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Bone marrow transplantation as a strategy for tolerance induction in the clinic

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1. ABSTRACT

The only way to overcome the need for life-long immunosuppression in a transplant recipient is to induce tolerance. Deletional tolerance can be reliably achieved with the induction of mixed chimerism through transplantation of donor bone marrow (BM). Despite the development of increasingly milder BM transplantation (BMT) animal models, BM engraftment in humans still requires considerably toxic conditioning and puts patients at risk for the development of GVHD. However, in a proof-of-concept trial, mixed chimerism and tolerance have been successfully induced in highly selected patients suffering from both end-stage renal disease and multiple myeloma. Meanwhile, there has been notable progress in developing advanced experimental BMT regimens, in particular through the use of costimulation blockers. Costimulation blockade in rodent models allowed the design of BMT protocols entirely devoid of irradiation. Costimulation blockers have also succeeded in more complex protocols in non-human primates. They are under clinical evaluation in renal transplantation as immunosuppressive therapy. Costimulation blockade may lead the way for the development of milder BMT protocols and broader application of mixed chimerism in organ transplantation.

2. INTRODUCTION

Despite satisfying rates of short-term survival after organ transplantation, long-term success remains poor. Patients require life-long immunosuppression which is associated with severe side effects, and current immunosuppressants cannot prevent chronic allograft rejection thereby limiting long-term graft survival (1-4).

The concept of tolerance through chimerism has a long history which is presented in detail elsewhere (5;6). Owen observed a naturally occurring state of mixed chimerism in fraternal bovine twins that share a common placental circulation (7). Subsequently, Medawar demonstrated that these chimeric twins were tolerant to skin grafts from their twin sibling (8;9), and was then also first to actively induce tolerance by injecting hematopoietic cells (10). Main and Prehn achieved full hematopoietic chimerism and tolerance through BMT and lethal irradiation (11). The concept of mixed chimerism – a state wherein hematopoietic cells of both the recipient and the donor co-exist in the recipient – was pioneered by Sachs and colleagues, and was first established after lethal irradiation (12) and subsequently after non-myeloablative conditioning (13). In addition to requiring less toxic host

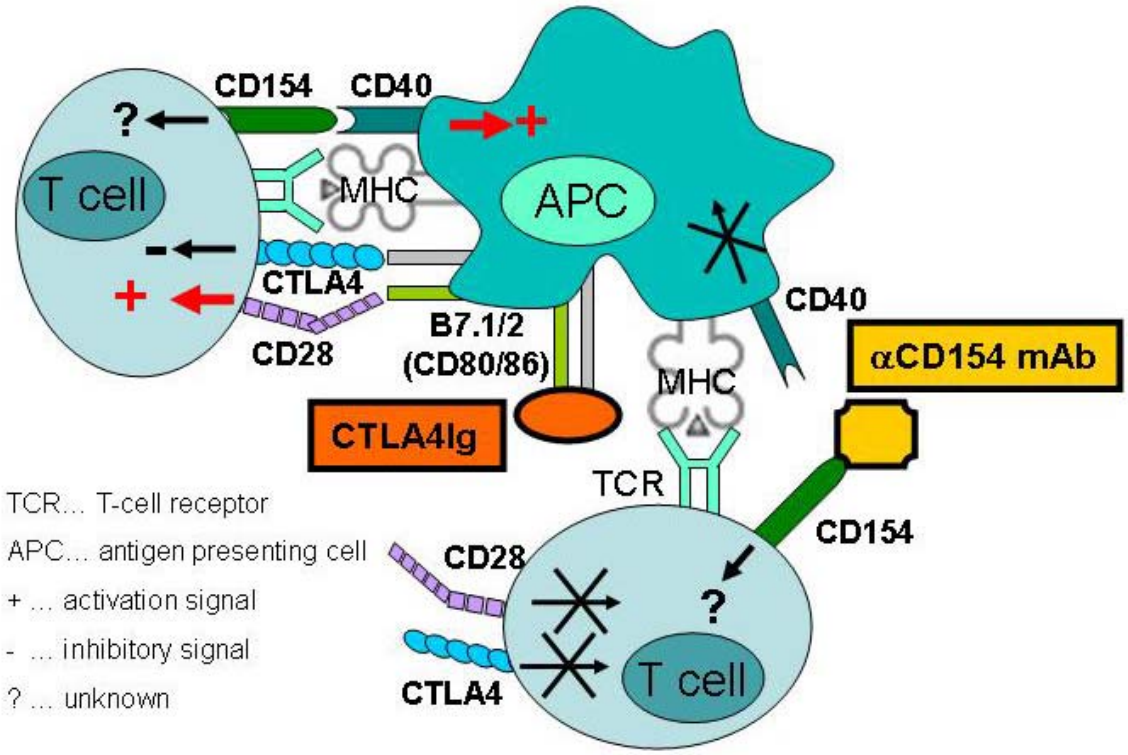

Figure 1. Schematic illustration of costimulatory blockade. (a) Interaction of T-cell and antigen-presenting cell. Antigens are presented via the MHC to the TCR, costimulation mediated by B7.1/2-CD28 and CD40/CD154 interaction induced activating signals whereas B7.1/2-CTLA4 induces inhibitory signals to the T cell. (b) Mechanism of costimulation blockers (anti-CD154mAb, CTLA4Ig): Activating signals are inhibited while allowing TCR-MHC interaction.

conditioning, mixed chimerism offers the advantage of improved immunocompetence (14;15) and reduced susceptibility to graft-versus-host disease (GVHD) (16;17).

The basic principle of tolerance via chimerism relies on educating the hematopoietic repertoire in central organs to become tolerant towards donor-antigen like it is tolerant to self-antigen. In the experimental setting, the key mechanism of this tolerance strategy is intrathymic deletion of newly-developing donor-reactive T cells, one of the major mechanisms by which also self-tolerance is maintained (10;18;19). A bone marrow chimeric individual deletes both self-reactive and allo-reactive T cells and thereby becomes tolerant to donor antigen. True tolerance makes immunosuppression dispensable. The robustness and specificity of tolerance has been firmly established by the acceptance of donor-specific skin - suggested to be the most immunogenic tissue and the most stringent experimental test – and the rejection of 3rd party grafts (12).

For the establishment of chimerism the individual needs to be prepared (i.e. conditioned) in a way that prevents rejection of the transplanted donor bone marrow cells (BMC) and that makes engraftment physiologically possible, i.e. stem cell homing to recipient BM niches. During the past two decades there has been substantial progress towards more selective and minimized conditioning in BMT.

3. MIXED CHIMERISM AND COSTIMULATION BLOCKADE

3.1. Progress in mouse models towards minimum conditioning

After years of having to wipe out the recipient's entire T cell repertoire in mixed chimerism models (e.g. by using depleting anti-CD4 and -CD8 mAbs) (13;20-23), substantially milder protocols were achieved through using costimulation blockers as part of BMT protocols (24-27). Costimulation blockers represent a targeted approach to modulate alloresponses, preventing activation signals at the early stage of costimulation between the antigen presenting cell (APC) and the T cell.

Anti-CD154 (CD40L) and CTLA4Ig - which are the most frequently used costimulation blockers - efficiently interfere with the CD28 and the CD40 pathways of T cell and APC activation (28;29). The fusion protein CLTA4Ig binds to B7 molecules on the APC as does its physiological counterpart CTLA4 expressed on the T cell surface. By affinity competition, the activating co-stimulation receptor CD28 is prevented from B7 ligation (Figure 1). Anti-CD154 is a monoclonal antibody that directly binds to and blocks the target ligand, i.e. CD154. Thereby it interferes with the CD40 pathway (Figure 1).

A variety of BMT protocols using costimulation blockade has been developed and improved within recent years and their underlying mechanisms have been extensively investigated (25-27;30-41). In addition to costimulation blockade most of them include low dose total body irradiation (TBI) (or non-myeloablative doses of busulfan) in order to allow engraftment of a clinically feasible dose of BMC (see Table 1). Substantial progress has been achieved towards minimum conditioning by omitting TBI when transplanting an approx. 10-fold higher than usual dose of BMC into the recipient (35;39;41). To date these high doses, however, are clinically not available. Even though syngeneic mobilized peripheral blood stem cells (PBSC) have been shown to engraft after non-myeloablative TBI (42), they turned out to be less tolerogenic in allogeneic models (Wekerle et al. unpublished). An increasing number of additional costimulation pathways have been described in recent years (43). The combined inhibition of multiple pathways has been found to act synergistically in non-BMT models (44). It appears likely that interference with several – positive and negative – costimulation pathways will allow further optimization of experimental BMT protocols, and indeed might be necessary for their clinical translation. So far experience in BMT models remains, however, limited. Recently, for instance, CD4 and CD8 co-receptor blockers (non-depleting mAbs) have shown encouraging results in BMT protocols without TBI when used as extensive recipient pretreatment (4 weeks) in specific strain combinations (45). No beneficial effect of OX40L blockade in addition to anti-CD154 and CTLA4Ig, in contrast, was found after non-cytoreductive allogeneic BMT (41). Furthermore, it has been shown that NK cell depletion strongly promotes BM engraftment, thereby allowing a reduction of the minimum conditioning requirements (40;46). These studies demonstrate that interventions like blocking of T cell co-receptors or eliminating NK cells help in making TBI dispensable.

3.2. Implications of model diversity on tolerance mechanisms

Notably, modes of actions of costimulation blockers differ between solid organ transplantation and BMT models (30). Besides, regarding the various BMT protocols that have already been developed (Table 1), it has become clear that tolerance mechanisms and the effectiveness or need of additional compounds often depend on the details of the regimen, some examples of which are discussed below.

3.2.1. Molecular mechanisms

BMT studies in CD154 knock out mice revealed that anti-CD154 is mainly a non-depleting blocking antibody (47). There is, however, also some evidence that anti-CD154 may deplete T cells in other models (48-50). Other data suggest that it might deliver an inhibitory signal to the T cell (51). A tolerogenic role of the tryptophan catabolizing enzyme indoleamine-2,3-dioxygenase (IDO) has been proposed in various settings of APC and Treg interaction (52). In an islet transplantation model the fusion protein CLTA4-IgG3 has also been shown to activate IDO (53). However, IDO activity was shown to be not critical in a BMT protocol using the clinically available human CTLA4-IgG1 (abatacept) (54).

3.2.2. Cellular mechanisms

The significance of progressive, thymus-independent deletion of pre-existing donor reactive T cells in the periphery upon BMT under costimulation-blockade has been firmly established (25;55-57). Clonal deletion is a welcome mechanism since it physically eliminates T cells with a certain antigen-reactivity. In costimulation-based protocols, there is additional evidence for regulatory mechanisms in the early phase after BMT (36;58;59). The specific subtypes of Tregs playing a role have not been established yet. For example, NKT cells have regulatory activity and their activation by alpha-galactosylceramide ameliorates autoimmune disease in some models. Stimulation of NKT cells in a mixed chimerism protocol, however, led to rejection. There is evidence that this may be due to NKT-mediated NK cell activation (Wekerle et al, unpublished). In rodent models using total lymphoid irradiation (TLI), in contrast, NKT cells have a beneficial role and help preventing GVHD (60). T cell tolerance is critical for inducing transplant tolerance. However, for complete tolerance to be accomplished it is important that NK cells and B cells are tolerized as well, which mixed chimerism models have been shown to achieve (40;61-63). Preliminary evidence suggests that cellular transfer of regulatory T cells or facilitating cells can have a beneficial role in mixed chimerism regimens (64-68).

3.2.3. Compatibility with immunosuppressants.

Short-term immunosuppression is necessary for ethical reasons when experimental protocols are translated into the clinical setting. Investigating the compatibility and benefit of different (combinations of) immunosuppressants in tolerance protocols is therefore required. The calcineurin inhibitors cyclosporine A (CsA) and tacrolimus abolished tolerance induction in a costimulation blockade based protocol (69). In contrast a four-week course of rapamycin-based immunosuppression improved murine mixed chimerism protocols by promoting BM engraftment (41). Another study, however, suggested that calcineurin inhibitors and corticosteroids are compatible which again may be due to protocol differences (70).

3.3. BMT protocols for large animals and non-human primates

A crucial hurdle for translating murine low toxicity protocols into the clinical setting are not only species differences but also the fact that experimental mice are usually kept under clean, protected conditions which prevents the physiologic maturation and ageing of the immune system (71). The T cell and B cell responses under physiologic conditions lead to a high frequency of memory cells reactive to alloantigen. Allo-reactive memory cells, however, are very difficult to tolerize. Furthermore, ongoing subclinical infections can also contribute to an augmented (allo-) response accelerating a rejection process (72;73). Pre-clinical protocols therefore require modifications of the conditioning regimen.

Table 1. Selected murine protocols for the induction of mixed chimerism and tolerance

strain combination	Myelo-suppression (TBI or cytotoxic drugs)	Recipient cell depletion	DST	MR1 (anti CD154mAb)	CTLA4Ig	BMC	mechanistical study	Ref
recipient: C57BL/6; donor: B10.A	d 0; 3 Gy TBI	d-6/17/14; antiCD4(2mg) antiCD8(1,4mg)	x	x	x	15 x 10^6; d0	T cell depleting Ab can replace Thymic irr.	22
recipient: C57BL/6; donor: B10.A	d 0; 3 Gy TBI	x	x	d0; 0,45mg	d2; 0,5mg	15 x 10^6; d0		25
recipient: C57BL/6; donor: B10.A	d 0; 3 Gy TBI	d-5; antiCD4(1,8mg) antiCD8(1,4mg)	x	d0; 0,45mg	d2; 0,5mg	15 x 10^6; d0		24
recipient: C57BL/6; donor: B10A	none	x	x	d0; 0,45mg	d2; 0,5mg	200 x 10^6; d0		39
recipient: C57BL/6; donor: Balb/c	none	x	x	d0/2/3/6/14/ 38/60/90; 0,5mg	x	20 x 10^6; d0/2/3/6/14/38/60/90		35
recipient: Balb/c, ; donor: C57BL/6	d0; 4 Gy TBI	x	x	d0/3; 0,5mg	x	25 x 10^6; d0		33
recipient: C57BL/6; donor: Balb/c	d5; busulfan 20mg/kg	x	Yes (initial BM dose)	d0/2/4/6; 0,5mg	d0/2/4/6; 0,5mg	T cell depleted: 20 x 10^6; d0/6		26
recipient: C57BL/6; donor: B10.A	3 Gy TBI; d0	d-1 anti CD8 (0,35mg)	x	d0; 0,5mg	x	20 x 10^6; d0	CD8 cells are responsible for resistance to alloBM engraftment under costimulatory blockade	31
recipient: C57BL/6 CD40L-/-; donor: B10.A	3 Gy TBI; d0	d-1 anti CD8 (0,35mg)	x	x	x	T cell depleted: 20 x 10^6, d0	MR1 blocks IA between CD40 and CD40L, antiCD40LmAbs need not play a role in CD4 signalling/deletion	35
recipient: C57BL/6; donor: Balb/c	d-1; 2 Gy TBI	x	x	d-1/0/1; 0,2mg	x	40 x 10^6; d0		27
recipient: B6; donor: Balb/c	d0; 1Gy TBI	x	d-10; 10 x 10^6 spleno-cytes	d-10/-7/-3/0/3; 1,6mg	x	40 x 10^6; d0		32
recipient: C57BL/6; donor: Balb/c	d-1; busulfan 20mg/kg	x	x	d0/2/4/6; 0,5mg	d0/2/4/6; 0,5mg	T cell depleted: 20 x 10^6; d0		38
recipient: Balb/c, ; donor: C57BL/6	x	x	d-7; 10 x 10^6 splenocytes	d-7/-4/0/3; 0,5mg	d-7/-4/0/3; 0,5mg	50 x 10^6, d0		34
recipient: C57BL/6; donor: Balb/c	d-1; 1Gy TBI, d0-28; rapamycin(0,2mg/kg), MP(10mg/kg), MMF(20mg/kg)	x	x	d0; 1mg	d2; 0,5mg	15-20 x 10^6, d0	chimerism can be increased by short-time immunosuppression	69
receipient: B6 CD45.1; donor: B6 CD45.2	d0; 7Gy local irradiation	x	x	x	x	200 x 10^6, d0	local irradiation enhances donor PHSC engraftment	37
recipient: C57BL/6; donor: Balb/c	d-1; 3Gy TBI	antiCD25 or anti IL2	x	d0; 1mg	d2; 0,5mg	15-20 x 10^6; d0	Peripheral deletion and regulation are essential in early phase of tolerance induction	36
recipient: C57BL/6; donor: Balb/c	none	d-5/-1; antiNK1.1(0.25mg), antiCD8(0,25mg)	x	d0; 0,5mg	x	30 x 10^6; d0	chimerism can be increased by NK cell depletion	46
recipient: C57BL/6; donor: Balb/c	d0-27; rapamycin(0,2mg/kg), MP(10mg/kg), MMF(20mg/kg)	x	x	d0; 1mg	d2; 0,5mg	50 x 10^6; d0		41
receipient: CBA, Balb/c; donor: B10	none	first stage(4weeks preBMT), second stage(d0/2/4); antiCD4(1mg), anti CD8(1mg) (non-depleting)	x	first stage(4weeks preBMT), second stage(d0/2/4); 1mg	x	T cell depleted: 10 x 10^6; d0		45
receipient: B6 CD45.1; donor: B6 CD45.2	d-1; 1Gy TBI	x	x	x	x	mPBMC 20 x 10^6; d0	mPBSC can induce chimerism in a nonmyeloablative congenic model	42
recipient: C57BL/6; donor: Balb/c	d5; busulfan 20mg/kg	d-2/-1/0; antiNK1.1(0.2mg) or anti LFA1(0.2mg)	yes (initial bone marrow dose)	d0/2/4/6/14/28; 0,5mg	d0/2/4/6/14/28; 0,5mg	15-20 x 10^6, d0; 2 x 106, d6	chimerism can be increased by NK cell depletion and by LFA-1 blockade	40

These challenges notwithstanding, there has been notable progress towards reduced conditioning in large animals. A porcine protocol devoid of thymic irradiation (TI) and TBI induced stable chimerism and donor-graft tolerance upon administration of depleting antibodies and transient CsA therapy (74). Conditioning consisted of T cell depletion, mobilized peripheral blood stem cells (mPBSC) and a post-transplant course of CsA. In dogs receiving MMF and CsA after hematopoietic stem cell transplantation (HSCT), a one-week injection course of

CTLA4Ig together with donor peripheral blood mononuclear cells (PBMC) helped to reduce TBI down to 1 Gy (75). A single injection of anti-CD154 on day -5 plus donor specific infusion (DSI) on day -4 significantly facilitated engraftment and BM survival (76).

In non-human primates a prototypical regimen involves non-myeloablative TBI (preferably fractionated so that side effects are reduced), anti-thymocyte globulin (ATG), thymic irradiation (TI), splenectomy, allogeneic BM and a 4-week-course of cyclosporine. It has been demonstrated that transient chimerism can be sufficient to induce allo-specific tolerance in this setting, as it is in humans (77). Kidney, heart and pancreatic islet allografts showed long-term survival despite weaning of CsA (78-80). Mixed leukocyte reactions (MLRs) and skin grafting confirmed a state of donor-specific tolerance (78). Costimulation blocker anti-CD154 enhanced mixed chimerism and allowed a reduced regimen, by the omission of splenectomy (81). Simultaneous blockade of CD28 and CD154 together with non-myeloablative doses of busulfan, anti-CD25 and sirolimus showed a further improvement in chimerism levels and durability, and provides one of the most advanced non-human primate tolerance protocols to date (82).

4. MOVING COSTIMULATION BLOCKERS AND MIXED CHIMERSIM TO THE CLINIC

4.1. Clinical trials for costimulation blockers without BMT

Costimulation blockers have been of considerable interest as immunosuppressants in solid organ transplantation and in T cell-mediated autoimmunity due to their encouraging success in animal studies (28). However, selective biologic agents may need to be re-designed for various indications. Abatacept (CTLA4Ig) was effective in rheumatoid arthritis and has been approved for this indication by the Food and Drug Administration (FDA). As abatacept proved unsatisfactory for organ transplantation in non-human primate studies, a series of mutants was screened and belatacept (formerly known as LEA29Y) was found to have sufficient efficacy in non-human primate kidney transplantation (83;84). Belatacept has subsequently been shown to be effective and safe in a calcineurin inhibitor-free immunosuppressive regimen in a clinical phase II kidney transplant trial (85) and is currently under evaluation in phase III trials.

In contrast to CTLA4Ig´s advance to clinical application, anti-CD154 therapy failed in clinical trials. Despite successful preclinical evaluation, thromboembolic complications were reported in early human trials leading to a halt in clinical development (86-88). However, recent approaches hold promise in targeting the CD40 pathway by other means such as monoclonal antibodies against the CD40 receptor. It has been proposed that this antibody (Chi220) not only blocks the CD40 pathway but also has agonistic properties. In combination with belatacept Chi220 has been shown to substantially prolong islet allograft survival in rhesus macaques (89).

4.2. Current status of clinical BMT – reducing toxicity

Despite successful and very mild protocols in animals, the major barriers for large-scale clinical application are toxicities and side effects of current BMT regimens in the clinic. Recent developments in clinical BMT have been reviewed in detail elsewhere (90-92).

Since classical BMT is often the only treatment option for patients suffering from hematological cancers or life-threatening genetic disorders, its side effects are endured. Apart from GVHD many acute and chronic severe side effects are associated with classical HSCT. Even in more recent reduced intensity conditioning regimens the problems of GVHD and the risk of infections remain (93;94). Thus progress towards the use of mixed chimerism for routine tolerance induction critically depends on advances in clinical BMT regimens.

4.3. Tolerance in a clinical pilot trial

A small prospective proof-of-concept pilot trial demonstrated that BMT can induce tolerance in human organ transplant recipients (95;96). Patients suffering from end-stage renal failure and multiple myeloma were conditioned with cyclophosphamide, ATG and thymic irradiation. Bone marrow and a kidney from an HLA-identical sibling donor were transplanted simultaneously, followed by a limited course of CsA. The outcome revealed that tolerance indeed ensued (at least) in some patients (e.g. one patient more than seven years post-KTX without immunosuppression has stable renal function). At the same time it demonstrated the current limitations of this approach, in particular as two patients developed GVHD (77). Macrochimerism was usually transient in this series of patients. The course of chimerism is thus similar to the experience in non-human primates underlying this regimen (78-81;97). While in rodents permanent mixed chimerism is required to achieve and maintain primary skin graft tolerance, in non-human primates (as described above) and humans transient macrochimerism – possibly followed by microchimerism – does suffice under specific circumstances to achieve tolerance towards a kidney graft. Consequently the tolerance mechanisms also differ between rodent mixed chimeras and humans (59;98;99). Thus, for now, we know how to render an organ recipient tolerant towards the graft, but we do not know yet how to do it in a way acceptable for widespread clinical use.

5. CONCLUSION AND PERSPECTIVES

Numerous protocols ranging from mice to man have proven the principle of establishing robust and reliable tolerance by transplanting donor BMC. Pre-conditioning may vary, but as soon as chimerism is established, organs derived from the bone marrow donor usually survive without any immunosuppressive medication of the host. However, BMT protocols that are currently in clinical use are unacceptably toxic to be offered to a transplant patient. Costimulation blockers allow mild host conditioning in rodents. Species differences and the immunological gap between selected mouse strains kept under protected conditions and humans constantly experiencing immunologic

challenges hamper direct translation of current low-toxicity animal protocols into the clinic. In order to further clinical progress, particularly stringent models and investigations on the tolerization of lymphocytes distinct from naïve T cells are required. Additionally, we may need to pay more attention to protocol differences before deducing conclusions on mechanisms or the compatibility and benefit of potential drugs. Cell types that resist to or promote tolerance induction, such as NK cells, NKT cells and regulatory T cells, are only partially defined and could offer new strategies for the induction of chimerism and tolerance.

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Abbreviations: BMT: bone marrow transplantation; TI: thymic irradiation; CTLA4Ig: cytotoxic T lymphocyte antigen -4 immuneglobulin; TBI: total body irradiation; CsA: Cyclosporine A; ATG: anti-thymocyte globulin; BMC: bone marrow cells; GVHD: graft versus host disease

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