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“Pathogenetic insights from the treatment of rheumatoid arthritis”

Iain B McInnes & Georg Schett

Correspondence to either author at:

IBM - Institute of Infection Immunity and Inflammation, University of Glasgow, Glasgow G128QQ, United Kingdom; iain.mcinnnes@glasgow.ac.uk

GS - Universitätsklinikum Erlangen Ulmenweg 18 91054 Erlangen, Germany; georg.schett@uk-erlangen.de

Search Terms

Pubmed search for articles up to February 2017 included rheumatoid, arthritis, biologic, mode of action, synovial biopsy, inflammatory synovitis. Articles were selected for their information around mode of action studies using therapeutics in rheumatoid arthritis.

Conflict of interest

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Abstract

Rheumatoid arthritis is a chronic autoimmune disease characterized by progressive articular damage, functional loss and comorbidity. The advent of effective biologic and small molecule kinase inhibitors in recent years has substantially improved clinical outcomes. Just as pathogenesis understanding lead in large part to the advent of such therapeutics, so mode of action studies of these specific immune targeted agents have shed new light on the immune pathways that drive articular inflammation and related co-morbidities. Thus cytokine inhibitors have definitely proven a hierarchically important position for TNF and IL-6 in disease pathogenesis with a likely role also for GM-CSF, but not IL-1, or IL-17. More recently, Janus kinase inhibitors have demonstrated that cytokine receptor families served by JAK/STAT dependent signaling are critical for disease, in part replicating the findings for IL-6 blockade but adding new knowledge as to the roles played by other cytokines e.g. the interferons. Finally, co-stimulatory blockade and B cell depletion demonstrate that the adaptive immune response, and other downstream pathways initiated by these cells, likely participate directly in synovial inflammation. Taken together, understanding the actions of specific immune interventions can elucidate definitive molecular or cellular nodes that are essential to maintain complex inflammatory networks that sub-serve disease.

Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by articular destruction associated increasingly over time with co-morbidities in vascular, metabolic, bone and psychological domains. RA affects up to 1% of the population, more often females and can present across the age decades. The primary aetiopathogenesis of RA is considered to be autoimmune, comprising a pre-RA phase in which systemic immune mediators e.g. autoantibodies and cytokines, are detected, leading thereafter to clinically evident, articular onset of 'early RA' that evolves into chronic inflammation ('established RA') associated with tissue remodeling and damage. Recent advances in the development of specific immune targeted therapeutics including biologics and kinase inhibitors have revolutionized clinical care and remarkably improved outcomes. Equally they have provided definitive proof of concept in unravelling the pivotal molecular and cellular nodes that reside within the complex inflammatory networks that propagate and perpetuate disease. In this short review we will summarize the key pathogenetic lessons learned and implications derived from the bio-therapeutic revolution of the last two decades in RA.

Considerations around RA pathogenesis

The pathogenesis of RA has been extensively reviewed recently and will be summarized only briefly herein [1-3]. The genetics of RA have been explored by conventional and genome wide approaches and the genetic architecture of the disease is well defined. Over 100 loci are associated with disease risk and progression and the majority implicate immune effector or regulatory gene products [4]. Prominent amongst these are the MHC class II locus, especially HLA DR01/04 implicating T cells recognizing autoreactive peptide, co-stimulatory pathways, including *CD28*, *CD40*, chemokines and cytokine receptors (e.g. *IL-6R*), post translational modification enzymes e.g. *PADI* and intracellular regulatory pathways e.g. *PTPN22*, *TNFAIP3*, *STAT3* all of which may alter the threshold for immune activation or failed regulation. It is recognized however that powerful epidemiologic effects operate upon this genetic background to promote disease. Extensive evidence supports a role for smoking (especially in *HLADRO1/04*⁺ individuals), and other pulmonary exposures including silica dust, together with additional variables e.g. vitamin D deficiency, obesity as pro-disease inducing factors [1,5]. In addition, there is evidence of a perturbed microbiome in the GI tract whereby long-term impacts on immune regulation and maintenance of host tolerance could be enacted, and also in the oral mucosa where *P. gingivalis*, or *A. actinomycetemcomitans*, have been proposed to promote disease via altered tissue citrullination [6,7]. Overall the earliest events in 'pre-RA' comprise altered innate immune reactivity and aberrant T cell / B cell cross regulation, likely in mucosal tissues, culminating in the emergence of autoantibodies that recognise a range of post translationally modified proteins that include those containing citrulline residues (anti-citrullinated autoantibodies -

ACPAs); though recent studies have also identified anti-carbamylated and anti-acetylated specificities.

How such autoreactivity transforms the normally acellular synovium into a chronically inflamed tissue is poorly understood. Articular localisation may arise from local microtrauma, microvascular insult, local complement activation, or may reflect direct activation of periarticular osteoclasts by circulating ACPAs that initiate bone damage and local IL-8 release to initiate synovitis [8,9]. The synovial lesion once established contains large numbers of infiltrating T cells, B cells, plasma cells, mast cells and macrophages, contained within a disrupted extracellular matrix and activated, stromal cells, especially synovial fibroblasts. Understanding the complex interplay of cells and soluble immune mediators particularly cytokine and chemokine families has been challenging, particularly when the dimension of disease duration is included. Animal models have been of some assistance but have not always faithfully reflect the human condition. As such it is timely to consider the invaluable opportunity offered by investigating the success and failure of specific targeted immune therapeutics in RA – in this sense they are serving as molecular scalpels to dissect mechanisms of disease.

TNF and IL-6 as critical disease effector pathways

Given their longevity in the field, the majority of studies have investigated the biology of TNFi in RA. Early studies identified rapid diminution of circulating IL-6 upon TNF blockade in RA consistent with existence of a functional cytokine hierarchy in vivo that in turn regulated the acute phase response [10]. Thereafter, a series of elegant studies using either arthroscopic or ultrasound guided synovial biopsies obtained prior to and after TNFi administration in patients demonstrated profound alterations including reduced cellular number and phenotypic composition, stromal cell activation, angiogenesis (including endothelial activation, neovascularization and adhesion molecule expression), increased lymphangiogenesis, altered cytokine and chemokine expression to favour regulatory pathways e.g. IL-10, altered matrix metalloproteinase / tissue inhibitor of MMP ratios and tissue macro-architecture [11-18]. Cellular imaging studies in vivo after TNFi similarly provided estimations for the first time of the tissue kinetics of monocytes in RA and demonstrated a pivotal role for TNF therein. Systemic studies investigating biomarkers have elucidated remarkable effects on bone turnover and metabolism, and also to some extent on lipid and glucose metabolism. These effects have now been examined at the transcriptional and metabolomics level in which rather exquisite effects are mediated, even within the TNFi class by distinct agents suggesting that more detailed mechanistic understanding may yet be possible in future studies with advanced molecular

and informatics approaches [19,20]. Nevertheless, this clinical evidence base, when combined with relevant ex vivo and in vitro studies that have liberally explored the biology of TNF in relevant tissue and cellular systems, clearly places TNF at the centre of RA pathogenesis. Moreover, they define the pleiotropic functional effects that TNF plays in this complex pathogenetic landscape.

Similar lessons are emerging from studies of IL-6Ri, primarily using tocilizumab. A majority of studies have examined the effects of IL-6Ri in circulating cell populations. Many have focused on the transcriptional changes that are mediated upon IL-6Ri. Primarily performed to seek biomarkers for response prediction, these nevertheless offer an insight into the pathways that are dependent upon IL-6 signaling in the disease and have so far correlated with interferon signal pathways in peripheral blood [21]. Synovial studies are more limited but demonstrate that IL-6R blockade leads to reduction in T cell activation markers and chemokine expression but also induced pro-repair gene sets [22]. These changes are discrete when compared with those noted upon B cell depletion and TNF inhibition suggesting some degree of specificity in synovial changes upon IL-6R blockade. That synovial IL-6 levels can also predict subsequent responses to IL-6R blockade has also been suggested suggesting that the absolute local IL-6 concentration is of pathologic importance [23]. Taken together these studies suggest that IL-6 occupies a pivotal position in regulating both T cell migration, and activation but also in the downstream inflammatory response. IL-6 also plays a pivotal role in comorbidity discussed below. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a pro-inflammatory soluble cytokine implicated by several previous studies in the pathogenesis of RA [24]. Via binding to GM-CSF receptor alpha (GM-CSFR α), GM-CSF activates neutrophils and macrophages in models of arthritis and macrophages and neutrophils in rheumatoid inflammatory tissues ex vivo; recent clinical trials have been successful providing human proof of concept for a pivotal role [25]. Functional studies in synovial tissues are awaited.

The recent advent of inhibitors of Janus kinases (JAK) has brought substantial new opportunity to investigate the activities of a broad range of cytokines in RA pathology. JAKs subserve the signal pathways of many cytokine receptors and as such allow examination of the inhibition simultaneously of a number of cytokines including IL-6, GM-CSF, type I interferons and the common γ -chain cytokines such as IL-7 and IL-15. Thus far tofacitinib, an inhibitor of JAK1/3, and very recently baricitinib, an inhibitor of JAK1/2 have received regulatory approval. Most mechanistic insights have been gained in evaluating tofacitinib bioeffects. A variety of ex vivo studies using cells purified from RA patients indicate that broad suppressive effects are mediated upon synovial T cells, B cells and fibroblast like synoviocytes by tofacitinib operating directly and via inhibition of local cytokine

dependent feedback loops [26-28]. Few studies have yet examined synovial responses. One elegant study demonstrated that after 4 weeks of tofacitinib treatment there were reductions in MMP expression and several chemokines including CCL2, CXCL10 and CXCL13 [29]. Tissue inflammation scores including T cell B cell and macrophage numbers were not altered perhaps reflecting a relatively early time point. STAT1 and STAT3 phosphorylation was also diminished consistent with the proposed mode of action of tofacitinib in synovitis. Future biopsy studies will be important to dissect the impact of varied JAK pathway selectivity across this emerging drug class.

It is worth considering the range of cytokine inhibitors that have been trialed unsuccessfully in RA. For example, IL-1 blockade achieves at best modest improvement as does IL-17A blockade, at least used as mono-biologic therapy [30]. Other studies demonstrated no benefit from IL-23 inhibition despite propitious pre-clinical data [31]. Thus the notion that RA is a 'Th17' disease, as demonstrated clearly for psoriasis and axial spondyloarthropathies is not sustained by the clinical evidence base. It is important to note however that this does not infer that such cytokines are not functionally active in RA synovium – they most likely are – but simply implies that these cytokines do not occupy a sufficiently vital role in the hierarchy of inflammation to lead to disease diminution upon their inhibition, or that they are targeted at a time point when their functional contribution is no longer pivotal [32].

T cells in RA

The biologic role of T cells in RA pathogenesis has long been debated. The strong genetic clues from MHC Class II and costimulatory pathway associations, critical role in animal models, and plausible place in driving host autoreactivity, including the identification of oligo clonal T cells in synovial biopsies are all suggestive of a beneficial role for T cell modulators [2]. However clinical trials in which T cells were depleted or functionally modified using ciclosporin, anti-CD4, anti-CD5 or Campath-1H did not yield robust clinical responses. In contrast the advent of abatacept as a costimulator inhibitor targeting the CD28 – CD80/86 pathway finally delivered a T cell targeted therapeutic of value. Robust clinical responses analogous to those achieved with TNFi are observed. Abatacept is a potent modulator of T cell activation in ex vivo studies though importantly it does not appear to enhance regulatory T cell responses. Abatacept also favorably reduces interactions between T cells, dendritic cells and macrophages to reduce downstream cytokine release [33, 34] and may inhibit osteoclastogenesis [33,34]. Murine models show that at least some of the beneficial effects of abatacept are mediated via reduction of reverse licensing of dendritic cells by T cells though such data are currently being investigated in human trials [35]. Synovial biopsy studies have

shown that abatacept leads to reduced cellular infiltration though this was most marked for B cells indicative of active cross talk between synovial T cells and B cells. Generalised reduction in inflammatory cytokine expression was also noted in one study; the consensus is that such changes may operate downstream from T cell costimulatory blockade [36,37]. These studies have refreshed interest in targeting T cell dependent effector and regulatory pathways for the future development of therapeutics.

B cells in pathogenesis of RA

The role of B cells in RA is highlighted by the autoantibody response comprising high affinity IgG antibodies against citrullinated, carbamylated and acetylated proteins. Generation of such antibodies requires T cell help and affinity maturation of B cells in lymphoid follicles leading to the formation of plasmablasts and autoantibody production. Apart from autoantibodies, biopsy studies have shown that B cells and plasma cells are abundantly present in the synovial membrane of RA patients and sometimes form aggregates or even tertiary lymphoid follicles suggesting local production of autoantibodies in the joints. Despite this robust evidence for the involvement of B cell mediated immune processes in RA, the profound anti-inflammatory potential of the B cell depleting treatment in RA was only partially expected, and reemphasised the role of adaptive immunity in RA at a time when T cell depletion had effectively failed [38, 39]

Treatment with rituximab, an antibody binding to the B cell specific surface molecule CD20 leads to sustained virtually complete depletion of peripheral B cells, while B cells at sites of inflammation such as the synovium are only partially affected [40]. Plasma cells, especially long-lived plasma cells, which lack CD20 expression, are not directly affected by rituximab treatment. Most clinical evidence suggests that rituximab in contrast to cytokine blocking agents works best in autoantibody-positive RA suggesting that rituximab indeed targets the adaptive immune dysfunction in RA [41]. Rituximab lowers autoantibody titres but this does not explain anti-inflammatory activity, which develops before the decrease in autoantibody titres is observed. Such effects are not fully understood but likely arise from rapid depletion of circulating B cells and a proportion of tissue B cells and plasmablasts. B cells are cytokine-producing cells and also have antigen-presenting properties, which are likely blunted in a fast and sustained manner by rituximab. On the other hand, the slow decrease in autoantibody titres in RA patients upon rituximab treatment suggests that these antibodies do not necessarily stem from long-lived plasma cells, but rather result from continuous B cells activation and plasmablast differentiation [42,43]. Extensive synovial biopsy studies have demonstrated depletion of tissue CD20+ B cells and plasmablasts, variable effects on

immunoglobulin synthesis, subsequent reductions in CD68+ macrophages and T cells, limited effects on lymphocytic aggregates and enhanced repair gene profiles [44-51]. Sustained low disease activity is associated with reduced B cell repopulation, that in turn has been associated with CXCL13 and CCL19 expression [52,53]. Intriguing pre-therapeutic gene signatures have been defined that predict rituximab responsiveness suggesting that there is a B cell defined effector pathway with hierarchical pre-eminence [54].

Based on these encouraging pathologic insights, other CD20-targeted antibodies (e.g. obinutuzumab, ibertumomab, ocaratuzumab) that could offer more potent depletion properties were considered but have either not started or advanced to larger scale trials in RA with the exception of the anti-CD20 antibody ocrelizumab which showed clinical efficacy in RA but was stopped due to increased infectious risk [55]. In contrast to complete B cell depletion a more subtle approach concerns inhibition of B cell modulatory cytokines. Atacicept, a fusion protein targeting two molecules required for B cell maturation and survival, BAFF und APRIL was studied in RA but failed to show significant anti-inflammatory activity despite lowering rheumatoid factor and circulating B cells levels [56]. Other interesting to target B cells in RA include development and focus on essential signalling molecules such as Brutons tyrosine kinase (Btk) and spleen tyrosine kinase (Syk). Syk is a critical signalling component downstream of the B cell- and Fc receptor and therefore important for a variety of cells involved in the pathogenesis of RA such as B cells, macrophages and osteoclasts. One Syk inhibitor (fostamatinib) advanced to phase 3 trials in RA, however efficacy was discouraging [57]. Btk is a kinase downstream of the B cell receptor and essential for B cell development and function and hence an interesting target in autoimmune diseases. Genetic absence of Btk in humans is associated with impaired B cell development and agammaglobulinemia. Small molecule inhibitors of Btk are available and currently used for the treatment of mantle cell lymphoma, while others are in early development for RA [58]. The variable responses thus far detected to kinase inhibitors however reflects our relatively limited understanding of the true cellular hierarchy of these pathways at the synovial pathogenetic level.

Taken together with the abatacept datasets alluded before, current evidence defines adaptive immune pathways as a non-redundant contributor in the RA inflammatory cascade. However, it remains possible that most clinically relevant effector pathways ultimately operate via 'downstream' TNF and IL-6 [59].

Targeting resident cells in the joint

RA is characterized by a profound resident tissue response associated with the formation of a hyperplastic synovial membrane, which acts as a cytokine producing tissue, facilitates the development of structural damage and most likely mediates the chronicity of the disease with high relapse rates when stopping treatment [60]. This process is based on a sustained activation of synovial fibroblasts, which proliferate and develop resistance to apoptosis. Epigenetic modifications of these cells by the inflammatory environment may allow sustained activation of the cells allowing the chronicity of disease and its substantial risk for recurrence when treatment is stopped [61]. Targeting of synovial fibroblasts in RA is in its infancy but may open new possibilities for long-term remission of disease and so far unprecedented possibilities to modify the diseases by rebooting the inflammatory microenvironment in the joint. Several approaches can be envisioned: (i) Formation of the hyperplastic synovial lining layer in RA requires homotypic cell assembly requiring the cadherin-11 adhesion molecule [62]. Blockade of cadherin-11 by neutralizing antibodies may effectively block or disrupt such hyperplastic synovial lining layer. Other approaches that can be envisioned is targeting molecules which are responsible for the proliferation of synovial fibroblasts such as synoviolin or Tyro-3 [63, 64]. Finally, targeting the epigenetic modifications of synovial fibroblasts in RA such as histone acetylation or bromodomain and extra-terminal (BET) proteins are interesting approaches, which may reverse the proinflammatory microenvironment, which continuously attracts immune cells to the joint [65-67]. Such approaches targeting resident cells in the joint are not necessarily anti-inflammatory requiring the modification of the design of respective trials in RA. Such data will also definitively place the stromal cell within the functional immune hierarchy of RA pathogenesis.

A potential future role for cellular therapy to treat inflammation in RA?

Modification of the inflammatory process by immune modulatory cells or cells inducing tolerance have always been an attractive vision to treat autoimmune diseases but have so far not progressed to clinical application in RA. This lag in the development of cellular therapies inducing tolerance has several reasons, which are based on challenges related to (i) absence of a single autoantigen triggering immunity in RA, (ii) challenges in feasibility and standardization of cell-based therapy approaches targeting joint disease and (iii) the success of biologic and small-molecule drug approaches to treat RA. Nonetheless cell-based therapeutic approaches could represent an attractive additional possibility to achieve long-term tolerance or suppression of inflammation in RA. Potential future approaches comprise the transfer of tolerogenic dendritic cells, immune regulatory mesenchymal stem cells or regulatory T cells. The principal feasibility of transfer of in vitro generated tolerogenic dendritic cells exposed to autologous antigens locally into the joints of RA

patients has been shown recently [68,69]. Furthermore, a small trial showed that administration of allogeneic adipose-derived mesenchymal stem cells has an effect on treatment-resistant RA [70].

Mechanisms of structural damage

RA leads to progressive damage as a result of continuous direct exposure of bone and cartilage to the inflammatory microenvironment. Bone loss starts very early in the disease course of RA [71]. It is driven by the induction of bone-resorbing osteoclasts by autoantibodies, leading to the first structural changes in the pre-disease phase and aggravated by the action of proinflammatory cytokines [72,73]. Presence of autoantibodies and duration of arthritis, resembling the exposure time of bone to inflammatory cytokines, therefore resemble the key factors determining bone and cartilage damage in RA. Therapeutic strategies to timely and effectively block the inflammatory synovial process have therefore proven effective to successfully retard structural bone and cartilage damage in RA [2]. Osteoclasts as the main bone resorbing cells are highly responsive to antibodies and inflammatory cytokines in particular TNF, IL-1 and IL-6/IL-6R complexes, which all induce osteoclast differentiation either directly or by inducing the master differentiation factors of osteoclasts, RANKL [74]. Therefore, treatment with cytokine inhibitors is particularly effective in preventing structural damage even in the absence of full control of inflammation. In support of this concept, the blockade of the downstream effector cytokine RANKL with denosumab inhibited the progression of bone erosions in patients with RA [75]. Direct down regulation of osteoclast function is also achieved by other modes of treatment such as the co-stimulation inhibitor abatacept, which binds osteoclasts via CD80 and CD86 and inhibits their differentiation [76]. Furthermore, JAKi may indirectly affect osteoclast function through blocking the action of IL-6/IL-6R signalling in osteoclasts and hence preserve bone form resorptive damage. Together these clinical trials have unequivocally implicated cytokine networks in pathogenetic bone damage in RA.

Mechanisms of major comorbidities

RA is associated with up to 2-fold increased risk of myocardial infarction and stroke independent from classical risk factors for atherogenesis [77]. The intrinsic elevation of cardiovascular risk in RA may be considered to result from systemic immune activation and inflammation. Chronically elevated serum levels of acute phase reactants such as C-reactive protein as well as proinflammatory cytokines, such as TNF and IL-6, are associated with enhanced atherogenesis and the development of cardiovascular events *per se* [78]. The duration and severity of inflammatory disease activity have been identified as factors leading to increased cardiovascular risk in RA. Furthermore, other factors such disease-associated functional disability and glucocorticoid intake

further increase the development of cardiovascular risk [79]. Several clinical studies have elucidated underlying mechanisms in the clinical context. Distinct changes in the lipid profile have been described in RA and other forms of chronic inflammation such as sepsis [80]; RA is associated with decreased levels in total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Furthermore, lipoprotein particles such as HDL change their composition with higher contents of acute phase proteins such as serum amyloid A (SAA) in RA patients. Particularly pronounced effects on the altered lipid metabolism in RA are found with the IL-6R blockade either by tocilizumab or downstream signaling blockade by JAK inhibition. Hence, decreased total cholesterol, high-density (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels and the disease-related changes of lipid particle composition revert upon IL-6R pathway inhibition [81]. Most importantly control of inflammation in RA has shown to affect cardiovascular risk: Methotrexate moderately lowers cardiovascular risk in RA [82]. Larger epidemiologic registers have also revealed a protective effect of TNF inhibitors on cardiovascular events in RA [83,84]. The MEASURE study definitively linked IL-6R signalling to these lipid particle assembly changes in RA [85]. Aside from cardiovascular disease RA is also complicated by central nervous system changes which are reflected in enhanced depression and central sensitization to pain. These effects are considered to result from the action of proinflammatory cytokines, particularly TNF and IL-6 on the central nervous system. In accordance, TNF inhibition rapidly reduces pain sensitization in the brain and also normalizes the dysbalance of the serotonin homeostasis in the brain of RA patients [86-88]. Similar effects are also emerging for IL-6R inhibition.

Prevention of disease and tolerance induction based on the foregoing?

Current treatment of RA aims for rapid and sustained suppression of inflammation once the inflammatory phase of the disease has started and patients present with joint swelling. Achieving immunological reset in RA patients is an ambitious future goal on the way to cure the disease, though such approaches are in their infancy. The observation that autoimmunity forms years before the inflammatory phase of the disease (often referred to as “pre-RA”), however, opens a therapeutic window for disease prevention [89-91]. Several approaches can be envisioned to induce immunological “reset” and gain immune tolerance: (i) Targeting T-/B-cell responses required for autoantibody production by drugs such as rituximab and abatacept could be used in the pre-RA phase with the aim to achieve seroconversion and prevention of disease onset. In this context, the Prevention of RA by Rituximab (PRAIRI) study which aims to retard or even prevent the onset of RA by a single-shot rituximab treatment is the most advanced study [92]. In addition, at least two trials with abatacept are underway addressing this concept. (ii) In addition, new concepts to modify the

pathogenic potential of antibodies with respect to their proinflammatory cytokine-inducing properties are being developed. (iii) Reduction of antigen expression and prevention of the formation of pathogenic immune complexes in RA is another promising approach to induce tolerance [93]. Cessation of smoking, which is the main environmental factor inducing the citrullination of proteins, is most likely the easiest and most cost-effective approach in this respect but other targeted approaches such as small-molecule inhibitors of peptidyl deiminases, the enzymes responsible for protein citrullination, are in development [94]. (iv) Induction of antigen-specific tolerance by cell-based treatment approach such as the tolerogenic dendritic cells loaded with autoantigens as described above or by the use of tolerogenic nanoparticles loaded with peptides involved in the autoimmune response of RA [95,96]. All such approaches do not primarily aim to target inflammation but rather the underlying immune dysregulation in RA. Hence, such approaches may be particularly useful in the preclinical phases of RA to prevent disease onset or in stable-remission phases of disease to prevent relapses after cessation of anti-inflammatory treatment.

In summary the revolution in immune and signal pathway targeted therapeutics have provided definitive immunologic check points or vulnerable nodes that place adaptive and downstream cytokine effector pathways at the centre of disease pathogenesis (Figure 1). The future challenge is to build on such observations to achieve the ultimate ambition namely achievement of immunologic homeostasis and drug free remission.

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