

Histocompatibility and Immunogenetics in Cord Blood Transplantation

Pablo Rubinstein and Christopher Hillyer

National Cord Blood Program, New York Blood Center 310 East 67th Street, New York, NY 10065, Phone: 212 570 3230, e-mail: prubinstein@nybloodcenter.org

ABSTRACT

This review of the immunogenetics of cord blood transplantation attempts to highlight the connections between classical studies and conclusions of the tissue transplantation field as a scholarly endeavor, exemplified by the work of Professor Hoecker, with the motivations and some recent and key results of clinical cord blood transplantation. The authors review the evolution of understanding of transplantation biology and find that the results of the application of cord blood stem cells to Transplantation Medicine are consistent with the careful experiments of the pioneers in the field, from the results of tumor and normal tissue transplants, histocompatibility immunogenetics, to cell and molecular biology. Recent results of the National Cord Blood Program of the New York Blood Center describe the functioning in cord blood transplantation of factors, well known in transplantation immunogenetics, like the F1 anti-parent effect and the tolerance-like status of donors produced by non-inherited maternal HLA antigens. Consideration of these factors in donor selection strategies can improve the prognosis of transplantation by characterizing "permissibility" in HLA-incompatible transplantation thereby increasing the probability of survival and reducing the likelihood of leukemic relapse.

Key terms: Umbilical Cord Blood, Hematopoietic Transplantation, Histocompatibility Genes, HLA matching.

INTRODUCTION

Tissue (mostly bone marrow) and solid organ transplantation (kidney, liver, heart) are well-established therapeutic technologies in current medical practice and are performed on many thousands of patients every year. Cord blood allotransplantation, more recently introduced to clinical medicine, has brought about a broad change in the attitudes of transplant physicians, mostly because HLA-mismatched unrelated donor cord blood can achieve engraftment with usually manageable levels of graft versus-host disease. Logistic advantages, such as donation free of risks, possibility of long term storage without inventory attrition, low prevalence of latent viral disease and prompt, safe availability, enhance its practical attractiveness. Yet, the immunogenetic properties of cord blood are predictable from its cellular composition and age in the context of an evolving histocompatibility system and are not surprising in the context of the evolving understanding of that system's biology. Although the broad immunologic bases of allotransplantation are reasonably well understood, important problems still remain to be solved properly. In this brief review of cord blood as source of hematopoietic tissue for bone marrow replacement (Rubinstein, 2005) and its immunogenetic underpinnings, we will examine the biological background of transplantation immunogenetics in general and some current challenges in overcoming the clinical problems of cord blood transplantation.

Early studies. The discovery of a genetic basis for the compatibility of tissue transplants was demonstrated by Little (1914) and quantitatively explored by Snell (1948), using transplantable tumors in inbred mouse strains. These Bar Harbor investigators disclosed the existence in mice of many genes whose shared presence in graft donors and recipients is required for the acceptance of tissue transplants. Snell showed that these genetic systems (he termed them "Histocompatibility" or "H" genes) segregated as Mendelian

traits, independently from each other and that they differed in "strength", with one H gene, later designated as H-2 (see below) being much stronger than any of the others. That an immune mechanism is responsible for the rejection of transplants, is, however, due to Gorer, who demonstrated the formation of antibodies with specificity for the donor's tissues (including, in mice, the erythrocytes) following allogeneic and xenogeneic transplantation (into rabbits) (1936, 1938).

Gorer encountered several red cell-agglutinating allo- and hetero-antibodies, whose reactivity with the red cells of mice of different strains allowed him to designate two of the corresponding antigens as "antigen I" and "antigen II". With genetic back-crosses between three inbred mouse strains, Gorer demonstrated that tumors of a strain carrying antigen II would grow only in back-cross animals carrying antigen II. He demonstrated that anti-II hemagglutinins were produced by mice rejecting A strain tumors (antigen II-positive) and that cells from A-strain tumors specifically absorb such anti-antigen II allo-hemagglutinins. In 1938, Gorer rephrased the genetic "laws" of transplantation in immunological terms: "Normal and neoplastic tissues contain isoantigenic factors that are genetically determined. Isoantigenic factors present in the grafted tissue and absent in the host are capable of eliciting a response that results in the destruction of the graft". He added that, "Under special circumstance the response may not be elicited or grafted tissues may not be destroyed thereby". Thus, the recognition of genetic differences in transplantation operated through the immunological recognition of the products of alleles at certainly one and possibly, several unlinked H genetic loci. In joint experiments reported in 1948, Gorer, Snell, et al. demonstrated that antigen II is one of the product(s) of the "strong H" (H-2) locus and uncovered the linkage of the H-2 genetic determinants to the Fused locus in the 9th linkage group of the mouse. Snell continued his investigation of this linkage group and discovered the existence in it of unrelated genetic loci determining polymorphic skeletal defects.

Shortly after this seminal collaboration, Gustavo Hoecker, then a young Chilean investigator, joined Gorer's Laboratory at Guy's Hospital in London with his wife, Dr. Olga Pizarro, and a year later, Snell's Laboratory in Bar Harbor. He then returned with Promethean offshoots (antibodies and breeding trios of congenic mice) from their research to start experimental immunogenetic work at the Universidad de Chile's Medical School as Chair of Biology and Genetics. Using Snell's congenic strains of mice (inbred lines that carried different H-2 alleles in a common genetic background) Hoecker was one of the first scientists to unravel the genetic and immunologic complexity of the H-2 gene. Thereafter, his was a life-long effort to understand the physiology of H-2 immunogenetic determinants and their biological role (Hoecker, 1986). He used classical and innovative tools, including immunization of F1-hybrid congenic animals and sequential absorption experiments, to make allo-antibodies of ever more restricted specificity. He was able in that way to describe the extended immune phenotypes of many H-2 alleles and the existence of distinct H-2 loci, by demonstrating crossing-over within H-2. Hoecker's serological work achieved great accuracy, earning him an admirable international reputation for reliability and helped pave the way to the forthcoming development of understanding in the field of human histocompatibility antigens and clinical transplantation. He also pioneered efforts to understand the role of H antigens in Medawar's Actively Acquired Tolerance by neonates (Billingham et al., 1953) by investigating their ontogeny, in the late fifties. Together with Olga Pizarro, he demonstrated that the H-2 antigens acquired full phenotypic expression late in intrauterine life, at, or within a few days of birth, depending on the strain (Pizarro et al, 1961; Hoecker and Pizarro, 1961). The nature of the relationship between delayed emergence of the H-2 antigenic expression and immunological tolerance was not, however, precisely established. In the case of hematopoietic tissue transplantation, in addition to the problem of incompatible graft rejection, donor tissue regularly carries reactive cells of the immune system. Such cells produce the syndrome designated by Morten Simonsen as "secondary" or "runt" disease (now, graft-versus-host disease) in recipients of allogeneic hematopoietic tissue (Simonsen, 1957). Interestingly, as also shown by DHW Barnes et al., 1958, F1 hybrid animals given adult hematopoietic transplants from either parental strain, regularly suffer a severe or fatal graft-versus-host reaction, but, in their words, "*The reduction and attenuation of 'secondary disease' which follows the use of foetal myeloid tissue is thought to be due to the acquisition of partial tolerance, by the maturing foetal cells, for host antigens*". Our own interest in cord blood transplantation, thus, stems from the report by Billingham et al., 1953, on acquired tolerance and from additional data reported by Simonsen (1957), DHW Barnes et al. (1958) and Hasek, et al. (1961).

Hoecker's work was, in several ways, very much in the mainstream of the research that led to the discovery of the Major Histocompatibility System of humans (van Rood, 1958; Payne and Rolfe, 1958; Dausset, 1959) now HLA (Human Leukocyte Antigen) and the enormous expansion of clinical transplantation, although this application was not, admittedly, his primary goal. In particular, his work antedated the extensive polymorphism of the HLA antigens

in humans and the realization that, just as in the mouse, HLA antigens are encoded by closely linked genes underlying different functions related to epitope presentation and activation of immune cells (reviewed by Bodmer, 1987). Bodmer (1987) and Benacerraf (1971) saw the complexity of MHC genes and their products as providing molecular clues for the study of the genetic control of immune responses. Initially, this study used chemically defined haptens and progressively more physiologic antigenic groups and permitted approaching the causes for the association of certain diseases with the presence or absence of specific HLA antigens.

Thus, the relation of H-2 and HLA to transplant acceptance or rejection is not only that of being molecular targets for tissue-rejecting cytotoxic alloantibodies and cells, but of presenting diverse kinds of epitopes (including those of alloantigens) for recognition by and clonal expansion of, previously naïve cells of the immune system. The major histocompatibility system is thus not only involved in transplantation, but functions as a major immunological control agency, in Bodmer's words, "a super supergene" (Bodmer, 1978).

CLINICAL TRANSPLANTATION

HLA-matched transplantation has led to solutions for previously intractable medical problems such as the terminal failure of solid organs (kidneys, hearts, livers and lungs) and also of bone marrow. As stated above, because of the extreme polymorphism of HLA loci, which is also ethnically stratified, finding matched donors for patients who need bone marrow transplants has become a worldwide effort. Vast national and international collaborations have permitted finding suitable donors for many patients even for some who have HLA types of vanishingly low frequencies (van Rood, 2007). In the solid organ field the problem exists too. The problem here is organizing a search for donors (largely cadaveric) and using HLA as a fast tool to find pre-registered patients that are matched to them. Originally proposed by van Rood (1967), international cooperation, in multiple ways, has become a necessary and standard tool. For patients with rarer HLA types, hierarchical rules (waiting lists) for access to scarce donors have been set up. (Such information is available in the Web sites of Transplant sharing organizations, such as UNOS, the Scientific Registry of Transplant Recipients, and the Organ Procurement and Transplantation Network).

Bone marrow transplantation is a particularly complicated form of transplantation because, in addition to the usual risk of (host vs.) graft rejection, the immune attack is potentially bilateral. Graft vs. Host reactivity underlies a very serious risk of graft versus host disease (GvHD), which, clinical practice shows, is a more frequent complication of bone marrow allografts than rejection, even with modern immunosuppression and GvHD prophylaxis. Thus, Registries of volunteer bone marrow donors, currently have over 14 million volunteer potential donors (a little less than the populations of Holland, or Chile, each about 16.5 million inhabitants in 2008) and donor recruitment continues. Despite these huge numbers and the fact that sibling donors (25% of whom are HLA-identical-by-descent) and other family members who are HLA-matched (although not

identical-by-descent) contribute importantly to the provision of well matched grafts, many patients in need of bone marrow grafts still cannot find an HLA-matched donor. Almost always, hematopoietic stem cell grafts are needed for patients with very poor prognosis (typically, with malignancies, but also with genetic conditions, like Fanconi anemia and some metabolic diseases, or acquired marrow insufficiencies, such as severe aplastic anemia). Therefore, most of these patients will die unless a suitable donor is found within a reasonably short time (a few months, or weeks, in some cases). Cord blood grafts, frozen and available "off the shelf" are importantly advantageous.

NIMA (maternal non-inherited HLA antigens): a form of Acquired Tolerance? The donor search might be facilitated by the observation that a substantial fraction of kidney patients fail to make antibodies against their maternal HLA antigens, not only those inherited, but, in a surprisingly high proportion of cases, the non-inherited as well. That HLA NIMAs can "condition" some patients so that they tolerate their presence in an incompatible kidney graft, was described by Claas et al., 1988. Because poly-transfused patients tend to become highly sensitized by blood leukocytes and may develop antibodies reactive with many HLA alloantigens, finding a cross-match-negative kidney donor may be extremely difficult. The survey by Claas et al. (1988) showed that 50 percent of highly sensitized patients were cross-match-negative (did not form antibodies) against their NIMAs (non-inherited maternal HLA antigens).

Apart from the obvious clinical implications regarding donor acceptability, Claas' data suggest that a long-lived human counterpart of murine neonatal tolerance may have been disclosed. Such a possibility has also been investigated in bone marrow transplantation and in regard to basic immunobiological mechanisms (van Rood and Class, 1990; van Rood et al., 2002; Burlingham, 2009; van Rood et al., 2005; Mold et al., 2008).

Umbilical cord blood transplantation: The practical utilization of cord blood as donor tissue for HLA matched or mismatched unrelated recipients (Rubinstein et al., 1993, Rubinstein, 2005; Locatelli et al., 2003; Querol et al., 2009) has grown in popularity and currently constitutes a substantial and growing fraction of all marrow transplantation (World Marrow Donor Association, 2009).

Cord blood grafts do have shortcomings, among which low total numbers of cells is the most difficult to overcome. In general, cord blood transplants provide 1/10 the total nucleated cell (TNC) dose of a usual bone marrow graft, except in small children. In comparison with bone marrow transplants, those of cord blood display delayed engraftment, increased probability of early graft failures and higher short-term transplant-related mortality (Rubinstein et al., 1998). However, the probability of severe GvHD is less and this accounts for a higher overall survival for patients at three years post-transplantation, particularly when the cord blood transplants do not present more than 2 HLA antigen mismatches (Eapen et al., 2007). Other advantages include the immediate availability of the frozen cell grafts when needed and a lower probability of transmission of latent infectious organisms (Rubinstein, 2005).

Most likely, 18,000 unrelated cord blood transplants have already been done worldwide, some 3,500 provided by

the National Cord Blood Program of the New York Blood Center as of March, 2010. Outcome data covering three years post-transplant is available in over 90% of cases transplanted with National Cord Blood Program grafts through 2006. These grafts were performed in over 100 different Transplant Centers worldwide since 1993, for different diseases, using different conditioning regimens and GvHD prophylactic routines and with donor selection schemes that varied in the relative importance assigned to histocompatibility and cell dose, the two most important donor variables (Rubinstein et al., 1998; Eapen et al., 2007; Querol et al., 2009; Barker et al., 2010). It is important to note that the degree of HLA typing resolution required in the case of bone marrow transplantation is full allele-level matching for HLA-A, -B, -C and -DR, while for cord blood transplantation matching HLA-A and -B matching only at antigen-level resolution and HLA-DR at allele-level remains the current practice. The lower resolution required for HLA-A and -B and the fact that HLA-C matching has not yet been found to influence the level of polymorphism currently to be considered in cord blood transplantation. Hence, "suitable" unrelated donors are routinely obtained more consistently than with bone marrow (despite the much lower number of typed donors available). Because of the interactions between the independent factors, histocompatibility and cell dose and their combined influence on Transplant-Related Mortality (TRM), our data are shown in Table 1 separated into sets defined by both, and thus split among groups with low, medium and high TNC doses and either 1 or 2 antigen mismatches, considering the three main HLA loci (HLA-A, -B and -DR). Our main conclusions from the data are:

1. We confirm that both the HLA match grade and the cell dose are separately associated with TRM in Cord Blood Transplantation.
2. Two HLA mismatches are associated with increased TRM compared to one, (RR = 1.9 vs. 1.7) independently of the cell dose.
3. In the presence of two HLA mismatches, graft TNC doses < 4.9 X 10⁷/Kg are associated with significantly higher TRM and should not be preferred when a better combination of match and cell dose is available.
4. A better HLA match and a higher cell dose reduce TRM rates, compared to lesser ones, for patients with HLA mismatches.

In clinical comparisons with other sources of hematopoietic stem cells, cord blood does quite well. In a recent study in children with acute leukemia (in cooperation with the International Bone Marrow Transplant Registry) (Eapen et al., 2007) we found that recipients of fully matched cord blood transplants had a lower incidence of TRM than recipients of bone marrow fully matched at allele level: relative risk = 0.26 (2/26 vs. 24/116), as well as lower relapse: relative risk = 0.68 (11/35 vs. 45/116) and fewer treatment failures: relative risk = 0.67 (13/35 vs. 45/116). Five-year leukemia-free survivals were: for allele-level fully-matched bone marrow, 38%; while for matched cord blood: 60% and for 1 antigen mismatched cord blood with more than 5 X 10⁷ nucleated cells, 45%. Because of the relatively low numbers of cord blood recipients of well-matched and high-cell dose grafts and the multiple comparisons tested, the results of these multivariate comparisons were not statistically significant.

Table 1

Interaction between HLA and Cell Dose IN cord blood transplants: (Single CB Transplants with outcome data, N = 1667)

Number of Mismatches	TNC Dose Range x 10 ⁷	Transplant Related Mortality @ 1 yr.	Relative Risk	P
2	< 2.5	74%	1.9	<0.001
1	< 2.5	65%	1.7	0.002
2	2.5-4.9	58%	1.4	0.009
1	2.5-4.9	48%	0.96	0.8
2	> 5	41%	Reference	
1	> 5	34%	0.8	0.091

Notes:

Patient-Donor Match HLA Level: CB units and patients were typed for HLA-A, -B, and -DRB1 using serological and DNA-based methods. Match grades for HLA-A and -B were assigned at low-intermediate resolution level (antigen level) and for DRB1 at high resolution (allele level) and expressed as mismatched for 0 (matched), 1, or 2 antigens.

Transplant-Related Mortality (TRM) is the count of patients who died after transplantation while in remission.

Statistical Analysis. Cox (or "proportional hazards") Regression performed with SPSS (2004)

(A full report on these data has just appeared on line (Barker et al., 2009)

Data in adults, from both our Program together with IBMTR (Laughlin et al., 2004) and from Eurocord (Rocha et al., 2004) indicate that the overall differences between the outcomes from cord blood and bone marrow transplantations are becoming smaller and that long term survival is improving markedly for both types of grafts recently, in part due to improved HLA typing methodology but also from other, still not well defined, causes.

HLA and the F1 effect (unidirectional mismatches): Current efforts to further improve the stem-cell transplantation of patients with these dread diseases, include the F1-anti-parent effect, discovered almost a century ago in the tumor transplantation field: when F1 hybrid animals between two H-2-different inbred strains of mice receive grafts from either parental strain donor: the recipient is unable to reject them (Little and Tizzer, 1916; AD Barnes and Krohn, 1957).

When the grafts are hematopoietic tissue or simply immune system cells, the lack of rejection allows the grafted tissue to mount an immune response against the host, resulting in potentially lethal graft-versus-host disease. When the parental inbred strain tissue donors (H-2 homozygous) are embryonic or neonatal, however, their immunological "immaturity" reduces the impact of graft-versus-host disease against the heterozygous recipients, which may, thus, engraft and remain H-2-chimeric and free of graft-versus-host disease. For this reason, we retroactively explored the results of HLA-homozygous donor cord blood grafts to heterozygous recipients (I.E., AA→AB) as the sole HLA-mismatch (GVH-only mismatches) (Table 2). Consequently, both HLA mismatching and the direction of mismatching affect the relative risks (RR) and the

significance (p) for clinical endpoints. The directional effect is particularly interesting in the case of leukemic relapse, where rejection-only mismatches increase the probability of relapse (compared to bilateral mismatches) with a P > 0.001; while GvHD-only mismatches lead to significant clinical improvement in engraftment and lower mortality. Because the numbers of homozygotes are small, the P values usually don't differ significantly for transplants with two mismatches from the reference value (with one mismatch) although they display a trend towards significant clinical improvement.

It is important to note that, because ~20% of donors in our inventory are homozygous for one or more HLA antigens, the probability of finding donors who are HLA mismatched only by lacking one (or more) recipient antigen(s), is not too small. Overall, accepting GvHD-only, one-way, mismatches would almost double the chance of finding donor grafts that perform as well as fully matched ones and would yield improved survival data. Furthermore, avoiding mismatches for HLA loci that are homozygous in leukemic patients (rejection-only mismatches) would also reduce significantly (and dramatically) the probability of relapse.

Non-inherited maternal antigens (NIMA): Another potentially most important modification of outcomes by immunogenetic effects is the demonstration that the already mentioned NIMA effect is operational in cord blood transplantation. Thus, NIMA matched HLA mismatches (antigens shared by the graft donor's mother and the recipient, but absent from the donor and the graft itself) result in remarkable reductions or absence of the cord blood's reactivity to maternal non-inherited HLA antigens present in the recipient. In collaboration with van Rood's group, the National Cord Blood Program of the New York Blood Center has encountered evidence that a defined HLA mismatch between donor and recipient may not decrease engraftment or survival and thus, be "permissible", when the mismatched HLA antigen of the recipient was present in the donor's mother's HLA type as a NIMA (van Rood et al., 2009). In this clinical situation, a transplant where the one- or two-HLA mismatch co-existed with a NIMA match, had significantly faster engraftment (p=0.043) and better survival: transplant-related mortality (TRM) (p=0.012) and overall survival (p=0.029), especially in patients >10 years old. The "protection" conferred by a single NIMA-match against decreased patient survival in transplants with two HLA mismatches was less than in those with a single HLA mismatch and similar to those of patients with a single HLA mismatch and no NIMA match (van Rood et al., 2009). The probability of these differences is currently not very strong, as only small numbers of patients happened to have been transplanted with HLA mismatches that were NIMA matches. The study was done (and transplant NIMA-status determined) retrospectively, because not all mothers had been HLA-typed up front. The number of transplants with NIMA-matched donors was, therefore, much smaller than it would have been if the HLA type of all mothers (and thus the pre-transplant ascertainment of NIMAs) had been part of the transplant selection criteria. The results are just sufficiently strong to support the proposal of a prospective trial in which donor NIMA information is used in the selection of the best possible cord blood match for patients.

Table 2

Cord blood Transplants with bidirectional or unidirectional HLA mismatches (MM): endpoint analyses by Cox Regression.
(See notes below)

A. Engraftment by Day 77: [RR>1 = higher chance of engraftment]			
A.1 Presence or absence of HLA Mismatch:			
Reference = 1 Mismatch		(N = 358).	
Test groups	0 MM	(N = 54):	RR: 1.4, P = 0.036
	2/3 MM	(N = 550/62):	RR and P, NS
A.2 Direction of HLA Mismatch:			
Reference = Bidirectional Mismatch		(N = 893)	
Test Groups	GvHD Only	(N = 45):	RR: 1.4, P = 0.050
Rejection Only	(N = 32)	RR and P, NS	
B. Acute Grade 3-4 GvHD by Day 150: [RR<1 = lower chance of grade 3-4 GvHD]			
B.1 HLA Mismatch:			
Reference = 1 Mismatch		(N = 344)	
Test Groups	0 MM	(N = 54):	RR: 0.1 P = 0.004
	2/3 MM	(N = 533/60)	RR and P, NS
B.2 Direction of HLA Mismatches:			
Reference = Bidirectional		(N = 859)	
Test Groups	GvHD/Rejection	(N = 46/32)	RR and P, NS
C. Transplant Related Mortality (First Five Years): [RR<1 = lower chance of TRM]			
C.1 HLA Mismatch:			
Reference = 1 Mismatch		(N = 379)	
Test Groups	0	(N = 58):	RR: 0.5, P = 0.011
	2/3 MM	(N = 570/66)	RR and P > NS
C.2 Direction of HLA Mismatches:			
Reference = Bidirectional		(N = 933) [
Test Groups	GvHD/Rejection	(N = 47/35)	RR and P = NS
D. Relapse Rate Over Five Years in 693 Patients with Leukemia or Lymphoma: [RR>1 = higher chance of relapse]			
D.1 HLA Mismatch:			
Reference = 1 Mismatch		(N = 220)	
Test Groups	0/2 or 3 MM	(N = 32/395/46),	RR and P = NS
D.2 Direction of HLA Mismatches:			
Reference = Bidirectional		(N = 617)	
Test Groups	GvHD Only	(N = 24),	RR and P = NS
Rejection Only		(N = 20),	RR = 3.0, P < 0.001
E. Overall Mortality @ Five Years post-Transplant: [RR<1 = lower chance of death]			
E.1 HLA Mismatch:			
Reference = 1 Mismatch		(N = 380)	
Test Groups	0 MM	(N = 58),	RR = 0.5, P = 0.011
	2/3	(N = 570/66),	RR and P = NS
E.2 Direction of HLA Mismatches:			
Reference = Bidirectional		(N = 934)	
Test Groups	GvHD Only	(N = 47),	RR = 0.6, P = 0.025
Rejection Only		(N = 35),	RR and P = NS

Notes.

Endpoints:

A. Time to myeloid engraftment was defined by the first of 3 consecutive days of absolute donor-type neutrophil cell blood count (ANC) $\geq 500/\mu\text{L}$.

B. Acute GVHD grade 3-4 (categorized by transplant center assessment)

The probability and relative risk of acute GVHD was assessed only in engrafted

C. Transplant-Related Mortality (TRM) (See Note to Table 1)

D. Relapse rate reflects the proportion of cases with re-occurrence of leukemia after transplantation.

E. Overall mortality includes deaths from all causes.

Statistical analysis by Cox Regression: RR (Relative Risk) and P (probability) are highlighted in Table II when data has $p < 0.05$.

In practical terms, if the information obtained in the small proportion of mismatched transplants that had a NIMA for a mismatched HLA antigen were confirmed and could be used prospectively, the number of better matched grafts available would increase by five- to ten-fold. The reason is that any potential HLA mismatch for which the donor has a NIMA match (say, patient HLA-A1, -A2; donor -A1, -A3; but where the donor's mother's type was HLA-A2, -A3) would be a "permissible mismatch". Because any recipient's HLA mismatched antigen could be present in a given donor's mother as a NIMA, disclosing the maternal NIMA haplotype would identify NIMA matches for any single A, B or DR mismatch. Then, NIMAs would extend dramatically the probability of finding appropriate donors for patients within relatively small donor panels, as it is likely to be the case for ethnic minority patients. The potentially multiplicative effect of NIMA on the probability of ascertaining excellent donors promises to be a further major contribution of Immunogenetics research to Medicine by accelerating the attainment of sufficiently large donor panels for graft selection (and reduce the cost of such panels). More importantly, it will also contribute to understanding the biology of mother-child immunological influences during pregnancy, by investigating the molecular specificity of the tolerogenic interactions that take place.

CONCLUSIONS

The use of immunogenetics technology in the definition of the broad mechanisms of transplant acceptance and rejection and their dependence on histocompatibility matching, has provided very important improvements on the probability of success in clinical transplantation. This short review of issues relevant to selecting unrelated donors for patients needing transplants, attempts to highlight the contribution that basic science has already had. The remarkable insights of the pioneers whose contributions built the current clinical matching capabilities, have led to our capability of selecting matched donors and also to the identification of mismatched donors whose mismatches are "permissible" (in the context of current immunosuppressive therapies). The latter may improve the odds of finding donors appropriate to specific recipients by virtue of NIMA influences and at least for cord blood, by advantageously utilizing unidirectional mismatches with donors homozygous at one of more HLA loci. After their confirmation and broad application in donor selection, cord blood could revolutionize the clinical results of hematopoietic transplantation and extend access to many more patients. This superior contribution to Medicine and public health will additionally help define the mechanisms and consequences of the polymorphism of major histocompatibility systems in the development of self-non-self immune discrimination.

ACKNOWLEDGMENTS

The original results described in this review have been obtained by the staff of the National Cord Blood Program from information relayed back by more than 100 Transplant Centers, worldwide. Their invaluable collaboration in producing the grafts, sharing clinical data and performing analyses is gratefully acknowledged.

REFERENCES

- BARKER JN, SCARADAVOU A, STEVENS CE (2009) Combined effect of total nucleated cell dose and HLA-match on transplant outcome in 1061 cord blood recipients with hematological malignancies. *Blood* (on-line pre-publication December 22, 2009 doi:10.1182/blood-2009-07-231068). Printed (2010) *Blood* 115: 1843-1849.
- BARNES AD, KROHN PL (1957) The estimation of the number of histocompatibility genes controlling the successful transplantation of normal skin in mice. *Proc R Soc B* 146: 505-26.
- BARNES DWH, ILBERY PLT, LOUTIT JF (1958) Avoidance of secondary disease in radiation chimaeras. *Nature* 181: 488.
- BENACERRAF B (1971) The role of MHC gene products in immune regulation and its relevance to alloreactivity. pp: 597-623, In: JAN LINDSTEN (ed) (1992) Nobel Lectures, Physiology or Medicine 1971-1980. World Scientific Publishing Co., Singapore.
- BILLINGHAM RE, BRENT L, MEDAWAR PB (1953) "Actively acquired tolerance" of foreign cells. *Nature* 172: 603-606.
- BODMER WF (1978) HLA: a super supergene. *Harvey Lect* 72:91-138.
- BODMER WF (1987) The HLA system: structure and function. *J Clin Pathol* 40: 948-958.
- BURLINGHAM WJ (2009) A lesson in tolerance maternal instruction to fetal cells. *N Engl J Med* 360: 1355-1357.
- CLAAS FH, GIJBELS Y, VAN DER VELDEN-DE MUNCK J, VAN ROOD JJ (1988) Induction of B cell unresponsiveness to noninherited maternal HLA antigens during fetal life. *Science* 241: 1815-1817.
- DAUSSET J (1959) Iso-leuco-anticorps. *Acta Haematol* 20: 156-66.
- EAPEN M, RUBINSTEIN P, ZHANG MJ, STEVENS C, KURTZBERG J, SCARADAVOU A, LOBERIZA FR, CHAMPLIN RE, KLEIN JP, HOROWITZ MM, WAGNER JE (2007) Comparison of Outcomes after Transplantation of Unrelated Donor Umbilical Cord Blood and Bone Marrow in Children with Acute Leukemia. *Lancet* 369: 1947-1954.
- GORER PA (1936) The detection of hereditary differences in the blood of mice by means of human group A serum. *J Genetics* 32: 17-32.
- GORER PA (1938) The genetic and antigenic basis of tumour transplantation. *J Path Bact* 44: 691-697.
- GORER PA (1938) The antigenic basis of tumour transplantation. *J Path Bact* 47: 231-252.
- GORER PA, LYMAN S, SNELL GD (1948) Studies on the genetic and antigenic basis of tumour transplantation. *Proc R Soc B* 135: 499-505.
- HASEK M, LANGEROVA A, HRABA T (1961) Transplantation Immunity and Tolerance. In: TALIAFERRO WH and HUMPHREY JH (eds) *Advances in Immunology*. Academic Press. pp: 1-66.
- HOECKER G (1986) Recollections of a Chilean member of the very exclusive H-2 club. *Immunogenetics* 24: 345-9.
- HOECKER G, PIZARRO O (1961) The Histocompatibility Antigens. In: CRISTOFFANINI AP, HOECKER G (eds) *Proceedings of International Symposium on Tissue Transplantation* (1962). Universidad de Chile. pp: 54-71.
- LAUGHLIN ML, EAPEN M, RUBINSTEIN P, WAGNER JE, ZHANG MJ, CHAMPLIN RE, STEVENS C, BARKER JN, GALE RP, LAZARUS HM, MARKS DI, VAN ROOD JJ, SCARADAVOU A, HOROWITZ MM (2004) Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 351: 2265-2275.
- LITTLE CC (1914) A possible mendelian explanation of a type of heredity apparently non-mendelian in nature. *Science* 40: 904-906.
- LITTLE CC, TIZZER EE (1916) Further experimental studies on the inheritance of susceptibility to a Transplantable tumor, Carcinoma (J. W. A.) of the Japanese waltzing Mouse. *J Med Res* 33: 393-453.
- LOCATELLI F, ROCHA V, REED W, BERNAUDIN F, ERTEM M, GRAFAKOS S, BRICHARD B, LI X, NAGLER A, GIORGIANI G, HAUT PR, BROCHSTEIN JA, NUGENT DJ, BLATT J, WOODARD P, KURTZBERG J, RUBIN CM, MINIERO R, LUTZ P, RAJA T, ROBERTS I, WILL AM, YANIV I, VERMYLEN C, TANNOIA N, GARNIER F, IONESCU I, WALTERS MC, LUBIN BH, GLUCKMAN E (2003). Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood* 101: 2137-2143.
- MOLD JE, MICHAELSSON J, BURT TD, MUENCH MO, BECKERMAN KP, BUSCH MP, LEE TH, NIXON DF, MCCUNE JM (2008) Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science* 322: 1562-1565.
- PAYNE R, ROLFE MR (1958) Fetomaternal leukocyte incompatibility. *J Clin Invest* 37: 1756-1763.

- PIZARRO O, HOECKER G, RUBINSTEIN P, RAMOS A (1961) The distribution in the tissues and the development of H-2 antigens in the mouse. *Proc Natl Acad Sci* 47: 1900-1906.
- QUEROL S, RUBINSTEIN P, MARSH SG, GOLDMAN J, MADRIGAL JA (2009) Cord Blood Banking: 'providing cord blood banking for a nation'. *Br J Hematol* 147: 227-235.
- ROCHA V, LABOPIN M, SANZ G, ARCESE W, SCHWERDTFEGER R, BOSI A, JACOBSEN N, RUUTU T, DE LIMA M, FINKE J, FRASSONI F, GLUCKMAN E (2004) Transplants of Umbilical-Cord Blood or Bone Marrow from Unrelated Donors in Adults with Acute Leukemia. *N Engl J Med* 351: 2276-2285.
185. RUBINSTEIN P, ROSENFELD RE, ADAMSON JW, STEVENS CE (1993) Stored placental blood for unrelated bone marrow reconstitution. *Blood*, 81: 1679-1690.
- RUBINSTEIN P (2005) Transplante de células troncales de sangre de cordón umbilical de donantes no relacionados: tecnología y resultados clínicos. In: PALOMO I, PEREIRA J, PALMA J (eds) *Hematología: Fisiopatología y Diagnóstico*. Universidad de Talca, Chile. pp: 417-432.
- RUBINSTEIN P, CARRIER CM, SCARADAVOU A, KURTZBERG J, ADAMSON J, MIGLIACCIO AR, BERKOWITZ RL, CABBAD M, DOBRILA NL, TAYLOR PE, ROSENFELD RE, STEVENS CE (1998) Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *New Engl J Med* 339: 1565-1577.
- SIMONSEN, M (1957) The impact on the developing embryo and newborn animal of adult homologous cells. *Acta Pathol Microbiol Scand* 40: 480-500.
- SNELL GD (1948) Methods for the study of histocompatibility genes. *J Genet* 49:87-108. SPSS® 13 Statistical Software (2004) SPSS Inc., Chicago, IL
- VAN ROOD JJ (1967) A proposal for international cooperation in organ transplantation: Eurotransplant. In: CURTONI ES, MATTIUZ PL, TOSI RM (eds) *Histocompatibility Testing 1967*. Munksgaard, Copenhagen. pp: 451-452.
- VAN ROOD JJ, EERNISSE JO, VAN LEEUWEN A (1958) Leucocyte antibodies in sera from pregnant women. *Nature* 181:1735-6.
- VAN ROOD JJ, CLAAS FH (1990) The influence of allogeneic cells on the human t and B cell repertoire. *Science* 248: 1388-93.
- VAN ROOD JJ, LOBERIZA FR, ZHANG MJ, OUDSHOORN M, CLAAS F, CAIRO MS, CHAMPLIN RE, GALE RP, RINGDEN O, HOWS JM, HOROWITZ MH (2002) Effect of tolerance to noninherited maternal antigens on the occurrence of graft-versus-host disease after bone marrow transplantation from a parent or an HLA-haploidentical sibling. *Blood* 99: 1572-157.
- VAN ROOD JJ, ROELEN DL, CLAAS FH (2005) The effect of noninherited maternal antigens in allogeneic transplantation. *Semin Hematol* 42: 104-111.
- VAN ROOD JJ, OUDSHOORN M (2008) Eleven million donors in Bone Marrow Donors Worldwide! Time for reassessment? *Bone Marrow Transplant* 41: 1-9.
- VAN ROOD JJ, STEVENS CE, SMITS J, CARRIER C, CARPENTER C, SCARADAVOU A (2009) Reexposure of cord blood to noninherited maternal HLA antigens improves transplant outcome in hematological malignancies. *Proc Natl Acad Sci USA* 106:19952-7.
- WORLD MARROW DONOR ASSOCIATION (2009) Cord Blood Banks/Registries Annual Report 2008 (<http://www.worldmarrow.org>)

