

Maternal-Fetal Relationship, Natural Chimerism and Bilateral Transplantation Tolerance as the Basis for Non-Myeloablative Stem Cell Transplantation

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Abstract

Bone marrow transplantation (BMT) which represents an important clinical tool for treatment of patients with a wide variety of malignant and non-malignant diseases, however, the procedure is associated with procedure-related toxicity and mortality as well as unavoidable late complications. Many of the undesirable consequences of BMT are caused directly or indirectly by the intensive conditioning administered during the pre-transplant period. However, if the main goal of the BMT procedure is to enable immunotherapy by alloreactive donor lymphocytes, the conditioning prior to BMT needs to be reconsidered, because transplantation tolerance across major histocompatibility complex (MHC) occurs spontaneously in nature, as evidenced by the fact that pregnant females do not reject their conceptus. In fact, as shown by Owens in the 1940s, placental parabiosis in utero leads to permanent mixed chimerism and bilateral transplantation tolerance. These observations followed by experiments carried out in the 1950s by Billingham et al. suggested that infusion of parental stem cells into neonates with no exogenous immunosuppressive treatment resulted in mixed chimerism and permanent transplantation tolerance to donor alloantigens. Thus, a window of opportunity provided shortly after delivery, was sufficient for induction of tolerance without the need for heavy conditioning. Tolerant recipients were shown to be chimeras with only a small proportion of donor cells. However, without corroborating evidence that transplantation tolerance could be intentionally induced, the approach could not be applied in clinical practice for immunocompetent recipients. Starting in 70s, we documented the feasibility of establishing bilateral transplantation tolerance by mixed chimerism following non-myeloablative conditioning in immunologically mature recipients across MHC in mice, rats and dogs. Several studies have shown that reduced intensity conditioning can be very useful for immunoregulation whereas more intensive the pre-grafting immunosuppression resulted in more aggressive the GVHD. These and other findings suggested that lower intensity conditioning may be sufficient for engraftment of donor stem cells, thus suggesting that immunosuppression without myeloablation may be sufficient for prevention of allograft rejection. Following engraftment of donor stem cells, donor lymphocytes infused with bone marrow or mobilized blood stem cells can eradicate residual hematopoietic cells of host origin, occasionally non-hematopoietic tumor cells of host origin as well. Whenever indicated, donor lymphocytes infusion (DLI) can be used at a later stage post BMT to eradicate residual malignant cells of host origin or for the treatment of residual or recurrent disease. Taken together, ongoing clinical studies suggest that high-dose, myeloablative chemoradiotherapy, could be safely replaced with non-myeloablative conditioning (NST).

1. Introduction

Unfortunately, bone marrow transplantation (BMT) which represents an important clinical tool for treatment of pa-

tients with a wide variety of malignant and non-malignant diseases is associated with high rates of procedure-related toxicity and mortality as well as unavoidable late complications [1]. Many of the undesirable

consequences of BMT are caused directly or indirectly by the intensive conditioning administered during the pre-transplant period. Following allogeneic stem cell transplantation, acute and chronic graft-versus-host disease (GVHD) on the one hand, and immunodeficiency while the immune system recovers or as a consequence of pre-transplant conditioning and post transplant immunosuppression for prevention and/or treatment of GVHD remain major problems to be solved towards improving the outcome following BMT.

2. Induction of Transplantation Tolerance by Mixed Chimerism

Induction of unresponsiveness by elimination of alloreactive T cell clones is a default of premature T cells since this is the basis for induction of self tolerance. Therefore, under the proper conditions induction of transplantation should be feasible by transplantation of stem cells as a source of uncommitted progenitor cells that can give rise to premature T cells. Thus, premature T cells of host origin, following adequate immunosuppression while avoiding myeloablative treatment are likely to accept allogeneic stem cells, thus leading to establishment of mixed chimerism and bilateral transplantation tolerance in mice [2-8] and man [9-13]. Similarly, following transplantation of T cell depleted stem cells or purified stem cells, deletion of host reactive T cells is anticipated, thus leading to engraftment of donor hematopoietic system while avoiding GVHD using no post transplant immunosuppressive therapy [14]. When the immune system matures in the thymus, antigens presented to premature T cells lead to antigen-specific clonal deletion. Similarly, presentation of foreign antigens in the thymus also result in development of unresponsiveness to such antigens, thus suggesting that presentation of donor alloantigens in the thymus is the mandatory requirement for deletion of antigen reactive cells. Following BMT, donor alloantigens can be easily presented in the thymus after thymic migration through the circulation, thus explaining the role and efficacy of donor hematopoietic cells in induction of donor specific transplantation tolerance.

Based on the conclusions from experiments done by 'mother nature' we have assumed that it should be possible to simplify the BMT procedure, since induction of unresponsiveness to donor stem cells, which seems to be the key element for successful and safe BMT, is one of the most frequent events in Nature, occurring in every pregnancy as self tolerance is established. Likewise, later during pregnancy, as soon as the circulation of two fraternal twins mixes in utero, as was originally described by Owen and colleagues, newborn calves are tolerant of each other [15]. Post nately, if donor stem cells are inoculated into the fetus early enough during the prenatal period, mixed chimerism results and consequently, bilateral transplantation tolerance develops with no additional conditioning. Based on these experiments by 'mother nature', it seems rather evident that induction of clonal deletion is a default in T cell onto-

geny. Hence, it was anticipated that uncommitted stem cells could acquire unresponsiveness to neoantigens, alloantigens included, thus suggesting that similar degree of unresponsiveness may be accomplished when the immune system is temporarily suppressed, thus giving a chance to uncommitted stem cells to acquire unresponsiveness to alloantigens presented to them at *status nascendi* in the thymus of a new host, the recipient. The resulting unresponsiveness results in recognition of recipient alloantigens as 'self' through a mechanism of clonal deletion, thus mimicking the normal sequence of events that take place during the ontogeny of the immune system. Consistent induction of unresponsiveness rather than continuous immunosuppression is the holy grail of transplant biologists and in the era of stem cell plasticity, induction of transplantation tolerance to alloantigens seems highly desirable.

3. The Development of Non-myeloablative Stem Cell Transplantation (NST)

Until recently, BMT was considered a most hazardous procedure associated with mandatory conditioning with myeloablative chemoradiotherapy. Hence, this procedure was reserved for patients with life threatening hematologic malignancy, after remission induction failure, or for patients relapsing following administration of all possible alternative approaches. Thus, in addition to the risks associated with the BMT procedure, many of the patients are in poor performance status, heavily beaten with prior chemotherapy combinations, frequently heavily colonized with infectious agents such as aspergillosis and candidiasis, and thus prone to additional risks, especially after being exposed to myeloablation and long-term immunosuppressive treatment before and for a long period of time after transplantation.

In principle, patients with high-risk hematologic malignancies in particular at the stage of minimal residual disease, could benefit from a curative procedure at an early stage of the disease, thus avoiding the need for long-term chemotherapy with all the associated complications, costs and discomfort. Patients with genetic diseases would also benefit at an early stage of their disease, when the chance for complete cure may be much higher. In enzyme deficiency diseases associated with central nervous system disease, replacement therapy with an early successful transplant can result in cure before irreversible changes occur in the CNS, while in patients with hemoglobinopathies, such as beta thalassemia major, replacement of genetically abnormal with normal hematopoietic stem cells may be accomplished before the patient is sensitized by numerous blood transfusions.

Starting in the 70s, we published a series of papers addressing the feasibility of establishing transplantation tolerance and bilateral unresponsiveness by mixed chimerism in immunologically mature recipients across MHC [2-5]. However, the mechanisms responsible for the balanced equilibrium in mixed chimeras were not understood. The principle of using mixed chimerism for induction of transplantation tolerance was confirmed by

Ildstad and Sachs [16] and subsequently by Sykes and Sachs [17-19]. In the 80s, we have shown that relapse following myeloablative BMT could be frequently reversed by donor lymphocyte infusion (DLI), thus confirming that the major therapeutic benefit induced by the allogeneic bone marrow transplant procedure was mediated predominantly by alloreactive donor lymphocytes [20,21]. This has led into the working hypothesis that the transplant procedure may be required for induction of host-versus-graft transplantation tolerance and that once engrafted, donor lymphocytes mediate the graft-versus-malignancy (GVM) effects which could be applied for eradication of a large number of host hematopoietic cells in malignant and non-malignant diseases [11].

In the 90s, following clinical application of reduced intensity conditioning, non-myeloablative stem cell transplantation or the so called 'mini transplant' approach, several groups following the same principles developed well tolerated regimen for the treatment of patients with malignant and non-malignant diseases [9-13]. It was soon realized that using NST, a larger number of patients might benefit from BMT, which could be offered to elderly patients, with no upper age limit, and also to patients with poor performance status that normally would not be considered eligible candidates for BMT. For young patients, BMT may be offered with no risk of impairment of growth and development, sterility and multiple endocrine adenopathy, currently unavoidable with conventional myeloablative procedures.

4. Conclusions

Experimental and clinical application of different immunosuppressive yet non-myeloablative regimen may be used to suppress the immune system of an allograft recipient, thus enabling donor stem cell engraftment. Considering the powerful cytoreductive potential of donor lymphocytes to eliminate malignant or otherwise abnormal host cells or their progeny, under conditions of host-versus-graft transplantation tolerance, cytoreduction by myeloablative chemoradiotherapy may be replaced with safer and well tolerated conditioning as a platform for subsequent cell-mediated immunotherapy with alloreactive donor lymphocytes. Based on this working hypothesis, the goal for future protocols is to aim for safer immune regulation, trying to mimic rather than to resist Nature, as a basis for safe and well-tolerated allogeneic stem cell transplantation to be followed by cell therapy. As soon as the mechanisms of maternal-fetal tolerance across MHC will be better understood, safer approaches may become available for the treatment of malignant and non-malignant diseases, using immune cells to eliminate, replace or control malignant and non-malignant cells.

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