© 2003 American Society for Blood and Marrow Transplantation
1083-8791/03/0905-0001$30.00/0
doi:10.1016/S1083-8791(03)00086-7

# Antigen-Specific Immune Function after Hematopoietic Stem Cell Transplantation

*Robertson Parkman*

Division of Research Immunology/Bone Marrow Transplantation, Children's Hospital Los Angeles; Department of Pediatrics, Keck School of Medicine/University of Southern California, Los Angles, California

Correspondence and reprint requests: Robertson Parkman, MD, Division of Research Immunology/Bone Marrow Transplantation, Children's Hospital Los Angeles, 4650 Sunset Blvd, Los Angeles, CA 90027
(e-mail: rparkman@chla.usc.edu).

Received March 2, 2003; accepted March 7, 2003

## ABSTRACT

**Hematopoietic stem cell recipients are characterized by an immunodeficiency of varying severity and duration. The present review focuses on the antigen-specific function of recipients with the hypothesis that the acquisition of antigen-specific function is predictive of the recipient's capacity to resist lethal infection with environmental pathogens.**



## KEY WORDS

Thymopoiesis • T-cell excision circle • Antigen-specific function • Graft-versus-host disease • Immunodeficiency

## INTRODUCTION

The first organized effort to evaluate antigen-specific immune function after hematopoietic stem cell transplantation (HSCT) was the assessment of patients with chronic graft-versus-host disease (GVHD) [1,2]. Because normal individuals do not have any pre-existing immunity to bacteriophage, patients were immunized with bacteriophage ϕX174. Patients with chronic GVHD showed (1) a reduced primary IgM antibody response and (2) reduced immunoglobulin class switching to IgG antibody compared with long-term HSCT recipients without chronic GVHD. When patients with chronic GVHD were immunized with a pneumococcal carbohydrate vaccine, they did not have significant antibody titers. Thus, recipients with chronic GVHD had defects in their ability to respond to both a neo-antigen (ϕX174) as well as to a recall antigen (pneumococcal carbohydrate). The transplantation community, however, did not undertake additional evaluations of the post-HSCT antigen-specific immune function in patients without chronic GVHD because it was felt that the immune deficiency present in recipients with chronic GVHD was only part of the myriad of complications associated with chronic GVHD. Furthermore, because most patients with chronic GVHD were on immunosuppressive therapy, the ongoing immunosuppression could contribute to the patients' reduced antigen-specific immune responses.

The current interest in post-HSCT immune reconstitution was generated in part by the observations that the adult recipients of unrelated HSCT, who did not have chronic GVHD and were not on immunosuppression, were as likely to have severe infections as recipients who had chronic GVHD [3]. Thus, the adult recipients of unrelated HSCT had an increased likelihood of severe infections regardless of their chronic GVHD status. In addition, the Memorial-Sloan Kettering group reported that the adult recipients of unrelated HSCT were more likely to have life-threatening infections than the adult recipients of related transplants and that all adult recipients of HSCT, whether unrelated or related, were more likely to have life-threatening infections than children [4]. Thus, the recipients of unrelated transplants, especially adults, had significant deficits in their immune reconstitution after HSCT that left them at risk of severe and potentially life-threatening infections.

During the last decade, a significant effort has been made to evaluate the immune reconstitution of HSCT recipients. In addition to evaluating cellular and humoral antigen-specific function, the assessment of post-HSCT immune function has been aided by

the development of new techniques to quantify thymopoiesis and define antigen-specific populations [5-8]. Using a combination of immunophenotypic analysis, assessment of thymopoiesis, tetramer analysis, and antigen-specific function, we now have a clearer picture of post-HSCT immune reconstitution. This review focuses on our current knowledge of post-HSCT antigen-specific immune function. The preclinical and clinical attempts to improve immune reconstitution after HSCT will not be part of this review.

## INITIAL OBSERVATIONS

The first coordinated assessment of immune reconstitution after HSCT was in infants transplanted for severe combined immune deficiency (SCID) [9,10]. The assessments were first performed when monoclonal antibodies for the identification of T and B lymphocytes did not exist. Nevertheless, it was shown that PHA-responsive cells of donor origin (T lymphocytes) were detectable within 3 weeks after successful histocompatible HSCT. However, normalization of immune function took up to a year after HSCT with an initial inability of the new immune system to reject third-party skin grafts and produce normal levels of immunoglobulins. In light of our current understanding of T lymphocyte differentiation, it is likely that the T lymphocytes seen early after HSCT were derived from mature donor–derived T lymphocytes or committed lymphoid progenitors rather than HSCs [11].

With the development of monoclonal antibodies, immune reconstitution after HSCT could be further defined. The early emergence of natural killer cells generally was seen followed by the appearance of CD8 and then CD4 T lymphocytes [12-14]. Immunophenotypic B lymphocytes were present in normal numbers by 1 to 2 months after transplantation, although there was an increased proportion that was CD5 positive [15]. When techniques for successful T cell–depleted HSCT were developed, it became possible to separate the contribution of the T lymphocytes present in the HSC product from the T lymphocytes derived from donor HSC. After the T cell–depleted HSCT of infants with SCID, who received pretransplant chemotherapy, no immunophenotypic T lymphocytes were seen until 2 to 3 months after transplantation. This time frame is similar to that seen in fetal lymphoid ontogeny when T lymphocytes are not seen in the peripheral fetal circulation until 12 weeks of gestation [16]. These observations support the hypothesis that it takes 12 weeks for HSC to engraft, migrate to the thymus, undergo effective thymopoiesis, and produce detectable levels of circulating T lymphocytes. As in the case of fetal lymphoid ontogeny, the first immunophenotypic T lymphocytes are not functional and are unable to respond to stimulation with either mitogens (PHA) or specific antigens. After HSCT there is the sequential appearance of T lymphocytes capable of being activated by mitogenic stimulation but incapable of secreting interleuklin-2 (IL-2), followed by PHA responsive lymphocytes capable of IL-2 production [17]. The same hierarchy is seen for responses to specific antigens: first, antigen-specific T lymphocytes capable of being activated by specific antigen but incapable of producing IL-2 are followed by the emergence of antigen-specific T lymphocytes capable of IL-2 (and other cytokines) production after antigen-specific stimulation [18]. Soon after HSCT peripheral T lymphocytes with immunophenotypes only found in the adult thymus or the fetal peripheral blood can be detected [19].

## T-CELL–REPLETE HSCT

When non–T cell-depleted HSC products are utilized, immunophenotypic T lymphocytes of donor origin can be detected within 2 to 3 weeks after transplantation with bone marrow (BM) indicating that either mature T lymphocytes or committed lymphoid progenitors are responsible for early T lymphocyte reconstitution. Over the last decade, the use of mobilized peripheral blood cells (PBC) as a source of HSC has come into common usage. Significant differences exist between the rapidity and the nature of immune reconstitution after HSCT with PBC compared with BM. After HSCT with BM, antigen-specific T lymphocyte function as measured by antigen-specific in vitro blastogenesis is not detected until patients are re-immunized (to common vaccination antigens) or there is reactivation of endogenous DNA viruses (cytomegalovirus [CMV], varicella zoster virus [VZV], herpes simplex virus [HSV]). In the recipients of PBC, the sustained presence of antigen-specific blastogenesis is detected throughout the post-HSCT period including the responses to both vaccines (tetanus toxoid) and endogenous DNA viruses [20]. However, the sustained presence of donor-derived antigen-specific T lymphocytes has not resulted in a decrease in post-HSCT viral and fungal infections [21]. Part of the differences between the immune reconstitution in the recipients of PBC compared with BM is that 10 times as many immunophenotypic T lymphocytes are contained in PBC product compared with BM. There also may be differences in the state of activation of the T lymphocytes present in the PBC because of G-CSF stimulation, which results in the eschewing of immune responses to a Th2 type [22]. Activation-induced apoptosis of T lymphocytes is present after HSCT and may play a role in determining post-HSCT immune function [23]. The presence of activation-induced apoptosis after HSCT of BM has an inverse correlation with the rapidity of CD4 T lymphocyte reconstitu-

tion. Thus, patients with higher levels of activation-induced apoptosis have lower CD4 counts 6 months after HSCT. An unresolved question is whether granulocyte colony-stimulating factor (G-CSF) stimulation changes the sensitivity of the transplanted T lymphocytes to activation-induced apoptosis.

Histocompatible or alternative donor recipients with an absence of acute or chronic GVHD have antigen-specific T lymphocyte function by 6 to 12 months after HSCT to environmental DNA antigens and are able to respond appropriately to immunization with vaccines such as tetanus toxoid and inactivated polio virus [24-27]. However, patients with significant GVHD have delays in the development of normal antigen-specific T lymphocyte function. What was initially unclear was whether the lack of antigen-specific T lymphocyte function was caused by an absence of naive T lymphocytes or the dysfunction of the existing T lymphocytes. The development of immunophenotypic and chemical methods to assess human thymopoiesis has given new insights into post-HSCT immune reconstitution [5,6]. The immunophenotype of recent thymic emigrants was determined to be $CD4^+$, $CD45RA^+$. Although an absolute correlation between the immunophenotype and thymopoiesis is now questioned, the immunophenotype was a valuable tool to assess post-HSCT thymopoiesis. Recipient age inversely correlated with the absolute number of immunophenotypic thymic emigrants, paralleling the results in normal individuals [28,29]. Thus, older HSCT recipients had a reduced capacity to produce new T lymphocytes compared with younger patients. In addition, recipients with a history of acute or chronic GVHD had reduced numbers of recent thymic emigrants compared with patients without acute or chronic GVHD.

## T CELL EXCISION CIRCLE ANALYSIS

Because of the uncertainty as to whether all $CD4^+$, $CD45RA^+$ cells were recent thymic emigrants, the T cell excision circle (TREC) assay has represented a major step forward in the assessment of post-HSCT immune reconstitution [5,6]. TREC are episomal DNA. Thus, as peripheral T lymphocytes divide, the frequency of TREC-positive cells decreases as the number of cell division increases. In normal individuals, the frequency of TREC-positive cells in the thymus remains constant throughout life. Thus, on a per-cell basis, the capacity of the thymus to make new T lymphocytes is maintained. However, the absolute number of thymocytes produced decreases with age. Therefore, to maintain a constant number of peripheral T lymphocytes, increased extrathymic T lymphocyte proliferation is required resulting in a decreased frequency of TREC-positive cells.

When HSCT recipients were assessed for the frequency of TREC-positive cells, the results paralleled those previously reported in the immunophenotypic analyses of recent thymic emigrants ($CD4^+$,$CD45RA^+$) [6,30]. The frequency of $CD4^+$,$CD45RA^+$ cells correlated with the frequency of TREC-positive cells. Older recipients had significantly fewer TREC-positive cells than younger recipients. The production of TREC-positive cells after T cell depleted–transplantation (by SBA agglutination and E rosette formation) was delayed as compared with unmanipulated BM, suggesting that the T cell depletion removed lymphoid progenitors that contribute to thymopoiesis post-HSCT. An assessment of the impact of acute and chronic GVHD on post-HSCT thymopoiesis showed that in the presence of chronic GVHD, TREC-positive cells, either CD4 or CD8, were not present [31]. Furthermore, a history of acute GVHD, in the absence of chronic GVHD, also had a significant negative impact on thymopoiesis. These clinical results confirm the original murine experiments of Lapp et al. [32], who showed that acute GVHD had a negative impact on thymopoiesis. Thus, acute and particularly chronic GVHD reduce the capacity of the thymus to support the differentiation of the newly engrafted donor HSC or common lymphoid progenitors.

## T CELL FUNCTIONALITY

After nonablative chemotherapy without HSCT, thymopoiesis is reduced. The recovery of thymopoietic function after chemotherapy was inversely correlated with patient age, and the capacity of the thymus to produce $CD4^+$ T lymphocytes was predicted by an increase in thymic volume [33]. Thus, in the non-HSCT setting, chemotherapy and presumably irradiation have a direct negative impact on the capacity of the thymus to produce new T lymphocytes in an age-dependent fashion.

The development of tetramer technology has permitted the identification of antigen-specific CD8 T lymphocytes (using class I tetramers) and CD4 T lymphocytes (using class II tetramers) [7,8]. Besides enumerating the frequency of antigen-specific T lymphocytes after HSCT, tetramer binding has permitted the evaluation of antigen-specific T lymphocyte function after HSCT. Whereas the majority of CD8 antigen-specific T lymphocytes in normal individuals are capable of cytokine production after antigen stimulation, heterogeneous responses to clinically relevant antigens like CMV are seen in HSCT recipients [8]. The functional capacity of CMV-specific T lymphocytes after HSCT predicts the likelihood of clinically significant CMV infections. Patients in whom the majority of their CMV-positive CD8 T lymphocytes were unable to produce tumor necrosis factor alpha (TNF-α) after antigen stimulation, were at increased risk of CMV infection compared with individuals

whose tetramer-positive CD8 T lymphocytes were capable of cytokine production. Further, the addition of steroids to standard post-HSCT immunosuppression resulted in the selective loss of the functional CMV-specific CD8 T lymphocytes. Currently, little is known about the regulation of antigen-specific differentiation after HSCT. It is clear, however, that the presence of antigen-specific T lymphocytes does not always predict protective immunity and resistance to infections. The tetramer binding results confirm previous observations that CMV-specific cytolytic T lymphocytes were not reproducibly detected until a year after HSCT [34].

Many life-threatening infections that occur in unrelated recipients both with and without chronic GVHD are caused by encapsulated respiratory bacteria, suggesting defects in antibody production rather than cellular immunity [3,35]. To evaluate the response of HSCT recipients to a naturally occurring bacterial carbohydrate antigen, recipients were assessed for their spontaneous antibody production to polyribosophosphate, the capsule antigen of *Hemophilius influenza* type b, which cross reacts with the K100 strains of *Escherichia coli*,which are normally found in the gastrointestinal tract. In normal infants, protective levels of antibody (>100 μg/mL) are found by 18 to 24 months of age [27]. When autologous HSCT recipients were evaluated, the kinetics of anti-PRP antibody production paralleled those of normal infants, with the majority of patients having protective levels of antibody by 18 to 24 months after HSCT. When histocompatible transplant recipients without chronic GVHD were assessed, the majority of patients had protective levels of antibody by 2 years after HSCT [36]. However, there was a minority of patients without detectable chronic GVHD who did not have protective levels of antibodies for as long as 9 years after HSCT. Thus, some histocompatible recipients have a prolonged inability to make protective levels of anticarbohydrate antibodies. When the recipients of unrelated HSCT were assessed, 90% of recipients were unable to produce protective levels of anticarbohydrate antibodies for up to 10 years after HSCT. The few patients who were able to produce protective levels of antibodies were children who had undergone transplantation at less than a year of age. In spite of their inability to produce anticarbohydrate antibodies, all recipients had protective levels of antibodies to tetanus toxoid after immunization. Therefore, their inability to produce anticarbohydrate antibodies is not part of a generalized antibody deficiency syndrome, but represents a specific defect. With follow-up of almost 10 years, it suggests that the recipients of unrelated transplants, regardless of their chronic GVHD status, have a prolonged and potential permanent inability to produce protective levels of antibodies to carbohydrate antigens, predisposing them to severe infections with respiratory bacteria. Because in normal lymphoid ontogeny the development of anticarbohydrate antibodies is the most differentiated antigen-specific function, it is not surprising that the inability to produce anticarbohydrate antibodies is the most frequently found immune deficit in HSCT recipients.

## CONCLUSION

The immune reconstitution of HSCT recipients is more complicated than initially realized. Whereas initially only patients with chronic GVHD were felt to have prolonged immunodeficiency, it is now realized that many patients, after HSCT with both related and unrelated donors, have significant prolonged and potentially permanent defects in their immune reconstitution, which predisposes them to severe infections. Because HSCT for neoplastic diseases is successful in reducing the frequency of relapse, ongoing research to reduce HSCT-related morbidity and mortality caused by post-transplant immunodeficiency will be necessary to improve the overall survival rates of HSCT recipients.

## ACKNOWLEDGMENTS

Supported in part by the NIH NCRR GCRC grant MO1 RR-43 and performed at the GCRC at Childrens Hospital Los Angeles.

## REFERENCES

1. Noel DR, Witherspoon RP, Storb R, et al. Does graft-versus-host disease influence the tempo of immunologic recovery after allogeneic human marrow transplantation? An observation on 56 long-term survivors. *Blood.* 1978;51:1087-1105.
2. Witherspoon RP, Storb R, Ochs HD, et al. Recovery of antibody production in human allogeneic marrow graft recipients: influence of time posttransplantation, the presence or absence of chronic graft-versus-host disease, and antithymocyte globulin treatment. *Blood.* 1981;58:360-368.
3. Ochs L, Shu XO, Miller J, et al. Late infections after allogeneic bone marrow transplantations: comparison of incidence in related and unrelated donor transplant recipients. *Blood.* 1995;86: 3979-3986.
4. Small TN, Papadopoulos EB, Boulad F, et al. Comparison of immune reconstitution after unrelated and related T-cell depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions. *Blood.* 1999;93:467-480.
5. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature.* 1998;396:690-695.
6. Douek DC, Vescio RA, Betts MA, et al. Assessment of thymic output in adults after hematopoietic stem-cell transplantation and prediction of T-cell reconstitution. *Lancet.* 2000;355:1875-1881.
7. Foster AE, Gottlieb DJ, Sartor M, Hertzberg MS, Bradstock KF. Ctyomegalovirus-specific CD4+ and CD8+ T-cells fol-

low a similar reconstitution pattern after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2002;8:501-511.
8. Ozdemir E, St John LS, Gillespie G, et al. Cytomegalovirus reactivation following allogeneic stem cell transplantation is associated with the presence of dysfunctional antigen-specific CD8+ T cells. *Blood.* 2002;100:3690-3697.
9. Parkman R, Gelfand EW, Rosen FS, Sanderson A, Hirschhorn R. Severe combined immunodeficiency and adenosine deaminase deficiency. *N Engl J Med.* 1975;292:714-719.
10. O'Reilly RJ, Keever CA, Small TN, Brochstein J. The use of HLA-non-identical T-cell-depleted marrow transplants for correction of severe combined immunodeficiency disease. *Immunodeficiency Rev.* 1989;1:273-309.
11. Hao QL, Zhu J, Price MA, et al. Identification of a novel, human mulitlymphoid progenitor in cord blood. *Blood.* 2001; 97:3683-3690.
12. Keever CA, Small TN, Flomenberg N, et al. Immune reconstitution following bone marrow transplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts. *Blood.* 1989;73:1340-1350.
13. Forman SJ, Nocker P, Gallagher M, et al. Pattern of T cell reconstitution following allogeneic bone marrow transplantation for acute hematological malignancy. *Transplantation.* 1982; 34:96-99.
14. Atkinson K. T cell sub-populations defined by monoclonal antibodies after HLA-identical sibling marrow transplantation. II. Activated and functional subsets of the helper-inducer and the cytotoxic-suppressor sub-populations defined by two colour fluorescence flow cytometry. *Bone Marrow Transplant.* 1986;1: 121-132.
15. Ault KE, Antin JH, Ginsburg D, et al. Phenotype of recovering lymphoid cell populations after marrow transplantation. *J Exp Med.* 1985;161:1483-1502.
16. Parkman R, Merler E. Discontinuous density gradient analysis of the developing human thymus. *Cell Immunol.* 1973;8:382-331.
17. Toribio ML, Alonso JM, Barcena A, et al. Human T-cell precursors: involvement of the IL-2 pathway in the generation of mature T cells. *Immunol Rev.* 1988;104:55-79.
18. Levin MJ, Parkman R, Oxman MN, et al. Proliferative and interferon responses following transplantation in man. *Infect Immunol.* 1997;20:678-684.
19. Rappeport JM, Dunn MJ, Parkman R. Immature T lymphocytes in the peripheral blood of bone marrow transplant recipients. *Transplantation.* 1983;36:674-680.
20. Ottinger HD, Beelen DW, Scheulen B, Schaefer UW, Gross-Wilde H. Improved immune reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. *Blood.* 1996;88:2775-2779.
21. Storek J, Dawson MA, Storer B, et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. *Blood.* 2001;97:3380-3389.
22. Volpi I, Perruccio K, Tosti A, et al. Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. *Blood.* 2001;97: 2514-2521.
23. Bhushan V, Singleton KL, Lin M-T, et al. Monitoring therapy for graft-versus-host disease (GVHD) following hematopoietic stem cell transplantation (HSCT). Response to glucocorticosteroids predicted by anti-CD3-induced T cell apoptosis. *Blood.* 2002;100:851a.
24. Gratama JW, Verdonck LF, Van Der Linden JA, et al. Cellular immunity to vaccinations and herpes-virus infections after bone marrow transplantation. *Transplantation.* 1986;41:719-724.
25. Ljungman P, Wilczek H, Gahrton G, et al. Long-term acyclovir prophylaxis in bone marrow transplant recipients and lymphocyte proliferation responses to herpes virus antigens *in vitro*. *Bone Marrow Transplant.* 1986;1:185-192.
26. Parkkali T, Kayhty H, Ruutu T, et al. A comparison of early and late vaccination with Haemophilus influenzae type b conjugate and pneumococcal polysaccharide vaccines after allogeneic BMT. *Bone Marrow Transplant.* 1996;18:961-967.
27. Gandhi MK, Egner W, Sizer L, et al. Antibody responses to vaccinations given with in the first two years after transplant are similar between autologous peripheral blood stem cell and bone marrow transplant recipients. *Bone Marrow Transplant.* 2001;28: 775-781.
28. Weinberg K, Annett GM, Kashyap A, et al. The effect of thymic function immunocompetence following bone marrow transplantation. *Biol Blood Marrow Transplant.* 1995;1:18-23.
29. Storek J, Witherspoon RP, Storb R. T cell reconstitution after bone marrow transplantation into adult patients does not resemble T cell development in early life. *Bone Marrow Transplant.* 1995;16:413-425.
30. Lewin SR, Heller G, Zhang L, et al. Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. *Blood.* 2002;100:2235-2242.
31. Weinberg K, Blazar BR, Wagner JE, et al. Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. *Blood.* 2001;97:1458-1466.
32. Seddik M, Seemayer TA, Lapp WS. T cell functional defect associated with thymic epithelial cell injury induced by a graft-versus-host reaction. *Transplantation.* 1980;29:61-66.
33. Mackall CL, Fleisher TA, Brown MR, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med.* 1995;332:143-149.
34. Quinnan GV, Kirmani N, Rook AH, et al. Cytotoxic cells in cytomegalovirus infection: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone marrow transplant recipients. *N Engl J Med.* 1982;307:7-13.
35. Atkinson K, Storb R, Prentice RL, et al. Analysis of late infections in 89 long-term survivors of bone marrow transplantation. *Blood.* 1979;53:720-731.
36. Kapoor N, Chan R, Weinberg KI, Burotto F, Parkman R. Defective anticarbohydrate antibody responses to naturally occurring bacteria following bone marrow transplantation. *Biol Blood Marrow Transplant.* 1999;5:46-50.