review article

stored placental blood for unrelated bone marrow reconstitution

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bone marrow transplantation (bmt) is indicated for selected genetic and hematologic diseases and for hematopoietic reconstitution in cases of iatrogenic or accidental ablation of bm.1,2 additionally, allogeneic bmt may contribute to the therapy of leukemia through graft-versus-leukemia effects (gvl), which are associated with, but possibly independent of, chronic graft-versus-host disease (gvhd).3-15 hla identical sibling donors offer the best graft compatibility, but hla-identical unrelated donors may also provide acceptable grafts.16 hence, the organization, in the united states, europe, and elsewhere, of large registries of hla-typed individuals who volunteer to donate bm for recipients lacking hla-identical sibling donors.17-20

placental blood, as an alternative source of hematopoietic stem cells for bm reconstitution, has recently been shown to yield successful sibling-donor placental blood "grafts" in children.21-24 since, furthermore, the numbers of stem/progenitor cells in placental blood25-28 are in the range associated with successfully transplanted adult bm,29-31 placental blood has the potential to overcome some of the limitations of the current system of registries for unrelated marrow donor procurement. "banks" of cryopreserved placental bloods would not depend, for example, on the recruitment and continued collaboration of large numbers of volunteer potential donors and on compensating for the unavoidable attrition caused by retired volunteers. systematic studies of the feasibility of using banked placental blood for bm reconstitution of unrelated recipients on a large scale seem, therefore, timely and warranted. at least three such studies have been proposed, including our own.32

this report examines the issues confronting the establishment of a panel of hla-typed and fully tested placental blood units and the evaluation of their efficacy in transplantation, contrasted with the registry system of volunteer potential donors.

rationale for using placental blood to reconstitute ablated bm

after bmt, bm graft rejection and severe gvhd are caused by incompatibility for tissue alloantigens, most importantly, for those encoded by the hla system. thus, the best bmt results are obtained with sibling donors who share both hla haplotypes with the recipient.4,33-36 unrelated hla-identical donors may also provide reasonably compatible marrow for patients lacking hla-identical sibs, but graft rejection and severe gvhd are more frequent.17,37-40 the difference in transplant prognosis between hla-identical sibling donors and hla identical unrelated volunteers implies the existence of incompatibility, either for non-hla histocompatibility loci,41-47 for antigens at hla loci other than the routinely tested hla-a, -b and -dr,48-49 or for undetected variants of the defined hla antigens.50-53

finding unrelated individuals who are hla-identical to some patients is very difficult because of the extreme polymorphism of most hla loci. therefore, large registries of volunteers have had to be organized as cooperating national centers in most western countries.17-20,54 after recruitment, the candidate donors' hla-a and -b types are entered in a computerized database to facilitate comparison with those of potential recipients. when hla-a and -b identical matches are found for a patient, the potential donors are recalled and dr-typed. candidate donors who are hla-a, -b, -dr identical to a patient are recalled again for confirmatory hla typing and, generally, for mixed lymphocyte cultures (mlc) with the prospective recipient, and for infectious disease testing. because bm donation requires hospitalization and general or spinal anesthesia, extensive counseling of candidate donors is necessary. all of these steps prolong the time between the initiation of a successful search and the actual donation. reflecting recent improvements, the median time is at present between 4 and 5 months, still too long for some patients with rapidly evolving disease.

the difficulties inherent in registering volunteers, locating, testing, retyping, counseling, and eventually extracting bm from unrelated donors, contrast with the ease and speed with which typed, tested, and frozen placental blood could be made available when needed.25 empirical demonstration that placental blood "transplants" are able to support the reconstitution of bm in an adult would give banked placental blood critical advantages over registered volunteer bm donors:

1. placental blood is an abundantly available and currently discarded source of hematopoietic stem and progenitor cells that can be harvested without risk to mother or infant.
2. ethnic balance can be maintained automatically in heterogeneous populations.
3. important infectious agents, particularly cytomegalovirus (cmv), are much less common in the newborn than in adults.
4. placental bloods, frozen and stored in a repository, can be made available on demand, eliminating the delays and uncertainties that now complicate the collection of marrow from unrelated donors.
5. frozen placental blood units are easily shipped and thawed for use when needed, whereas freshly donated bm has a limited shelf-life necessitating rigorous coordination between harvesting surgeons, transportation facilities, and transplantation teams.

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from 0066-4971/93/8107-0045$3.00/0

blood, vol 81, no 7 (april 1), 1993: pp 1679-1690

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(6) In a large inventory, placental blood units with common HLA types might be added only until an adequate number of replicates is frozen; further replicates could allow the selective culling of units, for example, by infectious disease risk status. Thus, the proportion of these common units would be kept to an optimum while that of uncommon units would continue to increase.

(7) There would be an undistorted accumulation of the HLA types encountered because, unlike volunteer donors who eventually retire from the Registry, stored placental bloods suffer no attrition other than by clinical use or by culling and substitution.

NUMBERS OF STEM/PROGENITOR CELLS IN PLACENTAL BLOOD

The number of hematopoietic precursor cells required for the repopulation of ablated marrow has not been determined convincingly thus far, although successful engraftment of BM transplants correlates roughly with the total numbers of nucleated cells in the donated BM. Thus, it is standard practice to infuse some 1 to 3 \( \times 10^6 \) nucleated cells per kilogram of recipient, but the amounts minimally required may vary with degree of donor/recipient HLA compatibility, anti-HLA sensitization of the patient, age, etc, variables which might also affect placental blood grafts. Recently, the number of granulocyte/monocyte colony-forming cells (GM-CFCs) has been suggested as a more specific correlate of the probability of a successful graft. In some studies, GM-CFC numbers in the range of 1 to 10 \( \times 10^6 \) per kilogram were associated with successful and reasonably rapid engraftment. It is encouraging that the numbers of GM-CFC recovered in placental blood “units,” 2 to 2.5 \( \times 10^6 \), compare favorably with the total collected in marrow donations for adults and that the proportion and absolute numbers of earlier precursors may be higher in placental blood than in BM collections. Because of the well-known variability of stem/progenitor cell assays, however, these results should be interpreted with appropriate caution.

IMMUNOGENETIC ATTRIBUTES OF THE HLA SYSTEM

Several characteristics of the HLA system result in the need for a large pool of volunteer donors. First is the polymorphism, or multiplicity of alleles, of most HLA loci. Each allele encodes an alloantigen that comprises a unique combination of epitopes. A given epitope may be present in otherwise distinct antigens, causing serologic cross-reactivities that may result in varied degrees of clinical incompatibility. Serologically inapparent microvariations also occur; related antigens may differ in only a few such epitopes and yet be recognized by T lymphocytes with potentially serious clinical consequences.

Second is complexity: the several HLA loci are inherited together, as genetic blocks of closely linked genes on chromosome 6. These multigene blocks are called HLA “haplotypes” and constitute the effective units of inheritance. Complexity increases the polymorphism of the system because the haplotype is composed of several discrete polymorphic HLA loci.

Third, the population frequencies of some haplotypes significantly exceed (or fall short of) the products of the frequencies of the individual alleles that comprise them, resulting in positive (or negative) linkage disequilibrium. Linkage disequilibrium may involve alleles at two neighboring loci only, eg, the class I HLA-A and -B loci, or at both class I and class II HLA loci, eg, HLA-A, -B, and -DR. In the latter type, generally called “extended haplotypes” or “supratypes,” the class III genes located between HLA-B and -DR are also in linkage disequilibrium. Because of linkage disequilibrium, individuals who possess antigens known to belong to an extended haplotype, such as A1, B8, and DR3, are most likely to have inherited them as a haplotype. There is a high probability that alleles at most or all other relevant HLA genes will also be the same in such extended HLA haplotypes. Thus, HLA compatibility between unrelated donor/recipient pairs matched for six antigens that are normally in two distinct extended HLA haplotypes may be similar to that between HLA-identical sib pairs and would be expected to produce the most successful unrelated grafts. Because such extended haplotypes are more frequent than expected, the probability of finding an unrelated donor match is also higher and should be found in relatively small donor panels.

Conversely, HLA types composed of antigens that do not maintain linkage disequilibrium are less common than expected and represent different combinations of haplotypes. Generally, for patients whose HLA antigens are not in linkage disequilibrium, matched unrelated donors are rare, may only be found in large donor registries, and are more likely to be incompatible at untested HLA loci. If other HLA loci were indeed important, unrelated HLA matches involving the less common phenotypes would yield a disproportionately high number of unsuccessful grafts. Evidence of the negative effects of incompatibility for one of these “other” HLA loci, HLA-DR, was reported and confirmed, though also disputed.

The common extended haplotypes differ among ethnic groups, making the current, mostly Caucasoid, volunteer donor pool less able to provide matched donors for black, Hispanic, and Oriental patients. This effect was formally demonstrated with a simulated population sample, including Caucasoid and Japanese individuals, but should be even greater in ethnic “melting pots.” Thus, HLA-matched donors for individuals who carry ethnically restricted extended haplotypes from different ethnic groups may only be found among his or her particular genetically admixed group.

THE CHANCE OF FINDING HLA-MATCHED UNRELATED DONORS

Matching for haplotypes and not merely for antigens, as suggested above, may be important for the success of BM transplants. This possibility would be inconsistent with a major premise of volunteer donor registries, namely, that good results depend on donors who have the same HLA-A, -B, and -DR antigens as the recipient, regardless of their haplotype combinations. A second premise is that the probability of finding matched donors increases continuously and cumulatively as a function of registry size. Analysis demonstrates that the probability of finding useful HLA matches only rises
to a plateau well below 100%. The plateau, ie, the maximum overall chance of finding matches, was estimated at 40% to 60% for Caucasoid patients, given a panel size of 1 to 3 million volunteers. However, reality is likely to be less favorable for at least four reasons:

1. More HLA antigens have been defined than were used in the calculations, and, hence, there are many more possible combinations (phenotypes) and the frequencies of most phenotypes are lower than estimated.

2. The populations of the United States and other Western countries contain distinct genetically isolated subsets and large numbers of persons with ethnically mixed ancestry. Subsets of the American population, of African, Hispanic, and Asian descent, for example, differ significantly from each other and from Caucasoid in the structural identity of some HLA antigens and in the antigenic composition and frequencies of their major extended HLA haplotypes. Some HLA phenotypes may be common in one ethnic group and either rare or absent in others and, because registered volunteers are disproportionately Caucasoid, the search for unrelated donors matching many non-Caucasoid HLA phenotypes is generally unsuccessful. The problem could be overcome by establishing large pools of volunteer donors of each ethnic group and each ethnic assortment, a probably unrealistic undertaking.

3. HLA-types in current volunteer BM donor registries are not in Hardy-Weinberg equilibrium, ie, the observed frequencies of homozygotes and heterozygotes for alleles at each locus do not correspond to those estimated from the respective gene frequencies (J.J. van Rood, November 1991, personal communication). The possible causes of this deviation include ethnic stratification and typing errors, both of which would reduce the chances of finding true matches.

4. Donor attrition, estimated at about 1% per month, has a distorting effect on the accumulation of HLA types in a registry. When volunteer donors with rare phenotypes retire, their HLA types are unlikely to be encountered again. Thus, the number of HLA types available in the registry is not strictly cumulative, as would be the case if marrow donations from the volunteers had been cryopreserved and stored.

THE FEASIBILITY OF USING PLACENTAL BLOOD

Theoretical advantages of placental blood as donor tissue for BM reconstitution have been described above. Testing whether these potential advantages are actually realized in practice, and bringing placental blood into clinical use, will require establishing a relatively large repository of HLA-typed preserved placental blood units, assessing the impact of immunologic differences and various infectious and genetic diseases that distinguish placent al blood from marrow donated by adults, and evaluating the outcome of transplants to unrelated recipients.

Immunologic Perspectives

Possible presence of maternal lymphocytes in placental blood. The potentially dire consequences of this possibility have been thoughtfully articulated. Transplacental passage of maternal lymphocytes may be inferred from the chimerism present in more than 25% of children with severe combined immunodeficiency disease (SCID) and Richard O'Reilly, personal communication, November 1992. Thus, it is possible, though not yet demonstrated, that such putatively intratruncation placent of maternal lymphocytes also occurs in normal neonates. On the other hand, detectable maternal blood cells or their DNA in the neonatal circulation is reportedly uncommon. For example, we have searched for DNA encoding noninherited maternal HLA antigens by means of gene amplification with the polymerase chain reaction (PCR), using locus- and allele-specific oligonucleotide primers for HLA-DQA1, -DRB3, or -DRB5 sequences. Under conditions in which “contamination” with one “maternal” cell per 10,000 was easily detectable in artificial mixtures, we found no maternal DNA in any of 17 consecutive cord bloods (P.R., unpublished observations, June 1992).

Thus, unless maternal cells promptly leave the fetal circulation, transplacental passage of maternal cells into normal newborns would appear to be quantitatively minimal or to occur infrequently, if at all. However, clinical reports of massive maternal-to-fetal bleeds suggest that occasional neonates may have important levels of maternal cells in their circulation. Therefore, it may be relevant that the one reported placent al blood transplant which failed to engraft came from an extraordinarily large unit (282 mL), nearly double the next largest unit collected by the same worker. However, a small number of T-cell clones raised from this unit were all fetal, and there was no other evidence of maternal cells in this collection. Continued search for maternal lymphocytes in placental blood and investigation of their possible influence on engraftment and GVHD, therefore, seems warranted.

Transplacentally passed maternal anti-HLA antibodies. Up to 20% of mothers form specific HLA antibodies to the paternal antigens inherited by their offspring. Such maternal antibodies may traverse the placenta without apparent ill effects on the fetus, but their consequences, in the context of allostransplantation of hematopoietic cells in placental blood, are as yet unknown. Some maternal antibodies can result in destruction of fetal cells, as in hemolytic disease of the newborn, neonatal thrombocytopenia, and neonatal neutropenia, but these syndromes usually result from immunization to tissue-specific alloantigens. Thus, severe hemolytic disease of the newborn is more often caused by erythrocyte-restricted Rh antigens than the more commonly incompatible but widely distributed ABO blood group antigens. Transplacentally acquired HLA antibodies do not reduce lymphocyte, platelet or granulocyte counts in placental bloods, although, in the mouse, maternal anti-H-2 antibodies do bind to most tissues of the hybrid fetus, including thymus and spleen. However, it has not been reported whether maternal HLA or other relevant antibodies were present in the plasma of the placental blood grafts already performed. If a substantial amount of plasma is included in the placental blood unit transplanted, the presence of maternal antibodies and the possibility that they may interfere with the engraftment and functioning of hematopoietic precursors should be investigated. Maternal antibodies would
be of lesser consideration if the plasma of placental blood units were removed during freeze-thaw processing.

Lack of protective immunity. The fetus will not have been exposed to the vaccines routinely given in childhood nor to the common infections against which adult marrow donors are usually immune. Thus, recipients of placental blood for BM reconstitution would become immunologically unprotected against some important agents and vaccination may be required. The possibility that in vitro immunization of placental blood lymphocytes might be developed to optimize the capacity of the graft to protect the host from infection and, possibly, from malignant disease recurrence, has been suggested (Wade Parks, 1991, personal communication).

Possibly lower immune responsiveness: GVHD, GVL, and graft rejection. The sibling-donor placental blood transplants performed up to now suggest that allore cognition and GVHD may be less intense than in recipients of similarly compatible BM. However, there are positive correlations between GVHD and GVL and negative correlation between GVHD and graft rejection suggesting that less severe GVHD with placental blood grafts might lead to lower GVL, decreasing its effectiveness in the treatment of leukemic patients and increasing the probability of marrow rejection. Only two leukemic patients grafted with placental blood have been reported to date. One patient with juvenile chronic myelocytic leukemia, conditioned with busulfan rather than whole-body irradiation, suffered disease recurrence probably caused by refractory leukemia, 7 months postplacental blood transplant. A subsequent marrow transplant from the same donor of the placental blood resulted in severe acute and chronic GVHD (Hal Broxmeyer, 1992, personal communication). The other, a patient with advanced acute B-cell leukemia, was reconstituted with placental blood from a one-haplotype mismatched sibling and was reported disease-free 1 year posttransplant. The latter patient had minimal and transient erythematous reactions suggestive of GVHD. There has been no evidence of graft rejection among the sibling placental grafts reported thus far, but the numbers are small and both reduced GVL and graft rejection must be closely monitored, particularly in future unrelated grafts.

On the other hand, reduced GVHD capacity of placental blood lymphocytes, if confirmed in the unrelated recipient setting, would be a clear advantage of placental blood transplants. Several possible mechanisms may underlie such a phenomenon. For example, some degree of natural specific immunologic tolerance to noninherited maternal antigens has been recognized in dogs, mice, and humans. Experimental exposure of fetal and early neonatal mice to major histocompatibility complex (MHC)-incompatible lymphocytes also results in specific immunologic tolerance. The latter experimental evidence has an intragenic parallel in humans: fetuses and neonates who receive allogeneic blood transfusions as therapy for Rh hemolytic disease may undergo engraftment of viable transfused cells, sometimes leading to GVHD and even to specific tolerance of skin grafts. In exploring an in vitro correlate of allore cognition, we found that cord blood-derived mononuclear cells are less responsive in MLC than are those of their HLA-identical siblings (P.R., 1990, unpublished observations). Similarly low MLC responsiveness was observed recently by Buckley (quoted in reference) and by Harris et al. Furthermore, allospecific T-cell–mediated lytic activity is much less inducible in placental than in adult bloods. However, because placental blood reportedly has more alloreactive cytotoxic T-lymphocyte precursors than does adult blood, high suppressor-cell activity may underlie the reduced allore cognition exhibited by placental blood lymphocytes.

If partial tolerance to alloantigens were a consistent feature of neonatal lymphocytes, placental blood transplants into HLA-incompatible recipients might have less risk of severe GVHD. Placental blood grafts might be especially appropriate for recipients whose incompatible HLA antigens are noninherited maternal antigens for the donor. In this connection, the one-haplotype–mismatched transplant reported by Viller et al may be exceptionally important: their donor/recipient sibling pair shared both HLA-DR alleles but differed in the maternally derived HLA-A and -B alleles. If confirmed by further observations, this phenomenon would vastly improve the chances of finding an adequate donor for a larger proportion of patients. Clearly, careful documentation of incompatible grafts and their outcome in both related and unrelated placental blood grafts is essential.

Infectious Disease Considerations

Infectious organisms that can be transferred to recipients with allogeneic marrow also may produce congenital infections or accidentally contaminate placental blood during collection and, therefore, might infect the eventual recipients of placental blood units. The overall risk of congenital infections depends on the prevalence and incidence of the specific infection in pregnant women and on the variable capacity of the infectious agent to cross the placental barrier. Some congenital infections, such as toxoplasmosis, syphilis, rubella and CMV, can be transmitted at any time during gestation. However, even when pregnant women have active infections with such organisms, not all of their “at-risk” infants will become infected. For example, only 40% of infants whose mothers have a primary CMV infection during pregnancy will be congenitally infected. Other organisms, such as hepatitis B virus (HBV), rarely cross the placenta during gestation and are usually acquired perinatally. Because precise routes of transmission are usually not known for perinatally contracted infections, whether organisms are present in placental blood also is not known. One possible route, involving leakage of infectious organisms across the placenta during labor and delivery, could be an important source of contamination for placental blood units. However, small concentrations of the agent, which may suffice to cause infection, may elude direct detection by current techniques. Additionally, during vaginal deliveries, the surface of the placenta and cord will come into contact with maternal blood and with the nonsterile mucosa of the cervix and vagina and with perineal skin. Therefore, collection procedures must be designed to minimize the chances for contamination with bacteria, viruses and candidiasis, a particularly important organism for immunosuppressed transplant recipients.
Relevant aspects of the more common congenital blood-borne infections that are important to patients requiring BM reconstitution are summarized below, to illustrate possible approaches and current difficulties of detecting infectious organisms in placental blood.

**Neonatal bacterial sepsis.** Sepsis occurs in 1 to 8 per 1,000 live births and usually is caused by organisms from the mother's vagina, most commonly group B streptococcus or Escherichia coli. Routine bacterial culture of placental bloods should detect sepsis as well as possible bacterial contamination of the unit during collection.

**CMV.** CMV, the most common life-threatening infection in BM transplant recipients, affects about 1% of newborns in the United States (range 0.2% to 2.0%), half from women who have primary CMV infections during pregnancy and the others from women who are already seropositive and have latent or recurrent infections. The virus can be reliably detected by tissue culture methods in the urine or saliva but not the blood of congenitally infected infants. Therefore, tissue culture would not be practical for screening placental blood for a large-scale repository. IgM antibody to CMV in the newborn (or in placental blood) is diagnostic, because passive maternal IgG antibodies to CMV would be more accurately detected by the virus or viral nucleic acids present in 40% to 60% of all placental bloods. Therefore, a more accurate test is needed to increase the sensitivity of detecting CMV in placental blood.

**Human immunodeficiency virus, type 1 (HIV-1).** HIV-1 is transmitted to about one third of infants born to HIV-1 infected women. However, only some of these infections appear to occur in utero. HIV-1 has only rarely been successfully cultured from blood collected within the first week of life, suggesting that a significant proportion of HIV-1 infections are acquired perinatally or postnatally. Recently, however, PCR-detectable HIV-1 sequences have been found in the peripheral blood of about two thirds of newborns later documented to be infected with the virus. However, the relative contributions of perinatal and postnatal routes of transmission to the probability of infection in at-risk infants remains controversial. The prevalence of HIV-1 infection in pregnant women in the United States varies from negligible to 2% to 3% in high-risk areas. Black and Hispanic women in certain urban areas have had especially high HIV-1 infection rates in association with intravenous drug use by themselves or their sex partners. Serologic testing for antibody will identify most infants who are at risk, except for those whose mothers have early HIV-1 infections and do not yet have detectable antibody. Testing for antibody to HIV-1 may be problematic in areas where laws prohibit testing without prior counseling and written informed consent, if the identities of the infant and/or mother are retained.

**HBV.** HBV is usually transmitted to infants in the perinatal period from mothers who have acute HBV infections at the time of delivery or are chronic carriers. The risk that the infant will be infected depends on the level of maternal viremia. The incidence of perinatal HBV infection in the United States varies from about 1 per 10,000 for whites to 5% to 8% for some Asian immigrants. Because hepatitis B surface antigen (HBsAg, the serologic marker of HBV infection) generally does not cross the placenta, it is currently necessary to test mothers' bloods to identify infants at risk. Sensitivity and specificity of diagnostic tests for the virus or viral nucleic acids would help establish the actual presence of HBV in a given placental blood unit.

**Hepatitis C virus (HCV).** HCV is present in about 5 per 1,000 US volunteer blood donors, and hepatitis caused by HCV has been reported as a complication of BMT. Although there is evidence that some women transmit the virus to their infants, the frequency, time, and route of transmission are not yet clear. However, seroconversion during the first year of life suggests that most infections are transmitted perinatally or postnatally. Assays for IgG antibody to HCV are available and a positive result is generally indicative of infection, although, as with IgG-based assays for other agents, its presence in placental blood would not distinguish between maternal and fetal infection. There are, as yet, no assays for HCV-specific IgM antibody nor routine methods to detect the virus.

**Human T-lymphotropic virus (HTLV).** HTLV is a highly-cell-associated retrovirus that is readily transmitted by the cellular components of blood. Breast feeding is a risk factor for maternal-infant transmission, but it is also likely that HTLV may be transmitted across the placenta, although how often and when are not known. While the prevalence of HTLV infection in the United States is generally very low, individuals from certain endemic areas may have rates as high as 2% to 3%. Detection of IgG antibody to HTLV in placental blood will indicate infection in the mother, but would not distinguish between maternal and fetal infection.

**Epstein-Barr virus (EBV).** EBV infection is emerging as an important complication of BMT associated with a rapidly progressive lymphoma, especially in congenital immune deficiencies. Importantly, EBV does not cross the placenta and congenital EBV infection is extremely rare. Syphilis. *Treponema pallidum* invades the placenta and from there enters the fetal circulation. As a consequence of the resurgence of syphilis among heterosexual adults, the incidence of congenital syphilis has increased dramatically over the past 5 years. In New York City, for example, the annual incidence of reported congenital syphilis increased from about 2 to 3 per 10,000 births in the early 1980s to 75 per 10,000 in 1990. The detection of IgG antibodies in placental blood would identify most infants at risk for syphilis, except for the few maternal infections contracted shortly before the infant's birth. However, positive test would not distinguish infants whose mothers might transmit the agent from those born to women who had already been treated. The IgM-FTA-ABS test (fluorescent treponemal antibody, absorbed with nonpallidum treponemes) might help identify infected infants, but has a substantial false-negative rate in asymptomatic congenital syphilis and up to 10% false positives.

**Toxoplasmosis.** *Toxoplasma gondii*, the most common congenital parasitic infection, also infects the placenta. The US incidence of congenital toxoplasmosis has been estimated...
recently at about 1 per 10,000 live births.\textsuperscript{154,155} Congenital toxoplasmosis is difficult to diagnose: parasites can be isolated directly or toxoplasma antigen can be detected by enzyme-linked immunosorbent assay (ELISA) in less than half of infected infants.\textsuperscript{156} The presence of IgM-specific antibody is diagnostic, but again, the best assay available is estimated to have a false-negative rate of 20%.\textsuperscript{155,157}

Despite the possibility of congenital and perinatal transmission of infectious agents, placental blood offers potential advantages, from the infectious disease perspective, for BM restoration. The frequency of latent and chronic infections, which tend to accumulate throughout an adult's lifetime, should be lower: only 1% of units would be infected with CMV compared with 40% to 60% of marrows from adult donors, for example, and virtually no placental bloods would be infected with EBV. On the other hand, most BM donor registry volunteers are middle-class Caucasoids, who are often regular blood donors and, thus, repeatedly tested and found to be negative for HIV-1 and several other blood-borne organisms. These infections are apt to be more prevalent in less privileged socioeconomic groups and, therefore, in some minority ethnic groups whose availability constitutes a particular advantage of the proposed placental blood resource.

In summary, strategies used for detecting infection in adult BM donors may not be entirely appropriate in placental blood. In the latter case, one must cope not only with the presence of passive maternal IgG antibodies that are not indicative of fetal infection\textsuperscript{158} but also with variation in the time of transmission from mother to fetus and its influence on the amount of the agent that may be present and on the development of fetal immune responses. While the presence of maternal antibodies to specific infectious agents might identify infants at risk of infection, testing placental blood for this purpose may not always be reliable. Because transplacental transport of maternal IgG is relatively slow,\textsuperscript{159-162} passively transferred maternal antibodies may not reach detectable levels in placental blood when the maternal infection occurs late and seroconversion ensues near the time of delivery. Another difficulty is the inconsistency of serologic evidence of infection in the fetus\textsuperscript{166}, up to 20% of infants with common congenital infections have no specific IgM antibodies detectable in placental blood.

These complexities of detecting infection in placental blood dictate that, at least initially, maternal as well as placental blood should be investigated to ensure state-of-the-art detection of the risk of infection, even though collecting blood specimens from every mother could be an onerous requirement for a future large-scale placental blood repository. Methods using only placental blood specimens should be rigorously validated before abandoning the parallel testing of mother's blood. At present, it may be reasonable to discard all units when a risk of infection is detected for agents such as HIV-1, even if most would not contain the agent. However, some of these units will have unique HLA genotypes that might be permanently unavailable if they were automatically discarded. Decisions in these cases may depend on the clinical implications of the specific infectious agent. Tests capable of directly detecting infectious organisms in placental blood, especially in the quantities likely to be present in perinatal infections, may help solve this issue.

\textbf{Relevant Genetic Diseases}

A disadvantage of placental blood compared with donated adult BM is that newborns may carry undiagnosed genetic diseases that would have automatically excluded adults from entering a donor registry. In general, genetic conditions affecting the hematopoietic system should disallow the use of affected individuals as donors of hematopoietic stem cells for marrow reconstitution. Among these conditions are hemoglobinopathies (sickle cell anemia, the thalassemias, etc), deficiencies of erythrocytic enzymes (such as glucose-6-phosphate dehydrogenase, adenosine deaminase, dihydrofolate reductase, pyruvate kinase, formaminotransferase), congenital anemias (Fanconi's anemia, the dyserythropoietic syndromes, Rh-null disease and its variants, etc), and congenital immunologic defects (such as SCID, other agammaglobulinemias, and the bare lymphocyte syndrome).

Specific questions to the mother may help to focus on these diseases when there are previous cases in the family, as will the ethnic stratification of certain conditions like sickle cell anemia and the thalassemias. Routine screening tests will disclose most of the hemoglobinopathies and others such as Rh-null disease will be revealed by blood typing; however, pretransplant testing should be considered for the most common of these entities.\textsuperscript{163,164}

\textbf{Practical Issues}

\textbf{Harvesting placental blood.} Placental blood is easily recovered through the umbilical vein. One method is to allow blood to drain from the severed end of the cord during the third stage of labor.\textsuperscript{24-26,165} Blood collection before delivery of the placenta takes advantage of uterine contractions and allows the recovery of some 80 to 160 mL of blood, but must be done rapidly and may interrupt the obstetrician's routine activities. Moreover, while this open system is simple, it is subject to contamination with maternal blood and microorganisms. We prefer to collect the blood into a closed system using a sterile donor blood collection set, shortly after the separation of the placenta. The latter method yields similar blood volumes (90 to 200 mL) with better control of the procedure and, thus, reduced opportunity for contamination. In bleeding the delivered placenta, it is important to consider that the umbilical vein and its tributaries easily collapse under even minimal external or internal (negative) pressure. To avoid this, we hold the placenta at its edges and suspend it from a specially designed frame, with the fetal aspect and the cord down. The cord is cleaned with gauze and ethanol-iodine or a similar disinfectant solution and the blood is drained as in a standard gravity phlebotomy. A similar method, which also perfuses the placental vasculature, has been proposed recently.\textsuperscript{166} Because the onset of blood clotting in umbilical cord and placental veins is relatively slow (R.E.R., 1976, unpublished data), a slight delay in retrieving the blood is permissible. Therefore, collection can be accomplished outside of the delivery room without disturbing mother and infant care activities. ACD (acid, citrate, dextrose) is an excellent anticoagulant but the ratio between its volume and that of
the blood must be kept within a relatively narrow range. CPD (citrate, phosphate, dextrose), on the other hand, is much less affected by variations in collected blood volumes. Studies suggest that hematopoietic precursor cells are not significantly reduced in number by storage for up to 48 to 72 hours at room temperature, sufficient time for transfer to a central processing site. In our experience, progenitor cell recovery remains highest if placental blood is not refrigerated before processing.

Freezing placental blood. Physical separation of mononuclear cells from the red blood cell (RBC) mass in placental blood seems to result in pronounced losses of progenitor cells and presumably of stem cells. Therefore, at present, the whole placental blood unit must be frozen without fractionation. Dimethyl sulfoxide is used as a cryoprotectant and the cooling rate (−1°C per minute) is optimized for leukocyte viability rather than for erythrocytes. This cooling rate can be obtained with a computerized controlled-rate freezer or with a mechanical −80°C freezer using a liquid heat sink, a method in use for over 10 years in our laboratory. Although considerable RBC lysis occurs upon thawing, no ill effects are seen after the transfusion of these units. Frozen placental blood units are best kept in the liquid phase of liquid nitrogen to prevent the changes in temperature that occur in the gas phase when opening the lids of laboratory liquid nitrogen refrigerators. The recovery of hematopoietic precursors from unseparated frozen placental blood after 5 years of storage was excellent in comparison with the same samples when assayed before freezing. Similarly, specimens tested after up to 7 years of storage were also in the range observed with fresh samples (D. Harris, in preparation). It should also be noted that some of the procedures, specifically those that depend on freezing placental blood units or fractions thereof, have been patented in the United States (rights assigned to Biocyte Corp, New York, NY) and licensing might be required.

Samples for future testing. The repository must anticipate the need for additional testing before a unit is used and, therefore, should maintain frozen aliquots of serum, DNA, and cryoprotected sterile mononuclear cells, for testing certain genetic and, possibly, also infectious disease markers. In addition, the frozen unit should include aliquots in satellite reservoirs that are integral to the frozen unit but which can be separated without removing the unit from the liquid nitrogen refrigerator or sacrificing its sterility. Without thawing the whole unit, these aliquots would allow confirmation of the identity of a unit whose recorded type matches a potential recipient.

Testing of mothers. In the course of our feasibility study, the mother will be interviewed and asked to answer questions relevant to her child’s ethnicity and risks of genetic and infectious diseases. She will also be asked to donate a blood sample, which would permit us to resolve certain types of HLA ambiguities and would also provide a firmer basis for some infectious disease tests. For example, the mother’s sample would help in assigning antigens to haplotypes and will be especially useful in resolving “blanks” in the child’s HLA phenotype that can represent either a homozygote or a heterozygote carrying an unidentified antigen. The mother’s blood sample also would help in discerning the risk for infections that may not be easily identified in placental blood and would also allow us to assess the efficacy of testing for infectious disease markers in placental blood alone. Furthermore, typing of the mother’s HLA and other genetic markers would help focus the search for maternal lymphocytes in the placental blood and permit the evaluation of the effects of incompatible noninherited maternal antigens when fully matched BM or placental blood units are not available.

Follow-up of infants. During the feasibility study we will maintain a record of all diagnoses made on the infant before discharge. This information will identify some genetic diseases, verify some congenital infections, and help distinguish congenital sepsis from inadvertent contamination of the placental blood unit during collection.

Confidentiality. To prevent possible breaches of confidentiality, we propose that all identifying information be discarded as soon as infectious disease testing is completed and any pertinent information has been relayed to the mother’s physicians. Discarding the identifiers of both mother and infant would preclude any possibility that these individuals could be approached in the future for a BM donation as a consequence of their participation in this study.

EVALUATION OF PLACENTAL BLOOD AS SOURCE TISSUE FOR HEMATOPOIETIC RECONSTITUTION

The utility of establishing a large-scale system of placental blood repositories for BM reconstitution in unrelated patients will depend on the answers to several questions. These questions, suggested by the discussions above, relate to issues of the adequacy and quality of the repository material, to the outcome of actual transplants, and, ultimately, to whether such a repository might supplement or even replace the volunteer donor registry system. For example, to establish the adequacy of placental blood as a source of hematopoietic tissue we need to clarify:

Quantitative aspects. Can adequate volumes of placental blood be collected routinely without contamination by infectious organisms? Are stem/progenitor cells consistently present in sufficient numbers to reconstitute older children and adults? Will it be possible to isolate and freeze stem/progenitor cell-containing suspensions of white blood cells from the whole blood unit and thereby minimize the space required for storage?

Qualitative aspects. Are maternal lymphocytes present? If so, how often, at what levels, and with what adverse clinical consequences? How will the differences in the prevalence of infection impinge on the suitability of units collected from ethnic subsets of the population? Can the relevant infectious agents be detected directly in placental blood with sufficient reliability? Will it be possible to streamline data collection and testing of specimens so that it will not be necessary to collect blood from the mother?

Clinical characteristics. How rapid and stable is the reconstitution of hematopoiesis by placental blood transplants? What is the level of GVHD in HLA-matched placental blood grafts? Is there a GVL effect even in the absence of GVHD? What degree of HLA mismatching is tolerated? Are nonin-
herited maternal antigens especially well tolerated? What are the infectious complications in recipients of placental blood?

Accurate answers to such questions should emerge through systematic, well-documented collection and testing procedures with scrupulous attention to record keeping and data management. Complete assessment of the outcome of unrelated placental blood transplants will require comprehensive reporting of the results of each case. Solid data must exist before a decision can be made on whether to establish large placental blood repositories for unrelated marrow reconstitution.

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