resulted in a substantial increase in the number of patients with SCD in adult hematology practice. However, in a recent prospective survey of the patients in CSSCD who survived beyond 20 years, the median age at death was 42 years for men and 48 years for women, implying a 25- to 30-year reduction in life expectancy compared with the general black population [2]. More importantly, most deaths were related to SCD. In contrast to the younger cohort in CSSCD, in which the mortality peaked in the first few years of life and was mostly due to infections, the causes of death in the adult population were less predictable [2,3]. Overt organ failure was the cause of mortality in 18%, and another 33% died of acute painful crises, mostly acute chest syndrome [2]. The mortality tended to increase sharply every decade after 20 years.

These findings have serious implications in the treatment of adult SCD patients. The clinical course often worsens in adulthood, even if symptoms were mild and organ functions were optimum in childhood. Chronic organ damage is often not evident before the third or fourth decades of life. Acute crises are less well tolerated in the face of ongoing insidious vascular damage to vital organs. Treatment of adult SCD patients is further complicated by lack of compliance with long-term drugs such as penicillin and hydroxyurea, overuse of narcotics and dependence, loss of productivity, and psychological symptoms in adulthood.

Table 1. *Results of Conventional Allogeneic Transplantation for Sickle Cell Disease in Children*

Variable	Belgian [7] (n = 50)	French [8] (n = 60)	United States [6] (n = 59)
Median age, y (range)	8 (1.7-23)	8.8 (2.2-22)	10.1 (3.3-15.9)
Graft rejection/disease recurrence	10%	6.6%	9%
Acute GVHD grade I-II	38%	NA	4%
Acute GVHD grade III-IV	2%	6.6%	3.8%
Chronic GVHD	20%	NA	4%
Stable mixed chimerism	12%	NA	22%
Overall survival	93%	90%	93%
Event-free survival	82%	82%	84%

NA indicates not applicable.

Hydroxyurea has been effective in reducing painful crises in adult SCD patients and thereby reducing hospitalization and mortality [4]. However, its potential for reducing chronic organ damage is unclear. Moreover, protracted treatment with hydroxyurea is associated with poor compliance. There has been little progress in the treatment of these patients that could stop or reverse the underlying process and result in improvement of survival and quality of life. Currently, the only curative option for patients with SCD is allogeneic stem cell transplantation (SCT). In the following sections, we discuss the evolution of transplantation in children with SCD and how we could explore the new developments in allogeneic transplantation to the benefit of adult SCD patients in light of the pediatric experience.

ALLOGENEIC SCT FOR SCD

The first report on cure of SCD was in a patient who underwent bone marrow transplantation (BMT) for acute myeloid leukemia [5]. Since then, more than 160 patients have undergone transplantation for SCD, but this has been performed almost exclusively in patients younger than 16 years old who have severe manifestations of SCD. Table 1 summarizes the results of allogeneic transplantation reported by 3 major groups [6-8]. Even though the more severely affected patients underwent allogeneic transplantation, the overall survival was 90% to 94%. The disease-free survival varied between 82% and 84%. Secondary graft rejection and disease relapse occurred in 10% to 15% of patients. However, with additional immunosuppression, the problem of graft rejection was minimized. Graft-versus-host disease (GVHD), both acute and chronic, although observed in a much smaller proportion of patients than in comparable age groups who underwent transplantation for malignant diseases, has been the most significant complication and cause of death after allogeneic transplantation.

A unique posttransplantation problem encountered in SCD patients has been an increased incidence of neurologic complications in the early posttransplantation period, particularly in patients with prior stroke [9]. The most common neurological complications were intracranial hemorrhage and seizures. However, with prolonged anticonvulsant prophylaxis, strict control of hypertension, prompt magnesium supplementation, maintenance of higher hemoglobin and platelet count, and avoidance of cyclosporine toxicity, these complications have been substantially reduced [6].

All patients with stable engraftment had a complete resolution of sickle-related symptoms (anemia, painful crises, acute chest syndrome, and stroke). Cerebral magnetic resonance imaging showed stable and even improved results [6]. Pulmonary functions were also stable or improved [6]. Growth and splenic function had also improved in those not receiving heavy immunosuppression for GVHD [6,7]. The major concern has been the effect of conditioning regimens on gonadal functions. The follow-up has not been long enough in most studies to evaluate the late effects, such as secondary malignancies, except for the Belgian study, which reported 1 case of acute myelogenous leukemia 4 years after transplantation [7].

However, despite the success of allogeneic BMT in the phenotypic cure of SCD in children, the procedure has not been advocated for adult SCD patients. In the following sections, we raise the possibility and investigate the prospects of using allogeneic SCT in adults in the light of several observations made in the pediatric population and the recent developments in the field of allogeneic transplantation.

FEASIBILITY OF ALLOGENEIC TRANSPLANTATION IN ADULT SCD PATIENTS

A previous study indicated that approximately 18% of patients with SCD would have an unaffected HLA-identical sibling, but taking into account the eligibility criteria and parental consent, only 1% to 2% of the total population of SCD patients would undergo an allogeneic transplantation as a child [10]. Thus, there would be a proportion of adult SCD patients with potential family donors who did not undergo transplantation as children because of either parental refusal or a failure to meet the eligibility criteria [10,11]. The question remains as to whether these patients should be offered an allogeneic transplantation and, if so, what the eligibility criteria and the modality of transplantation should be.

A few patients older than 16 years (up to 23 years) have undergone allogeneic BMT in France and Bel-

gium, and the effect of age was not discernible because of extremely small numbers [7,8]. However, the adult clinical trial of the Seattle consortium was halted because of toxic death in the first 2 patients [12]. Adult patients with SCD are more likely to have chronic vascular damage to vital organs such as kidneys, lungs, brain, and heart and would thus be less likely to tolerate full-intensity conditioning. Concerns about the feasibility of conventional conditioning in adult SCD patients raises issues about the timing of transplantation and the modality.

THE RATIONALE FOR NONMYELOABLATIVE CONDITIONING

Animal studies have demonstrated the feasibility of achieving durable engraftment with a reduction in the intensity of the conditioning if adequate immunosuppression is used [13]. Subsequent clinical trials in which nonmyeloablative conditioning was able to induce stable engraftment in patients with advanced malignancies otherwise deemed unsuitable for full-intensity conditioning have substantiated the concept [13-16].

Adult patients with SCD are less likely to withstand full-intensity conditioning because of the underlying incipient organ damage inflicted by the vascular damage and the inflammatory state. If the transplant-related mortality in children is considered to be 5% to 10%, in adults, after conventional conditioning with busulphan and cyclophosphamide, transplant-related mortality is likely to increase severalfold. The other considerations are rejection and GVHD. Because of multiple transfusions, these patients are more likely to experience graft rejection, which was exemplified by rejection in 4 of 12 patients in the French study who were conditioned without antithymocyte globulin (ATG) [8]. Patients with SCD have an underlying inflammatory state, marked by the high circulating levels of tumor necrosis factor-α and endothelin-1 [17], but whether that aggravates the host-versus-graft reaction is not known. The high circulating levels of tumor necrosis factor-α are likely to upregulate major and minor histocompatibility antigens, with an enhanced recognition of these antigens by alloreactive T cells, and thus increase the likelihood of severe GVHD [18]. Moreover, GVHD is an age-dependent process and is likely to be a greater problem in adult SCD patients. Previous reports have suggested a higher incidence of GVHD in the African-Caribbean population, which could be due to a greater degree of minor HLA antigen mismatch or a difference in the pharmacokinetics of anti-GVHD drugs [19,20].

MIXED CHIMERISM AND SCD

Reduced-intensity conditioning was originally proposed as a means of achieving mixed chimerism, which would allow stable engraftment and reduce the incidence of GVHD [13,15]. Mixed chimerism was documented in several patients with SCD after conventional transplantation. In the multicenter study from the United States, stable mixed chimerism was documented in 13 of 50 SCD patients with stable engraftment [21]. The level of donor chimerism varied between 90% and 99% in 8 patients but was much lower in 5 patients. The donor chimerism varied between 11% and 74% in these 5 patients, and even with 11% donor chimerism, the hemoglobin was stable and the sickle cell hemoglobin (HbS) was only 7%. In the Belgian study, 3 of 50 patients had <10% host chimerism, and another 3 patients had 30%, 35%, and 50% host chimerism, all with normal hematology [7]. The conditioning regimens used in these studies were not designed to produce mixed chimerism, but mixed chimerism could have resulted from either a robust recipient immune system or in vivo T-cell depletion of the graft by the circulating ATG that was used in conditioning. Whatever the mechanism might be, these observations raise the possibility of phenotypic cure of SCD by the induction of stable mixed hematopoietic chimerism.

However, these studies did not investigate lineage-specific chimerism, and 2 recent murine studies have facilitated our understanding of the chimerism of various lineages and cure of SCD. In lethally irradiated healthy mice, Iannone et al. [22] studied the effect of reconstitution with varying ratios of T cell–depleted marrow from normal and transgenic sickle cell mice. In their model, 25% normal myeloid chimerism resulted in >90% normal hemoglobin in blood. Reducing the HbS levels to <80% resulted in progressive normalization in both hematologic and histologic parameters in a linear fashion until HbS reached 0%. Although 40% myeloid chimerism resulted in elimination of sickle red blood cells from circulation, 70% chimerism was required to eliminate anemia. Liver infarcts were observed with HbS of 16.8% even though complete hematologic correction was achieved. These findings suggest that mixed red blood cell chimerism corrects hematologic indices but not organ pathology. However, the major caveat in interpreting these results is that the recipient mice lacked the sickle genotype and could not be representative of the effects of mixed chimerism in an SCD recipient.

Kean et al. [12] studied the effect of major histocompatibility complex–mismatched T cell–depleted BMT in transgenic knockout sickle mice from BALB/c and SJL mice with low-dose busulphan and co-stimulation blockade of CD28/B7 and CD40/CD40L pathways. Ten of 13 mice achieved complete red blood cell chimerism and mixed white blood cell chimerism (43%) without developing GVHD; this resulted in normalization of red cell morphology and hematologic parameters and also of renal and spleen

Table 2. *Nonmyeloablative Transplantation for Sickle Cell Disease*

Variable	Iannone et al. [23] (n = 6)	van Besien et al. [24] (n = 2)	van Besien et al. [25] (n = 2)	Krishnamurthi et al. [26] (n = 1)	Schleuning et al. [27] (n = 1)
Conditioning	Flu/200 cGy TBI/ATG (n = 2)	Flu/Mel/ATG	Flu/Mel/alemtuzumab	Flu/Bu/ATG/TLI	Flu/Cyclo
Age, y	3 to 20	40 to 56	19 and 24	8	22
Primary engraftment	5	2	2	1	1
Graft rejection/disease recurrence	All	None*	1	No*	No*
Acute GVHD	Grade II (n = 1)	Both	None	No	No
Chronic GVHD	None	1	None	No	Yes
Stable mixed chimerism	Transient	NA	1	Yes	Yes
Death/cause	None	GVHD (both)	None	No	No

Flu indicates fludarabine; TBI, total body irradiation; TLI, total lymphoid irradiation; Mel, melphalan; Bu, busulphan; Cyclo, cyclophosphamide; ATG, antithymocyte globulin; NA, not applicable; GVHD, graft-versus-host disease.
*Short follow-up or early death.

pathology. The higher level of donor red cells compared with the white cells suggested a survival advantage of normal over sickle red blood cells. The survival advantage of normal red cells in SCD patients was further substantiated by the documentation of 10% to 54% red blood cell chimerism and <2.5% white cell chimerism in sickle mice conditioned without busulphan. However, the murine models are unlikely to be fully representative of the human SCD setting, particularly when the mice were not presensitized by previous transfusions, as would be the case with most SCD patients eligible for an allograft. There is little doubt that engraftment across HLA barriers in multiply-transfused adult SCD patients is likely to be more challenging.

The lesson we could derive from these studies is that red blood cell engraftment is essential for cure of SCD, which could be achieved with mixed lymphoid or myeloid chimerism. The issue is whether we could reliably induce mixed chimerism in a clinical setting with nonmyeloablative conditioning.

RESULTS OF NONMYELOABLATIVE TRANSPLANTATION IN SCD

Several anecdotal reports of nonmyeloablative transplantation for SCD have been published in the last couple of years that have included a few adult patients. Table 2 summarizes these results. There have been 3 main approaches to nonmyeloablative transplantation. The Seattle group used low-dose total body irradiation (TBI) and a cyclosporine/mycophenolate-based approach in patients with advanced hematologic malignancies [13,14]; this resulted in successful engraftment in heavily pretreated patients, but not in others, such as those with chronic myeloid leukemia. Contrary to the animal model, a reduced-intensity regimen failed to induce stable mixed chimerism [14]; however, it is worth noting that the animals had a more robust immune system because of the lack of any prior immunosuppression. This protocol has also been explored in SCD patients. The addition of fludarabine, with or without ATG, to a low-dose TBI–based regimen failed to induce sustained engraftment in 6 patients with SCD [23] (Table 2). Tapering of posttransplantation immunosuppression was associated with a loss of donor graft in most patients even 12 months after transplantation. However, the regimen was associated with no toxicity, and the rejections were nonfatal with autologous reconstitution after weaning of immunosuppression. The interesting observations in this study were maintenance of improved hematologic parameters and an HbS level <30%, despite the decline in donor chimerism [23,28]. In vitro studies on the posttransplantation marrow samples demonstrated an overrepresentation of donor erythroid progenitor cells over the myeloid counterpart, suggesting that the clinical benefit from mixed chimerism is derived both from an extended life span of mature donor red cells and a selective advantage of donor red cell progenitors in the marrow [28].

The other approach has been the combination of fludarabine and an alkylating agent with or without ATG or alemtuzumab (Campath-1H; Schering Health Care Ltd, West Sussex, UK) [15,16,29,30]. Similar to the experience with malignant diseases, most patients with SCD achieved sustained engraftment after such conditioning regimens (Table 2). Use of melphalan and ATG along with fludarabine resulted in sustained engraftment and cure in 2 adult SCD patients treated with this regimen, but both died of GVHD [24]. The same group achieved successful engraftment without GVHD by replacing ATG with alemtuzumab in a 24-year-old man [25]. The combination of fludarabine, busulphan, ATG, and total lymphoid irradiation (500 cGy) was also successful in achieving progressively improving donor chimerism without GVHD in an 8-year-old girl at 14 months of follow-up [26]. A German group reported successful engraftment with limited chronic GVHD in a 22-

year-old patient with 11 months of follow-up who had initial mixed chimerism that converted to full donor chimerism [27]. Although initial engraftment has been feasible with these protocols, the follow-up is too short to comment on sustained engraftment, given the fact that late graft rejections up to 6 to 12 months after transplantation have been documented in the low-dose TBI protocol [23].

The third approach is aimed at reducing GVHD by the use of in vitro T-cell depletion of the graft. In vitro T-cell depletion has resulted in a high incidence of secondary graft failure in patients who have undergone transplantation for malignant diseases with reduced-intensity conditioning [31,32]. However, if engraftment was sustained, stable mixed chimerism was frequently documented [32]. One patient with SCD who received in vitro T-cell depletion after reduced-intensity conditioning lost the graft by 28 days [25].

HOW CAN WE SUCCESSFULLY DEVELOP A NONMYELOABLATIVE CONDITIONING REGIMEN FOR ADULT SCD PATIENTS?

Limited experience with the 2 predominant approaches indicates that minimal conditioning based on the Seattle protocol is unlikely to result in sustained engraftment in most adult SCD patients. However, use of fludarabine and an alkylating agent with or without ATG could circumvent the problem of engraftment but cause an unacceptable degree of GVHD. Unlike with malignant diseases, an alloreactive process is undesirable in SCD. GVHD is poorly tolerated by adult SCD patients, who already have sustained serious compromise of organ functions. Thus, 2 issues need to be addressed to successfully perform transplantation in an adult SCD patient. First, the conditioning should enable sustained engraftment, and GVHD should be minimal or absent. Mixed myeloid and lymphoid chimerism at an early posttransplantation period is desirable. The murine studies and the early data from the Seattle group suggest that erythroid lineage is likely to engraft faster and more completely than the myeloid lineage [12,28]. Early erythroid engraftment, on one hand, helps in the rapid amelioration of sickle-related problems, and, on the other hand, mixed lymphoid chimerism at an early posttransplantation period is likely to prevent the development of severe GVHD. Unfortunately, most of the existent nonmyeloablative protocols are associated with unstable chimerism that either rapidly converts to full donor chimerism with a high incidence of GVHD or loses donor cells with autologous reconstitution [14-16].

In the United Kingdom, the use of a regimen consisting of alemtuzumab in vivo has resulted in a very low incidence of GVHD, with sustained engraftment in almost all patients [30]. Sustained mixed chimerism was documented in approximately 30% of the patients [33]. The major problems associated with this protocol have been a delayed immune reconstitution, which increased the susceptibility to viral infections, and a lack of early graft-versus-leukemia effect in high-risk malignancies [34]. The delayed immune reconstitution observed in that study [34] was attributed to the prolonged half-life of alemtuzumab, which not only results in depletion of T cells of the infused graft, but also hampers the early lymphocyte recovery [35]. A dose de-escalation study on alemtuzumab is currently under way to determine the right balance between immune reconstitution and prevention of GVHD. The only other protocol that has been associated with a low incidence of GVHD has been a combination of fludarabine and cyclophosphamide, and this has been a consistent finding in both clinical and experimental studies [29,36].

Ideally, T-cell depletion of the graft is the most reliable method for prevention of GVHD. In SCD, in vitro T-cell depletion will significantly increase the probability of graft rejection. How best to balance the degree of host immunosuppression and the extent of T-cell depletion in the setting of nonmyeloablative transplantations is currently unclear. The Pessaro group [37] has achieved successful engraftment in adult thalassemic patients with fludarabine and reduced doses of busulphan and cyclophosphamide, preceded by preconditioning with hydroxyurea and azathioprine. In 14 adult thalassemic patients treated with this protocol, the overall survival was 70% and event-free survival was 57%.

We propose to incorporate all 3 of these elements into our suggested conditioning protocol for adult SCD patients. The conditioning protocol would incorporate fludarabine 30 mg/m^2 for 5 days, cyclophosphamide 30 mg/kg for 2 days, and alemtuzumab 60 mg over 3 days from day -6 to day -4, preceded by hydroxyurea 1 to 2 g/d and azathioprine 50 to 100 mg daily for 6 weeks. The preferred choice would be granulocyte colony-stimulating factor–stimulated peripheral blood stem cells from a HLA-matched family donor with a target dose of 5×10^6 CD34 cells per kilogram. However, it needs to be borne in mind that any reduced-intensity protocol will have to go through phase I trials in adult SCD patients and that any experimental protocol should not be used outside the framework of a clinical trial.

Fertility is a major issue in patients with SCD. To make the nonmyeloablative protocol acceptable to adult SCD patients, the effect of the conditioning protocol on fertility should be carefully considered and studied. We expect the proposed protocol to have less of an effect on gonadal function than busulphan- or melphalan-containing protocols.

PATIENT SELECTION

If we consider allogeneic transplantation to be a curative option for SCD, it should be offered to all willing patients. However, the main problem with the approach is the fact that a significant proportion of patients would be exposed to the morbidity and mortality currently associated with the procedure. It cannot be assumed, unless proven, that a particular nonmyeloablative regimen will be associated with less toxicity than conventional transplantation. Thus, the eligibility criteria for allogeneic transplantation with reduced-intensity conditioning needs to be outlined with a degree of rigorousness essential for clinical trials. Although such guidelines exist for pediatric transplantation, it is more difficult to identify a suitable adult population that is likely to benefit from an allogeneic transplantation with low regimen-related toxicity.

It is also important to be aware of the perception, expectation, and acceptance of such procedures in the patient group. A survey was performed on 100 adult SCD patients in Chicago to assess the their perception of allogeneic transplantation, by using a standard reference gamble paradigm [38]. Twenty-eight patients were unwilling to accept any risk of short-term mortality to be cured of SCD. Sixty-three patients were willing to accept a risk of short-term mortality of >5%. Of these patients, 20 patients were ready to accept a risk >30%, and 12 patients were willing to accept a risk of ≥40%. More importantly, there was a lack of concordance between the willingness of healthcare providers to offer an allogeneic transplantation and the patients' decisions. Acceptance of a high-risk curative option might differ according to the healthcare system and the perceptions in a given community. We are currently undertaking a similar survey at our center to determine the patient-related factors and the proportion of adult patients with possible matched family donors. Our current recommendations for inclusion in the reduced-intensity allogeneic transplantation trial are detailed in Table 3.

Table 3. *Proposed Eligibility Criteria of Adult SCD Patients for Nonmyeloablative Transplantation*

Patients with matched family donors without major hemoglobinopathy; age <40 y; with one or more of the following despite treatment with hydroxyurea:
More than 3 hospital admissions with painful crises in 1 y
Chronic severe debilitating pain from SCD
More than 1 episode of acute chest syndrome in 2 y
History of stroke or neurologic event lasting >24 h
Refractory leg ulcers
Early evidence of organ damage (eg, renal, pulmonary, or cardiac)
Transfusion dependence
Without evidence of
End-stage pulmonary or renal disease
HIV infection
Other irreversible comorbid condition

HIV indicates human immunodeficiency virus.

TIMING OF TRANSPLANTATION: EARLIER RATHER THAN LATER

The clinical trials investigating the role of allogeneic SCT in SCD have included only patients with more severe disease. However, the Belgian study included 14 asymptomatic or minimally symptomatic patients in their study, with the argument that they were to return to their country of origin where the supportive care was not optimal [7]. The outcome in this group of patients was remarkable, with 100% overall survival, 93% event-free survival, and no instance of grade III or IV acute or extensive chronic GVHD. The popular concept that allogeneic transplantation is too toxic to be offered to less symptomatic patients is challenged by these findings. There is little doubt that if the preexisting organ damage and underlying inflammation are minimal, the probabilities of conditioning-related toxicity, rejection, and GVHD would be reduced. If transplantation is being considered for an adult SCD patient, these factors should be prime considerations. These patients have already been subjected to 2 decades of insidious or obvious organ damage, which is reflected in the sharp increase in mortality every decade after 20 years and which is often compounded by long-term blood transfusion and resultant iron overload [39]. Allogeneic transplantation is more likely to be successful if it is considered earlier than later, and the eligibility criteria in adult patients should be sought in a different setting than children with SCD.

ALTERNATIVE DONOR TRANSPLANTATION

One of the major barriers to the development of allogeneic transplantation in SCD is the lack of fully matched family donors in most [10,11]. The major challenge lies in the development of alternative donor transplantation if most SCD patients are to benefit from an allogeneic transplantation.

Unrelated donor (UD) bone marrow or peripheral blood transplantation has been developed as an effective alternative to family donor grafts for the treatment of hematologic malignancies, but not for nonmalignant disorders or hemoglobinopathies, in particular. Recently, the Pessaro group [40] demonstrated that with extended haplotype matching, the outcome of UD transplantation in patients with thalassemia can be significantly improved. However, the major problem in developing a UD transplant program is an underrepresentation of the ethnic groups with SCD in the national and international donor registries.

UD cord blood might be a more readily available source of stem cells for SCD patients. Initial results of related cord blood transplantation in children with SCD are extremely encouraging [41]. The Eurocord transplant group reported the outcome of 33 patients with thalassemia and 11 patients with SCD who received related cord blood transplantation (median age of all patients, 5 years; range, 1-20 years). At a median follow-up of 24 months, the overall survival in both disease groups was 100%, with 90% disease-free survival in SCD patients (1/11 SCD patients rejected the graft). The probability of developing acute and chronic GVHD in all patients was 11% and 6%, respectively. The immunologic naivety of cord blood cells could be advantageous when HLA-mismatched transplantation is considered. However, the major problem in adult transplantation is obtaining the optimum stem cell dose, given the overriding importance of cell dose in achieving engraftment [42]. It is worth noting that in the Eurocord study, a high cell dose was obtained (median, 4×10^7/kg; range, 1.2-10×10^7/kg), which might not be a consistent feature of unrelated cord units. In this study, the use of methotrexate in GVHD prophylaxis adversely affected event-free survival. A major impediment in developing unrelated cord blood transplantation for adult SCD patients is a greater likelihood of rejection for grafts with a lower cell dose and fewer alloimmune T cells due to a stronger host-versus-graft effect, as discussed previously.

The most readily available donor in any disease condition is usually an HLA haplotype-mismatched relative, generally a parent or sibling. Considering the paucity of matched related donors for patients with SCD and the poor availability of suitable UDs from registries, a mismatched family donor is the best option for an adult SCD patient in terms of availability. The recent developments in the field of haplotype-mismatched transplantation have been a landmark in the evolution of allogeneic transplantation [43]. The Perugia group [43] has demonstrated that successful engraftment could be achieved if the donor was conditioned with fludarabine, thiotepa, total body radiation, or melphalan and ATG. $CD34^+$ selected grafts containing megadoses of $CD34^+$ cells resulted in an almost complete absence of GVHD. However, the major problem has been a significant delay in immune reconstitution and resulting fatal infections. Moreover, adult SCD patients are unlikely to tolerate such intensive conditioning. The same investigators have further explored the role of natural killer cell alloreactivity in engraftment and survival [44]. In murine models, natural killer cell infusion has resulted in sustained engraftment with minimal conditioning [45]. The detrimental effects of extensive T-cell depletion might be overcome by infusion of antigen-specific T cells [46,47] or L-leucyl-L-leucine methyl ester–treated T cells, which prevents GVHD by depleting dipeptidyl peptidase I–expressing cytotoxic cellular subsets, yet preserves the response to recall antigens [48]. A recent report from a Japanese group suggested that engraftment with an acceptable degree of GVHD can be achieved without T-cell depletion if the haploidentical donor is the mother or a noninherited maternal antigen–mismatched sibling, on the basis of the concept of tolerance developed because of fetomaternal chimerism [49]. Although it is possible that the limited diversity of HLA antigens in the Japanese population might have a favorable influence on the outcome of such transplantations, this concept, if proven to be clinically viable in larger studies in other populations, may circumvent many problems associated with T cell–depleted haploidentical grafts.

Several other approaches of reducing GVHD and yet achieving engraftment and maintaining the reactivity of T cells to third-party antigens are currently being explored. Additional co-stimulatory signals are required for sustained T-cell activation, and blocking co-stimulatory signals have been evaluated as potential measures to reduce GVHD [49]. The administration of CTLA4 immunoglobulin or anti-CD28 antibodies, which block the interaction of CD28 on T cells and B7 molecules on antigen-presenting cells, and anti-CD40L (CD154) monoclonal antibody, which can interfere with the interaction of CD154 on T cells and CD40 on antigen-presenting cells, has been shown to reduce the severity of GVHD in animal models [50,51]. Guinan et al. [52] reported successful engraftment with a low incidence of GVHD after infusion of haploidentical donor bone marrow incubated with CTLA4 immunoglobulin. Thus, blockade of co-stimulation pathways provides a potential for inducing anergy and reducing GVHD without T-cell depletion, yet retaining a normal antigenic response. The use of mesenchymal cells [53] and keratinocyte growth factors [54] has been shown to reduce GVHD, promote engraftment, and reduce conditioning-related tissue damage in preclinical and phase I studies.

CONCLUSION

The major obstacles to the development of allogeneic transplantation for SCD are the paucity of matched family donors and the morbidity and mortality associated with the procedure. If engraftment and stable mixed chimerism can be induced by reduced-intensity conditioning, their combination may significantly reduce conditioning-associated toxicity and GVHD and result in cure of sickle-related symptoms. Limited experience of reduced-intensity conditioning in SCD patients has been dismal. The best way to achieve stable mixed chimerism is currently unclear, and clinical trials are ongoing. Attempts to

increase the ethnic representation in the UD registries and similar clinical trials on UD bone marrow and cord blood transplantation are warranted. Further developments in the field of haploidentical donor transplantation might offer the best chance of cure for most patients with SCD.

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