THERAPEUTIC T-CELL MANIPULATION IN RHEUMATOID ARTHRITIS: PAST, PRESENT AND FUTURE

John D Isaacs. Professor of Clinical Rheumatology and Director, Wilson Horne Immunotherapy Centre.

Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University

4th Floor Catherine Cookson Building

Framlington Place

Newcastle-upon-Tyne

NE2 4HH

Tel. 0191 222 5337

Fax. 0191 222 5455

j.d.isaacs@ncl.ac.uk

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ABSTRACT

Accumulating evidence suggests that rheumatoid arthritis (RA) is a T-cell mediated autoimmune disease. Early attempts at disease modulation using strategies such as CD4 monoclonal antibodies (mAbs) were severely hampered by a lack of biomarkers of autoreactivity. Recently, however, costimulation blockade has emerged as an effective treatment for RA. Alongside a greatly improved mechanistic understanding of immune regulation, this has rekindled hopes for authentic and robust immune programming. The final pieces of the jigsaw are not yet in place for RA but, in other disciplines, emerging treatment paradigms such as non-mitogenic anti-CD3 mAbs, autoantigenic peptides, and even cellular therapies are providing hope for a future in which immunopathology can be specifically and vigorously curtailed.

KEYWORDS

Rheumatoid arthritis; T-cell; therapeutic tolerance; anti-CD3; immune modulation; monoclonal antibody; immunotherapy; costimulation blockade; autoimmunity; regulatory T-cell

INTRODUCTION

Models of rheumatoid arthritis (RA) pathogenesis have undergone several major revisions since the discovery of rheumatoid factor (RhF) spawned a model focussed on B-cells and immune complexes [1]. Subsequently T-cells took centre stage [2], only to be usurped by models focussed on cytokine dysregulation [3]. Recently a B-cell resurrection has taken place [4] but most now accept a complex pathogenetic model incorporating multiple aspects of innate and acquired immunity, whose relative contribution varies according to disease stage and probably between patients. Targeted therapies and associated pharmacogenetic analyses will play an important role in unravelling the complex pathological tapestry of RA [5]. This review will focus on the likely role of T-cells, and their potential for therapeutic manipulation.

EVIDENCE FOR T-CELL INVOLVEMENT IN RA

T-cells are one of the most abundant cell types in RA synovium, comprising 30-50% of synovial tissue cells [6]. The majority are CD4+, although CD8+ T-cells are also present and may be of pathogenic importance [7]. A significant contributor to the demise of the T-cell model of RA was the paucity of TH1-type cytokines in the rheumatoid synovium. The discovery of T-cell subsets led to immunopathological paradigms characterised by 'immune deviation' away from a healthy balance of T-cell derived cytokines. TH2 excess (IL-4, IL-5, IL-10) underpinned allergic or 'humoral' diseases such as atopic eczema and asthma, whereas TH1 excess was implicated in cellular pathologies such as diabetes, multiple sclerosis and RA. However, levels of T-cell cytokines, such as interferon gamma (IFN-γ) and interleukin-2 (IL-2), were low in RA synovium in contrast to monokines such as TNF-α and macrophage colony stimulating factor (M-CSF) [8]. It was subsequently demonstrated that synovial T-

cells activated macrophages via membrane interactions [9] but, at the time, a dogma evolved that T-cells were merely innocent bystanders in RA synovium, attracted by the inflammatory milieu. Recently IL-17, produced by imaginatively named TH17 T-cells, has been implicated in human autoimmune inflammation [10]. Although discovered some 10 years ago, a potentially key role in diseases such as RA has only recently been attributed to IL-17 [11]. TH17 T-cells mature from naïve T-cells, and survive, in an environment characterised by IL-6, TGFβ and IL-23 [12]. IL-17 is a highly pleiotropic cytokine with effects on a variety of cell types including monocytes, fibroblast-like synoviocytes, chondrocytes and osteoclasts. Direct or indirect effects include inflammation, angiogenesis, osteoclastogenesis, and breakdown of bone and cartilage. A number of studies have demonstrated elevated levels in blood and synovium of RA patients, with correlations between synovial levels and joint damage. [13-15]. Nonetheless, the biology of TH17 cells differs in important ways between mouse and human and the relative contribution of TH1 and TH17 T-cells to RA pathogenesis remains to be formally established [16-19].

Recent studies of genetic predisposition to RA have bolstered evidence for T-cell involvement in its pathogenesis. For many years, the only consistent genetic linkage was with the HLA-DRβ1 locus. Alleles containing the so-called ‘shared epitope’ at residues 70-74 associated with more severe RA, and a ‘double dose’ with an even worse prognosis [20]. Although this locus underpinned class II HLA polymorphism, and therefore guided the presentation of foreign and self antigens to T-cells, consistent autoreactivity to potential autoantigens proved surprisingly difficult to demonstrate. Recently, more powerful genetic studies have implicated the gene PTPN22 in RA predisposition [21]. There is weaker evidence linking CTLA-4 [22]. PTPN22 helps to set T-cell activation thresholds, whereas CTLA-4 downregulates activated T-cells – critical determinants of T-cell responses to foreign antigens but

also for both central and peripheral tolerance to self antigens. Recent epidemiologic studies have incorporated these genetic factors into credible models of RA aetiology. Cigarette smoking, which increases the rate of protein citrullination in the lung, appears to predispose to anti-CCP autoantibody development in individuals who carry the shared epitope [23]. Anti-CCP autoantibodies associate with destructive RA [24] although are also found in healthy individuals prior to the onset of synovitis [25]. An individual's relative risk of developing RA is further heightened if they carry the RA-linked PTPN22 allele. Indeed, in some (although not all) populations, the most adverse set of factors are associated with a relative risk of developing RA of 20-25 times background [26,27]. Furthermore, the gene encoding peptidyl arginine deiminase 4 (PADI4), an enzyme responsible for citrullination of proteins, has been associated with RA in studies of Asian populations, although inconsistently in Europeans [28-30]. These data significantly strengthen the case for T-cell involvement in RA, at least in the early stages of anti-CCP positive disease. Additionally, STAT4 is a transcription factor downstream of both the IL12 and IL23 receptors on T-cells, whose target genes include both interferon-γ and IL-17. Recent studies have implicated the gene encoding STAT-4 in RA susceptibility in North American, European and Asian populations [31,32].

The aetiological factors implicated in anti-CCP positive RA do not appear to predispose to anti-CCP negative RA [26]. Whilst this may seem to reduce the likelihood of T-cell involvement in those patients, no alternative aetiological models currently exist. The fact that RA T-cells are hypo-proliferative, or anergic, is one factor that has complicated attempts to demonstrate T-cell autoreactivity [33,34]. However, assays that rely on T-cell cytokine secretion rather than proliferation have started to show consistent examples of autoreactivity. For example, T-cell responses against the cartilage (auto)antigen human glycoprotein (hGP)-39 are associated with

production of immunoregulatory IL-10 in DRβ1*04-positive healthy controls but pro-inflammatory cytokines in DRβ1*04-positive RA patients [35]. It is also important to appreciate that pathogenic T-cells may not always resemble conventional, antigen-activated T-cells. For example, some T-cells in RA appear to be cytokine- rather than antigen-activated [36]. There is also good evidence that 'exhausted' or senescent, CD28-, T-cells play an important role in RA pathogenesis [37], and other unusual subsets of T-cells have also been identified [38,39]. In general these atypical T-cells appear to have autoreactive potential and are therefore legitimate targets. Increasingly their biology is becoming elucidated, revealing novel pathways and molecules for therapeutic intervention [40-42].

SMALL MOLECULE INHIBITORS OF T-CELLS

A number of conventional disease-modifying anti-rheumatic drugs interfere with T-cell function. For example, ciclosporin is a calcineurin antagonist. Interleukin-2 production is central to T-cell activation and proliferation, and calcineurin is a critical upstream signalling intermediary. Consequently, ciclosporin inhibits IL-2 production and is a potent immunosuppressive drug, widely used in solid organ and bone marrow transplantation to treat graft rejection and graft-versus-host disease. It was introduced as a treatment for RA during the early 1990s, its use underpinned by clinical trials that demonstrated both reduction in symptoms and signs as well as slowing of radiographic progression [43-45]. It continues to be used as a second or third-line DMARD, particularly in combination regimes [46,47], but dose-limiting toxicity has restricted its widespread application. Renal toxicity, in particular, has been problematic [48].

Leflunomide inhibits dihydroorate dehydrogenase (DHODH), a central enzyme in de novo pyrimidine biosynthesis, and a pathway that is important for the proliferation of

activated T-cells [49]. Clinical trials have shown an acceptable safety profile and efficacy that matches methotrexate (MTX) and sulphasalazine, which includes retardation of joint damage [50,51]. It has a long half life and slow onset of action but regimes that incorporate a loading dose tend to be associated with a higher incidence of toxicity, particularly gastro-intestinal. Regular monitoring of blood counts, liver function and blood pressure is required during routine use. In cases of toxicity, the clearance of leflunomide can be accelerated with cholestyramine.

MTX is the most widely prescribed drug for RA. Even at the doses used to treat RA it inhibits purine and pyrimidine synthesis but only for a brief time after each administration. Therefore although MTX does inhibit antigen-induced T-cell proliferation temporarily after each dose, it is uncertain what contribution (if any) this makes to its therapeutic efficacy [52].

THE PAST: THERAPEUTIC TOLERANCE AND THE HISTORY OF T-CELL TARGETING IN RA

The discovery of monoclonal antibodies (mAbs) in the 1970s was followed by a period of intense investigation of their potential to prevent and ameliorate undesirable immunopathology. The rationale was that helper T-cells were the ‘master regulators’ of immune responses but that their programming could be plastic. Subsequent studies, using mAbs to target cell surface antigens, demonstrated their potent ability in this regard. For example, it was possible to generate transplant tolerance (the acceptance of a foreign organ graft without a requirement for chronic immunosuppression) by targeting CD4+ (± CD8+) T-cells for a brief period around the time of transplantation [53]. Of more relevance to rheumatology, autoimmunity could be prevented and ‘switched off’ using similar regimes [54]. Initial regimes utilised depleting mAbs but even more powerful effects were demonstrated with non-depleting mAbs, including the emergence of regulatory T-cells. The associated

phenomena of linked and bystander suppression, whereby induced tolerance to a particular peptide extended to other epitopes derived from the same protein and even to peptides derived from distinct proteins in the same microenvironment, promised powerful and revolutionary therapies for autoimmunity. A number of rules and models were generated to explain the experimental findings although the underlying cell biology was not elucidated. Of relevance to human disease, a greater bulk of pathogenic T-cells required more intensive immunomodulatory regimes. In the transplant setting not all MHC barriers were amenable to tolerance induction, and others required combinations of depleting and non-depleting mAbs administered over several weeks [55]. Similarly some autoimmune models (eg murine SLE) appeared more amenable to immune modulation therapy than others (eg. collagen-induced arthritis). A number of distinct T-cell surface molecules were permissive for tolerance induction, and the ‘war analogy’ model postulated that treatment merely ‘blindfolded’ pathogenic T-cells, preventing their interaction with neighbours, resulting in a default state of self-tolerance and regulatory T-cell generation [56].

The first biological therapies to be applied to RA targetted T-cells. These ranged from lymphocytotoxic depleting therapies such as CAMPATH-1H (alemtuzumab) to non-depleting CD4 mAbs. This area has recently been reviewed [57] and will not be covered in detail. However, three key limitations hampered development of these therapies.

1. It was assumed that lymphocyte depletion would be followed by full reconstitution with a newly generated repertoire. Indeed, a driving force behind this mode of therapy was the concept of an emerging, self-tolerant, immune system. The adult human immune system was severely limited in its capacity for reconstitution, however, particularly in RA. Lymphocyte depletion was followed by long-lasting lymphopenia, although without clear detriment

[58,59]. A number of potential contributors to poor reconstitution have been identified subsequently [38,60].

2. The *in vivo* biological activity of mAbs that targeted human T-cells proved extremely difficult to predict. The possible consequences of binding included depletion, modulation, shedding, activation and coating; the precise outcome was determined by features of the mAb and its target that were only partly understood. *In vitro* assays proved to be poor models of the *in vivo* (micro)environment and the information provided by animal models was severely limited by inter-species differences in both target antigen and Fc-gamma receptors (FcγR) [61]. These limitations were vividly and catastrophically illustrated by the phase I study of TGN1412, in which 6 healthy volunteers received a T- cell 'superagonist' mAb that had been administered safely to cynomolgus monkeys. The life-threatening cytokine storm experienced by the human volunteers was difficult to replicate *in vitro* even after the event and has resulted in further safeguards during mAb development [62,63].
3. The aforementioned limitations of animal and *in vitro* testing meant that the tolerogenic potential of mAbs could not be predicted. Furthermore, because the cell biology of therapeutic tolerance was not understood, biomarkers of therapeutic tolerance induction were not available. Compounding these limitations, a tolerogenic mAb was not necessarily anti-inflammatory, and a lack of short-term improvement in a disease like RA need not represent treatment failure – yet anti-inflammatory drugs could antagonise tolerogenic effects [64,65]. Hence without biomarkers to guide dose and duration of therapy, therapeutic tolerance was truly a 'holy grail', success as likely to represent luck as judgement. It is quite plausible that potentially tolerogenic drugs were prematurely and inappropriately abandoned and, when truly non-depleting mAbs were finally developed, plans to administer these for

prolonged periods at high dosage were complicated by unpredicted side effects such as skin rashes [66]. Inflammation itself is likely to inhibit tolerance generation, and other regimes combined biologic agents to suppress inflammation prior to the administration of anti-T cell therapy [67].

These limitations curtailed the initial development of T-cell mAbs in RA. Indeed, the prevailing sentiment was that biological therapies in general could have limited utility in autoimmunity and the entire therapeutic area was endangered. Fortunately the parallel development of TNFα blockade provided much needed optimism.

THE PRESENT: COSTIMULATION BLOCKADE

It was, of course, possible that CD4 was not a tractable target in human RA or that RA was not susceptible to therapeutic tolerance induction. Certainly late, refractory disease (the patient group chosen for many clinical trials) presented several undesirable characteristics such as high levels of inflammation, prior therapy with multiple drugs and, potentially, intrinsic defects of immune regulation. However, a (then) recently described concept suggested a key event in T-cell activation to be the interaction of T-cell co-stimulatory molecules with their ligands on antigen presenting cells (APCs). Disruption of these interactions not only prevented T-cell activation but was also associated with tolerance induction [68]. The best known costimulatory molecule/ligand interactions were those between CD28, and B7.1 and B7.2 (now known as CD80 and CD86), and CD40 with CD40L. Animal models again emphasised the potential potency of so-called costimulation blockade [69,70]. Early trials of a CD40L mAb in patients with SLE provided encouraging efficacy signals but were complicated by unexpected thromboembolic events which halted further development [71]. The CD28 interaction with CD80/86 proved more amenable to therapeutic intervention, however. The strategy that was finally adopted utilised a fusion protein between CTLA4 and a modified human IgG1 Fc, CTLA4-Ig. CTLA4 is

a negative regulator of T-cells that is up-regulated after T-cell activation. It has a higher affinity for CD80/86 than CD28, thereby displacing it and acting as a T-cell 'brake'. CTLA4-Ig retains the affinity of CTLA4 and therefore interrupts the costimulatory signal, preventing and potentially reversing T-cell activation. The Fc modification prevents complement activation, potentially reducing the incidence of infusion reactions [72].

Abatacept is administered by intravenous infusion. Loading doses on days 1, 15 and 29 are followed by monthly infusions of approximately 10mg/kg, a dose that showed significant efficacy at the 6 month primary endpoint of a phase IIB study [74]. Responses to placebo and 10mg/kg abatacept respectively were 35% and 60% (ACR20); 12% and 36% (ACR50); and 2% and 16% (ACR70). Quality of life also improved with abatacept and benefits were sustained at 12 months, including significantly higher rates of DAS28 remission compared to placebo (10% vs 35%), and clinically relevant improvements in function [75]. Two phase III clinical trials of abatacept have confirmed these benefits. In the Abatacept In Methotrexate inadequate responders (AIM) trial ACR20, 50 and 70 response rates at 12 months were superior with abatacept compared to placebo: 73% vs 40%, 48 vs 18% and 29% vs 6% respectively [76]. There were also improvements in function (HAQ-DI) and health-related quality of life (SF36) [77]. Abatacept therapy was additionally associated with a slowing of radiographic progression at 2 years, and improvement in symptoms and signs has now been documented to 3 years [78,79]. The Abatacept Trial in Treatment of Anti-TNF INadequate responders (ATTAIN) study confirmed the benefits of abatacept in patients with a current or previous inadequate response to TNFα blockade [80][81]. Efficacy of abatacept is sustained for at least 2 years in this patient group [82].

In the AIM study, overall adverse event (AE) rates were similar with abatacept or placebo although abatacept recipients had a higher incidence of acute infusion reactions (8.8 vs 4.1%) and pre-specified serious infections (2.5 vs 0.9%) [76]. In ATTAIN acute infusion reactions and infections were non-significantly higher in the active treatment arm but rates of serious adverse events (SAEs) and serious infections were equivalent to the rate with placebo [80]. Therefore, to date abatacept has demonstrated an acceptable safety profile when added to MTX or other conventional DMARDs, and its immunogenicity has been low [83,84]. In particular there does not appear to be a significantly enhanced risk of infection or malignancy. In the Abatacept Study of Safety in Use with other RA thErapies (ASSURE) trial, however, its combination with TNFα blockade led to a doubling of SAEs, with no corresponding improvement in efficacy [85]. Even when combined at the lower dose of 2mg/kg with etanercept the SAE rate was 16.5% (abatacept plus etanercept) vs 2.8% (abatacept plus placebo), and serious infection rate 3.5% vs 0% respectively [86]. Therefore abatacept should not, at this time, be combined with other biologic therapies.

.Abatacept is now licensed for the treatment of RA. The quality of response may increase with time on the drug, and long-term extension studies suggest a relatively low rate of secondary treatment failure. There is a sustained rate of response at 2 and 3 years by intention-to-treat analysis and, intriguingly, an apparent continued improvement in response rate according to the less robust ‘as-observed’ analysis [82]. Radiographic studies also suggest further slowing of joint damage in the second year of therapy [78]. Whilst these data may suggest a gradual ‘switching off’ of disease, only time can tell whether abatacept induces true tolerance – in which case a patient in remission on abatacept should be able to stop therapy without loss of benefit.

Recently, the ATTEST trial (Abatacept or infliximab versus placebo, a Trial for Tolerability, Efficacy and Safety in Treating RA) provided an indirect comparison of treatment with infliximab and abatacept [87]. Patients with an inadequate response to MTX were randomised to infliximab (3mg/kg every 8 weeks), abatacept (10mg/kg every 4 weeks) or placebo infusions in a double-blind, double-dummy, placebo- and active (infliximab)-controlled trial (MTX was continued). Both abatacept and infliximab were superior to placebo for efficacy throughout the 12 month study period and the response rates for the two active arms were similar at 6 months. By 12 months, however, DAS low disease activity (35.3 vs 22.4%), DAS remission (18.7 vs 12.2%), good EULAR response (32.0 vs 18.5%) and HAQ-DI (57.7 vs 52.7%) all favoured abatacept, whereas.SAEs (9.6 vs 18.2%) and serious infections (1.9 vs 8.5%) were more frequent with infliximab. Therefore, notwithstanding the fact that this was not designed as a head-to-head comparison of the biologic drugs, over the 12 month study period the therapeutic ratios favoured treatment with abatacept.

Although marketed as a costimulation blocker, abatacept's precise mode of action remains undefined. For example, some evidence suggests that binding directly to APCs upregulates indoleamine dioxygenase and tryptophanyl-tRNA-synthetase production, which will indirectly inhibit T-cell proliferation by limiting tryptophan availability [88].

THE FUTURE: NOVEL TREATMENT APPROACHES

Non-mitogenic CD3 mAbs

CD3, the 'defining' surface marker on T-cells, was one of the first antigens to be targeted for immune modulation in the 1980s. The murine mAb OKT3 was administered in brief courses as an immune suppressant to treat refractory rejection episodes, particularly in renal transplantation [89]. Although highly effective, its initial administration to a patient was usually complicated by a problematic 'first-dose' or

‘cytokine release’ reaction. The syndrome comprised a constellation of symptoms ranging from high fever and chills to aseptic meningitis and respiratory distress [90]. Corticosteroid prophylaxis allayed the severity of the reaction but this potential toxicity, and the inherent immunogenicity of a murine mAb, limited the application of OKT3 to graft-threatening rejection episodes and its use in autoimmunity was not explored.

First-dose reactions with OKT3 were the consequence of massive, synchronised T-cell activation. This occurred when OKT3, bound to the T-cell surface, was ‘cross-linked’ by FcγR-bearing accessory cells such as NK cells or monocytes. Such a reaction, on an even greater scale, underpinned the disastrous reaction to TGN-1412 referred to earlier [62]. Because the interaction between mAb and FcγRs is predicated by a small number of critical residues in the mAb Fc, genetic engineering techniques have been used to create non-FcγR binding (non-activating or non-mitogenic) CD3 mAbs. In animal models, equivalent mAbs are potent immunodulators without invoking cytokine release. For example, autoimmune diabetes was permanently switched off, in association with regulatory T-cell generation [91]. As with CD4 mAbs, the cell biology of tolerance induction in this setting is incompletely understood. Some theories suggest a requirement for partial T-cell signalling and activation, whereas others imply a ‘blindfolding’ and environmental isolation of the T-cell by mAb coating [92].

Three non-activating CD3 mAbs have entered the clinic in non-rheumatological diseases: teplizumab, otelixizumab and visilizumab. Teplizumab, a direct descendent of OKT3, and visilizumab have two amino acid alterations in the Fc, that reduce FcγR binding. In contrast otelixizumab incorporates a single mutation, that prevents N-linked glycosylation of the Fc (glycosylation being essential for FcγR binding and

complement activation). Like OKT3 before them, both teplizumab and the aglycosyl otelixizumab have been used as immunosuppressants to reverse acute allograft rejection [93,94]. More impressively, brief courses of therapy in type I diabetes (T1D) resulted in disease stabilisation for over two years [95-97]. Repeated courses have been administered with some evidence that therapy induces favourable immunoregulatory mechanisms [98,99]. In a phase I/II study of psoriatic arthritis, 8-10 days of treatment with teplizumab was followed by a reduction in tender and swollen joints for at least 3 months [100]. First-dose reactions were reported to be absent or mild in these studies. Inflammatory bowel disease and graft versus host disease have been the disease targets for visilizumab. In a phase I study in steroid-refractory ulcerative colitis, doses of 10-15μg/kg were associated with clinical benefit although mild to moderate cytokine release was observed even at these low doses [101,102].

Other T-cell surface molecules

A variety of other T-cell mAbs are approved for the treatment of non-rheumatological indications. Efalizumab is a humanised CD11a mAb that blocks interactions between the integrin LFA-1 and cell adhesion molecule ICAM-1. In this way efalizumab inhibits tight lymphocyte adhesion to inflamed endothelium [103]. Administered by weekly subcutaneous injection, it is licensed for the treatment of moderate to severe psoriasis, and has also shown encouraging data in severe atopic dermatitis [104,105]. Alefacept is a fusion protein between the extracellular domain of LFA-3 and human IgG1 Fc. Because CD2, the ligand for LFA-3, is expressed more densely on memory T-cells alefacept has the potential advantage of preferentially targeting this disease-associated T-cell subset. The CD2/LFA-3 interaction provides costimulation to T-cells but this mAb is also cytotoxic, therapy reducing the number of circulating memory T-cells. As with many biological therapies, the most relevant mechanism of action is uncertain [106,107]. However, administered by weekly intramuscular injection, it is approved for the treatment of moderate to severe plaque

psoriasis and a recent clinical trial demonstrated efficacy in psoriatic arthritis [108,109]. Natalizumab, a mAb of human IgG4 isotype, targets the α4 integrin chain and blocks the α4β1/VCAM-1 interaction at inflammatory sites as well as the α4β7/MADCAM-1 interaction at mucosae. In both locations therapy interferes with lymphocyte trafficking although a recent publication also highlights its ability to mobilise haematopoietic stem cells [110]. It is approved for the treatment of multiple sclerosis (MS) and is also effective for remission induction in Crohn's disease [111,112]. Development of natalizumab for MS was temporarily suspended following reports of progressive multifocal leucoencephalopathy in three recipients but it has now been reinstated under a restricted programme [113].

Peptide therapy

An important qualification to the treatments discussed so far is their potential to target most or all T-cells, which may increase the risk of non-specific immune suppression associated with their use. Regulatory T-cell subsets will also be targeted, with potentially beneficial (as postulated for non-activating CD3 mAbs) or detrimental consequences. The ideal immunomodulatory therapy may, therefore, be one that specifically targets autoreactive T-cells. This concept has been proven repeatedly in animal models but these are generally induced autoimmune diseases of inbred mouse strains where, by definition, the autoantigen is known. In contrast pathogenic T-cell clones have proved elusive in RA. Furthermore, and not surprisingly for an outbred population, several autoantigens have been identified with only partial overlap of autoreactivity between patients. However, antigen-specific therapies have produced encouraging data in human allergic diseases and in T1D.

Fel d 1 is an antigen present in animal dander that is an allergen in atopic asthma. In cat allergic subjects, six fortnightly intradermal injections of a Fel d 1 peptide mixture

improved symptoms and quality of life. There was an associated reduction in the late asthmatic reaction and cutaneous hypersensitivity to whole cat dander [114]. *In vitro* data showed reduced TH1 and TH2 responses and the induction of an IL-10-secreting, suppressive, regulatory cell population [115]. Mechanistic studies suggest that the dose and length of peptide are important determinants of peptide therapy efficacy but optimal dosing regimes have yet to be defined [116]. In recent onset T1D, three subcutaneous injections of a modified peptide derived from human heat shock protein 60 (hsp60), spaced over 6 months, led to preservation of insulin secretion when compared to placebo, and improved metabolic control lasting for 18 months [117,118]. There was associated *ex vivo* evidence for TH2 immune deviation. A small, dose-ranging study confirmed the beneficial effects on pancreatic function but could not reproduce the benefit in terms of insulin requirement or metabolic control [119].

Hsp peptides have also been studied as immunomodulators in juvenile idiopathic arthritis (JIA). A series of peptides derived from human and bacterial hsp60, predicted by computer algorithm to bind a range of human DR subtypes, were incubated *in vitro* with PBMC from JIA patients and healthy controls. Higher T-cell proliferative responses were observed using JIA PBMC, in association with a higher IL-10:IFNγ ratio in culture supernatants [120]. A further *in vitro* study suggested that, in JIA, the quality of the T-cell response to peptides derived from the dnaJ hsp depended on their origin: bacterially-derived peptides evoked a pro-inflammatory response whereas human sequences evoked regulatory responses [121]. In a related study, RA patients with *in vitro* T-cell reactivity to a peptide derived from bacterial dnaJ (with sequence homology to the shared epitope) were treated orally with this peptide for 6 months. No clinical outcomes were reported but treatment was associated with reduced *in vitro* T-cell proliferation to the peptide, with reduced

secretion of IFNγ, TNFα, and IL-2 but increased IL-4 and IL-10. FoxP3 expression was also increased in post-treatment blood, possibly suggestive of an immunoregulatory effect of treatment [122].

The precise role of hsps in autoimmunity remains unclear, and therapies such as those described above may have complex modes of action. For example, the hsp peptide utilised in the T1D studies signals via TLR2 as well as presumably binding the TCR. The former activity is associated with both a reduction in chemokine-induced chemotaxis and may also enhance regulatory T-cell function [123,124]. As alluded to earlier in this article, the cartilage glycoprotein hGP39 has been implicated as an RA autoantigen [35,125]. An altered peptide ligand (APL) has been identified, based on an immunodominant hGP39 epitope [126]. APLs can act as TCR antagonists, and are therefore of potential therapeutic benefit. A TCR antagonist administered to multiple sclerosis patients resulted in disease flares, however, due to the unpredicted recruitment of novel, autoreactive T-cell specificities [127]. The hGP39 APL has a distinct, non-classical, design and should not have this potential. Phase I RA studies have also studied nasal administration of an hGP39-derived peptide as well as infusions of a soluble complex of HLADR4 with a different hGP39-derived peptide [128,129].

Cellular therapies

Regulatory T-cells are now recognised and, to some extent, validated as important players in the maintenance of immune tolerance and the control of immunopathology. There are several regulatory T-cell subsets, some induced and others naturally occurring [130]. The best characterised are the thymically-derived, FoxP3-expressing 'natural' regulatory T-cells (T_{reg}) [131]. Inherited deficiency of this subset is associated with a range of autoimmune phenomena in animals and in man [132]. Furthermore, in animal models, manipulation of T_{reg} numbers and/or function can influence

susceptibility to autoimmunity and have profound effects in established disease. Critically, adoptive transfer of these cells can be an effective therapy, and some of the treatments discussed earlier in this review may influence T_{reg} function [133]. The situation in human autoimmunity is less clear but there are now a number of publications attesting to a defect of T_{reg} number or function in these conditions, including rheumatoid arthritis [130,134-136]. Indeed, TNFα blockade appears to boost T_{reg} function in RA, in addition to inducing development of a novel regulatory T-cell subset [137,138]. Furthermore, expansion of T_{reg} may underlie the long-term responses to autologous stem cell transplantation observed in juvenile idiopathic arthritis [139]. In animal models T-cell superagonists, that activate T-cells through CD28, expand T_{reg} with beneficial effects in a range of immune pathologies [140,141]. Unfortunately, it was one such mAb (TGN1412) that produced a massive first-dose cytokine storm with serious sequelae for the participants in a phase I study [62]. As with OKT3, modifications to the mAb Fc could almost certainly produce a safer product that retains efficacy, but such an approach is unlikely in the near future. Another novel approach for boosting T_{reg} function is to combine strategies, such as tolerogenic peptide administration with non-mitogenic anti-CD3 or TNFα blockade [142,143].

Ultimately it may be possible to treat human autoimmunity by the isolation, expansion and reinfusion of T_{reg} from patients. Such an approach is effective in animal models although the most potent effects occur when cellular expansion involves the use of a relevant autoantigen [144]. The low frequency of T_{reg} in peripheral blood, combined with their lack of a unique surface phenotype, further complicates this approach and it may prove more feasible to generate regulatory T-cells by pharmacological manipulation of peripheral blood [145]. Autologous cellular therapy requires GMP facilities and would be significantly more expensive than even current biologic

therapies. The overall feasibility and acceptability of this approach will depend strongly on the induction of a powerful and reproducible immunomodulatory effect [146,147].

Recently, a potentially beneficial immunomodulatory response was obtained by vaccinating RA patients with expanded, activated and irradiated autologous synovial fluid T-cells [148]. This was an open study of 16 patients and the reported efficacy must be confirmed in a formal trial. Nonetheless treatment was associated with expansion of CD4+_and CD8+ T-cells, many of which expressed the Vβ2 T-cell receptor chain. Some were anti-idiotypic, responding specifically to vaccine T-cells with production of IL-10 (CD4+ cells) or granzyme B (CD8+ cells). A broader regulatory response, however, was directed towards activated T-cells in general, specifically against peptides derived from the IL2 receptor alpha chain (so-called anti-ergotypic T-cells). The latter response may be critical to harness in a disease where the precise autoantigen and pathogenic T-cell clones are not readily identifiable, perhaps explaining why a previous attempt at T-cell vaccination using TCR-derived peptides has not been pursued [149].

CONCLUSIONS

Over the past 20 years, the development of T-cell targeted therapies for autoimmunity has come full circle. The pessimism that surrounded early clinical trials of CD4 mAbs has been replaced by optimism surrounding costimulation blockade and more novel approaches to immunomodulation. In large part this new confidence has been fuelled by an enhanced understanding of immune regulation, and particularly of regulatory T-cell function. However, we continue to require better biomarkers of tolerance induction in order to exploit these novel modalities to their full potential. We are also better at recognising and defining autoantigens, potentially moving us closer to our ultimate goal of inducing antigen-specific tolerance via safe

and innocuous treatments. Ultimately established RA remains a complex disease, and it remains to be seen whether any immunomodulatory therapies will be sufficiently potent to correct many years of cumulative immune dysregulation and pathology. As safer treatments and more informative biomarkers allow us to move into earlier disease, however, the potential for cure should become a reality.

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CONFLICT OF INTEREST STATEMENT

The author consults for and/or sits on advisory boards of Roche, Bristol-Myers Squibb, GlaxoSmithKline and Almirall. He is in receipt of current research funding from Abbott. He is named as co-inventor on a European patent relating to the use of a non-mitogenic anti-CD3 mAb for inflammatory arthritis.

KEY MESSAGES

1. Accumulating evidence incriminates T-cells in RA pathogenesis.
2. Co-stimulation blockade is efficacious in RA.
3. Better biomarkers and novel immunomodulatory strategies may soon enable us to ‘switch off’ autoimmunity.