

Cell-Based Therapy in Allergy

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Abstract IgE-mediated allergy is an immunological disorder occurring in response to otherwise harmless environmental antigens (i.e., allergens). Development of effective therapeutic or preventive approaches inducing robust tolerance toward allergens remains an unmet goal. Several experimental tolerance approaches have been described. The therapeutic use of regulatory T cells (Tregs) and the establishment of molecular chimerism are two cell-based strategies that are of particular interest. Treg therapy is close to clinical application, but its efficacy remains to be fully defined. Recent proof-of-concept studies demonstrated that transplantation of syngeneic hematopoietic stem cells modified in vitro to express a major allergen leads to molecular chimerism and robust allergen-specific tolerance. Here we review cell-based tolerance strategies in allergy, discussing their potentials and limitations.

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1 Introduction

IgE-mediated allergic disorders are immunological hypersensitivity reactions. Allergic immune responses occur against environmental, otherwise innocuous antigens, known as allergens. Genetic and environmental factors influence susceptibility to allergic hypersensitivity (Akdis 2006; Vercelli 2008).

The only causative treatment available so far is allergen-specific immunotherapy (SIT), a vaccination strategy (Valenta 2002). Increasing doses of allergens (mostly in the form of crude extracts) are administered to allergic individuals with the goal to induce “desensitization”. In this context, immunological tolerance in allergy was suggested to be defined as persistence of efficacy after treatment (Akdis and Akdis 2009). Numerous mechanisms have been suggested to show how SIT alters B cell and T cell responses. Mechanisms may vary depending on treatment protocol, route of administration and allergen preparation (Larche et al. 2006). SIT induces de novo humoral responses dominated by the production of protective allergen-specific IgG antibodies, mainly IgG4 (Nouri-Aria et al. 2004; Wachholz and Durham 2003). A Th2 to Th1 shift and induction of Tregs also contribute to effectiveness (Bohle 2008). SIT is, however, associated with limited effectiveness and considerable risks, as exemplified by anaphylactic reactions or therapy-induced sensitization to additional allergens when crude extracts are used (Valenta 2002). In addition to the need for more effective allergen-specific therapeutic strategies, prevention of allergy by prophylactic induction of tolerance is a major unmet need (Hamelmann et al. 2007).

Several experimental tolerance strategies have been described, with cell-based approaches having gained particular attention in recent years. This review discusses induction of tolerance through cell therapies as a possible approach to prevent and treat allergy.

2 Prevention of Allergy by Induction of Peripheral Tolerance

Peripheral, i.e., extrathymic, T cell tolerance is a critical mechanism of self-tolerance and can either occur by peripheral clonal deletion (Webb et al. 1990), anergy (Fathman and Lineberry 2007) or suppression (Sakaguchi 2005).

Potent tolerance models relying on peripheral tolerance have been developed. Conceptually, they suffer, however, from the drawback to have no mechanism in place to tolerize newly developing T cells and thus their effects are usually limited in duration (Wekerle et al. 2003).

2.1 Induction of Tolerance via the Mucosal Route

Specific non-responsiveness to inhaled and ingested allergens is a major goal in IgE-mediated allergy. Mucosal tolerance is induced through administration of antigens via the oral or nasal route (Faria and Weiner 2005). This phenomenon was reported very early as protection of systemic anaphylaxis was observed upon injection of hen's egg protein in guinea pigs having been fed with the antigen (Wells 1911). Two primary mechanisms of mucosal tolerance have been described. Low-dose antigen-feeding induces active suppression by generation of Tregs, high-dose feeding regimens induce anergy (Friedman and Weiner 1994). TGF β (transforming growth factor beta)-producing T helper type 3 (Th3) cells are a Treg subtype specifically induced upon antigen-feeding (reviewed in Faria and Weiner 2005). Beside T cell-mediated immunoregulation, induction of allergen-specific IgA antibodies is a prominent mechanism of oral tolerance (Challacombe and Tomasi 1980).

Mucosal tolerance via the respiratory tract is more relevant to inhalant allergens. High-dose intranasal exposure of one single dominant T cell epitope of the house dust mite allergen Der p 1 was sufficient to induce T cell anergy toward the whole Der p 1 allergen by linked suppression, a phenomenon prominently described in transplantation (Hoyne et al. 1993, 1999; Qin et al. 1993). Dominant T cell epitopes of different allergens and in different combinations have been investigated for intranasal application, resulting in reduction of allergen-specific IgE and IgG responses. However, allergen-specific isotypes are not completely eliminated and only short-term follow up was reported in these models (Hufnagl et al. 2005, 2008; Marazuela et al. 2008; Wild et al. 2007). Involvement of Tregs and a Th2 to Th1 shift are important mechanisms in these protocols (Hufnagl et al. 2008; Marazuela et al. 2008; Winkler et al. 2006).

2.2 Induction of Tolerance by Intradermal Peptide Application

Systemic subcutaneous administration of T cell peptides of the major cat allergen Fel d 1 (without adjuvant) successfully led to peripheral T cell tolerance in experimental models. Translation to the clinical setting employing intradermal administration of T cell peptides of Fel d 1, however, induced late asthmatic reactions in some patients (Briner et al. 1993; Haselden et al. 1999; Oldfield et al. 2002). T cell responses were diminished, but humoral responses toward the full

length allergen persisted (Briner et al. 1993). In a novel transgenic mouse model of asthma mimicking the human situation systemic intradermal application of one Fel d 1 immunodominant peptide in pre-sensitized mice resulted in resolution of airway pathology through linked suppression, possibly through generation of Tregs (Campbell et al. 2009). To allow full assessment of the potential of T cell peptides as therapeutic vaccines, several parameters still need to be determined, such as optimum length and dose of peptides (Larche and Wraith 2005).

2.3 Tregs as Cell-Based Therapy in Allergy

Regulatory T cells play a critical role in maintaining self-tolerance (Sakaguchi and Powrie 2007). Numerous, incompletely defined, subpopulations of Tregs have been described, with CD4+CD25+FoxP3+ natural Tregs arising from the thymus, and adaptive/induced Tregs generated in the periphery playing major roles (Feuerer et al. 2009). Tregs regulate T cell and B cell responses and also innate immunity. Whether memory T cells are amenable to regulation by Tregs remains controversial (Levings et al. 2001; Yang et al. 2007). The therapeutic exploitation of Tregs has attracted enormous interest in recent years, fueled by hopes of developing antigen-specific treatments for transplant recipients, autoimmune diseases and allergies (Akl et al. 2008; Hutchinson et al. 2008; Riley et al. 2009). So far, however, the physiology of Tregs remains incompletely understood and their therapeutic potential largely unexplored (Schiopu and Wood 2008).

Several lines of evidence reveal a prominent role of Tregs in regulating, or preventing, respectively allergic immune responses. A role for T regulatory type 1 (Tr1) cells in modulating allergic immune responses has been found by comparing immune responses upon allergen exposure between healthy individuals and allergic patients (Akdis et al. 2004). Natural high exposure of venom allergen (phospholipase A2) induces a switch of specific Th1 and Th2 cells to Tr1 cells (Meiler et al. 2008). Tr1 cells are of special therapeutic interest, as they are currently under clinical investigation in bone marrow (BM) transplant recipients, as are natural Tregs (nTregs) (reviewed in Roncarolo and Battaglia 2007; Roncarolo et al. 2006; Schiopu and Wood 2008; Riley et al. 2009). First clinical trials using freshly isolated or expanded donor nTregs show encouraging results in the prevention of lethal graft versus host disease (GVHD) (Trzonkowski et al. 2009; Brunstein et al. 2011; Di Ianni et al. 2011). nTregs express the transcription factor forkhead box P3 (FoxP3) (Miyara et al. 2009) and play an indispensable role in maintaining self-tolerance (Sakaguchi et al. 2008). FoxP3-deficient patients suffering from immunodysregulation, polyendocrinopathy and enteropathy X-linked syndrome (IPEX) are affected by atopic disease, resulting in increased serum IgE levels, eosinophilia, eczema-like skin lesions and enhanced Th2 responses. This provides clear evidence that Fox P3-expressing Tregs play an essential role in suppression of Th2-driven immune responses in humans (Chatila 2005).

In experimental models in allergy, the therapeutic application of nTregs abolished allergic airway inflammation (Kearley et al. 2005, 2008; Leech et al. 2007). Transfer of nTregs resulted in reduction of allergen-specific IgE and a boost of IgG1 immunomodulation after nasal administration of Der p 1 in an IL-10 independent manner (Leech et al. 2007). In another model, ova-specific CD4+CD25+ T cells suppressed T cell responses in the lung but IgE responses were not altered in a preventive approach (Kearley et al. 2005). Features of chronic allergen-induced inflammation were resolved but established remodeling in the lung was not reversed and humoral responses to allergen were not altered (Kearley et al. 2008). Thus, the use of natural Tregs has a suppressive effect on allergic airway inflammation, but seems to be less effective in preventing IgE responses. Application of FoxP3-transduced polyclonal CD4+ cells is effective in models of autoimmunity and allo-transplantation (Chai et al. 2005; Jaeckel et al. 2005; Pilat et al. 2010). Using polyclonal transduced Tregs together with the application of a recombinant grass pollen allergen in preventive and therapeutic murine models, we observed that allergen-specific IgE was not significantly reduced and T cell responses were not suppressed (Baranyi, Pilat, Wekerle et al., unpublished data).

In summary, Treg therapy is of considerable interest for treating allergic diseases. Numerous basic biological questions still need to be resolved in this new field of investigation. Only then can the clinical potential of Treg therapy be adequately assessed.

3 Tolerance Induction by Hematopoietic Stem Cell Transplantation (HSCT)

Self-tolerance is mediated mostly by various subpopulations of hematopoietic cells (von Boehmer and Kisielow 2006). Chimerism-based tolerance strategies emulate this fundamental characteristic of physiologic self-tolerance with the aim of inducing tolerance toward introduced, disease-causing antigens (Fehr and Sykes 2008; Wekerle and Sykes 2001). Such chimerism protocols are the only strategies to reliably establish central tolerance, i.e., the intrathymic clonal deletion of antigen-specific thymocytes. Thereby they provide mechanisms not only for tolerizing pre-existing mature T cells through peripheral mechanisms (Bigenzahn et al. 2005; Wekerle et al. 2002), but also for continuously tolerizing the newly developing T cells constantly arising in the thymus (Wekerle et al. 1998; Wekerle and Sykes 2001). Moreover, chimerism is capable of inducing T cell, B cell and NK cell tolerance (Sykes et al. 1998), and also tolerance in pre-sensitized recipients (Colson et al. 2000). Tolerance induced by chimerism is thus of particular robustness and durability.

Two types of hematopoietic chimerism can be distinguished: cellular and molecular, with mixed chimerism being the most commonly used subtype of cellular chimerism. Allogeneic donor BM (containing HSC) is transplanted to establish mixed chimerism. In the case of molecular chimerism autologous (i.e., syngeneic in

the rodent setting) HSC are transplanted after genetic modification *ex vivo* to express the disease-causing antigen(s) (Bagley et al. 2002a).

Advanced experimental protocols of cellular hematopoietic chimerism have been systematically developed for several decades. Translation to large animal models and the clinical setting have been accomplished for transplantation tolerance (Fehr and Sykes 2008). Thus, chimerism-based tolerance is conceptually and empirically an attractive tolerance strategy with demonstrated clinical potential.

3.1 Mixed Chimerism

Following transplantation of donor BM into appropriately conditioned recipients, donor and recipient hematopoiesis co-exists ($>1 < 100\%$ donor cells), leading to donor-specific tolerance (reviewed in Sykes 2001; Wekerle and Sykes 2001).

Subsequent to the early observation of chimerism as a natural phenomenon in cattle, the concept has been translated to numerous experimental models (Billingham et al. 1953; Owen 1945). At first myeloablative recipient conditioning was used (Ildstad and Sachs 1984), which has been extensively modified so that substantially milder regimens have since become available (Pree et al. 2007). Mixed chimerism was found to be preferable over full chimerism as it reduces the risk of graft versus host disease (GVHD) and is associated with superior immunocompetence (Singer et al. 1981; Sykes et al. 1988). The use of co-stimulation blockers (mostly CTLA4Ig and anti-CD40L) as part of BM transplantation (BMT) protocols allowed chimerism and tolerance to be induced with the mildest, least toxic conditioning protocols developed to date (Durham et al. 2000; Pree and Wekerle 2006; Sنانoudj et al. 2006; Wekerle et al. 2000). Permanent engraftment of conventional BM doses without recipient irradiation, remained however, an important goal. Either unrealistic mega doses of BM (Blaha et al. 2005; Durham et al. 2000; Wekerle et al. 2000), or recipient myelosuppression (by irradiation or cytotoxic drugs) have been necessary (Adams et al. 2001; Blaha et al. 2003; Koporc et al. 2008; Takeuchi et al. 2004). Co-transplantation of recipient Tregs allowed for the first time the engraftment of conventional doses of fully allogeneic BM without any myelosuppressive recipient conditioning (Pilat et al. 2010; Pilat and Wekerle 2010).

The clinical potential of the mixed chimerism strategy has recently been emphasized by two pilot trials. In the first series, patients suffering from chronic renal insufficiency plus concomitant myeloma were simultaneously grafted with a kidney and with BM from an HLA-identical sibling (Bühler et al. 2002; Fudaba et al. 2006). Patients accepted the renal grafts without maintenance immunosuppression. In a subsequent trial, HLA-mismatched BM plus a kidney were transplanted into recipients with chronic renal failure (without concomitant malignancy) (Kawai et al. 2008; LoCascio et al. 2010). Four out of five patients became tolerant, with long-term preserved kidney graft function (reported follow up of up to 5 years; one patient lost his graft due to humeral rejection). These

results provide clinical proof-of-concept that transplantation tolerance can be achieved through mixed chimerism (Pilat et al. 2009). Widespread application of this regimen is, however, prevented by the extensive myelosuppressive host conditioning which is required to allow even transient engraftment of HLA-mismatched BM and which is associated with substantial risks and toxicities. Therefore, more advanced, milder protocols that already exist in the rodent setting need to be translated to clinical use for routine application of the mixed chimerism strategy to become realistic.

3.2 *Molecular Chimerism*

Transplantation of syngeneic HSC that have been genetically modified in vitro to express disease-causing antigen(s) back into the same individual induces tolerance toward the introduced antigen(s). This gene therapy has been successfully employed in experimental models of allo- or xeno-transplantation and some selected autoimmune diseases. Autologous HSCT has the advantage compared to allogeneic HSCT that the risk of GVHD is avoided (Copelan 2006), and that the lower immunological barrier would allow milder host conditioning (Bagley and Iacomini 2003).

The safety issues associated with gene therapy, however, are a major drawback of molecular chimerism. Retro- or lentiviral vectors currently used for gene introduction into HSC cause substantial toxicity due to immune reactions or oncogenesis (Baum et al. 2003; Hacein-Bey-Abina et al. 2008). Recently, progress toward safer gene delivery has been reported (Aiuti et al. 2009; Mitsuyasu et al. 2009) and it is anticipated that acceptably safe vector systems will become available some time in the future.

3.2.1 **Introduction of Allo- and Xeno- Genes to Establish Tolerance**

Major histocompatibility complex (MHC) molecules are the primary target of immune responses in allotransplantation (Davidson and Diamond 2001; LeGuern 2007). Expression of single donor MHC class I and class II antigens can be sufficient for inducing tolerance toward fully allogeneic donors under certain circumstances, due to linked suppression and “infectious tolerance” (Frasca et al. 1997; Qin et al. 1993). Besides, certain alleles of MHC class I and II are strongly associated with the risk of developing autoimmune disease.

Donor MHC class I or class II genes transduced into recipient cells in vitro and transferred into recipients led to prolonged cardiac allografts survival (Madsen et al. 1988). Similarly, swine MHC class II DRB was integrated by retroviral transfer into murine and swine BM cells (BMC) in vitro (Emery et al. 1993; Shafer et al. 1991). By translating this method to a porcine in vivo model, prolonged renal allograft survival was achieved by transducing a single allogeneic MHC class II gene into recipient BMC (Emery et al. 1997; Sonntag et al. 2001).

In contrast, MHC class I gene transfer seems to be more difficult due to poor peptide presentation of MHC class I peptides (LeGuern 2007). The congenic mouse strains B10.AKM (H-2K^k)-B10.MBR (H-2K^b)—differing in only one H-2K allele—serve as a model of allogeneic molecular chimerism. Several studies introducing the MHC class I (K^b) into murine BMC (K^k) showed hyporesponsiveness and prolongation of skin allografts (K^b). In these protocols low transduction efficiency at first resulted only in prolongation of skin grafts but not durable tolerance (Bagley et al. 2000; Fraser et al. 1995; Mayfield et al. 1997). By improving transduction efficiency of BMC by a different retroviral gene transfer system, stable multilineage molecular chimerism and long-term skin graft tolerance was achieved (Bagley et al. 2002b). Mechanistically, central thymic deletion plus peripheral mechanisms, including regulation by CD4+CD25+ Tregs, were shown to play a role in this model (Forman et al. 2005, 2006; Kang and Iacomini 2002) and existence of molecular blood chimerism is required for durable tolerance (Tian et al. 2006).

In xenotransplantation (pig to human), natural xenoantibodies against the carbohydrate epitope Gal α -1, 3-Gal (α gal) are an additional barrier. Thus both B cell and T cell tolerance are required for long-lasting acceptance of xenografts. Humans, Old World monkeys and Apes possess a diminished function of α 1, 3-galactosyltransferase (α GT), the enzyme producing α gal (Galili et al. 1988). The α GT knockout mouse (GT⁰) model (having IgG and IgM antibodies toward α gal) is used to study natural antibodies in xenotransplantation. By introduction of α GT into syngeneic BMC B cell tolerance through molecular chimerism can be studied. Long-term molecular chimerism and B cell tolerance were achieved even when mice were pre-sensitized with pig cells and cardiac grafts from α GT expressing mice were accepted in GT⁰ mice (Bracy and Iacomini 2000, 2002; Bracy et al. 1998, 2001; Kearns-Jonker et al. 2004; Mitsuhashi et al. 2006). Autologous transduced BMC expressing α GT was also transplanted into rhesus monkeys and xenoantibody production was strongly reduced after sensitization with porcine cells (Fischer-Lougheed et al. 2006).

3.2.2 Introduction of Auto-Antigens to Establish Tolerance

Molecular chimerism has also been studied in selected autoimmune models (Alderuccio et al. 2003). In experimental autoimmune encephalomyelitis (EAE), a rodent model to study multiple sclerosis (Zamvil and Steinman 1990), genes encoding for myelin oligodendrocyte glycoprotein (MOG), phospholipid protein (PLP), myelin basic protein (MBP) and antigens inducing EAE, were introduced into BMC and established tolerance in EAE (Chan et al. 2008; Xu and Scott 2004; Xu et al. 2006). In one study introduction of MBP resulted in worsening of the disease, possibly due to insufficient MBP expression (Peters et al. 2000). Introduction of one peptide epitope of MOG (MOG_{40–55}) fused to a murine MHC II-associated invariant chain into syngeneic BMC resulted in tolerance induction in preventive and therapeutic models reported recently (Eixarch et al. 2009).

In the NOD type I diabetes model autoreactive T cells respond to islet antigens (insulin, glutamic acid decarboxylase (GAD) and others). The MHC class II region in NOD mice encodes a single MHC class II molecule I-A^{g7} (homologue to human HLA-DQB1 lacking a charged amino acid at position 57 associated with susceptibility to develop type 1 diabetes) which confers susceptibility to spontaneous type I diabetes (Atkinson and Leiter 1999). Induction of molecular chimerism through transplantation of BM expressing a ‘disease-resistant’ MHC class II molecule prevented type I diabetes in NOD mice and was sufficient to restore normoglycemia after islet transplantation and led to central thymic deletion of autoreactive T cells (Tian et al. 2004a, 2007a).

3.3 Hematopoietic Stem Cell Transplantation and Allergy

Cases in which BM transplanted from allergic donors induced allergies in the recipient have been reported. In reverse, allergies have been described to be cured in allergic recipients receiving a BMT from a healthy donor, suggesting that BMT is an effective treatment for allergies (though of course not indicated for this disease). No systematic, prospective evaluation of these issues, however, is available (Hourinane et al. 2005; Khan et al. 2009; Storek et al. 2011).

4 Induction of Tolerance via Molecular Chimerism in Allergy

Molecular chimerism is an effective strategy for tolerance toward allo- and auto-antigens. We recently started to investigate this strategy for tolerance in allergy. As most clinically relevant allergens have been identified and are available as cDNAs (Valenta and Kraft 2002), and as only a limited number of allergens is relevant in any given patient (and even geographical) region, allergies seem particularly suited for the molecular chimerism approach.

4.1 Prevention of Allergy by Induction of Tolerance via Molecular Chimerism

In proof-of-concept studies, we developed a preventive experimental model for induction of tolerance via molecular chimerism (Baranyi et al. 2008). A clinically relevant grass pollen allergen, Phl p 5 (Vrtala et al. 1993), was employed as model allergen. Phl p 5 is highly immunogenic upon subcutaneous immunization with the adjuvant aluminiumhydroxide in wild type mice (BALB/c) (Linhart et al. 2007). For cell surface expression on HSC Phl p 5 was fused to a signal peptide and a

transmembrane domain and ligated into a retroviral expression vector. Syngeneic BMC were transduced with recombinant retroviruses integrating Phl p 5 and thereafter Phl p 5-expressing BMC were transplanted into myeloablated syngeneic recipients. Mice transplanted with Phl p 5-transduced BM and mice of control groups were repeatedly immunized with recombinant (r) Phl p 5 and an unrelated allergen (rBet v 1) post BMT. BMT of Phl p 5-transduced cells resulted in stable long-term multilineage chimerism (follow-up approximately 9 months). Transduction of true HSC, already suggested by long-term multi-lineage chimerism, was confirmed through successful transplantation of BM taken from chimeras into secondary recipients (Down and Ploemacher 1993).

In this model durable tolerance was demonstrated by the complete absence of Phl p 5-specific IgE, IgA and IgG antibodies throughout follow up while high antibody responses to Bet v 1 were preserved. Tolerance at the effector cell levels in vitro and in vivo toward Phl p 5 was also achieved. T cell tolerance toward the introduced allergen was demonstrated by unresponsiveness in proliferation assays. These results have been confirmed by transplanting BM transduced with a distinct, unrelated allergen (Bet v 1) (Gattringer, Wekerle et al., unpublished). Again lasting molecular chimerism and allergen-specific tolerance were observed. Therefore molecular chimerism is uniquely potent in establishing complete and permanent tolerance at the T cell, B cell and effector cell levels in experimental type I allergy (Baranyi et al. 2008, 2009).

4.2 Anticipated Impediments to Translate Molecular Chimerism to the Clinical Setting and Possible Solutions

Safety aspects have high priority in prophylactic approaches in allergy (Kulig et al. 1999). Therefore non-toxic protocols are a pre-requisite for translation. The experimental protocol employed in our proof-of-principle study entails several components that cannot be applied clinically, such as retroviral gene transfer, irradiation and others. We propose that solutions to these problems can be envisioned (Fig. 1).

4.2.1 Moving to Safer Protocols

Retro- or lentiviral vector-based delivery to integrate transgenes is efficient but associated with oncogenicity and mutagenesis (Thomas et al. 2003). The hyperactive *Sleeping Beauty* DNA transposon was shown to integrate transgenes in a robust and stable manner into CD34+ HSC reconstituted in mice and was even successful to modify T cells before transfer in humans in a gene therapy approach (VandenDriessche et al. 2009). Therefore DNA transposons are a potential safer alternative for integrating genes into HSC.

In contrast to murine models, in which chimerism needs to persist in order to maintain robust tolerance (Tian et al. 2006), transient mixed chimerism was

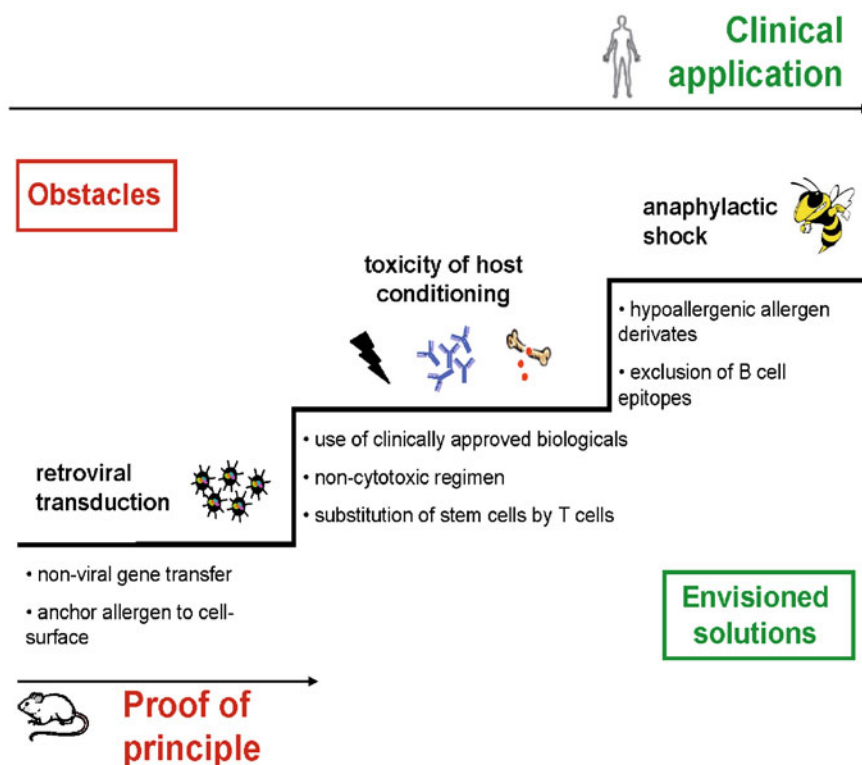


Fig. 1 Obstacles and envisioned solutions of cell based therapy by molecular chimerism, describing the hurdles on the way to clinical application starting with a proof-of-principle experiment in the mouse model

sufficient for tolerance in monkey models and clinical trials (Kawai et al. 2008; Ochiai et al. 2007). Thus, if transient chimerism suffices episomal, non-integrating delivery can be used, or antigens can be anchored directly to the cell surface (Belting and Wittrup 2009; Thomas et al. 2003; Yolcu et al. 2002).

In our proof-of-principle study, mice received a myeloablative dose of irradiation. Reduction of irradiation resulted in lower chimerism levels, but mice were still tolerant long-term (Baranyi, Wekerle et al., unpublished). In other models of molecular and mixed chimerism very mild experimental protocols have already been developed and could be translated to the allergy system in the future (Blaha et al. 2003; Eixarch et al. 2009; Forman et al. 2005; Tian et al. 2008; Wekerle et al. 2000; Pilat et al. 2010).

Application of the co-stimulation blocker anti-CD40L (anti-CD154) turned out to be very efficient in mixed chimerism rodent models (Wekerle et al. 1998). In a molecular chimerism model anti-CD40L was reported to enhance BM engraftment (Bagley et al. 2002c). Anti-CD40L is also effective in preventing sensitization to an allergen (Linhart et al. 2007). Unfortunately, this antibody induces severe

thromboembolic side effects in large animals and humans, and is therefore not used in the clinical setting (Kirk et al. 1999; Sidiropoulos and Boumpas 2004). Studies recently published show successful substitution of anti-CD40L by blockade of CD40 in murine models in allo-transplantation (Gilson et al. 2009), but clinical applicability remains uncertain. Blocking alternative co-stimulation pathways may also allow avoidance of anti-CD40L.

HSC engraftment requires—at least some—recipient conditioning. The transplantation of mature T cells instead of HSC in a murine model of molecular chimerism (crossing a MHC I barrier) led to long-lasting central tolerance by re-entry of mature T cells into the thymus (Tian et al. 2004b, 2007b, 2008). Thus, the use of allergen-expressing mature T cells, or other hematopoietic cell populations (McCurry et al. 2006), is an attractive alternative to be explored as a substitute for HSC.

4.2.2 Avoiding Anaphylaxis

Expression of full length allergens on transplanted BM—as in our proof-of-concept studies—implies the risk of anaphylaxis, mostly in the therapeutic but also in the preventive setting. Intracellular expression of an antigen was sufficient to induce T cell tolerance in mice (Tian et al. 2003). Expressing dominant T cell epitopes of allergens on the surface of HSC would conceivably avoid the risk of anaphylaxis, provided that T cell tolerance is sufficient to induce B cell tolerance, as it has been described (Li et al. 2008). Use of small peptides would also facilitate fusion of several dominant T cell epitopes of various allergens to achieve protection toward several allergens at the same time.

5 Conclusion

Preventive and therapeutic tolerance strategies are an important goal for improved medical management of allergies. Cell-based therapies are attractive candidates for development and substantial progress has been achieved in this field at the experimental level. Evaluation of Treg therapy has begun for certain indications and will allow a better appreciation of its usefulness in the treatment of allergy. Molecular chimerism is a powerful experimental approach leading to particularly robust allergen-specific tolerance. Its development is currently at an early proof-of-concept stage, but further progress toward clinical application in the long-term future can be envisioned.

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