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ORIGINAL PRE-CLINICAL SCIENCE

Blockade of adhesion molecule lymphocyte function–associated antigen-1 improves long-term heart allograft survival in mixed chimeras

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BACKGROUND: The mixed chimerism approach for intentional induction of donor-specific tolerance was shown to be successful in various models from mice to humans. For transplant patients, the approach would obviate the need for long-term immunosuppression and associated side effects; moreover, it would preclude the risk of late graft loss due to chronic rejection. Widespread clinical application is hindered by toxicities related to recipient pre-conditioning. Herein we aimed to investigate a clinically relevant protocol for tolerance induction to cardiac allografts, sparing CD40 blockade or T-cell depletion.

METHODS: B6 mice were conditioned with non-myeloablative total body irradiation, fully mismatched BALB/c bone marrow cells, and short-term therapy, based on either anti– lymphocyte function–associated antigen-1 (anti–LFA-1) or anti-CD40L. Multilineage chimerism was followed by flow-cytometric analysis, tolerance was assessed with skin and heart allografts from fully or major histocompatibility complex–mismatched donors. Mechanisms of tolerance were investigated by analysis of donor-specific antibodies (DSAs), mixed lymphocyte reaction (MLR) assays, and deletion of donor-reactive T cells.

RESULTS: We found that the combination of cytotoxic T-lymphocyte antigen 4 immunoglobulin (CTLA4Ig) and rapamycin with LFA-1 blockade enhanced bone marrow engraftment and led to more efficient T-cell engraftment and subsequent tolerization. Although fully mismatched skin grafts were chronically rejected, primarily vascularized heart allografts survived indefinitely and without signs of chronic rejection, independent of minor antigen mismatches.

CONCLUSIONS: We have demonstarted a robust protocol for the induction of tolerance for cardiac allografts in the absence of CD40 blockade. Our findings demonstrate the potential of a clinically relevant minimal conditioning protocol designed to induce lifelong immunologic tolerance toward cardiac allografts.

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Induction of tolerance in clinical heart transplantation would not only eliminate toxic side effects of long-term immunosuppressive therapy, but it would also prevent immune-mediated cardiac allograft vasculopathy (CAV), which is the main cause of late graft loss. Although

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numerous strategies have been successfully implemented to induce donor-specific tolerance in murine models, almost all of them have failed when being translated to large animals and non-human primates (NHPs). The mixed chimerism approach is the only tolerance strategy that has been successful across species and has been implemented in a series of human renal transplantation trials.[1] However, there are 2 major hurdles preventing widespread clinical application in human organ transplantation.[2] First, for several reasons, most experimental murine protocols are not ready for translation into NHPs or humans. Cytotoxic recipient conditioning is often considered too risky for (renal) transplant recipients; moreover, humans tend to develop only transient mixed chimerism or full chimerism, which is associated with an increased risk of graft-vs-host disease (GVHD).[3] Furthermore, anti-CD40L, the backbone of most non-myeloablative mouse models, is not available in the clinical setting due to life-threatening thromboembolic complications.[4] The second hurdle for clinical application of the mixed chimerism approach in human organ transplantation is that those NHP studies with successful tolerance protocols for kidney allografts cannot translate and extend to heart, lung, or islet allografts.[5–8] Murine models mostly use skin transplantation, which is considered to be the most stringent approach. However, this approach is not ideal when it comes to translation to NHP and human solid-organ transplantation, as skin grafts are not primarily vascularized and trigger a different immune response due to altered donor T-cell migration.[9]

We believe that organ-specific tolerance approaches are urgently needed to bring the mixed chimerism approach to clinical application. In NHPs and humans, reduced-intensity protocols mostly lead to transient chimerism in a human leukocyte antigen (HLA)-mismatched setting, unlike in mice, where durable and multilineage mixed chimerism can easily be achieved across major histocompatibility complex (MHC) barriers.[10] T-cell chimerism has been shown to be a major predictor of successful tolerance induction[11]; however, in a human combined kidney and bone marrow (BM) transplantation (BMT) trial, T-cell chimerism was lost as early as 2 weeks post-BMT.[12] Whereas kidney and liver have been proposed to have tolerogeneic potency, heart allografts may need a more robust approach to tolerance to ensure survival and prevention of CAV.

The adhesion molecule lymphocyte function–associated antigen-1 (LFA-1; CD11a) is important for T-cell homing and has recently been shown to influence T-cell differentiation, thereby fine-tuning effector functions.[13] LFA-1 signaling via ligation of LFA-1 and its target intercellular adhesion molecule (ICAM) has been shown to impact allograft survival in multiple mixed chimerism and operational tolerance models, although most of them utilized additional CD40 blockade.[14–17] We hypothesized that blockade of LFA-1 signaling may benefit BM engraftment by targeting costimulatory blockade-resistant T cells,[18] particularly memory T cells,[17] and by inhibition of natural killer (NK)-cell activation[19,20] even in the absence of CD40 blockade. Rapamycin, a mechanistic target-of-rapamycin (mTOR) inhibitor, is known for its potent immunosuppressive functions, which are commonly ascribed to inhibition of T-cell proliferation. Moreover, rapamycin plays a major role in modulating innate and adaptive immune responses by favoring the development of regulatory T cells (Tregs), and thereby creating a tolerogeneic environment.[21] In this study, we were able to show that anti–LFA-1 treatment in combination with short-course rapamycin not only enhances BM engraftment in a clinically relevant BMT protocol sparing CD40 blockade, but is also superior to anti-CD40L for induction of tolerance toward fully mismatched heart allografts. Chimerism levels remained stable throughout follow-up, and acute rejections, as well as CAV, were successfully prevented.

Methods

Ethics statement

All experiments were approved by the local review board of the Medical University of Vienna and were performed in strict accordance with national and international guidelines of laboratory animal care. All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). The experiment described in this proposal are covered by ethics vota of the Austrian Federal Ministry of Science, Research and Economy BMWF-66.009/0195-II/10b/2009 and GZ 66.009/0230-II/3b/2011 (according to "Tierversuchsgesetz" 2012, BGB I No. 114/2012). (Mortality rates associated with the various procedures are presented in Table S1 in the Supplementary Material, available online at www.jhltonline.org/.)

Animals

Female C57BL/6 (B6, recipient, H-2^b), BALB/c (donor, H-2^d), and C3H/HeNCrl (C3H, third party, H-2^k) mice were purchased from Charles River Laboratories (Sulzfeld, Germany). B10.D2 (H-2^d, minor antigen matched to B6) and CNCr.129P2-Cd40^{tm1Kik}/J (CD40$^{-/-}$; H-2^d, BALB/c background) mice were purchased from Jackson Laboratories (Bar Harbor, Maine) and bred at our own facility. All mice were housed under specific pathogen-free conditions in ventilated filter cages (up to 5 mice). Recipient mice were used at 6 to 8 weeks of age, with an average weight of 18 to 20 g. Animals were provided with sterilized water and rodent chow (Sniff, Soest, Germany) ad libitum.

BMT protocol

Groups of age-matched B6 recipients received non-myeloablative total body irradiation (TBI) using a Xylon X-ray unit[22] (2 to 3 Gy, Day −1) and costimulation blockade with T-lymphocyte antigen 4 immunoglobulin (CTLA4Ig; 0.5 mg, Day 2) with or without anti-CD40L (MR1; 1 mg, Day 0), anti–LFA-1 monoclonal antibody (MAb; anti-CD11a, M17/4, 0.5 mg, Days −1 and 2), and rapamycin (5 mg/kg, Days −1, 0, and 2) as indicated. In indicated mice, NK cells were depleted with anti-NK1.1 MAb (0.25 mg on Days −1 and 2; Clone PK136). In vivo antibodies were injected intraperitoneally (IP) in a volume of 250 to 500 μl (diluted in