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Review

Immune monitoring of transplant patients in transient mixed chimerism tolerance trials

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ABSTRACT

This review focuses on mechanistic studies performed in recipients of non-myeloablative bone marrow transplant regimens developed at Massachusetts General Hospital in HLA-identical and HLA-mismatched haploidentical combinations, initially as a platform for treatment of hematologic malignancies with immunotherapy in the form of donor leukocyte infusions, and later in combination with donor kidney transplantation for the induction of allograft tolerance. In patients with permanent mixed chimerism, central deletion may be a major mechanism of long-term tolerance. In patients in whom donor chimerism is only transient, the kidney itself plays a significant role in maintaining long-term tolerance. A high throughput sequencing approach to identifying and tracking a significant portion of the alloreactive T cell receptor repertoire has demonstrated biological significance in transplant patients and has been useful in pointing to clonal deletion as a long-term tolerance mechanism in recipients of HLA-mismatched combined kidney and bone marrow transplants with only transient chimerism.

1. Introduction

The studies of combined kidney/bone marrow transplantation (CKBMT) across HLA barriers in patients without malignant disease at MGH [1,2] (see article by Tatsuo Kawai et al.) built on a series of translational studies in murine models, non-human primates and patients with hematological malignancies. The translational aspect of these studies is schematized in Fig. 1. Studies in the murine model for the purpose of two different goals each involved initial induction of mixed chimerism. The original mixed chimerism model upon which all of the studies are based was designed for the purpose of inducing allograft tolerance [3] (Fig. 1C). It involved treatment of mice with depleting anti-CD4 and anti-CD8 mAbs along with low-dose (3 Gy) TBI in order to overcome allograft rejection and make "space" for marrow engraftment, respectively. The addition of thymic irradiation was found to be necessary for the achievement of T cell chimerism, which correlated with durable chimerism [3] and was later shown to be required to eliminate intrathymic alloreactivity that otherwise rejected donor progenitors as they entered the thymus, precluding a donor contribution to central deletional tolerance of newly-developing thymocytes [4–6] . Later, using the model represented in Fig. 1A, we utilized mixed chimerism induction in mice as a platform for delayed donor lymphocyte infusion (DLI), which we had shown could mediate potent graft-vs-leukemia (GVL) effects without causing graft-vs-host disease (GVHD) when inflammatory stimuli induced by conditioning had been given sufficient time to subside before DLI administration [7–12]. This "lymphohematopoietic GVH response (LGVHR)" remained confined to the lymphohematpoietic system due to the absence of local inflammatory stimuli in the epithelial GVHD target tissues. We had found that such inflammation provided a critical checkpoint for trafficking of GVH-reactive T cells to these tissues, where they caused disease [11,12]. The conditioning regimen in these mice was tailored to the treatment of indolent lymphoid malignancies by replacing the low-dose TBI in Fig. 1C with pre-transplant cyclophosphamide [10,13], which has cytoreductive effects on lymphoid malignancies.

The protocol in Fig. 1A was translated directly into clinical studies in patients with refractory hematologic malignancies in whom all other treatment modalities had failed (Fig. 1B). While this protocol excluded

Abbreviations: APC, antigen-presenting cell; BMT, bone marrow transplantation; CKBMT, combined kidney and bone marrow transplantation; CML, cell-mediated lympholysis; DLI, donor leukocyte infusions; GVHD, graft-vs-host disease; GVL, graft-vs-leukemia/lymphoma; LDA, limiting dilution analysis; LGVHR, lymphohematopoietic graft-vs-host response; MiHA, minor histocompatibility antigen; MLR, mixed lymphocyte reaction; TBI, total body irradiation; TCR, T cell receptor; Treg, regulatory T cell

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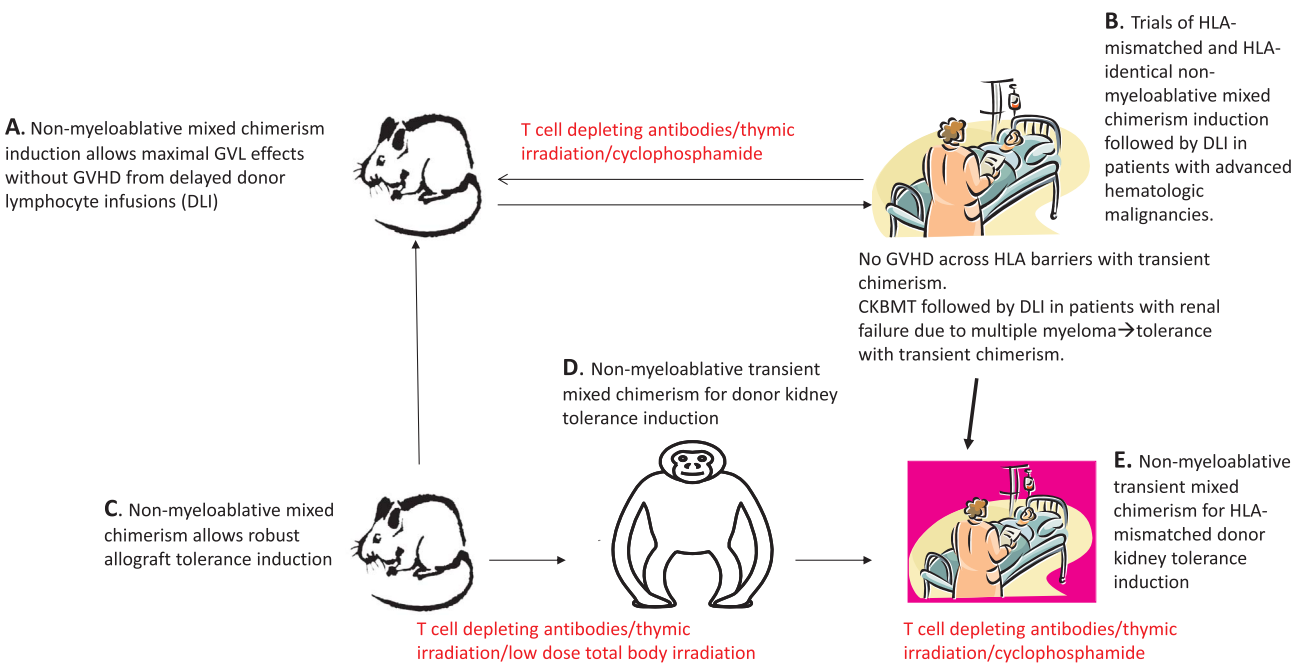

Fig. 1. Schematic representation of translational studies that led to the MGH ITN CKBMT trials. A. A murine model for non-myeloablative mixed chimerism induction was established with a combination of pre-transplant cyclophosphamide, T cell-depleting antibodies and local thymic irradiation. Mixed chimerism was used as a platform for delayed donor lymphocyte infusions, which mediated potent GVL effects without causing GVHD; B. The murine model in A was translated into clinical trials in patients with advanced hematologic malignancies, aiming to achieve mixed chimerism with non-myeloablative conditioning in both HLA-identical and HLA-mismatched haploidentical related donor settings, followed by DLI to achieve anti-tumor effects. Conditioning was similar to that in A, except a short course of cyclosporine was given post-transplant to compensate for the incomplete donor and recipient T cell depletion achievable with the *in vivo* antibody treatments given; C. Murine models using low-dose total body irradiation (TBI), T cell-depleting antibodies and thymic irradiation were used to establish durable mixed chimerism across full MHC barriers; D. Efforts to translate the model in C to a non-human primate model were successful in achieving renal allograft tolerance if the donor kidney and bone marrow were given simultaneously, although only transient chimerism was achieved. Conditioning included low-dose TBI, thymic irradiation, peri-transplant T cell-depleting antibodies and a short course of cyclosporine following the transplant to compensate for incomplete T cell depletion; E. The achievement of transient chimerism without GVHD across HLA barriers in hematologic malignancy patients in B, combined with the renal allograft tolerance achieved in monkeys with transient chimerism in D, permitted evaluation of a regimen developed in B for the induction of renal allograft tolerance across HLA barriers in patients without malignant disease.

the indolent types of malignancies for which the approach was intended, it nevertheless was associated with remarkable tumor responses and even cures in patients with bulky, refractory lymphomas and myelomas who lacked any other hope for survival [14–20]. These potent anti-tumor effects most likely reflected the ability of recipient-derived professional antigen-presenting cells (APCs) to trigger GVH alloreactivity, which is associated with improved GVL effects compared to those elicited in full chimeras lacking recipient APCs [8,18,21–23]. Thus, the strategy of using mixed chimerism as a platform to achieve potent GVL effects without GVHD was successfully translated into the clinic, both in the HLA-identical and HLA-mismatched settings. In the case of HLA-identical transplants, stronger GVL effects in mixed chimeras [17,24] may be explained by the expectation that the number of recipient miHAs presented on host APCs likely exceeds the number that can be presented on donor APCs through the exogenous antigen processing pathway, especially for CD8 allorecognition. These studies provided seminal demonstrations that: 1) durable mixed chimerism could be achieved with non-myeloablative conditioning in humans, even with HLA-mismatched donors [14–20], albeit not as reliably as desired. An example of durable mixed chimerism in an HLA-mismatched transplant recipient is illustrated in Fig. 2; 2) durable or transient mixed chimerism achieved under these conditions could occur without GVHD [14–20], though not as reliably as desired. As is discussed below, transient chimerism achieved across HLA barriers with one of these regimens was reliably NOT associated with GVHD, providing a key safety feature that permitted exploration of this approach for CKBMT in patients without malignant disease; and 3) delayed DLI could convert mixed to full chimerism, even across HLA barriers, without inducing GVHD [14–20], though not as reliably as desired.

The bottom part of the translational scheme presented in Fig. 1 represents the studies of mixed chimerism solely for the purpose of organ allograft induction, which were closely interrelated with those in the top part of the figure. Early efforts were made to translate the non-myeloablative tolerance protocol in Fig. 1C to a large animal, non-human primate model (Fig. 1D) [25]. This model is discussed in more detail in the paper in this issue by Kawai et al. In my view, intermediate large animal models are ethically necessary before embarking on clinical trials of early immunosuppression withdrawal, which denies the patient standard-of-care treatment. Non-human primates, for the most part, model the hurdles to tolerance induction in patients quite faithfully. As is discussed by Kawai et al., achievement of durable mixed chimerism was elusive in this non-human primate model, but key observations were made that permitted the clinical trials in Fig. 1E to be carried out. In particular, it was found that a high proportion (about 60–70%) of animals receiving non-myeloablative CKBMT achieved long-term tolerance to their kidney grafts, despite the transient nature of their chimerism [25,26].

Meanwhile, the non-myeloablative mixed chimerism/DLI studies in patients with lymphoid malignancies (Fig. 1B) had shown an acceptable safety profile and were extended to a group of patients who had renal failure due to multiple myeloma and an available HLA-identical sibling donor. These first human CKBMT studies revealed that both transient and durable mixed chimerism could be associated with tolerance to donor kidneys [27–29]. Remarkably, those patients with transient chimerism, like others in the lymphoma trials discussed above, often enjoyed remarkable tumor responses [19,27,28], suggesting that the initial engraftment followed by rejection of donor marrow could elicit anti-tumor immunity, a hypothesis that was tested and shown to be valid in the murine model [30–33].

Thus, clinical studies in patients with hematological malignancies provided key safety and efficacy data that permitted testing of HLA-mismatched related donor CKBMT in patients with renal failure and no