Published in final edited form as:
Curr Opin Rheumatol. 2014 March ; 26(2): 219–227. doi:10.1097/BOR.0000000000000029.

Prospects for Therapeutic Tolerance in Humans

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Abstract

Purpose of review—To provide an overview of recent advances and future possibilities for therapeutic tolerance.

Recent findings—Allograft survival despite complete immunosuppressant withdrawal has been demonstrated in selected renal transplant recipients with haematopoietic chimerism. Early clinical trials of mesenchymal stromal cell therapy have shown promising results in several autoimmune diseases. Regulatory T-cells show potential benefit in graft versus host disease, although challenges to *ex vivo* expansion remain. Targeted modulation of T-cell function *in vivo* with monoclonal antibodies have shown beneficial effects in phase II/III trials of multiple sclerosis (alemtuzumab) and type I diabetes mellitus (teplizumab, otelixizumab). Emerging data from animal models suggests an important role for the commensal microbiome in the maintenance and disruption of immune tolerance with parallels in human studies.

Summary—After years of slow progress, recent research has reduced the translational gap between animal models and clinical therapeutic tolerance. Early detection of autoimmunity, potentially at preclinical stages, offers a window of opportunity for tolerogenic therapy. Reliable biomarkers of tolerance are urgently needed to provide objective measurements of the effectiveness of tolerogenic therapies, and to allow for intelligent immunosuppressant withdrawal in patients whose autoimmune disease is stable.

Video Abstract

Keywords

therapeutic tolerance; immune tolerance; autoimmune; regulatory T-cell; therapy

Introduction

Immunosuppression has been the mainstay of treatment for autoimmune diseases for the past half century. Traditional therapeutics, such as corticosteroids or cytotoxic agents, generally provide blanket suppression of the immune response. More recently, monoclonal antibodies have provided the opportunity to target specific aspects of the immune cascade at

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No conflicts of interest

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a molecular level. However, these immunosuppressant approaches fail to distinguish between responses to self and non-self antigens and hence carry a burden of increased infection, and in certain cases, malignancy.

The resetting of immune tolerance to self antigens, or the exploitation of tolerance towards alloantigens in the setting of transplantation, without the need for immunosuppression has been termed “therapeutic tolerance” [Figure 1]. This concept has been demonstrated in numerous animal models, although translation to humans has historically been lacking. In this review, we will focus on recent advances towards clinical therapeutic tolerance [Figure 2].

Cellular therapies

Four main cellular therapies have shown promise in human tolerogenesis - haematopoietic chimerism, mesenchymal stromal cells, regulatory T cells, and dendritic cells. The latter is the focus of a detailed review in this issue, and hence will not be discussed further here.

Haematopoietic chimerism

The pioneering work of Medawar and Owen over 60 years ago established that therapeutic tolerance in the transplant setting could be conferred by prior recipient conditioning during foetal life with donor haematopoietic cells [1, 2]. The key requirement for success was the achievement of haematopoietic chimerism, with a “dual” immune system derived from both donor and recipient haematopoietic elements. Later work identified that this could also be achieved in adults by prior myeloablation; however, initial protocols achieved this through extreme whole-body irradiation with severe and often fatal side-effects. Combined with the risks of graft versus host disease (GvHD), these limitations have severely limited the adoption of bone marrow transplantation (BMT) for tolerance induction in humans [3].

An exception to this is in the context of haematological malignancy, where the toxic effects of BMT are an acceptable price to pay for cure and where graft versus leukaemic effect is beneficial. Several small clinical studies in patients with multiple myeloma and end-stage renal failure confirmed the possibility of drug-free renal allograft survival in combination with BMT [4]. These promising results have provided impetus for the development of less toxic myelodepletive conditioning in solid organ transplantation outside of the setting of haematological malignancy. Indeed, several such recent proof-of-concept human studies have demonstrated complete withdrawal of immunosuppressive drugs in significant proportion of recipients [Table 1], and long term follow-up data are eagerly awaited.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are multipotent stem cells which can differentiate into a range of mesodermal lineages including haematopoietic cells, adipocytes, chondrocytes and osteocytes [8]. Initially studied for their regenerative properties, MSCs have been the recent focus of intense interest due to their immunomodulatory properties both *in vitro* and *in vivo* in several animal models of human autoimmune disease. The exact mechanisms by which MSCs regulate the immune response are unclear, though studies suggest soluble factors (such as transforming growth factor β (TGFβ), prostaglandin E2, indoleamine 2,3-

dioxygenase (IDO) and human leukocyte antigen (HLA) G5) as well as direct cell-contact are important [8]. Traditionally MSCs are harvested from bone marrow samples [9], although they can now also be obtained by less invasive approaches including from adipose tissue [10], oral mucosa [11] and umbilical cord [12].

The safety of intravenous MSC infusion in autoimmune disease has been confirmed in several early-phase clinical trials, with promising suggestions of beneficial therapeutic effects in these small studies [13] Table 2]. However, observations that MSCs can differentiate to sarcoma cells *in vitro* [21] and can potentiate the immune evasion of breast cancer cells [22] raise significant safety concerns. Furthermore, the *in vivo* longevity of MSCs may be limited. In a recent human autopsy study of patients who had received intravenous MSC infusions before death, 9 of 13 patients had detectable donor MSC DNA where the infusion was given within the past 50 days, compared to only 2 of 8 patients who had received the infusion more than 50 days previously [23]. Nevertheless, it is possible that a transient induction of tolerance could be maintained far beyond the life-span of the MSC through mechanisms such as infectious tolerance, as will be discussed later.

Regulatory T cells

T cell depletion and functional anergy underpinned early theories of tolerance induction. A revolutionary paradigm shift came with the recognition of “dominant” tolerance mechanisms, whereby regulatory T cell (T_{reg}) subsets actively and specifically suppress or regulate potentially harmful immune responses. In addition to offering a potentially powerful *in vivo* effector mechanism for tolerogenic therapies [3], an alternative approach is the *ex vivo* isolation of T_{regs}, either from the patient or an allogenic donor, and subsequent use as a therapeutic agent *per se* [24]. Autologous *ex vivo* expanded T_{reg} therapies circumvent the potential negating “host versus graft” effect, although the bespoke manufacturing process is significantly more time-consuming and costly compared to their “off-the-shelf” allogenic T_{reg} counterparts. Indeed, intravenous infusions enriched with either donor-specific or allogenic T_{regs} have shown potentially beneficial effects in the prevention of GvHD in human BMT [25, 26], although this is yet to be translated to other human diseases.

Much of the difficulty in this translation lies with a lack of reliable T_{reg} surface markers, with an over-reliance on functional assays and the potential risk of unintentional inclusion of pro-inflammatory T cells within therapeutic isolates [24]. However, recent epigenetic studies have suggested that expansion of naïve CD45RA+ T_{regs} may provide a more stable regulatory phenotype compared with unselected T_{reg} populations [27]. Furthermore, *in vivo* animal studies and *in vitro* studies with human cells have confirmed the ability of T_{regs} to potentiate a regulatory phenotype in other effector T cells in a process termed “infectious tolerance’ [28], which may amplify the therapeutic effect. However, the *in vivo* stability of a regulatory phenotype in human T_{reg} therapy remains to be established, and observations that T_{regs} can potentiate the metastatic spread of breast cancer in mouse models [29] raises potential safety concerns. Both issues will require careful consideration and will likely be critical to the success of T_{regs} as a future cellular tolerogenic therapy.

T-cell modulation

In addition to the use of exogenous T_{regs} as a cellular therapy, it is also possible to manipulate the existing T cell population *in vivo* towards a tolerogenic state. One of the earliest and least subtle approaches is the mass depletion of lymphocytes through the use of animal-derived anti-thymocyte globulins and monoclonal antibodies such as alemtuzumab (anti-CD52). These agents are now routinely used as induction agents in solid organ transplantation, reducing the incidence of acute allograft rejection [30] and enabling graft survival with reduced maintenance immunosuppression in a phenomenon termed "*prope* tolerance" [31]. In recent clinical trials, alemtuzumab has been shown to reduce relapse rate in multiple sclerosis [32], though at the expense of a paradoxical increase in other forms of autoimmunity during immune reconstitution, most notably autoimmune hyperthyroidism [33].

In a similar approach, monoclonal antibodies directed against the T-cell receptor (anti-CD3: teplizumab and otelixizumab) have shown promise in the treatment of type I diabetes mellitus. When used in early disease, anti-CD3 therapy can improve endogenous insulin secretion and reduce exogenous insulin requirements as demonstrated in phase I/II clinical trials [34]. Pre-clinical data suggest an induction of T_{regs} following anti-CD3 treatment [35], though the precise mode of action remains elusive. Randomised controlled phase III trials have however failed to reach primary endpoints, possibly reflecting under-dosing and heterogeneity in clinical response [34], and further research continues.

Co-stimulation blockade

The priming of a naïve T-cell response requires the presence of both an antigen-specific signal via the T-cell receptor, and a co-stimulatory signal provided by the antigen-presenting cell (APC). Both of these signals are mediated by receptor-ligand interactions at the so-called "immunological synapse" [36]. By manipulating the co-stimulatory signals, it is possible to abrogate the priming of an effector T-cell response or even shift priming towards a T_{reg} phenotype [37].

Two of the best-characterised co-stimulatory interactions take place between CD80 and CD86, which are expressed on the surface of APCs, and CD28 on the surface of T cells [37]. Abatacept and belatacept are CTLA-4:Ig Fc fusion proteins which compete with CD28 for CD80/86 and hence block this co-stimulatory signal. Abatacept is licensed for use in rheumatoid arthritis, and has also been trialled in several other autoimmune diseases including inflammatory bowel disease [38], multiple sclerosis [39] and systemic lupus erythematosus [40] though with less promising results.

Given the pivotal role of co-stimulatory signals in the initial activation of T cells, therapy aimed at co-stimulatory blockade may be most effective when delivered early in the establishment of autoimmune disease. Indeed, in a recent randomised double-blinded trial of 112 patients with a recent (<100 days) diagnosis of type I diabetes, abatacept delayed the reduction in endogenous insulin secretion by around 9 months, although subsequent disease progression in both groups was largely similar [41]. Furthermore, in patients with early undifferentiated inflammatory arthritis, 6 months of abatacept therapy reduced the rate of

progression to rheumatoid arthritis at 2 years by 20%, though falling just short of statistical significance in this small study [42]. The enhanced detection of autoimmunity at very early or even preclinical stages may therefore provide a “window of opportunity” for co-stimulatory blockade and other approaches in future tolerogenic strategies [43].

Interleukin-2

Interleukin-2 (IL-2) is a key player in T-cell activation and proliferation, and has been the focus of recent interest as a possible target for therapeutic tolerance. Early phase clinical trials of daclizumab, a monoclonal antibody directed against the α-subunit of the IL-2 receptor (CD25), have shown promise in the treatment of multiple sclerosis [44]. There is a surprising heterogeneity of action observed at a cellular level - daclizumab not only blocks IL-2 binding to CD25 on the surface of T-cells, but has also been shown to block the *trans*-presentation of IL-2 by binding to CD25 on the surface of dendritic cells [45] and also appears to upregulate natural killer (NK) cell mediated T-cell destruction [46]. Furthermore, IL-2 is important for the proliferation of T_{regs} [47], with deficiency of CD25 resulting in autoimmunity in humans [48]. In a recent phase 1/2a clinical study of hepatitis C virus-induced cryoglobulinaemic vasculitis, low-dose IL-2 led to an *in vivo* expansion of $CD4^{+}CD25^{hi}FOXP3^{+}$ T_{regs} with associated clinical improvements [49]. With such diverse and at times opposing functions, it is unlikely that unselective IL-2 blockade will provide a reliable tolerogenic strategy in humans, though may prove to be a useful tool in the conditioning of cellular therapies *ex vivo*.

Allergen-specific immunotherapy (ASIT)

Allergic reactions are characterised by a Th_2 skewed response to a specific allergen, mediated largely by IgE bound to IgE receptors on the surface of innate immune cells including mast cells and basophils. Upon contact with the cognate allergen, IgE cross-linking triggers the release of chemokines and vasoactive compounds, leading to an immuno-inflammatory cascade resulting in rapid bronchoconstriction and widespread vascular permeability with potentially fatal consequences [50]. Although mechanistically different from autoimmunity, recent advances in allergy immunotherapy may hold important lessons for therapeutic tolerance.

The traditional management of allergic disorders has focussed on allergen avoidance, and the use of drugs to counter the allergic response such as anti-histamines, corticosteroids and adrenaline. An alternative approach is desensitisation therapy with graded allergen challenge. Numerous studies have identified the central importance of IL-10 production by T cells (termed Tr1 cells) in this process, with consequent down-regulation of pro-inflammatory cytokine production and shifting of B cell antibody production away from IgE and towards IgG4 [51]. Furthermore, so-called “regulatory B-cells” may also play a role in IL-10 production and allergen presentation, though this relatively novel concept is somewhat controversial [52].

Precisely which factors promote the differentiation and stimulation of Tr1 cells in this context remain elusive, though low followed by gradually escalating allergen doses and sustained allergen exposure appears to be important, particularly if delivered by the oral

route. In two randomised placebo-controlled and double-blinded trials of oral ASIT therapy for children with peanut allergy [53, 54], patients in the treatment arms showed significantly reduced skin-prick responses and loss of atopy to oral allergen challenge after 1 year of ASIT compared to placebo. One study also demonstrated higher levels of FoxP3+CD4+CD25+ T_{regs} in the ASIT group [54]. Similarly, in an observational study of hen's egg oral ASIT [55], allergen tolerance was associated with an increase in a hypo-proliferative CD4+CD38+CD45RO- T cell subset.

Although not equivalent to tolerance induction, ASIT provides proof-of-principle for the potential to therapeutically modulate antigen-specific responses *in vivo*. In terms of tolerance, the induction of an immunoregulatory response to one autoantigen can catalyse a similar response to other autoantigens in a process termed "epitope spreading". Therefore, although the nature of the antigens that drive human autoimmunity remain elusive, antigen-specific therapy may yet hold promise.

The microbiome in autoimmunity and tolerance

Genetic predisposition to autoimmunity is important but by no means absolute - for example, monozygotic twin studies show a concordance of only 12-15% for rheumatoid arthritis [56]. Recent interest in identifying the environmental triggers and perpetuators of autoimmunity has focussed on interactions with the human microbial flora, or "microbiome" [57]. It is estimated that there are at least 10 times as many microbes within the gut than cells within the human body [57], and recent advances in high-throughput techniques have revealed approximately 65% variation in gut microbial genes between individuals [58].

Strong evidence from animal models supports a role for the microbiome in the breakdown of immune tolerance. In the K/BxN murine arthritis model, arthritis activity is greatly attenuated in mice reared in germ-free environments, combined with a reduction in autoantibody titres and pro-inflammatory Th17 T-cell populations [59]. However, subsequent introduction of a single species of gut microorganism (segmented filamentous bacteria) was sufficient to recreate autoimmunity, and this effect could be inhibited by antibiotics effective against the microbe [59]. In human observational studies, colonisation by specific bacteria such as *Porphyromonas gingivalis* has been shown to correlate with the presence of anti-citrullinated protein antibodies in rheumatoid arthritis [60], and antibodies against citrullinated *P. gingivalis* peptides have been shown to cross-react with citrullinated self-proteins [61].

Contrary to the above, there is also evidence to support a pro-tolerogenic role for the microbiome in preventing autoimmunity. Colonisation by a mixture of *Clostridium* species has been demonstrated to increase colonic T_{reg} populations in mice, which were subsequently resistant to chemical-induced colitis [62]. Furthermore, murine colonic T_{regs} have been demonstrated to show antigen-specificity for microbial antigens in vitro [63], and the recent findings of specific T_{reg}-promoting receptors on the luminal surface of gut lamina propria in mice raises the intriguing possibility of microbial ligands for host immunoregulatory mechanisms [64].

Given its emerging importance in the regulation of immunity, microbiome modulation offers an enticing target for therapeutic tolerance. The simplest form of microbiome-based therapies are antibiotics, which have been shown to afford benefit, albeit limited, in rheumatoid arthritis [65] and inflammatory bowel disease [66]. A more intelligent and perhaps more powerful approach would be a focussed rebalance of a pathogenic microbiome towards a tolerogenic state, such as by the use of faecal matter from healthy individuals in a process termed “faecal transplantation” [67, 68]. Faecal transplants have proven efficacy in the treatment of *Clostridium difficile* colitis [67], and there are increasing numbers of case reports of beneficial effects in small cohorts of patients with autoimmune disease such as inflammatory bowel disease and multiple sclerosis [67, 68]. In a recent pre-clinical study, *Clostridium* isolates from healthy human stool were shown to increase intestinal T_{reg} populations in germ-free mice, and also provided a protective effect against experimentally-induced colitis [69]. Randomised controlled clinical trials in this rapidly evolving field are awaited with keen interest.

Looking to the future - biomarkers of tolerance

A major obstacle to the advancement of tolerogenesis in human studies is the lack of reliable biomarkers that distinguish immune tolerance from a state of immunosuppression. Such biomarkers would allow for objective and direct measurements of the effectiveness of tolerogenic therapies, and also would allow for intelligent withdrawal of immunosuppression in patients whose autoimmune disease is stable [70].

Intriguing observations in the field of transplantation have shown that immune tolerance is perhaps more achievable than previously believed. A minority of transplant patients will not reject their allograft despite, often unintentional, cessation of their immunosuppressive medication, yet can still mount potent immune responses to other non-self antigens in a phenomenon termed “operational tolerance”[71]. Encouraged by these findings, prospective immunosuppression weaning studies have demonstrated the ability to achieve operational tolerance in as many as 40% of stable liver transplant recipients [72, 73], albeit with strict exclusion criteria. Studies of international cohorts of operationally-tolerant renal and liver transplant patients have identified distinct tolerance signatures of gene expression in peripheral blood microarray analyses (reviewed by Chandrasekharan *et al*. [70]), which are able to identify operationally tolerant individuals with high specificity. Interestingly, comparisons of operational tolerance in renal and liver transplant patients have shown non-overlapping gene expression signatures, with a predominance of NK cell gene enrichment in liver transplantation compared to B-cell signatures in renal transplantation [74]. Although the analysis of small microarray samples is statistically challenging [71], this does raise the distinct possibility of allograft-specific tolerance mechanisms. Some of these may indeed act in the local allograft microenvironment and be undetectable at the systemic level, as suggested by murine transplantation experiments [75]. Nevertheless, the results from these observational studies are encouraging, and the validation of putative biomarkers in prospective immunosuppressant withdrawal studies is awaited with much interest.

Conclusion

Immune tolerance has long been attainable in animal models, although translation to humans has been frustratingly elusive. However, recent evidence from human transplantation provides clear proof-of-principle that therapeutic tolerance is an attainable target, and a diverse range of tolerogenic strategies have shown promise in early clinical trials. It is increasingly apparent that the mechanisms of immune tolerance are multifaceted, with the relative dominance of each mechanism dependent on the disease-specific state of immune dysregulation. Key to the advancement of tolerogenesis is the use of tolerance biomarkers, allowing for disease-specific and patient-tailored therapy. With such continuing advances it is surely possible to move immune tolerance away from the current horizon of therapeutic possibility and towards future clinical reality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Funding Disclosure: K.F.B. is supported by a Wellcome Trust and Newcastle University Clinical Research Fellowship in Translational Medicine and Therapeutics. Work in the Musculoskeletal Research Group is supported by the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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Key Points

- Recent research has helped to narrow the translational gap between experimental animal models and clinical therapeutic tolerance
- The survival of transplanted allografts despite complete withdrawal of immunosuppression is possible with haematopoietic chimerism, and is observed sporadically in a small number of transplant recipients in a phenomenon termed “operational tolerance”
- Cellular therapies including regulatory T cell and mesenchymal stromal cell transfer show promise in the treatment of several autoimmune diseases in recent early-phase clinical trials
- The enhanced detection of autoimmunity at very early or even preclinical stages can provide a window of opportunity for therapeutic tolerance
- Reliable biomarkers of tolerance are urgently needed to provide objective measurements of the effectiveness of tolerogenic therapies, and to allow for intelligent immunosuppressant withdrawal in patients whose autoimmune disease is stable.

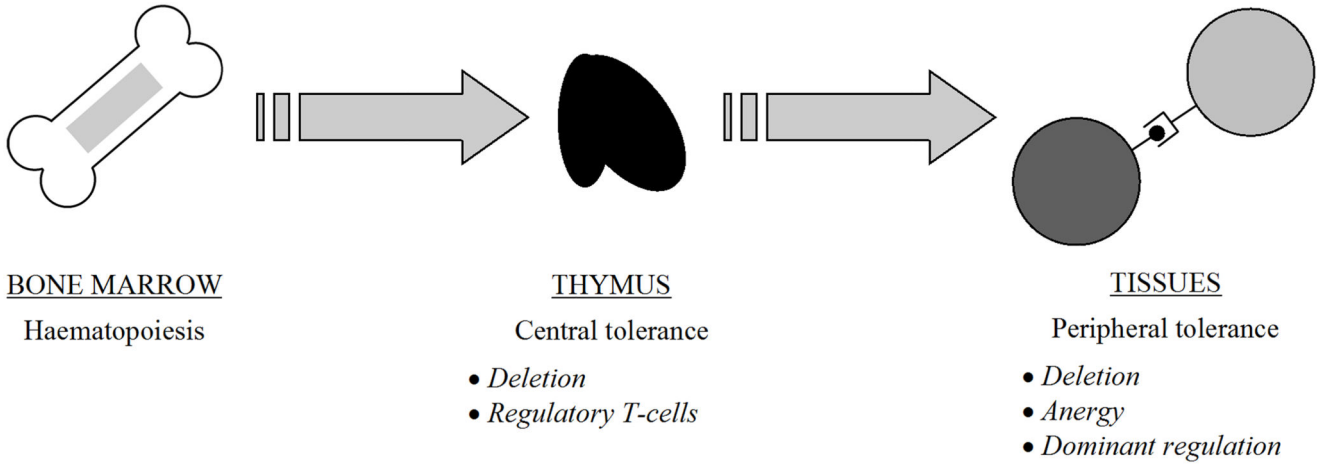

Figure 1.

Overview of central and peripheral tolerance mechanisms. Thymocytes are produced by the bone marrow from early foetal life and travel to the thymus for maturation. In the thymus, promiscuous expression of self-antigens from a range of tissue types (mediated by the AIRE gene) and affinity-based mechanisms result in deletion of autoreactive T cells and the generation of regulatory T-cells. In the periphery, further deletion and anergy can occur if T cells encounter antigen in the absence of sufficient co-stimulatory “danger” signals. Furthermore, unwanted autoreactivity can be directly suppressed by regulatory T-cells (dominant regulation).

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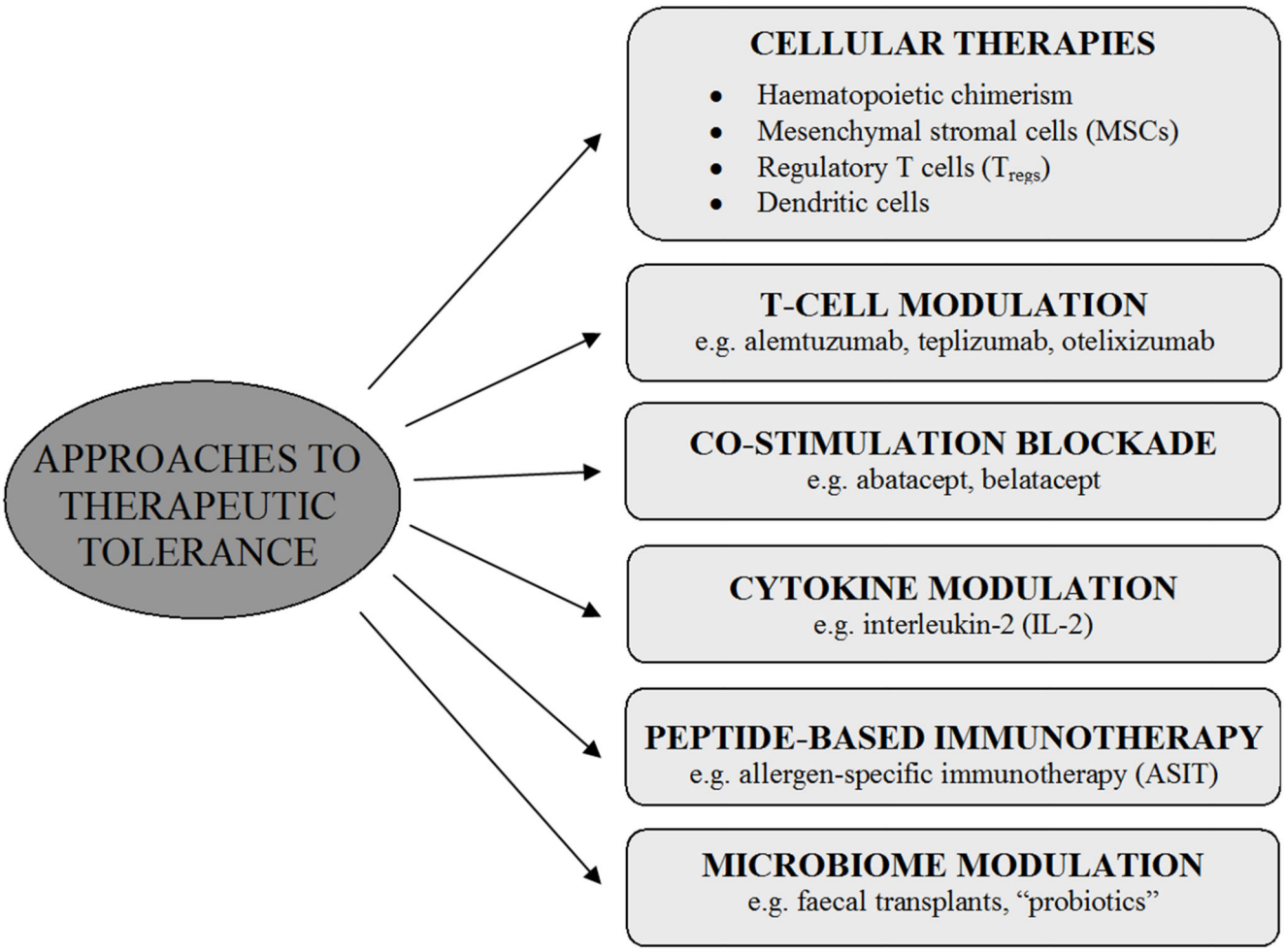

Figure 2.
Approaches to therapeutic tolerance induction.

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Table 1

Recent studies of haematopoietic chimerism in human solid organ transplantation.

Study	Organ	Number of patients	Protocol	Outcomes
Scandling *et al.* (2012) [5]	HLA-matched living donor kidney transplant	16	Conditioning: ATG, CS, TLI (10 × 80-120cGy) HSCT (CD34 enriched) day +11 MI: MMF, CYS	12 patients achieved durable peripheral blood donor chimerism, of which 11 have been fully withdrawn from immunosuppression (median 21 months, range 1 - 40) No GvHD, no graft loss 1 patient suddenly died at 42 months, 1 patient developed breast cancer (possibly unrelated)
Leventhal *et al.* (2013) [6]	HLA-mismatched living donor kidney transplant	15	Conditioning: FLU, CYC, TBI (200cGy) HSCT (facilitating cell enriched) day +1 MI: TAC, MMF	9 patients achieved durable peripheral blood donor chimerism, of which 6 have been fully withdrawn from immunosuppression (median 13.5 months, range 6-22) No GvHD 1 patient developed bone marrow failure with graft loss and viral sepsis
Schneeberger *et al.* 2013 [7]	HLA-matched cadaveric upper limb transplant	4	Conditioning: ATZ, CS BMT (unmodified) day +14 MI: TAC	Graft survival in all 4 patients on TAC monotherapy (vs. standard TAC/MMF/CS regimen) No GvHD No patients achieved durable donor chimerism

ATG = anti-thymocyte globulin, ATZ = alemtuzumab, BMT = bone marrow transplant, CS = corticosteroid, CYC = cyclophosphamide, CYS = cyclosporin, FLU = fludarabine, GvHD = graft versus host disease, HSCT = haematopoietic stem cell transplant, MI = maintenance immunosuppression, MMF = mycophenolate mofetil, TAC = tacrolimus, TBI = total body irradiation, TLI = total lymphoid irradiation.

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Table 2

Studies of mesenchymal stromal cell (MSC) therapy for autoimmune disease in humans.

Study	Disease	Number of patients	MSC source	Outcomes
Ra *et al.* (2011) [14]	Various (multiple sclerosis, polymyositis, rheumatoid arthritis, atopic eczema, autoimmune hearing loss)	10	Autologous adipose-derived, intravenous delivery	Safety, some evidence of improved clinical outcomes
Duijvestein *et al.* (2010) [15]	Crohn's disease	10	Autologous bone marrow-derived, intravenous delivery	Safety, three patients achieved significant clinical response
Garcia-Olmo *et al.* (2009) [16]	Crohn's disease	49	Autologous adipose derived, topical delivery with fibrin glue	Safety, improved peri-anal fistula healing in fibrin-MSC versus fibrin-only control group (71% vs. 16% - study included non-immune causes of fistulation)
Ciccocioppo *et al.* (2011) [17]	Crohn's disease	12	Autologous bone marrow-derived, intra-fistula injection	Safety, complete fistula closure in 7 patients and reduced clinical disease severity
Connick *et al.* (2012) [18]	Multiple sclerosis	10	Autologous bone marrow-derived, intravenous delivery	Safety, improvement in visual acuity, visual evoked response latency and optic nerve area
Karussis *et al.* (2010) [19]	Multiple sclerosis	34	Autologous bone marrow-derived, intravenous (n=14) and intrathecal (n=34) delivery	Safety, improved neurological disability scores (study also included patients with amyotrophic lateral sclerosis)
Sun *et al.* (2009) [20]	Systemic lupus erythematosus	4	Allogenic living relative bone marrow-derived, intravenous delivery	Safety, significant improvements in SLEDAI scores and proteinuria levels

Adapted from Bernado & Fibbe [13]. SLEDAI = systemic lupus erythematosus disease activity index.