

Immunological Reconstitution and Immunoregulatory Cells in Hematopoietic Stem Cell Transplantation

Masahiro Imamura

Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Abstract

Analysis of cytokine gene expression in peripheral blood mononuclear cells from patients received allogeneic hematopoietic stem cells transplantation (allo-SCT) showed that type 1 helper T cells (Th1)-derived cytokines increased in severe graft-versus-host disease (GVHD) while Th2-derived cytokines such as IL-4, IL-10, and IL-13 increased in mild GVHD. These results indicate that Th2 cells suppress GVHD although Th1 cells augment GVHD. Chimerism analysis showed that mixed chimerism was often observed in younger (<30 years old) patients. Mixed chimerism in older (≥ 30 years old) patients were related to rejection and relapse while this situation is not the case in younger patients, thus indicating that mixed chimerism is an important prognostic factor in older patients. Among the chimerism of various cell populations, donor-derived CD56-positive cells are important in early engraftment when determined in allogeneic nonmyeloablative stem cell transplantation (allo-NST), regardless of the proportion of donor-derived CD3-positive cells. This result suggests that donor-derived CD56-positive cells are a more useful indicator for engraftment and rejection in early time period. Complementary-determining region 3 (CDR3) size spectratyping in T-cell receptor (TCR) chain subfamilies (V β) showed that high level of diversity in TCR V β repertoire is important for a late rejection and skewed TCR V repertoire is well correlated to occurrence of GVHD. Expression of inhibitory natural killer (NK) cell receptors such as CD158b and CD94/NKG2A on peripheral CD3-negative and positive cells were increased in parallel with GVHD. Interestingly, these molecules appeared to regulate GVHD while preserving graft-versus-leukemia (GVL) effect.

1. Introduction

The way of immunological reconstitution after allo-SCT is critical for favorable clinical outcome since engraftment failure, GVHD, relapse, and infection are closely affected by the recovery pattern of immunoregulatory cells and the diversity of TCR V β repertoire. It is therefore important to analyze immunological reconstitution in each cell population and TCR V β repertoire for regulating unfavorable post-transplant complications and obtaining better clinical outcome [1-4]. Although many immunoregulatory cells have been reported, NK cell receptor-positive cells have unique function which effectively inhibits GVHD but sustains GVL effect [5-7].

2. Cytokine Profile after Allo-SCT

Cytokines are an important factor to regulate immunological responses. It is therefore interesting to understand the expression profile of various cytokines after allo-SCT. Enzyme-linked immunosorbent assay was basically used to examine serum cytokine level, except radioimmunoassay for IFN- γ . The IL-6 levels increased in the sera shortly after allo-SCT regardless of the presence of acute GVHD [8]. In contrast, IFN- γ and TNF- α subsequently increased in patients with acute GVHD, thus indicating that a sequential increase and synergy in IL-6, IFN- γ , and TNF- α is necessary to induce acute GVHD. When acute GVHD disappeared, these levels decreased. Similar findings were observed in chronic GVHD. Although IL-2 was undetectable throughout the observation period even in patients with GVHD, soluble

IL-2 receptor levels increased at the engraftment period and the onset of acute and chronic GVHD, showing a good correlation to the disease status [9].

Cytokine gene expression in peripheral blood mononuclear cells was also analyzed by reverse transcription polymerase chain reaction (RT-PCR) method [10,11]. Expression of IFN- γ (Th1-type cytokine) and inflammatory cytokines (e.g., TNF- α , IL-6 and IL-1 β) increased in severe acute (\geq grade III) and chronic (extensive type) GVHD while Th2-type cytokines such as IL-4, IL-10, and IL-13 increased in mild acute (\leq grade II) and chronic (limited type) GVHD, thus indicating that the balance between Th1 and Th2 is an important prognostic factor for better clinical outcome in terms of GVHD.

3. Chimerism Analysis in Fractionated Cell Populations

Four types of microsatellites (D3S1359, D6S89, ACTBP2, and HGH) were used to analyze chimerism using capillary electrophoresis system [12]. Genomic DNA was extracted from peripheral blood mononuclear cells or CD3-, CD14-, CD15-, and CD56-positive cells in some case. Mixed chimerism was more often observed in younger (<30 years old) patients than in older (\geq 30 years old) patients. Graft failure and relapse developed more frequently in patients with mixed chimerism than in those with complete chimerism. This is more marked in older patients than in younger ones. One of the reason for this age difference may be due to readily established transplantation tolerance in younger patients who possess intact or almost normal thymic function since graft failure and acute GVHD were often seen in older patients with mixed chimerism.

In a patient with ALL, mixed chimerism was seen in mononuclear cells but not in whole blood population. In a relapsed patient with AML, mixed chimerism was seen in whole blood population and granulocytes. Dubovsky et al. [13] reported that detection of mixed chimerism in CD19-positive cells but not in whole blood was useful to predict lymphoid blast crisis in a CML patient. A similar finding was shown in ALL patients. These results suggest that chimerism analysis in fractionated populations are preferable to detect early relapse after allo-SCT.

Furthermore, in the setting of allo-NST one patient, who had once engraftment but finally had late rejection, showed almost complete donor type chimerism in CD56 positive cells but mixed chimerism in CD3-positive cells. This result suggest that donor-derived CD56-positive cells play an important role in ensuring engraftment. This speculation was confirmed by the fact that late rejection was seen in parallel with a decrease in the proportion of donor-derived CD56-positive cells and that the patient with high proportion of donor-derived CD56-positive cells had successful engraftment regardless of the proportion of donor-derived CD3-positive cells.

NK cells are known to recognize the class I major

histocompatibility complex (MHC) and kill the target cells which express quantitatively or qualitatively aberrant class I MHC through their receptors such as killer-cell inhibitory receptors (KIRs) and CD94/NKG2 [14-16]. In fact, it is reported that NK cells derived from the recipient can induce the marrow graft rejection in murine bone marrow transplantation model of parent into F₁ combination [17]. This phenomenon was originally called the hybrid resistance and NK cells derived from the recipient can recognize the class I MHC of the parent; namely, parental strain targets are lysed because they do not express all of the self class I antigens of the F₁ hybrid, failing to deliver inhibitory signals to all subsets of F₁ NK cells.

In this sense, donor-derived CD56-positive cells are more important to ensure engraftment than CD3-positive cells; therefore, it is necessary to monitor the proportion of donor-derived CD56-positive cells as well as CD3-positive cells. However, there exists one interesting and important problem. Future direction should be focused on how donor-derived NK cells can recognize the host-derived class I MHC through their receptors since HLA-matched sibling donors were selected in the present study on allo-NST. It is not yet confirmed that NK cells recognize minor histocompatibility antigens different from MHC. One possibility is that donor-derived NK cells may recognize nonclassical MHC such as HLA-E [18].

4. T-cell Repertoire Analysis

CDR3 size spectratyping is usually used to analyze T-cell receptor repertoire [19]. The oligoclonal expansion of T-cells occurs in c GVHD, GVL effect, immunological reconstitution, and viral infection; therefore, CDR3 size spectratyping is essential to understand the disease status after allo-SCT.

By using 24 TCR V β , TCR repertoire in peripheral blood was analyzed. Many TCR V β were skewed in the early stage. Although the skewed TCR V β was observed at the acute GVHD, V β 6 was skewed more often at the acute GVHD of the skin. In case of cytomegalovirus (CMV) infection, V β 2 and V β 6 were often skewed. The recovery of diversity in TCR V β repertoire appeared to be faster in the patients with acute GVHD than in those with chronic GVHD. Also, that appeared to lower in the patients with CMV infection than in those without such infection. There was a statistically significant difference between patients with chronic GVHD and those without chronic GVHD in the average of diversity in TCR V β repertoire at 3 months after allo-SCT. Namely, it was lower in the patients with chronic GVHD than in those without chronic GVHD, thus indicating that low diversity in TCR V β repertoire is predictive for chronic GVHD development. On the contrary, it turned out that high level of diversity in TCR V β repertoire was important for a late rejection.

5. Analysis of NK Cell Receptor-Positive Cells

When NK cell receptor-positive cells were examined in peripheral blood mononuclear cells from allo-SCT, NK cells with KIRs (CD158b-positive and CD3-negative) increased from early time period to 6 months [5, 20]. The percentage of CD158b-positive and CD3-negative cells decreased in patients with chronic GVHD compared with those without chronic GVHD and no severe acute GVHD was observed in the patients analyzed here, thus indicating that these cells appear to inhibit GVHD. Furthermore, CD158- and CD3-positive (mainly CD8-positive) cells also increased from 3 months to >6 months. Interestingly, these cells markedly increased in patients with chronic GVHD compared with those without chronic GVHD. Clinical course clearly showed that the high proportion of CD158b- and CD3-positive cells well correlated with the disappearance of chronic GVHD and vice versa [6].

CD158b specifically recognize HLA-Cw1, 3, 7, and 8 and belongs to the immunoglobulin superfamily [16]. The other type of NK cell receptor is a member of C-type lectins (i.e., CD94) which makes a heterodimer with NKG2 and recognize HLA-E. This receptor is also expressed on NK cells as well as T cells and inhibits the cytolytic function of T cells. The proportion of CD94-positive and CD3-negative cells increased in patients without chronic GVHD [20]. Even though chronic GVHD developed, the proportion of those cells was higher in patients with good prognosis than in those with poor prognosis. CD94- and CD3-positive (mainly CD8-positive) cells increased in patients with chronic GVHD who had good prognosis. The same results were obtained in NKG2A-positive cells. These results are consistent with the previous results on CD158-positive and CD3-negative cells and CD158- and CD3-positive cells. In any case, these cells appear to regulate GVHD.

6. Functional Analysis of CD94/NKG2A- and CD3-positive Cells

CD94/NKG2A-positive cells were induced by the stimulation of peripheral blood mononuclear cells by OKT3 and IL-15 [21]. Proliferative responses in mixed lymphocyte culture (MLC) showed that CD94-depleted cells responded to the stimulator cells better than did CD94-enriched cells and the response of the mixture of these two populations was comparable to the response of the unfractionated cells.

When G-CSF-mobilized peripheral blood mononuclear cells were used for MLC, the proportion of CD94/NKG2A-positive cells markedly increased with 2 week-allogeneic stimulation. CD14-depletion induced decreased proportion of such cells. Addition of CD14-positive cells recovered the proportion of CD94/NKG2A-positive cells while the intervening membrane inhibited this recovery, thus indicating that the cell to cell interaction is necessary to induce this effect. CD94-positive cells can kill K562 cells but not PHA-stimulated autologous lymphoblasts. This result suggests that CD94-positive cells in-

hibit GVHD while preserving GVL effect. A similar result was obtained against autologous leukemic cells from a patient with CML. NK cell receptor-positive T cells therefore must be one of the promising and ideal candidates for cellular therapy to leukemia patients in the future.

7. Conclusions

It is important to monitor immunological reconstitution for better understanding of the various complications after allo-SCT such as engraftment failure, rejection, GVHD, relapse, and infection. Fractionated cell populations and frequent examinations are required for the global recognition of the disease status. The information obtained by the above-mentioned analyses will be helpful to obviate and resolve various complications, thus resulting in the improvement of the clinical outcome of allo-SCT. Furthermore, functional analysis of immunoregulatory cells and their efficient generation will open a new avenue to the development of cellular therapy.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research and that on Priority Areas "Cancer" from the Ministry of Education, Culture, Sports, Science, and Technology. I would like to thank my colleagues, Drs. Juni Tanaka and Yutaka Tsutsumi, for their collaboration.

References

1. Tanaka J, Kasai M, Imamura M, et al. Evaluation of mixed chimerism and origin of bone marrow derived fibroblastoid cells after allogeneic bone marrow transplantation. *Br J Haematol.* 1994;86:436-438.
2. Oberkircher AR, Strout MP, Herzig GP, Fritz PD, Caligiuri MA. Description of an efficient and highly informative method for the evaluation of hematopoietic chimerism following allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 1995;16:695-702.
3. Yamanaka K, Kwok WW, Micelson EM, Masewicz S, Smith F, Nepom GT. Selective T-cell-receptor gene usage in allorecognition and graft-versus-host disease. *Transplantation.* 1993; 55:1167-1175.
4. Gorski J, Yassai M, Zhu X, Kissela B, Keever C, Flomberg N. Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. *J Immunol.* 1994;152:5109-5119.
5. Tanaka J, Mori A, Ohta S, et al. Expression of HLA-C-specific natural killer cell receptors (CD158a and CD158b) on peripheral blood mononuclear cells after allogeneic bone marrow transplantation. *Br J Haematol.* 2000;108:778-783.
6. Tanaka J, Tutumi Y, Mori A, et al. Sequential analysis of HLA-C-specific killer cell inhibitory receptor (CD158b) expressing peripheral blood mononuclear cells during chronic graft-versus-host disease. *Bone Marrow Transplant.* 2000;26: 287-290.
7. Albi N, Ruggeri L, Aversa F, et al. Natural killer (NK)-cell function and antileukemic activity of a large population of CD3+/CD8+ T cells expressing NK receptors for major histocompatibility complex class I after 'three-loci' HLA-incompatible bone marrow transplantation. *Blood.* 1996;87:3993-

- 4000.
8. Imamura M, Hashino S, Kobayashi H, et al. Serum cytokine levels in bone marrow transplantation: synergistic interaction of interleukin-6, interferon- γ , tumor necrosis factor- α in graft-versus-host disease. *Bone Marrow Transplant.* 1994;13:745-751.
 9. Kobayashi S, Imamura M, Hashino S, Tanaka J, Asaka M. Clinical relevance of serum soluble interleukin-2 receptor levels in acute and chronic graft-versus-host disease. *Leuk. Lymphoma.* 1997;28:159-169.
 10. Tanaka J, Imamura M, Kasai M, et al. Cytokine gene expression in peripheral blood mononuclear cells during graft-versus-host disease after allogeneic bone marrow transplantation. *Br J Haematol.* 1993;85:558-565.
 11. Tanaka J, Imamura M, Kasai M, et al. The important balance between cytokines derived from type-1 and type-2 helper T cells in the control of graft-versus-host disease. *Bone Marrow Transplant.* 1997;19:571-576.
 12. Tsutsumi Y, Tanaka J, Zhang L, et al. Analysis of chimerism in allogeneic stem cell transplanted patients using a capillary electrophoresis system. *Acta Haematol.* In press.
 13. Dubovsky J, Daxberger H, Fritsch G, et al. Kinetics of chimerism during the early post transplant period in pediatric patients with malignant and non malignant hematologic disorders: implications for timely detection of engraftment, graft failure and rejection. *Leukemia.* 1999;13:2060-2069.
 14. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunology Today.* 1990; 11:237-244.
 15. Biassoni R, Falco M, Cambiaggi A, et al. Aminoacid substitutions can influence the natural killer (NK)-mediated recognition of HLA-C molecules. Role of serine-77 and lysine-80 in the target cell protection from lysis mediated by "group 2" and "group 1" NK clones. *J Exp Med.* 1995;182:605-609.
 16. Moretta A, Biassoni R, Bottino C, et al. Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol Rev.* 1997;155:105-117.
 17. Bordignon C, Daley JP, Nakamura I. Hematopoietic histocompatibility reactions by NK cells in vitro: model for genetic resistance to marrow grafts. *Science.* 1985;230:1398-1401.
 18. Braud VM, Allan DSJ, O'Callaghan CA, et al. HLA-E binds to natural killer cell receptor CD94/NKG2A, B and C. *Nature.* 1998;391:795-799.
 19. Wu CJ, Chillemi A, Alyea EP, et al. Reconstitution of T-cell receptor repertoire diversity following T-cell depleted allogeneic bone marrow transplantation is related to hematopoietic chimerism. *Blood.* 2000;95:352-359.
 20. Tanaka J, Tutumi Y, Zhang L, et al. Increased proportion of HLA-class-I-specific natural killer cell receptors (CD94) on peripheral blood mononuclear cells after allogeneic bone marrow transplantation. *Acta Haematol.* 2001;105:89-91.
 21. Tanaka J, Tutumi Y, Zhang L, et al. Induction of CD94/NKG2A expression on T cells in MLC by CD14+ cells from G-CSF-mobilised peripheral blood mononuclear cells. *Br J Haematol.* In press.