TREATING TO RE-ESTABLISH TOLERANCE IN INFLAMMATORY ARTHRITIS - LESSONS FROM OTHER DISEASES

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Abstract

Therapeutic tolerance embraces the concept of 'switching off' immunopathology by specifically targeting elements of the immune system. It has been achievable in preclinical models of transplantation and autoimmunity for more than two decades, but previous attempts to translate to the clinic have been unsuccessful. However, an improved understanding of tolerance mechanisms, along with novel therapeutic agents and strategies, are starting to bear fruit in a number of disease areas. True tolerance is achievable in transplantation settings, and long-term remissions can be induced in various autoimmune and atopic conditions. Equivalent outcomes should be achievable in inflammatory arthritis although this may require an improved understanding of the immune dysregulation that is intrinsic to RA, and better definition of RA autoantigens. Biomarkers of tolerance induction would rapidly advance the field in all therapeutic areas. This chapter summarises the advances made in other therapeutic areas, and the lessons learned, that we can now apply to RA.

Key Words

Therapeutic tolerance
Immune modulation
Immunotherapy
Anti-CD3
Mixed chimerism
Autologous stem cell transplantation
Tolerance biomarkers
Tolerogenic dendritic cell
Mesenchymal stromal cell
Cellular therapy

Introduction

Current treatment paradigms for rheumatoid arthritis emphasise the need to treat inflammation aggressively to prevent joint damage and co-morbidities. Disease remission is the goal of therapy and can now be achieved in a proportion of patients, particularly in early disease. The question then arises as to whether therapy can be withdrawn or tapered but, certainly in established RA, treatment cessation leads to disease flare more often than if therapy is continued [1]. In essence the disease has been suppressed by therapy and re-emerges upon drug withdrawal. In contrast, immune tolerance is the natural state whereby the organism does not mount an immune response against self, whilst maintaining the ability to react to 'foreign' substances and tissues. By definition this process has broken down in autoimmune disease and the focus of this chapter is to ask whether it can be re-established by appropriate targeting of the immune system (therapeutic tolerance induction).

The first clear evidence that immune tolerance could be induced by a therapeutic intervention dates back to the 1980s. Several groups demonstrated that short courses of monoclonal antibody (mAb) therapy, generally targetted at CD4 ± CD8 on T-cells, could allow the transplantation of foreign tissue grafts or switch off established autoimmunity [2]. In both cases a brief intervention resulted in life-long tolerance, with no evidence of immune deficiency. Subsequently, equally dramatic results were obtained using other more specific interventions such as antigenic peptide administration (via a number of alternative routes) or by mucosal administration of autoantigen. The important concept that evolved from this work was that the immune system was 'plastic' and, to an extent, immune memory could be controlled and/or replaced. Furthermore, therapeutic tolerance was shown to be an active process, involving, *inter alia*, regulatory T-cell induction [3]. This underpinned phenomena such as infectious tolerance, linked suppression and epitope spreading, which ensured the robustness of tolerance induction [4].

Definitions and a brief historical perspective

To discuss therapeutic tolerance in terms of autoimmunity, definitions are paramount. In transplantation the situation is clear: if a foreign organ is accepted in the absence of immunosuppression in an otherwise immunocompetent host, then tolerance has been achieved. This occurs infrequently but immunosuppression is sometimes stopped in a transplanted patient (usually due to severe side effects or co-

existent disease) and, in occasional cases, the graft is not rejected (Figure 1A). In autoimmunity the situation is more complex and we are left with 'operational' definitions of tolerance. In RA, tolerance should manifest as an immunocompetent patient, in remission, on no immunosuppressive or anti-inflammatory therapy. However remission itself is a complex concept, and does occur spontaneously, for example in palindromic RA (Figure 1B)[5]. Whether this represents attempts by the immune system to reinstate self tolerance is unclear and a major limitation is our lack of biomarkers for the tolerant state (see Transplantation section below). In RA, true therapeutic tolerance induction should provide a disease 'cure', although physicians and patients alike would be content with an intermittent therapy that controlled disease for prolonged intervals without affecting immune competence.

Models of therapeutic tolerance induction generally propose a central role for the T-cell:antigen presenting cell (APC) interaction. Disruption and modulation of this key interaction underpins most tolerogenic interventions. Early preclinical protocols used brief courses of T-cell depleting mAbs to induce tolerance to foreign tissue or to reverse autoimmunity [2]. Subsequently, non-depleting mAbs were shown to be even more effective and it was demonstrated that a broad range of T-cell surface antigens could be targeted for tolerance induction. Models suggested that therapy acted by 'blindfolding' the key cells, preventing effective interactions and allowing the immune system to return to its natural state of self tolerance [6].

However, initial trials in RA patients failed to reproduce these exciting preclinical data [7]. A range of targets including CD4, CD5, and CD7 were selected but clinical improvement was generally transient or absent. In contrast anti-CD52 (CAMPATH-1H) resulted in deep depletion of several lymphocyte subsets, and was associated with significant improvement in open label trials [8, 9]. However therapy was associated with first-dose reactions, and immune reconstitution was very slow. This led to concerns over long-term immune competence, although this has not proven to be a problem in the longer term [10, 11]. A flurry of anti-CD4 trials failed to demonstrate consistent benefit and the advent of anti-cytokine therapy slowed development in this area. Recently CTLA4-Ig (abatacept) has shown that T-cells can be targeted in a clinically effective manner. Abatacept blocks costimulation, thereby interrupting the second signal required for full T-cell activation. Historically, signal 2 blockade was a highly effective means to induce tolerance in T-cells [12]. However abatacept is administered monthly, without longer breaks in therapy, preventing

conclusions being drawn regarding its tolerogenic potential. Nonetheless, long-term observational studies of abatacept administration hint at enhanced efficacy with time, perhaps suggesting a gradual turning off of disease [13].

Lessons from transplantation and other diseases

It is almost 20 years since initial attempts to induce tolerance in RA patients. This work continues but, in parallel, strategies have been studied in a range of other immunopathologies, ranging from transplantation to allergy. The remainder of this chapter will study these examples and extract lessons that might be applied to RA

1. Transplantation

Transplantation models were some of the first to demonstrate true therapeutic tolerance. Even animals that were actively rejecting skin could be tolerised, rejection being converted to tolerance, perhaps one of the most dramatic examples of immune plasticity [14]. Despite the success of modern immunosuppressive regimes chronic rejection, drug toxicity and opportunistic infections create the need for improved methods to combat alloreactivity. Two major strategies have been employed. In the first, lymphocyte depletion around the time of transplantation mimics, to an extent, successful murine tolerance protocols. In this way drugs such as alemtuzumab or anti-thymocyte globulin (ATG) have allowed the use of very significantly reduced post-transplant immunosuppression [15]. Calne has termed this ‘prope (near) tolerance’, but acute and chronic rejection still occurs in some cases [16]. Furthermore, homeostatic proliferation may expand memory T-cell clones capable of rejection (see Safety Lessons section below).

These observations suggest that lymphocyte deletion alone, at least to the sub-ablative levels achievable with mAb therapy, is unlikely to generate robust clinical tolerance. Presumably residual auto- or allo-reactive clones remain and these must be regulated in some way for true therapeutic tolerance induction. Several groups are therefore studying non-myeloablative (or mini) haematopoietic transplantation for tolerance induction. In conventional haematopoietic transplants, the recipient immune system is eradicated and replaced by the graft. In mini-transplants, conditioning is less stringent but still sufficient to allow engraftment of donor cells, resulting in a chimeric haematopoietic system, in which donor and host cells co-exist. The presence of donor cells, particularly APC, underpins the induction of both central and peripheral

tolerance to donor antigens, and subsequent solid organ transplantation (from the same donor) without a requirement for long-term immunosuppression [17]. Although such protocols generally use living-related donors, graft versus host disease (GvHD) occurs in a proportion of patients and this may interrupt central tolerance mechanisms. Furthermore although the results to date are highly impressive, the robustness of the tolerant state remains uncertain. In particular, chimerism is lost in a significant proportion of patients. This is not associated with immediate breakdown of tolerance but could enhance the likelihood of later rejection episodes triggered by extrinsic insults such as infectious agents [16].

Transplantation tolerance is obvious when immunosuppressive drugs have been withdrawn but we cannot currently identify tolerant patients who remain on anti-rejection medication. It is not appropriate to stop these drugs to 'test' tolerance, although the risk is less for some organs than for others. What are needed are biomarkers of tolerance that identify patients in whom therapy can be stopped. These could be present in serum, urine, or as a 'molecular signature'. Recent attempts to define such biomarkers have produced interesting and mostly unexpected results. For example, B-cells, NK T-cells and γδ T-cells have featured as prominently as T-cells. Furthermore, potential biomarkers have differed according to the organ transplanted, and also with comorbidities [18]. Most remain to be validated but a tolerogenic biomarker would signal a massive advance for the identification of patients in whom immunosuppressive therapy may be safely discontinued.

Tolerance biomarkers are even more essential in autoimmunity, where anecdotal reports have suggested long-term disease modulation following apparently unsuccessful trials of tolerogenic therapy [19]. Critical issues to consider here are that tolerance takes time to develop and that tolerogenic therapies may not have short-term anti-inflammatory effects [20]. Furthermore, because tolerance induction is an active process some anti-inflammatory and immunosuppressive drugs may counter tolerance induction. This has been demonstrated for ciclosporin, potentially cyclo-oxygenase-2 inhibitors, and even corticosteroids [21]. Other drugs, such as rapamycin, may have pro- or anti-tolerogenic properties, depending on the precise setting [22, 23]. Therefore the period surrounding the administration of a potentially tolerogenic therapy in RA is a difficult one. Active inflammation may not be a sign that treatment is ineffective, yet the treatment of inflammation could be counter-productive, as could

uncontrolled inflammation itself, which is designed to ‘drive’ immunity. Under these circumstances it is easy to understand how a potentially effective treatment might be abandoned prematurely. In contrast, biomarkers of early tolerance induction might signal that treatment is ‘working’, even whilst active synovitis persists, encouraging perseverance rather than abandonment of therapy. Such biomarkers would also help to identify the effects of immunosuppressive and anti-inflammatory drugs on tolerogenic mechanisms. In the future we may therefore monitor two types of biomarker: those that signal inflammation, such as the CRP, and those that signal tolerance; there will be circumstances under which the two readings provide distinct signals.

In summary, transplantation has shown us that therapeutic tolerance is possible, albeit using sophisticated and powerful therapeutic regimes. It has also demonstrated the need for tolerance biomarkers to signal when immune suppression can be safely lifted. The need for such biomarkers is even stronger in inflammatory, autoimmune diseases.

2. Diabetes

During the last five years there has been encouraging progress in the application of tolerogenic therapies in type 1 diabetes. Most progress has been made with so-called non-activating, or non-mitogenic, anti-CD3 mAbs. OKT3, a murine anti-human CD3 mAb has been used for many years in the transplant setting to treat steroid-refractory allograft rejection. Its potent immunosuppressive properties usually reverse rejection but at the expense of toxicity, in particular a first-dose cytokine storm. This can manifest in many ways, including adult respiratory distress syndrome and aseptic meningitis, and can prove fatal [24]. This has prevented its application in autoimmunity, where the cost-benefit balance is deemed unfavourable, and led to the development of so-called non-activating or non-mitogenic equivalents. The first-dose cytokine storm is the consequence of T-cell activation, which follows the cross-linking of surface-bound anti-CD3 mAb by FcγR-bearing cells. Mutations in the mAb Fc region reduce or prevent cross-linking and hence significantly lessen first-dose reactions. Studies in preclinical models, such as diabetes-prone NOD mice, demonstrated a potent tolerogenic profile for $F(ab')_2$ fragment of anti-CD3 mAbs, which similarly cannot bind FcγR [25]. These reversed established diabetes and restored tolerance to islet antigens via a combination of effector T-cell silencing or

death, and TGFβ-associated regulatory T-cell induction [26]. Two groups have used non-mitogenic anti-CD3 mAbs, teplizumab and otelixizumab, to treat patients with recent-onset type 1 diabetes. Patients with an appropriate family history and circulating autoantibodies were followed at regular intervals until the onset of impaired glucose tolerance. They were then treated with a brief course of anti-CD3 therapy, in both cases non-mitogenic although via distinct Fc mutations [27, 28]. Recent follow-up of these patients has suggested a slowing of disease progression for at least 18 months and possibly up to 5 years [29, 30].

The most important lesson to emerge from this work is the key importance of timing of therapy. In the European trial efficacy correlated with residual beta cell function at the time of therapy, and the patients with lowest C-peptide levels derived minimal benefit. Whilst not entirely surprising, treatment was within 6 weeks of disease onset, suggesting that treatment in the pre-clinical phase may have been even more effective. Rheumatologists recognise the importance of early RA treatment in terms of preventing joint damage but timing could be even more critical from the perspective of immune modulation because tolerance may breakdown several years before clinical disease [31]. The heritability of RA is less than for type 1 diabetes, rendering pre-clinical disease harder to identify but a number of groups are starting to combine serology, genetics and demographic factors to potentially identify susceptible individuals.

Teplizumab administration was associated with the appearance of IL-10 secreting CD4+ T-cells and FoxP3+ CD8+ regulatory T-cells, as well as an increased frequency of autoantigen-reactive CD8+ T-cells [32-34]. These were not used to predict response to therapy but could provide important pharmacodynamic clues. Pre-clinical data suggest that antigen-specific regulatory T-cells can be potently induced by a combination of anti-CD3 and nasal pro-insulin, thereby combining the non-specific tolerising effects of anti-CD3 with an antigen-specific signal[35].

In summary, T1D provides a useful clinical corollary to RA. The pathogenesis of both diseases appears similar and it is clear that T1D can be modulated by non-depleting anti-CD3 therapy. However, treating similarly early disease will provide a challenge for rheumatologists and the concept of identifying ‘pre-RA’ remains in its infancy.

3. Safety lessons

If tolerogenic therapies are to be used in early, or even preclinical, disease then safety becomes paramount. A significant issue with many biological therapies, particularly mAbs, is that toxicity may be difficult or impossible to predict from pre-clinical models [7]. Toxicity may be a direct consequence of the therapy itself, or secondary to resultant perturbations of the immune system. First-dose cytokine release reactions are a good example of the former, and the unpredictability of such reactions was exemplified by the TGN1412 'Northwick Park' trial. A mAb that appeared very safe (and potently tolerogenic) in pre-clinical studies resulted in life-threatening side effects in four previously healthy subjects [36]. A potential explanation is the ability of human FcγRs to cross-link mAbs of human IgG4 isotype, thereby provoking T-cell activation. Even after the event, however, the precise mechanism proved very difficult to unravel [37]. The 'phenotype' of first dose reactions may also vary with disease. For example in multiple sclerosis patients treated with alemtuzumab, the first administration of drug was associated with a transient but worrying recurrence of previous neurological symptoms and signs, which correlated with circulating cytokine concentrations [38]. Not surprisingly, first-dose reactions are dose-related, as demonstrated by a recently aborted trial of teplizumab in T1D. In this study a change in drug packaging led to approximately 40% higher bioavailability, which appears to have been responsible for the enhanced toxicity [30]. As we start to think about biosimilars and 'generic' biologic drugs it becomes very important to recognise the many factors that can influence mAb PK and PD aside from simply the primary protein structure, such as the structure of the Fc-associated glycan [39]. Cytokine release reactions can also be provoked by extremely small mAb doses, as observed with a further anti-CD3 mAb, visilizumab. When used to treat refractory ulcerative colitis, doses as low as 10-15μg/kg were associated with first-dose reactions [40].

A good example of unpredicted secondary adverse events also emerges from alemtuzumab trials in MS. At present alemtuzumab looks to be one of the most potent therapies for MS. There may not be true tolerance induction but yearly pulses of this lymphocytotoxic therapy are highly effective at suppressing flares and curbing functional disability [41]. However, more than a quarter of patients subsequently develop secondary autoimmunity, most commonly of the thyroid gland [42]. Similar but less frequent events were also reported in patients receiving alemtuzumab for ANCA-positive vasculitis [43], Bechet's disease [44], and following renal

transplantation [45], and also in other lymphopenic settings, such as post bone marrow transplantation [46]. A recent study suggests these events are a complication of immune homeostasis, in which remaining, or newly developing, lymphocytes rapidly expand to fill the lymphopenic environment. Immune homeostasis depends on weak interactions with self-MHC, and so it is not surprising that autoimmunity can be a complication, particularly in individuals already predisposed to autoimmunity. Interestingly, in MS secondary autoimmunity is related to pre-treatment serum IL-21 levels, in turn predicted by polymorphism in the IL-21 gene [47]. Some drugs, such as rapamycin, appear to influence the expanding homeostatic repertoire in favour of immune regulation [48-50]. This has yet to be linked to improved outcomes, at least in solid organ transplantation, but suggests ways in which such phenomena might be manipulated for benefit [51].

Immunosuppression is a potential consequence of any immunomodulatory therapy, and one that rheumatologists are very familiar with. Immunosuppression should only be temporary with tolerogenic therapies, however. In the European T1D trial, reactivation of EBV occurred in most patients in association with a CD8+ T-cell lymphocytosis that occurred a few weeks after therapy. EBV titres fell as the lymphocytosis resolved, suggesting that these CD8+ T-cells were targeting EBV reactivation in B-cells, demonstrating potent and specific immunocompetence within a few weeks of anti-CD3 administration [28].

In summary although targeted therapies are more specific than conventional immunosuppressive and immunomodulatory drugs, side effects will occur. Even with tolerogenic therapies infections should be anticipated in the short-term but unexpected and even paradoxical side effects may also be encountered. As with first-dose reactions and secondary autoimmunity, a better understanding of these phenomena should result in better and targeted treatments.

4. Atopy

The therapies referred to thus far are antigen non-specific and, whilst these appear to be safe and to preserve immunocompetence, the ideal therapy is one that targets just the pathogenic clones of T-cells that are causing autoimmnity or transplant rejection. Atopy is a very common form of immunopathology, in which the inciting antigen is generally known. In atopic conditions it has proved possible to harness

powerful immunomodulatory effects by using whole antigen or antigen-derived peptides.

In allergic asthma, patients receiving by intradermal injection a combination of peptides derived from Fel d 1, the major cat allergen, demonstrated symptomatic improvement by a number of measures [52]. Reported studies are relatively small, however clinical responses generally correlate with surrogate response measures such as skin test reactivity and ex vivo measures of immune function. Thus, therapy is associated with reductions in TH1 and TH2-type responses and a shift towards an IL-10 secreting regulatory population [53]. Recently, the latter cell population has been associated with the phenomenon of 'linked suppression' in patients responding to therapy [54]. Linked suppression and epitope spreading describe the situation where tolerance to administered peptides 'spreads' to involve additional peptide sequences present in the same protein or in other proteins present in the same location. The induction of such mechanisms will be critically important in terms of tolerogenic therapy for human autoimmunity, because there is a broad repertoire of autoantigens by the time disease is clinically apparent. These ex vivo data also serve as important biomarkers of efficacy, and add considerable credibility to the clinical data.

Autoreactivity to heat shock proteins (HSP) has been demonstrated in a number of autoimmune conditions, including T1D, juvenile idiopathic arthritis (JIA) and RA. HSP peptide administration has provided some evidence of a biological effect in T1D, and ex vivo immunomodulatory effects have been suggested in rheumatic disease, but without clear clinical efficacy [55-57]. Because of the similarity between bacterial and human HSPs, such phenomena may implicate antigenic mimicry in autoimmune pathogenesis. However, the precise link between HSPs and autoimmunity remains unclear and the mode of action of HSP-based peptide therapies may differ from those in atopy. For example, some HSP peptides appear to exert a direct action via toll-like receptors [58].

Peptide-based therapy is assumed to operate by interfering with the T-cell receptor/MHC interaction. A related approach is to generate TCR 'antagonists', altered peptide ligands that differ from disease-associated peptides at key residues. In vitro and in pre-clinical models these appear to switch off autoreactive T-cells, perhaps by providing a partial signal, leading to tolerance induction. Trials of such a peptide in MS led to disease flares, however, apparently via the recruitment of pathogenic T-cell specificities [59].

Our mucosae are continuously bombarded by foreign antigens in the air that we breathe and the food that we eat and yet diseases such as asthma and G-I intolerances are relatively rare. This is because our mucosal immune system is specifically designed to down-regulate immune responses to such substances. This phenomenon can be exploited to induce tolerance to autoantigens, so-called mucosal tolerance induction. In terms of tolerogenic therapies this is likely to represent one of the safest approaches, potentially applicable to very early disease or even pre-clinical disease. The intranasal route appears to be the most potent for mucosal tolerance induction but a recent phase 2 RCT of intranasal recombinant Human Cartilage glycoprotein-39 (HCGP-39), a potential RA autoantigen, failed to demonstrate benefit after 13 weeks [60]. There are a number of potential explanations for this result, relating to trial design and other factors, including the possibility that HCGP-39 is not an autoantigen in RA. Again, the lack of tolerance biomarkers severely restricts interpretation of these data. In contrast, a recent phase I/II study in T1D employed a plasmid that encodes pro-insulin. Details of the trial, including route of administration, are limited but therapy is reported to have stabilised C-peptide and reduced glycosylated haemoglobin levels [61].

The lessons to be learned from atopy are that, if we can identify a robust autoantigen, interventions based around this antigen may have powerful effects, with the added advantage of providing useful surrogate markers of efficacy and potential biomarkers of tolerance induction. Unfortunately T-cell autoantigens have thus far proved elusive and inconsistent in RA, and the few clinical trials that have taken place have provided negative results. However, the potential rewards of antigen-specific therapy are great, in particular the possibility of applying mucosal tolerance approaches (which should be very safe) in early or pre-clinical disease.

5. Juvenile Idiopathic Arthritis (JIA)

Tolerance is an active process and therefore attempts to induce therapeutic tolerance will require a minimum level of immune functionality. For example, a functioning thymus is needed to generate a new T-cell repertoire following depleting therapies, and effective regulatory T-cell function appears essential for robust and lasting tolerance induction. Autologous stem cell transplantation (ASCT) as a treatment for autoimmunity involves aggressive lymphocyte depletion using drugs such as cyclophosphamide and anti-lymphocyte antibodies followed by the infusion

of T-cell depleted autologous stem cells, harvested from the patient's peripheral blood at an earlier timepoint. The rationale behind this relatively aggressive approach is to enable a new, non-autoreactive, immune system, to fill the space vacated by the eradicated, diseased one. There are several caveats to this approach: conditioning is unlikely to eradicate every autoreactive lymphocyte; similarly the stem cell 'graft' may contain autoreactive cells; and the graft clearly carries genes that predispose to autoimmunity, so disease could recur. Earlier discussions around delayed reconstitution, and immune homeostasis, are also clearly relevant in this setting. Indeed, in RA patients ASCT was associated with a period of symptomatic improvement, not dissimilar to that previously achieved with alemtuzumab [62]. In contrast, long-term disease remissions have been noted in other conditions such as SLE, MS and, notably, JIA. In JIA therapy was followed by the appearance of regulatory T-cells, at least some of which appeared to be newly generated in the thymus. The regenerating immune system also exhibited a less inflammatory cytokine profile [63, 64]. Similarly, in SLE and MS long term remissions have followed ASCT, in association with profound changes in the TCR repertoire and other evidence of immune regulation such as emergence of FoxP3+ T-cells, and normalisation of dysregulated B-cell homeostasis [65, 66]. These data suggest that an autoreactive immune system may not need to be completely eradicated and replaced to achieve long-term disease remission. Provided there is a degree of immune 'plasticity', immunomodulatory therapy may provide an environment or 'space' to enable dominant tolerance mechanisms to re-emerge and suppress any residual disease-related clones. In other words, the targetted immune system strives to regain its natural state of self-tolerance. This is an exciting concept because it does not rely on the rheumatologist or immunotherapist to understand and target every facet of the autoimmune diathesis. On the other hand it also suggests that the lack of plasticity may render tolerance harder to induce. For example, the sub-optimal response to ASCT in RA could reflect the existence of protective microenvironments or niches in the inflamed synovium for autoreactive clones [67]. Similarly, the immune system in RA patients displays a number of features that could limit its plasticity. These include suppressed thymic function, an 'exhausted' lymphocyte phenotype and a dysregulated cytokine environment that may affect immune homeostasis [68, 69]. Regulatory T-cells are also dysfunctional in RA, and B cell tolerance check points may also be defective [70, 71]. Therefore, whilst attempts to induce therapeutic tolerance in RA

must continue, this could be a disease where engraftment of an allogeneic immune system, perhaps combined with thymic ‘adjuvants’ may provide the best chance of success [72, 73].

In summary, ASCT in JIA has confirmed that our immune systems are intrinsically programmed to avoid autoreactivity, and appropriate therapy may provide an opportunity for endogenous tolerance mechanisms to re-establish themselves (Figure 2). On the other hand this renders it important to understand the immune defects present in diseases such as RA, and to target these as part of our tolerogenic regime.

6. Graft versus host disease and cellular therapies

As a potential alternative to haematopoietic transplantation, it may soon be possible to specifically target and replace malfunctioning cell types and subsets. Several groups are developing methods for expanding and reinfusing autologous Tregs, although these may need to be antigen-specific to fully harness their potential [74, 75]. An alternative approach may be to encourage the conversion of non-regulatory peripheral blood T-cell subsets to Tregs [76]. Tolerogenic dendritic cells (tolDC) are another cell type that could be used to instruct the development of a tolerant immune system, and plans are underway for a proof-of concept trial in RA [77]. As bespoke therapies, both of these will be expensive to produce and available only in specialised centres that contain Good Manufacturing Process (GMP) laboratories and the technical expertise to reliably generate these sophisticated products. Consequently such therapies may first enter the clinic to treat haematological conditions such as graft versus host disease (GvHD). The success of such strategies in rheumatic disease additionally requires the appropriate cell types to function normally under disease-associated conditions, which is not necessarily the case for Tregs in RA [70]. On the other hand mesenchymal stem cells (or mesenchymal stromal cells, MSC) could provide an off-the-shelf therapy capable of restoring tolerance. Derived from various sources, including bone marrow and adipose tissue, these cells display lineage markers suggestive of a stromal origin [78]. They are pluripotential, capable of giving rise to fat, cartilage, bone, muscle and other tissues when cultured under appropriate conditions. However, they are also potently immunosuppressive and potentially tolerogenic. Following activation they produce indole amine dioxygenase (IDO), an enzyme that converts tryptophan to kynurenine, thereby inhibiting T-cell proliferation

[79]. MSC appear to have multiple properties that could benefit established autoimmunity, including T-cell modulation and Treg induction [80]. Furthermore, their propensity to home to sites of inflammation and tissue damage could focus their activities, including a reparative potential, at appropriate sites [81]. MSC have already been applied to the management of severe, steroid-refractory graft versus host disease, with preliminary evidence of success [82]. Because they act in a non-MHC restricted fashion and are not themselves immunogenic, they can essentially be used 'off the shelf', potentially providing a therapy for a number of applications, extending from the management of autoimmunity to tissue engineering [82].

In summary, rheumatologists and haematologists have collaborated for many years in the context of ASCT for refractory autoimmune disease. This liaison is likely to strengthen as different cellular therapies approach clinical application. In terms of autoimmunity, MSC are likely to compete with tolDC to provide the first 'proof of concept' of a cellular therapy. However, many questions will need to be addressed, including cell dosage, route of administration, and antigen specificity, as well as optimal background therapies.

Current RA management and applying these lessons to trial design

Previous attempts to induce therapeutic tolerance in RA have been unsuccessful. However our general approach to therapy has changed since trials of ant-CD4 and similar mAbs were conducted. We now treat disease earlier, with more aggressive drug regimes, even starting to treat patients with early inflammatory arthritis but no firm label of RA [83, 84]. The immune dysregulation of established RA may mitigate against attempts at tolerance induction but, in early RA, even conventional drugs have lasting influences on disease progression, the so-called 'window of opportunity'. Therefore our best hopes for tolerance induction must be in early intervention trials and, whilst not futile, trials in established disease have a higher hurdle to jump. Trial design should also incorporate step-down regimes whereby the potentially tolerogenic drug is ultimately withdrawn, which is also aligned with some current practice. Interestingly, a small study of short-term infliximab use in newly diagnosed RA demonstrated that the drug could be withdrawn in many patients with maintained disease remission, although methotrexate was continued [85]. Infliximab would not generally be considered a tolerogenic drug but can boost regulatory T-cell function so we now need larger studies of similar design, incorporating drugs such as non-

activating anti-CD3 [70, 86]. Withdrawal design studies in which all participants initially receive active drug, followed by withdrawal in one group, should be an ethically acceptable way to test tolerogenic drugs. The question of 'bridging' therapy during the period of tolerance induction remains to be addressed. Would steroids be a suitable therapy or would they interfere with tolerance mechanisms? Alternatively, perhaps infliximab could safely suppress inflammation during that brief window? These are exciting times and it seems that current practice is moving towards a trial design that will enable us to robustly test the concept of therapeutic tolerance induction in inflammatory arthritis.

Summary

Over recent years, therapeutic tolerance has graduated from a pre-clinical concept to a clinical reality, at least in transplantation. Promising data are also emerging in atopy, where antigen-specific approaches are providing robust evidence for immune modulation. In autoimmunity, non-antigen-specific therapy looks promising in T1D and MS. In this chapter we have tried to summarise some of the salient lessons for RA that are emerging from work in these other conditions (Figure 3). Perhaps the most important is the need for robust biomarkers of tolerance induction, with which to guide therapy. However we have also learnt, optimistically, that therapeutic tolerance is not an 'all or none' phenomenon. The natural state of our immune system is one of self-tolerance and if drugs can provide a 'nudge' in the right direction and the diseased immune system retains sufficient residual plasticity, the task can be finished by the re-emergence of endogenous tolerance mechanisms, as seen following ASCT in JIA, SLE and MS. Finally, it is reassuring that our current approaches to RA management could be readily adapted to robust trials of tolerogenic drugs.

Practice Points

- Tolerance occurs 'naturally' in some transplant recipients, and can also be induced by creating 'mixed chimeras' with non-myeloablative haematopoietic transplantation.
- Long-term remission is achievable with autologous stem cell transplantation in JIA, SLE, and MS.

- Long-term remission has also been achieved in T1D and MS using non-depleting and depleting anti-T-cell mAbs respectively
- Peptide therapy can very specifically modulate the dysregulated immune response in atopic conditions.
- In general, tolerance induction should be easier to achieve in early disease.

Research Agenda

- Robust biomarkers are required to identify the truly tolerant patient and to help to design tolerance trials
- A better understanding is required of the effects of conventional anti-inflammatory and immunosuppressive drugs on tolerance induction
- We need a better understanding of immune function and dysfunction in RA, including a better definition of autoreactivity and identification of autoantigens.

Figure legends

Figure 1. Tolerance in autoimmunity and transplantation. Tolerance is readily recognisable in transplantation (left), when a recipient retains a foreign organ graft without the need for immunosuppression. The situation is less clear in autoimmune disease (right hand panel). Even when a patient is in remission on no therapy this could represent the natural pattern of disease with relapses and remissions. Biomarkers of tolerance are needed to distinguish these scenarios.

Figure 2. In the tolerant state, potential autoreactivity is balanced by immunoregulatory mechanisms (top panel). This balance becomes disturbed in immunopathology (middle panel). Tolerogenic therapy re-establishes a new balance. Because this is an active process the new set state may not be absolutely identical to the naturally tolerant state (bottom panel).

Figure 3. Lessons learned. This figure recapitulates the lessons highlighted in the main article.

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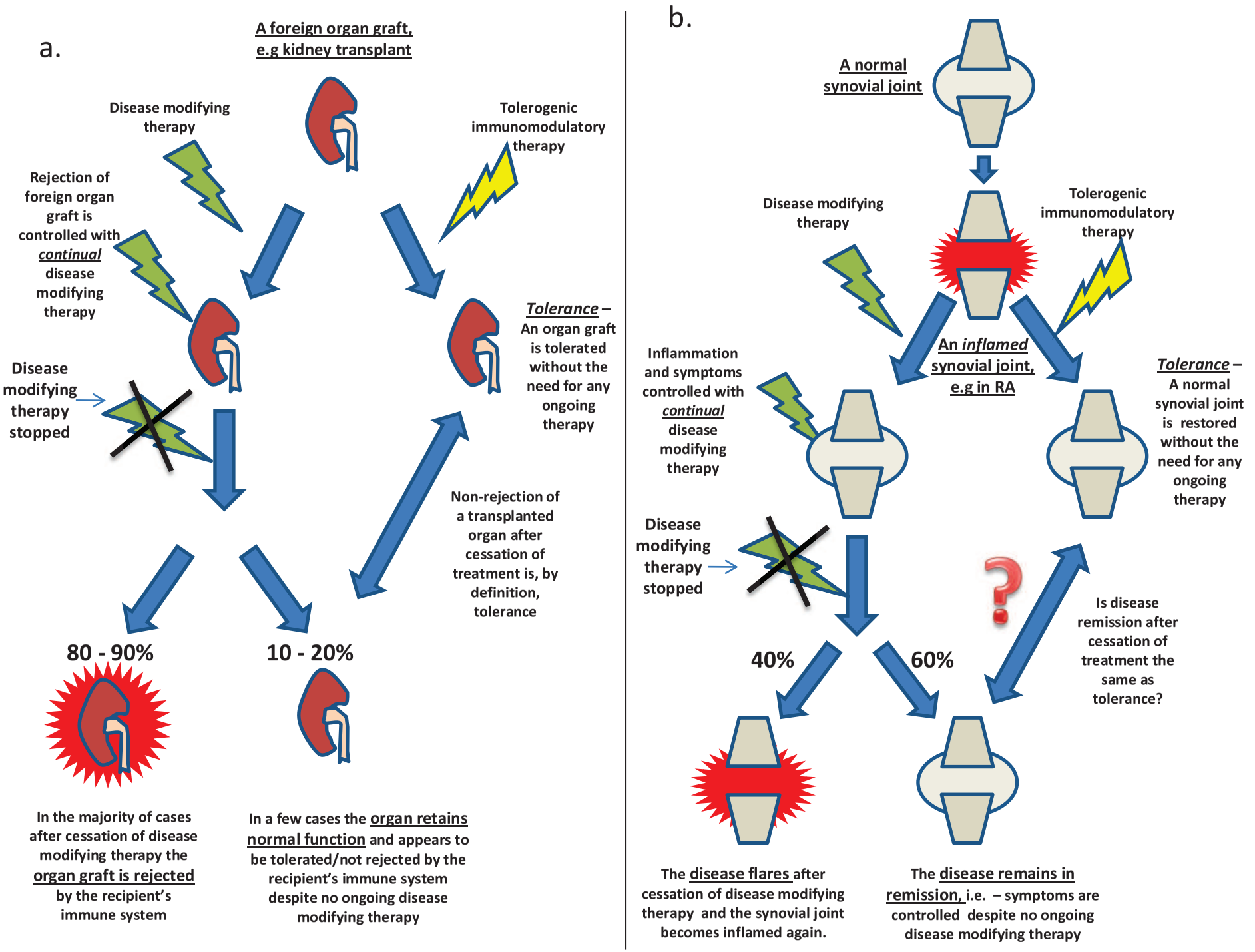
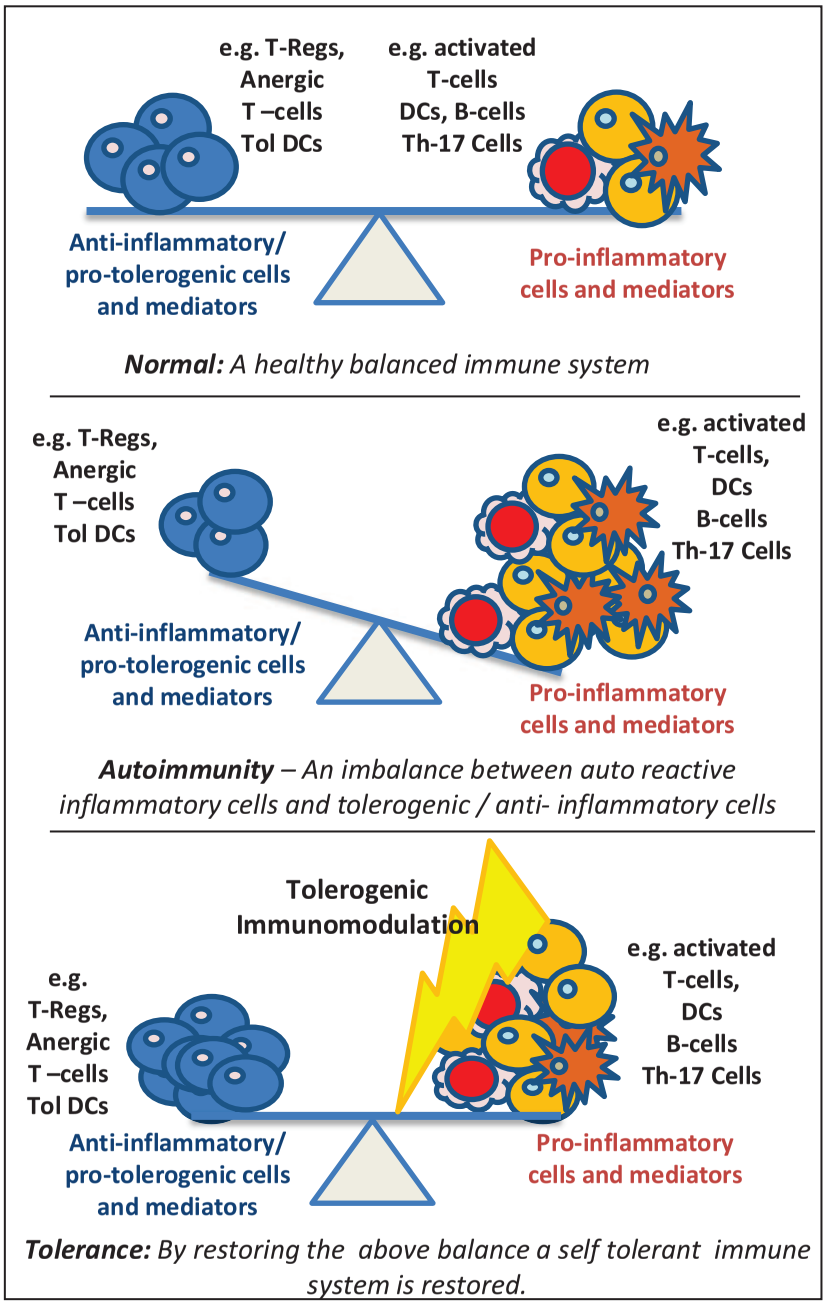

a.
A foreign organ graft, e.g kidney transplant
Disease modifying therapy
Tolerogenic immunomodulatory therapy
Rejection of foreign organ graft is controlled with continual disease modifying therapy
Disease modifying therapy stopped
Tolerance – An organ graft is tolerated without the need for any ongoing therapy
Non-rejection of a transplanted organ after cessation of treatment is, by definition, tolerance
80 - 90%
10 - 20%
In the majority of cases after cessation of disease modifying therapy the organ graft is rejected by the recipient's immune system
In a few cases the organ retains normal function and appears to be tolerated/not rejected by the recipient's immune system despite no ongoing disease modifying therapy
b.
A normal synovial joint
Disease modifying therapy
Tolerogenic immunomodulatory therapy
An inflamed synovial joint, e.g in RA
Inflammation and symptoms controlled with continual disease modifying therapy
Tolerance – A normal synovial joint is restored without the need for any ongoing therapy
Disease modifying therapy stopped
?
Is disease remission after cessation of treatment the same as tolerance?
40%
60%
The disease flares after cessation of disease modifying therapy and the synovial joint becomes inflamed again.
The disease remains in remission, i.e. – symptoms are controlled despite no ongoing disease modifying therapy


e.g. T-Regs,
Anergic
T –cells
Tol DCs
e.g. activated
T-cells
DCs, B-cells
Th-17 Cells
Anti-inflammatory/
pro-tolerogenic cells
and mediators
Pro-inflammatory
cells and mediators
Normal: A healthy balanced immune system
e.g. T-Regs,
Anergic
T –cells
Tol DCs
e.g. activated
T-cells,
DCs
B-cells
Th-17 Cells
Anti-inflammatory/
pro-tolerogenic cells
and mediators
Pro-inflammatory
cells and mediators
Autoimmunity – An imbalance between auto reactive
inflammatory cells and tolerogenic / anti- inflammatory cells
Tolerogenic
Immunomodulation
e.g.
T-Regs,
Anergic
T –cells
Tol DCs
e.g. activated
T-cells,
DCs
B-cells
Th-17 Cells
Anti-inflammatory/
pro-tolerogenic cells
and mediators
Pro-inflammatory
cells and mediators
Tolerance: By restoring the above balance a self tolerant immune
system is restored.

***Lesson 1*: It is better to intervene early during the pathological process**

Immune system

Immunomodulatory therapies

***Lesson 2*: Caution is required when using novel therapies**

Lessons

Immunomodulatory therapies

Plastic immune system, e.g. ? JIA

Following intervention the immune system is able to re-establish a healthy balance

Immunomodulatory therapies

Unhealthy / aged immune system e.g. ? RA

Following intervention the immune system struggles to re-establish a healthy balance

***Lesson 3:* Tolerance induction is an active process requiring a plastic immune system**

Concurrent pharmacological therapies, e.g. cyclosporin , rapamycin, COX-2 inhibitors etc

Anti-inflammatory/ Pro-tolerogenic cells and mediators

Pro-inflammatory cells and mediators

***Lesson 4*: Anti-inflammatory and immunosuppressive drugs may interfere with active tolerance mechanisms**

•Cellular therapies

•Anti CD3 and other monoclonal antibodies

•Peptide therapies

Immunomodulatory therapies

Anti-inflammatory/ Pro-tolerogenic cells and mediators

Pro-inflammatory cells and mediators

***Lesson 5:* Tolerance can be achieved in several ways, some specific and some less or non-specific**

Following the above interventions there is currently no way of assessing the immune balance and whether tolerance has been safely induced.

Anti-inflammatory/ Pro-tolerogenic cells and mediators

Pro-inflammatory cells and mediators

***Lesson 6:* Biomarkers are thus needed as a robust surrogate indicator of immune balance and tolerogenic potential**