Host natural killer immunity is a key indicator of permissiveness for donor cell engraftment in patients with severe combined immunodeficiency☆

Amel Hassan, MD,[a] Pamela Lee, MD,[b] Paraskevi Maggina, MD,[c] Jin Hua Xu, BSc,[a] Diana Moreira, MD,[c] Mary Slatter, MBBS,[d] Zohreh Nademi, PhD,[c] Austen Worth, BMBCh,[a] Stuart Adams, PhD,[a] Alison Jones, MBBCh,[a] Catherine Cale, MBBS,[a] Zoe Allwood, MPH,[a] Kanchan Rao, MBBS,[a] Robert Chiesa, MD,[a] Persis Amrolia, MBBS,[b] Hubert Gaspar, MBBS,[b] E. Graham Davies, MBBChir,[b] Paul Veys, MBBS,[a] Andrew Gennery, MBBS,[d] and Waseem Qasim, MBBS[b] *London and Newcastle upon Tyne, United Kingdom*

Background: Severe combined immunodeficiency (SCID) can be cured by using allogeneic hematopoietic stem cell transplantation, and the absence of host immunity often obviates the need for preconditioning. Depending on the underlying genetic defect and when blocks in differentiation occur during lymphocyte ontogeny, infants with SCID have absent or greatly reduced numbers of functional T cells. Natural killer (NK) cell populations are usually absent in the SCID-X1 and Janus kinase 3 forms of SCID and greatly reduced in adenosine deaminase deficiency SCID but often present in other forms of the disorder.
Objective: To determine if SCID phenotypes indicate host permissiveness to donor cell engraftment.
Methods: A retrospective data analysis considered whether host NK cells influenced donor T-cell engraftment, immune reconstitution, and long-term outcomes in children who had undergone nonconditioned allogeneic stem cell transplantation between 1990 and 2011 in the United Kingdom. Detailed analysis of T- and B-cell immune reconstitution and donor chimerism was compared between the NK^+ (n = 24) and NK^- (n = 53) forms of SCID.
Results: Overall, 77 children underwent transplantation, with survival of 90% in matched sibling donor/matched family donor transplants compared with 60% when alternative donors were used. Infants with NK^-SCID were more likely to survive than NK^+ recipients (87% vs 62%, $P < .01$) and had high-level donor T-cell chimerism with superior long-term recovery of CD4 T-cell immunity. Notably, 33% of children with NK^+SCID required additional transplantation procedures compared with only 8% of children with NK^-SCID ($P < .005$).
Conclusions: NK^-SCID disorders are highly permissive for donor T-cell engraftment without preconditioning, whereas the presence of NK cells is a strong indicator that preparative conditioning is required for engraftment of T-cell precursors capable of supporting robust T-cell reconstitution. (J Allergy Clin Immunol 2014;133:1660-6.)

Key words: *Severe combined immunodeficiency, conditioning, natural killer cells, chimerism, engraftment, adenosine deaminase deficiency*

Severe combined immunodeficiencies (SCIDs) are a heterogeneous group of genetic disorders with common clinical phenotypes presenting in early infancy with serious or recurrent infections and failure to thrive. For more than 40 years, allogeneic hematopoietic stem cell transplantation (allo-SCT) has provided curative therapy for these disorders.[1] There has been longstanding debate and controversy around how and when to best perform transplantations in infants given a diagnosis of SCID.[2] Conventionally, conditioning comprising myeloablative or submyeloablative chemotherapy is used in patients undergoing allo-SCT to both eradicate host cellular immunity and empty the bone marrow niche in readiness for donor stem cell engraftment. In small infants conditioning might carry notable morbidity and mortality, and thus it has been long argued that in the absence of host cellular immunity, sufficient donor cell engraftment can be achieved without ablative conditioning. Some centers strongly advocate infusion of unmanipulated donor grafts, arguing that in the HLA matched family donor (MFD) setting, sufficient T-cell engraftment can be achieved without preconditioning and that this can sustain long-term immune recovery.[3-5] In general, although these patients might not have significant levels of multilineage stem cell engraftment, adequate donor T-cell engraftment in combination with successful seeding of T-cell precursor niches sufficient to maintain thymopoiesis can support long-lived immune recovery. Engraftment of B-cell or myeloid precursors is usually low or absent, and although a number of children will recover antibody production, many will require immunoglobulin replacement therapy for life.[2] In the absence of an HLA identical donor, stringently T cell–depleted mismatched hematopoietic stem cell grafts can also mediate sustained thymopoiesis, although in the absence of mature donor T cells, recovery is slow and takes many months.

Analyses of SCID cohorts undergoing transplantation in Europe and North America[4,6-9] have given rise to the concept of

From [a]the Immunology and Bone Marrow Transplant Units, Great Ormond Street Hospital for Children, London; [b]Cellular & Molecular Immunology, Institute of Child Health, University College London; [c]the Bone Marrow Transplant Unit, Great North Children's Hospital, Royal Victoria Infirmary, Newcastle upon Tyne; and [d]the Institute of Cellular Medicine, University of Newcastle, Newcastle upon Tyne.

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Corresponding author: Waseem Qasim, MBBS, Institute of Child Health, 30 Guilford St, London WC1N 1EH, United Kingdom. E-mail: W.Qasim@ucl.ac.uk.

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Abbreviations used
ADA: Adenosine deaminase deficiency
allo-SCT: Allogeneic hematopoietic stem cell transplantation
JAK3: Janus kinase 3
MFD: Matched family donor
MSD: Matched sibling donor
NK: Natural killer
SCID: Severe combined immunodeficiency
TREC: T-cell receptor excision circle

permissive and nonpermissive host environments, and the presence of host natural killer (NK) immunity might be an important determinant in considering which infants require preconditioning. Aside from donor matching, there are 2 additional and interrelated aspects of host NK immunity that might be critical in determining successful outcomes in these infants. First, innate cellular immunity mediated by macrophages and NK cells might be a barrier against donor cell engraftment.[10] Data from animal studies suggest that NK cell immunity plays a crucial role in the rejection of allogeneic cells, particularly in the HLA-mismatched setting.[11] Second, underlying genetic defects largely determine when lymphoid differentiation arrests during ontogeny, and this in turn directly influences whether niches for T-cell and NK cell precursors are occupied. Thus SCID disorders caused by defective common gamma chain (SCID-X1)[12] and Janus kinase 3 (JAK3) SCID[13] have circulating B cells but no T-cell or NK cell immunity ($T^-B^+NK^-$), reflecting a defect that impedes common T/NK precursor development.

In other SCID disorders in which T-cell developmental arrest occurs at a later stage of differentiation, NK development is unaffected, and presumably the common T/NK precursor niche is occupied, which might result in competition during engraftment. SCID caused by adenosine deaminase (ADA) deficiency usually affects multiple lineages and is associated with NK deficiency (although less profound), and NK cell numbers might recover if children are detoxified with enzyme replacement therapy with PEG-ADA ahead of allo-SCT.

Previous surveys of transplantation outcomes for SCID considered the importance of the SCID phenotype based on the presence or absence of B cells, and T^-B^+ hosts fared better than T^-B^- recipients.[14] The importance of host NK cell immunity was widely suspected, but incomplete recipient characterization precluded detailed analysis. Here we report the United Kingdom experience of nonconditioned allo-SCT for SCID disorders and, for the first time, confirm that the presence of host NK immunity is a key indicator of whether the host SCID environment is likely to be permissive for donor cell engraftment.

METHODS

Patients' characteristics

Between 1990 and 2011, 77 infants (52 male) with SCID underwent allo-SCT without preconditioning at 2 United Kingdom pediatric centers commissioned to undertake such procedures (see Table E1 in this article's Online Repository at www.jacionline.org). All were T-cell deficient, with absent T cells or reduced T-cell numbers with absent or severely impaired proliferation responses to the mitogen PHA.

Age at transplantation was similar for the groups and ranged from 1 week to 17 months, with a median age of 3 months and a median follow-up of 3070 days for the NK^+ group and 3001 days for the NK^- group (range, 760-7300 days).

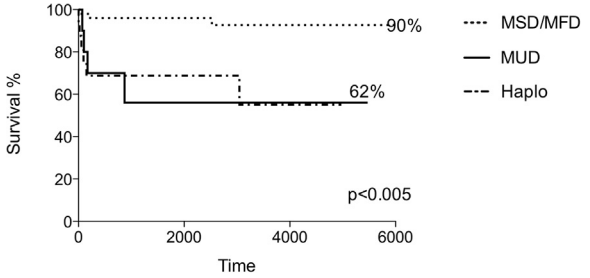

FIG 1. On the basis of donor type, overall survival of infants with SCID after nonconditioned allo-SCT with MSD or MFD donors (49/77 infusions) was 90%, and that after matched unrelated grafts *(MUD)* and haploidentical *(Haplo)* transplantations (28/77 infusions) was 62%.

All children received antimicrobial prophylaxis and immunoglobulin replacement therapy from the time of diagnosis. SCID phenotypes were determined on the basis of flow cytometry for T cells (CD3), B cells (CD19), and NK cells (CD16/CD56) and, where indicated, had defined molecular defects on the basis of abnormal or absent protein expression, metabolite analysis, or genetic mutations. Patients were grouped for analysis based on the presence ($>100 \times 10^6$/L) or absence ($<100 \times 10^6$/L) of NK cells at the time of transplantation. This threshold reflects the lower limit of normal for age range of NK cell numbers in healthy infants. Bone marrow or cord blood grafts were infused (n = 49) in the matched sibling donor (MSD) or fully HLA MFD setting. Bone marrow or cord blood was also infused from 11 HLA-matched unrelated donors. Haploidentical grafts were enriched for stem cells by using CliniMacs CD34 selection (n = 17). No prophylaxis against graft-versus-host disease was given to 35 infants, and the remainder received cyclosporine alone or in combination with mycophenolate mofetil, prednisolone, or both.

Event-free survival was defined as survival without resorting to second procedures, and where indicated, second procedures were undertaken with conditioning. Details are provided in Table E1, where second procedures are highlighted in gray below data for the primary infusion.

Engraftment and chimerism

Whole blood, granulocyte, or mononuclear cells were subjected to chimerism analysis in all subjects. Where indicated, lineage-specific chimerism was also determined for CD3 (T cells), CD15 (myeloid lineage), and CD19 (B cells) after magnetic bead selection from peripheral blood by using an AutoMACS Pro-Separator (Miltenyi Biotec, Bergisch Gladbach, Germany). The PowerPlex 16 system (Promega, Southampton, United Kingdom) was used to PCR amplify 16 short tandem repeat loci in these patient samples. The PCR products were then analyzed by using an AB3130 Genetic Analyser with Gene Mapper v4.0 software (Life Technologies, Carlsbad, Calif).

Statistical methods

Kaplan-Meier curves were used to analyze survival figures. The log-rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests were used to compare survival between different groups. Logistic regression was performed with SPSS software (SPSS, Chicago, Ill) to identify determinants of survival after hematopoietic stem cell transplantation in different groups.

RESULTS

Survival of infants with SCID after allo-SCT

Overall survival after nonconditioned allo-SCT procedures was 81%, with 90% survival after MSD or MFD infusions (49/77 procedures, Fig 1). Matched unrelated grafts and haploidentical transplantations (28/77 procedures) had less favorable outcomes, with survival of 62% in both groups. For comparison, over the same period, overall survival after conditioned allo-SCT for SCID was 72% (n = 148), although confounding differences in donor type and graft sources prevented controlled analysis against