Selected Summaries

Placenta: An Alternative Source of Hematopoietic Stem Cells

[Kurtsber J, Laughlin M, Graham ML, Smith C, Olsen JF, Halperin EC, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 1996; 335:157-166].

Successful allogenic bone marrow transplantation depends on the availability of an HLA matched donor and avoidance of severe graft-versus-host disease (GVHD). To overcome these two major constraints, the authors used partially HLA mismatched placental blood from unrelated donors as an alternative source to reconstitute hematopoiesis.

Patients were eligible for enrollment if there was neither an HLA identical related donor nor a related donor with two HLA mismatches available and if an HLA-matched, unrelated bone marrow donor could not be identified within six months. Twenty five consecutive patients including 24 children (median age *7.0* years) with a variety of malignant and non malignant conditions (ALL-12, ANLL-5, CML-1, Neuroblastoma-1, Kostmann's syndrome-1, Fanconi's anemia-2, Amegakaryocytic thrombocytopenia-1, Lesch-Nyhan disease-1 and myelodysplastic syndrome-1) were chosen. HLA matching was performed before transplantation by serologic typing for class I HLA antigens and low resolution molecular typing for class II HLA alleles. In donor-recipient pairs who differed by no more than one HLA antigen or allele, high resolution class II HLA typing was done retrospectively. For donor-recipient pairs who were mismatched for two HLA antigens or alleles, high resolution typing was used prospectively to select the best match for HLA-DRB1. Cryopreserved units of placental blood were thawed and infused over a period of 10-15 minutes for first three transplantations. For subsequent grafts, unit was washed with 10% dextran and 5% albumin before infusion.

All patients received placental blood from unrelated donors and were evaluated for hematologic and immunologic reconstitution and GVHD. Twenty four of the 25 donor recipient pairs were discordant for one to three HLA antigens. In 23 of the 25 transplant recipients, the infused hematopoietic stem cells engrafted. Acute grade III GVHD involving the skin and gut occurred in 2 patients and chronic GVHD in another two children.

Seven of the 19 patients undergoing transplantation for malignant conditions and 5 of the 6 with non malignant conditions survived uneventfully with Karno fsky scores above 90, with a median follow up of 12Vi months. The overall 100-day survival rate among these patients was 64% and the overall event free survival was 48%. It was concluded that HLA mismatched placental blood from unrelated donors is an alternative source of stem cells for hematopoietic reconstitution in children.

Comments

HLA-matched bone marrow is the principal choice of transplantable hematopoietic stem cells and progeniter cells in a variety of malignant and non-malignant conditions. In case, a suitable HLA

matched donor is not available, alternative sources of allogeneic hematopoietic stem cells need to be explored. Placental blood, which is routinely discarded may provide an answer to this problem. Successful transplants of placental hematopoietic cells taken from completely or partially (with a difference of a single antigen) matched sibling donors are on the records(l). In such cases, the recipient has minimal chances of GVHD.

The present report highlights the use of placental blood as a source of hematopoietic cells in unrelated and unmatched (by as many as three HLA antigens) recipients. The tranplants engrafted and produced all the major cell lines without a significant GVHD. The low frequency and mild degree of GVHD observed in this study could be related to the cellular immaturity of the placental blood or young age of the recipients(2).

The major advantages of using placental blood over bone marrow is that it is less likely to contain viruses, storage is feasible and procurement time is reduced from 6 months to 2 weeks; and patient preparation for transplantation takes lesser time. At the same time, stem cells and progeniter cells in placental blood have more capacity for proliferation and self renewal than those in marrow(3). Transfusion of placental blood is fraught with theroretical risk of transmission of a genetic disease and availability of only one unit for each transplantation procedure.

In the same issue of the journal, Silberstein and Jefferies(4) have raised the possibility of placental blood banking. Establishment of such banks is justified by the present study. Additionally, this may lead to better quality control for collection, processing and storage of placental blood to be used for hematopoietic reconstitution. It is also suggested that the placental blood be obtained only following the maternal consent and should be tested for infectious agents, genetic disorders and presence of drugs that may hinder hematopoiesis, prior to transplantation.

Future developments include the expansion of placental blood stem and progenitor cells, *ex vivo* to provide an additional back up unit for the same recipient or allow donation to more than one recipient at a time(5).

There is also a need for a comparative study of transfusion of hematopoietic stem cells from bone marrow and placenta in order to assess the utility, feasibility, morbidity and mortality associated with the two procedures.

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Antimalarial Vaccine

[Stoute JA, Slaqui M, Heppner DG, Momin P, Kester KE, Desmons P, et al. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against Plasmodium falciparum malaria. N Engl J Med 1997; 336: 86-91].

Currently malaria occurs in 90 countries, and according to the World Health Organization it causes 500 million cases and 2.7 million deaths per year. Hence a concerted effort to evolve a cost effective method like a vaccine for combating this deadly disease continues.

Three formulations of the recombinant vaccine (RTS,S) based on fusion of the circumsporozoite protein and hepatitis B surface antigen plus a potent adjuvant were evaluated in an unblinded trial in 46 subjects (age 18 to 45 years) who had not been exposed to malaria. RTS is a single polypeptide chain corresponding to aminoacids 207 to 395 of P. *falciuparum* that is fused to HBsAg (adw serotype). S is a polypeptide of 226 amino acids that correspond to HBsAg. Vaccine 1 consisted of RTS,S in a formulation containing alum and monophosphoryl lipid A (designated SBAS4); vaccine 2 consisted of RTS,S in an oil in water emulsion (SBAS3); vaccine 3 consisted of RTS,S in this emulsion plus the immune stimulants monophosphoryl lipid A and QS 21 (SBAS2). The standard dose of vaccine 1 was 1 ml and that of vaccines 2 and 3 was 0.5 ml; each dose delivered 50 microgram of RTS,S antigen. The third dose of vaccines 2 and 3 was reduced to 0.1 ml in response to adverse reactions after the second dose. Vaccines were administered intramuscularly in the deltoid region at 0, 4 and approximately 28 weeks. Twenty two subjects who received three doses of the vaccine agreed to a challenge with P. *falciparum* sporozoites, eight against vaccine 1, and seven each given vaccines 2 and 3. Cloned chloroquin sensitive P. *falciparum* parasites were used to infect laboratory reared Anopheles stephensi. Challenge occurred on one of three consecutive days approximately three weeks after, the third dose of vaccine. The challenge continued until five infected mosquitoes had successfully fed. Six unimmunized subjects served as controls and parasitemia developed in all of them, 11 to 13 days after sporozoie challenge. Malaria developed in seven of eight subjects given vaccine 1, with a mean prepatent period 12.6 days (range 11 to 18); five of seven subjects given vaccine 2 became infected with a mean prepatent period of 15.2 days (range 14 to 19). In contrast only one of seven subjects given vaccine 3 became infected giving a vaccine efficacy of 86%. The responses to HBsAg were clearly affected by preexisting hepatitis B immunity whereas all subjects who were negative for HBsAg before immunization seroconverted after a receiving a single dose of vaccine 2 or 3, most such subjects required two doses of vaccine 1 to seroconvert and three days to achieve maximal responses. On the other hand subjects who were already positive for HBsAg before immunization had early maximal responses after

the first dose itself. All initial doses were well tolerated causing only mild discomfort at the injection site. The second doses of vaccines 2 and 3 produced severe side effects like headache, malaise, myalgia in 4 subjects and hence the third doses of vaccine 2 and 3 were reduced in all.

Comments

Plasmodium falciparum is widely prevalent causing fatal infections. The morbidity caused by other species, namely, *P. vivax* is also immense. Hence intense research is on to develop a vaccine which is efficacious against all species of *Plasmodium* and causes few side effects in infants and children. The above vaccine which demonstrated considerable protection against P. *falciparum* in non immune adults and simultaneously protected against Hepatitis B infection, is a result of decades of research in this direction.

Various antimalarial vaccines have been developed but all are poorly immunogenic. These vaccines included those against sporozoites, intrahepatic form of the parasite and transmission blocking vaccines against gametocytes or later stages in mosquitoes(1). Among these only one vaccine SPF 66 targeted at erythrocytic phase has proved partially effective(2). Later radiation attenuated sporozoites were used for vaccination but these could not confer absolute protection(3). Exposing humans to irradiated mosquitoes infected with P. *falciparum* and P. *vivax* has demonstrated immunity to malaria but large scale implementation of this is impossible. The stumbling block against development of an effective vaccine is the inability to grow sporozoites *in vitro* in large amounts for vaccine manufacture. Hence an attempt to develop a vaccine based on circumsporozoite protein present in sporozoites and intrahepatic stages with various adjuvants for immune enhancement was made after elaborate trials in experimental animals. Antibodies against circumsporozoite antizen developed in all subjects who received two or more doses. Because of a small sample size, effects of prexisting hepatitis B immunity on antisporozoite responses could not be discerned.

Elaborate clinical trials are still needed to determine the overall efficacy of the vaccine. Its ability to protect against P. *vivax* infection is unknown. How effective it is against chloroquin resistant strains of P. *falciparum* needs to be elucidated. Its ability to protect adults not exposed to malaria is analogous to a situation in a neonate with no exposure. The nature and amount of immune response it might elicit in adults already exposed to malaria and its ability to prevent further infection is not clear. If it proves successful, then this vaccine conferring dual protection against malaria and hepatitis B would be a noteworthy advance.

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