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Dendritic cells, T cells and their interaction in rheumatoid arthritis

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Summary

Dendritic cells (DCs) are the key professional antigen-presenting cells which bridge innate and adaptive immune responses, inducing the priming and differentiation of naive to effector CD4$^+$ T cells, the cross-priming of CD8$^+$ T cells and the promotion of B cell antibody responses. DCs also play a critical role in the maintenance of immune homeostasis and tolerance. DC–T cell interactions underpin the generation of an autoimmune response in rheumatoid arthritis (RA). Here we describe the function of DCs and review evidence for DC and T cell involvement in RA pathogenesis, in particular through the presentation of self-peptide by DCs that triggers differentiation and activation of autoreactive T cells. Finally, we discuss the emerging field of targeting the DC–T cell interaction for antigen-specific immunotherapy of RA.

Keywords: antigen presentation, autoantigen-specific CD4$^+$ T cells, autoimmunity, dendritic cells, immunotherapy, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) has a strong human leucocyte antigen (HLA) class II association: patients carrying HLA-DR alleles that encode a 'shared epitope' (SE) five-amino acid sequence motif (including HLA-DRB1*04:01, *04:04, *04:05 and *01:01) exhibit a high risk of seropositive RA. In contrast, other HLA-DR alleles, including HLA-DRB1*03:01, are associated with seronegative RA and a milder disease course. The molecular mechanisms underpinning the association between particular HLA alleles and autoantibody positivity are increasingly appreciated, including the presentation of HLA class II-restricted autoantigens to autoreactive CD4$^+$ T cells. Dendritic cells (DCs) are the key professional antigen-presenting cells (APCs) for T cell priming. They also play a major role in immune tolerance. DCs discriminate between self- and non-self-antigens on the basis of associated innate immune activating or suppressive signals, after which DC antigen uptake and presentation promotes T cell activation or regulation. DCs and T cells collaborate in the pathogenesis of RA, particularly through the presentation of antigen that triggers the differentiation of autoreactive T cells, as well as innate immune effector functions.

Based on human and rodent model evidence, we propose a working model of RA, where autoantigen-specific CD4$^+$ T cells, including follicular helper T cells (Tfh), are primed by major histocompatibility complex (MHC) class II$^+$ DCs exposed to environmental inflammatory factors that enhance their maturation, as well as generation and presentation of neoepitopes. Autoreactive and potentially cross-reactive Tfh propagate autoimmune arthritis through activation of B cells with genetic tolerance defects, followed by germinal centre formation and affinity maturation and glycosylation of autoantibodies, which contribute to the effector phase of the disease through innate mechanisms. Although partially regulated, the autoimmune response persists due to ongoing stimulation of autoreactive T cell clones by a variety of synovial MHC class II$^+$ APCs and draining lymph node (dLN) DCs (Fig. 1). This paper reviews the evidence for the contribution of DCs and T cells to this model.

DC antigen presentation

DCs are specialized APCs which link the innate and adaptive immune responses, activating and priming effector CD4$^+$ T cells, cross-presenting antigen to CD8$^+$ T cells

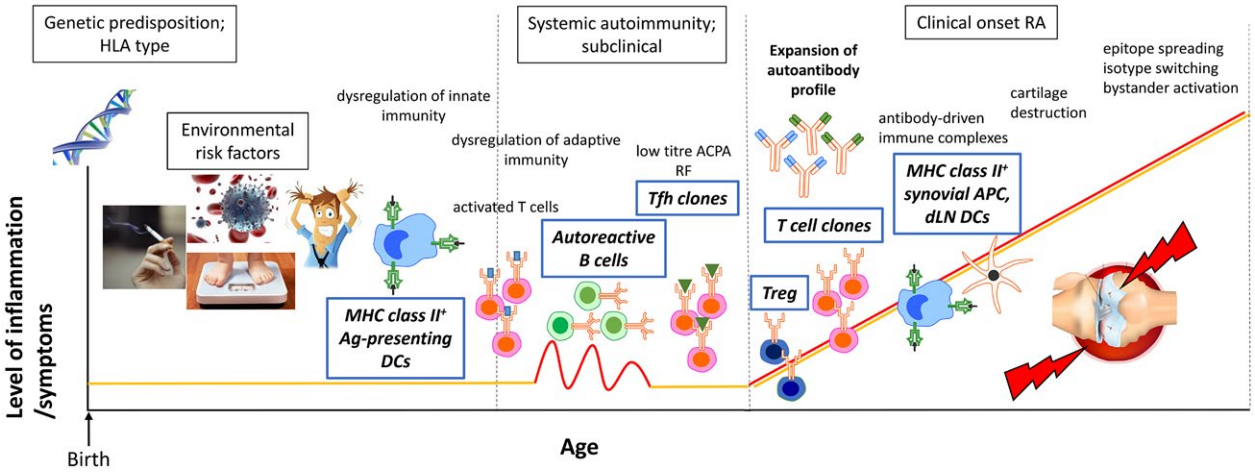

Fig. 1. A working model for the development of rheumatoid arthritis (RA). Both genetic and multiple non-genetic risk factors predispose to the development of RA. In this context, disease initiation probably involves dysregulation of innate and adaptive immunity. Mature major histocompatibility complex (MHC) class II$^+$ dendritic cells (DCs) probably induced by the environmal inflammatory milieu, prime autoantigen-specific CD4$^+$ T cells, including follicular helper T cells (Tfh) cells. Subsequent germinal centre formation, affinity maturation of B cells and expansion of the autoantibody profile may lead to antibody-driven immune complex formation and subsequent cartilage destruction coinciding with overt RA expression. Although partially regulated, the autoimmune response persists due to ongoing stimulation of autoreactive T cell clones by a variety of synovial MHC class II$^+$ antigen-presenting cells (APC) and draining lymph node (dLN) DCs. Downstream processes, including neoepitope formation, isotype switching and bystander activation, may further propagate autoimmunity.

and promoting B cell antibody production [1]. DCs also play important roles in the maintenance of immune tolerance. In RA, DCs are thought to drive the activation of self-peptide-reactive inflammatory T cells, Tfh and consequently B cells for stimulating autoantibodies [2–4].

DC subsets for antigen presentation

DCs are heterogeneous and can be divided into two major functionally distinct subsets: conventional myeloid DCs (cDCs) and plasmacytoid DCs (pDCs) [5]. Human cDCs express typical myeloid markers and may be subdivided into CD1c$^+$ and CD141$^+$ fractions, while pDCs typically lack expression of these cell surface markers. Functionally, CD1c$^+$ DCs prime CD4$^+$ T cells, while CD141$^+$ DCs cross-present antigen to prime CD8$^+$ and CD4$^+$ T cells, thus stimulating CD4$^+$ T cell help for cytotoxic T lymphocyte (CTL) generation and B cell activation [6]. In contrast to cDCs, pDCs have lower levels of MHC class II expression and reduced APC capacity; however, they are specialized to secrete type I interferons (IFNs), and are therefore important players in viral immunity [1,7]. A further monocyte-derived inflammatory DC population identified in ascites and synovial fluid co-expresses CD1c and CD14 [8,9].

DC antigen presentation in immune homeostasis

DCs constitutively phagocytose self-antigen derived from apoptotic cells during immune homeostasis in the absence of any overt inflammatory stimuli, and migrate from the periphery to draining lymph nodes via tissue lymphatics [10,11]. These apoptotic bodies actively promote regulation by stimulating transforming growth factor (TGF)-β production, suppressing DC maturation, enhancing self-antigen-specific CD4$^+$ peripheral regulatory T cell (T_{reg}) induction and CD8$^+$ T cell deletion. Immune tolerance is maintained through active suppression of inflammatory mediator production and DC activation [12–16].

DC antigen presentation in response to inflammatory signals

DC recognition of inflammatory stimuli – including pathogen-associated molecular patterns (PAMPs), endogenous cytokines or damage-associated molecular pattern molecules (DAMPs) – initiates a signalling cascade leading to activation of the nuclear factor kappa B (NF-κB) transcription factor pathway (Fig. 2). The NF-κB family consists of five binding proteins: p50, p52, RelA (p65), cRel and RelB, which can form homo- and heterodimers [17]. NF-κB activation comprises classical and alternative pathways, which are stimulated by different signals. The classical pathway (RelA-p50, cRel-p50) transcriptionally activates survival and inflammatory genes, and the alternative pathway plays additional roles in immune tolerance and activation of DCs and B cells for adaptive immunity [18,19]. RelB heterodimerizes with p50 for classical pathway or p52 for alternate pathway activation, while RelB-RelA heterodimers are suppressive.

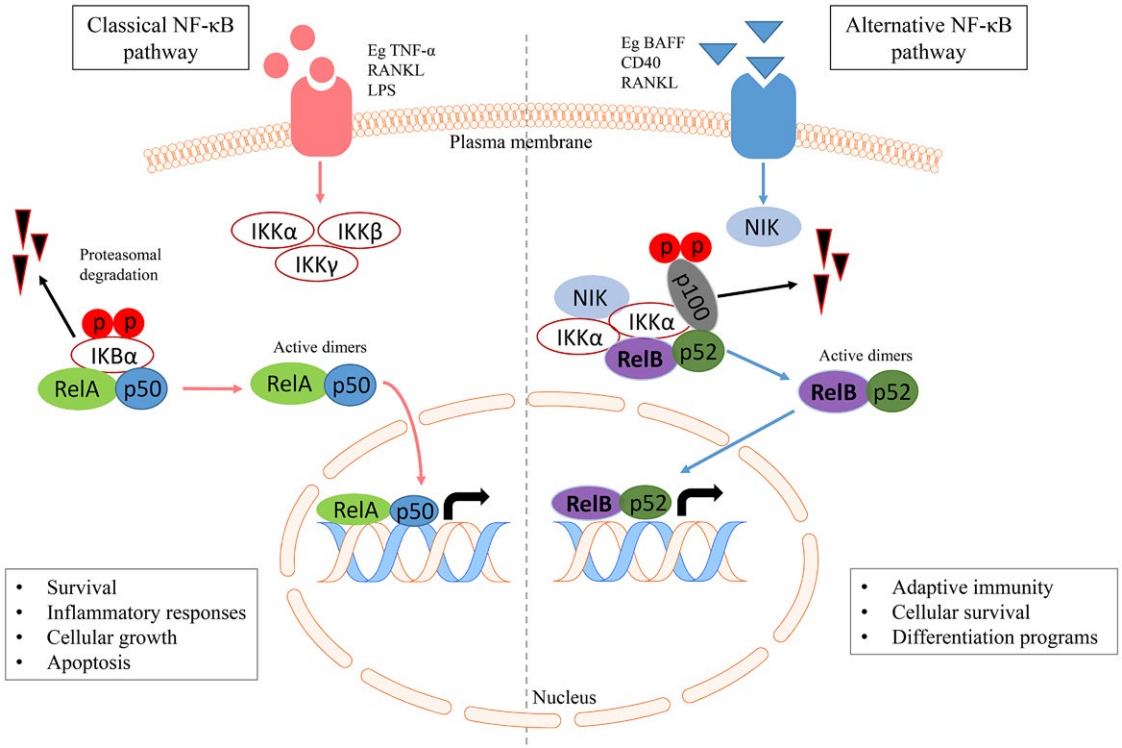

Fig. 2. The classical and alternative nuclear factor kappa B (NF-κB) pathways. The classical NF-κB pathway (left): activation is induced by many extracellular signals including cytokines such as tumour necrosis factor (TNF). Activation leads to phosphorylation of inhibitory I-κBα (I-κB kinase) by the IKK complex, resulting in its degradation. The RelA/p50 complex is freed from the inhibitory interaction with IκBα and is an active dimer, which translocates to the nucleus to activate the transcription of target genes. c-Rel and RelB also heterodimerize with p50. The alternative NF-κB pathway (right): activation of this pathway occurs after stimulation by a more restricted set of ligands, including CD40, B cell activating factor (BAFF) and receptor activator of NF-κB (RANK). Subsequently, NF-κB-inducing kinase (NIK) activation phosphorylates IKKα. pIKKα is crucial for phosphorylation of p100, leading to proteasome-dependent processing of p100 to p52, ultimately liberating active RelB-p52 dimers, which transactivate NF-κB responsive genes. This figure was created using the Motfolio PPT Drawing Toolkits (www.motfolio.com), and adapted from [147–149].

NF-κB proteins are retained in an inactive form in the cytosol, bound to an I-κB kinase (IKK) inhibitor protein. Activation of NF-κB-inducing kinase (NIK) and other kinases leads to phosphorylation of I-κB and subsequent nuclear translocation of RelB/p50 and RelB/p52 transcription factor dimers [17,20]. RelB has a unique and critical role in DC maturation, as RelB-deficient mice have normal numbers of undifferentiated immature DC precursors but are deficient in mature DCs [21,22]. After NF-κB pathway activation, mature DCs exhibit enhanced capacity for antigen presentation for priming of naive $CD4^+$ and/or $CD8^+$ T cells by up-regulating surface MHC class II expression as well as adhesion and co-stimulatory molecules, including CD40, CD80 and CD86, and secreting cytokines to support T cell differentiation [23–25]. RelB transcriptionally regulates CD40 and after CD40 ligation, RelB translocates to the nucleus of DCs for prolonged periods [26].

DC autoantigen presentation in RA

In RA patients, DCs are recruited in high concentrations to joint synovial fluid and tissues [27–29]. These synovial DCs are generally mature and NF-κB is over-expressed. Nuclear RelB expression correlates with disease activity, indicating that DCs are responsive to local inflammatory PAMPs, DAMPs and/or cytokines, including tumour necrosis factor (TNF) [19]. Multiple subsets of MHC class II^+ APCs are present in RA synovial tissue, including Toll-like receptor (TLR)-activated myeloid-derived cells, $CD11c^+CD20^+$ activated B cells and MHC class II^{hi} fibroblast-like synoviocytes [30]. Thus, after initiation of inflammatory arthritis, multiple APC types could perpetuate the presentation of synovial fibroblast or cartilage autoantigens such as vimentin, aggrecan, type II collagen (CII) and HCgp130 to synovial migratory memory T cells previously primed by DCs in lymph nodes draining the site of initial antigen presentation [4].

There are a number of sources for potentially immunogenic autoantigens presented by DCs in the context of RA-risk HLA-DR allomorphs, inducing the expansion of self-peptide-specific T cells prior to RA expression. These include infectious and self-protein antigens that are post-translationally modified by citrullination and

carbamylation. The latter processes, enhanced under conditions of stress and damage, expand the production of neoepitopes. The peripheral adaptive immune system may be particularly reactive to neoepitopes if they were not presented by the thymus to negatively select autoreactive T cells during development. Furthermore, histones are citrullinated during the process of neutrophil extracellular trap (NET) formation. In this process, decondensed DNA is expelled from dying neutrophils after their recruitment to sites of infection, resulting in containment of bacteria by a web of extruded material. This process releases extracellular citrullinated autoantigens and possibly also infectious antigens in synovial fluid [31,32]. Citrullination of self-epitopes derived from vimentin, fibrinogen, aggrecan, enolase, cartilage intermediate layer protein (CILP) and type II collagen (CII) enhances peptide binding to RA-associated HLA-DR alleles containing the shared epitope (SE) motif and promotes subsequent autoreactive T cell expansion. Thus, neoepitopes contribute to the molecular basis conferred by SE-positive (SE^+) HLA-DR alleles to RA susceptibility [33,34]. Furthermore, cross-presenting DCs taking up citrullinated necrotic antigen under conditions of stress or infection may be co-stimulated by citrullinated antigen-specific $CD4^+$ T cells that could provide help to citrullinated peptide-specific cytotoxic T lymphocytes (CTL) and anti-citrullinated protein antibodies ($ACPA^+$) B cell germinal centre (GC) differentiation.

DCs as master orchestrators of immune responses

$CD1c^+$ DCs, pDCs and moDCs are all consistently enriched in rheumatoid synovial fluid and tissue [28,35]. RA synovial DCs secrete chemokines that attract proinflammatory immune cells including macrophages, neutrophils and monocytes [4,36] (Fig. 3). DC secretion of chemokines chemokine (C-C motif) ligand 3 (CCL3), CCL17, chemokine (C-X-C motif) ligand 19 (CXCL19) and CXCL10 also recruits T cells to the RA synovium [37]. Enhanced expression of MHC class II and co-stimulatory molecules (CD40, CD80, CD86) by mature $CD1c^+$ DCs in RA patients is sufficient to trigger T cell activation *in vitro* [37]. In comparison with DCs from healthy controls, RA $CD1c^+$ DCs secrete increased amounts of proinflammatory cytokines interleukin (IL)-1β, IL-6, IL-23 and IL-12 [8,27,37,38]. These cytokines are known to induce $CD4^+$ T cell differentiation into activated T helper type 1 (Th1) (IL-12, IL-1) and Th17 (IL-1β, IL-6 and IL-23) cells, which are crucial players in RA pathogenesis. Thus, cDCs are partially responsible for the elevated inflammatory Th1 and Th17 cells in RA synovial fluid and tissue [39,40].

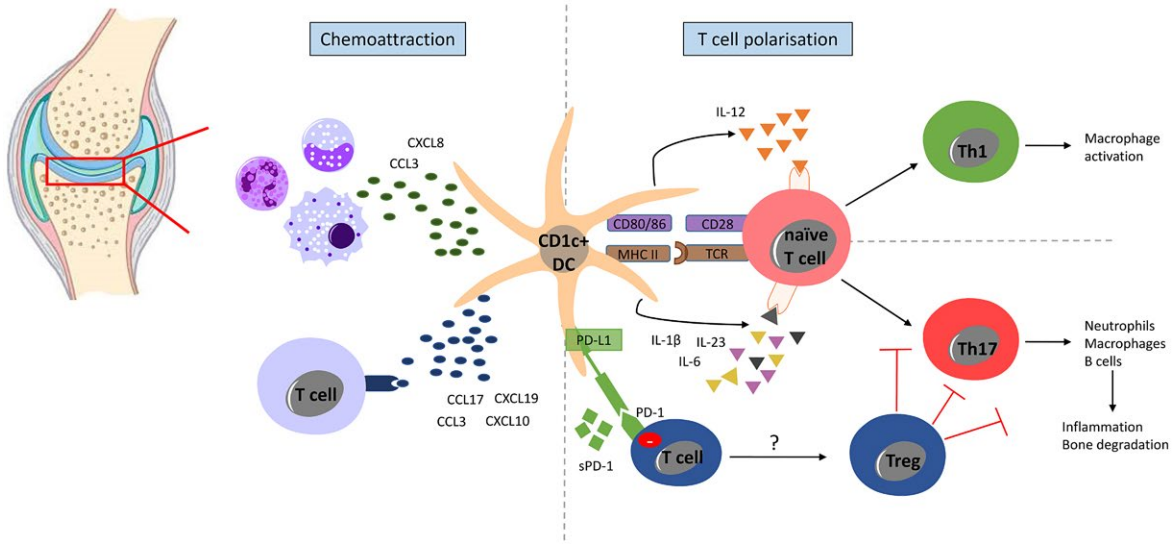

Fig. 3. Synovial $CD1c^+$ dendritic cell (DC)-mediated chemoattraction, T cell activation and polarization. Intra-articular DCs secrete multiple chemokines, including chemokine (C-C motif) ligand 3 (CCL3), CCL17, C-X-C motif chemokine ligand19 (CXCL19) and CXCL10, to induce T cell migration to the rheumatoid arthritis (RA) synovium. RA synovial $CD1c^+$ DCs also secrete chemokines (including CCL3 and CXCL8) that attract proinflammatory immune cells, including macrophages, neutrophils and monocytes. RA synovial $CD1c^+$ DCs have enhanced expression of human leucocyte antigen D-related (HLA-DR) and co-stimulatory molecules CD40, CD80 and CD86 required for T cell activation. Synovial $CD1c^+$ DCs from RA patients produce cytokines required for T cell polarization towards T helper type 17 (Th17) [interleukin (IL)-1β, IL-6, and IL-23] and Th1 (IL-12) differentiation. Synovial fluid from seropositive RA patients show high levels of serum soluble (s) programmed cell death 1 (PD-1), which may interfere with the PD-1/programmed death ligand 1 (PD-L1) inhibitory signalling loop. This figure was generated using the Motfolio PPT Drawing Toolkits (www.motfolio.com), and knee image was obtained from https://www.pearsonschoolsandfecolleges.co.uk/FEAndVocational/SportsStudies/ALevel/OCRALevelPE2008/Samples/SamplepagesfromOCRASPEStudentBook/chapter1_sample.pdf, page 7, figure 1.4.

DC-mediated activation and differentiation of these inflammatory T cells potentiate further synovial immune responses; for example, Th17 cells mediate neutrophil recruitment, B cell activation and osteoclastogenesis leading to bone resorption and cartilage degradation [41] and Th1 cells promote macrophage activation [42]. In addition, RA synovial DCs are capable of inducing T_{reg}, at least *in vitro* [43], potentially through prolonged engagement of regulatory T cell receptor programmed cell death 1 (PD-1) [43,44]. Briefly, PD-1 is expressed by activated T and B cells [45]. After engagement with its ligands programmed death ligand 1 (PD-L1) and PD-L2, which are widely expressed in non-lymphoid tissue, PD-1 signals a negative feedback loop that attenuates the T cell response and mediates peripheral tolerance [44,46]. Of interest, transcripts of the co-inhibitory PD-L1 ligand are highly expressed in RA synovium, but expression of PD-L1 protein appears to be blocked by serum soluble (s)PD-L1, which is present at high levels in seropositive RA, thereby down-regulating the PD-1/PD-L1 inhibitory signalling axis [47].

T_{reg} in RA

T_{reg} play an indispensable role in regulating self-peptide specific T cells. There are two classes of T_{reg} identified in humans: constitutively generated thymic T_{reg} (tT_{reg}) and peripherally induced T_{reg} (pT_{reg}). The transcription factor forkhead box protein 3 (Foxp3) is a critical regulator for $CD4^+$ T_{reg} development and function [48,49]. tT_{reg} can be further delineated in humans as resting T_{reg} ($Foxp3^{lo}CD45RA^+CD25^{hi}$ rT_{reg}) and activated T_{reg} ($Foxp3^{hi}CD45RA^-CD25^{hi}$ aT_{reg}) [50]. pT_{reg} may be $Foxp3^+$ antigen-specific cells induced in response to antigen presented by tolerogenic DCs, or $Foxp3^-$ $CD4^+$ T cells that acquire regulatory function in the periphery when exposed to inflammation. These pT_{reg} secrete IFN-γ and IL-10 (Tr1 cells) or TGF-β [51]. Induction of pT_{reg} represents an important mechanism for antigen-specific control of autoreactive T cells escaping selection and entering the periphery in the face of deficient central tolerance or age-related expansion of autoreactive effector-memory T cell clones [52].

T_{reg} may regulate in different ways, including deletion and anergy of effector T cells, and generation of new pT_{reg} ('infectious' tolerance). For example, murine self-reactive T cells acquired a folate receptor 4 $(FR4)^{hi}CD73^{hi}$ anergic phenotype in the presence of $Foxp3^+$ T_{reg} [53]. In RA patients, $CD4^+CD25^+Foxp3^+$ T_{reg} with high plasticity for IL-17 production accumulated in inflamed synovium [54,55]. Moreover, a subset of $Foxp3^+CD39^+$ T_{reg} in RA synovial tissue (ST) suppressed the production of IFN-γ, TNF and IL-17F but not IL-17A [56]. While peripheral blood (PB) T_{reg} in RA patients were shown to retain suppressive capacity, this capacity was lost in synovial fluid T_{reg} [57]. Furthermore, $Foxp3^+$ T_{reg} activated by an inflammatory cytokine milieu produced IL-17 [58]. Conversely, TNF inhibition induced a distinct population of T_{reg}, which enhanced regulatory function in RA via TGF-β [59,60]. Together these results suggest that the inflammatory cytokine milieu contributes to T_{reg} dysfunction, preventing efficient control of autoreactive T cells recognizing self-antigens.

T cell help for autoantibody production

In the inflamed RA synovium, follicular structures resembling germinal centres (termed ectopic lymphoid-like structures; ELS) show evidence of sustained activation-induced cytidine deaminase (AID) expression [61,62] – an enzyme critical for regulating somatic hypermutation and class-switch recombination of the immunoglobulin (Ig) genes [62]. The detection of class-switched ACPA, produced by mature B cells resident in ELS in RA synovium, implies cognate antigen-specific $CD4^+$ T cell help [61]. However, antigen-specific $CD4^+$ T helper cells in ELS have not yet been demonstrated. Moreover, RA ELS showing a synovial lymphoid "pathotype", characterized by high levels of T and B cell gene expression, were significantly associated with ACPA seropositivity and high disease activity [63,64].

As seropositive RA is characterized by prominent autoantibody production and T cell–B cell aggregates in the RA synovium, Rao *et al.* [65] hypothesized that synovial $PD\text{-}1^+$C-X-C motif chemokine receptor $(CXCR5)^-$ $CD4^+$ T cells observed at increased frequency in seropositive RA patients may be Tfh cells [65]. Typically, Tfh cells are identified by the co-expression of CXCR5, PD-1 and inducible T cell co-stimulator (ICOS), and may contribute to autoimmune diseases by facilitating the aberrant generation of autoantibodies and aiding the formation or maintenance of pathogenic ELS [66,67]. After Tfh activation, up-regulation of CXCR5 in concert with CCR7 down-regulation are crucial chemokine responsiveness changes that guide activated T cells toward the T cell–B cell border in the GC and to enter the B cell follicle [68]. PD-1, although inhibitory, controls GC positioning and helper functions of Tfh cells [69]. $PD\text{-}1^+CXCR5^-$ RA synovial cells, termed 'peripheral helper' T (Tph) cells, were non-exhausted, activated cells specialized to promote B cell help, plasma cell differentiation and to enhance antibody production. Tph were transcriptomically distinct from Tfh cells, in that they displayed a unique chemokine receptor expression profile, including CCR2, that direct migration to inflamed peripheral sites and thus endow Tph with a unique capacity to interact with B cells within non-lymphoid structures of the inflamed) synovium.

In RA sera and synovial fluid, ACPA of the IgG isotype have been shown to harbour N-linked glycans in the variable antigen-binding domains in both heavy and light chains, and to a much greater extent than other IgG molecules [70]. Interestingly, Fab glycosylation of

ACPA-IgG was also observed in patient samples collected at the time of RA diagnosis (disease duration < 1 year). However, none of the ACPA-IgG N-glycosylation sites were germline-encoded, thus suggesting that Fab glycosylation was introduced after extensive somatic hypermutation [70]. Furthermore, in contrast to ACPA-IgG, ACPA-IgM had no additional glycosylation in the variable region [71]. These results strongly suggest that glycosylation of the Fab region of ACPA-IgG results from somatic mutation during ACPA maturation, could be T cell-dependent, and may already develop before onset of RA [72].

T cell receptor (TCR) diversity for recognition of antigen

$CD4^+$ and $CD8^+$ surface TCRs recognize antigen processed by APCs and presented by MHC class II and class I molecules, respectively. The TCR is a heterodimer comprising an α and β chain, both of which are formed by somatic rearrangements of variable (V), diversity (D; β chain only) and junctional (J) gene segments [73,74] (Fig. 4a). Recombined gene segments are spliced together with the constant region (C) to form a functional TCRαβ complex of a single specificity [74].

These somatic gene segment rearrangements result in combinatorial diversity [73,74]. Further random insertion/deletion of nucleotides at the V, (D), J junction during recombination creates junctional diversity of the hypervariable complementarity-determining region 3 (CDR3) [74,75] (Fig. 4b). As a result, the CDR3 region of the TCR is unique and can serve as a molecular fingerprint of each T cell clone. The current dogma proposes that the CDR3 loops make the greatest contact with antigenic peptides presented by HLA [76–78] (Fig. 4c). It is likely that the TCR evolved to position the highly diverse CDR3 residues to where they have maximal contact with HLA-presented antigen in order to recognize the enormous diversity of antigenic peptides, which is essential for avoiding blind spots during pathogen recognition and for distinguishing between foreign and self-antigens [74,79].

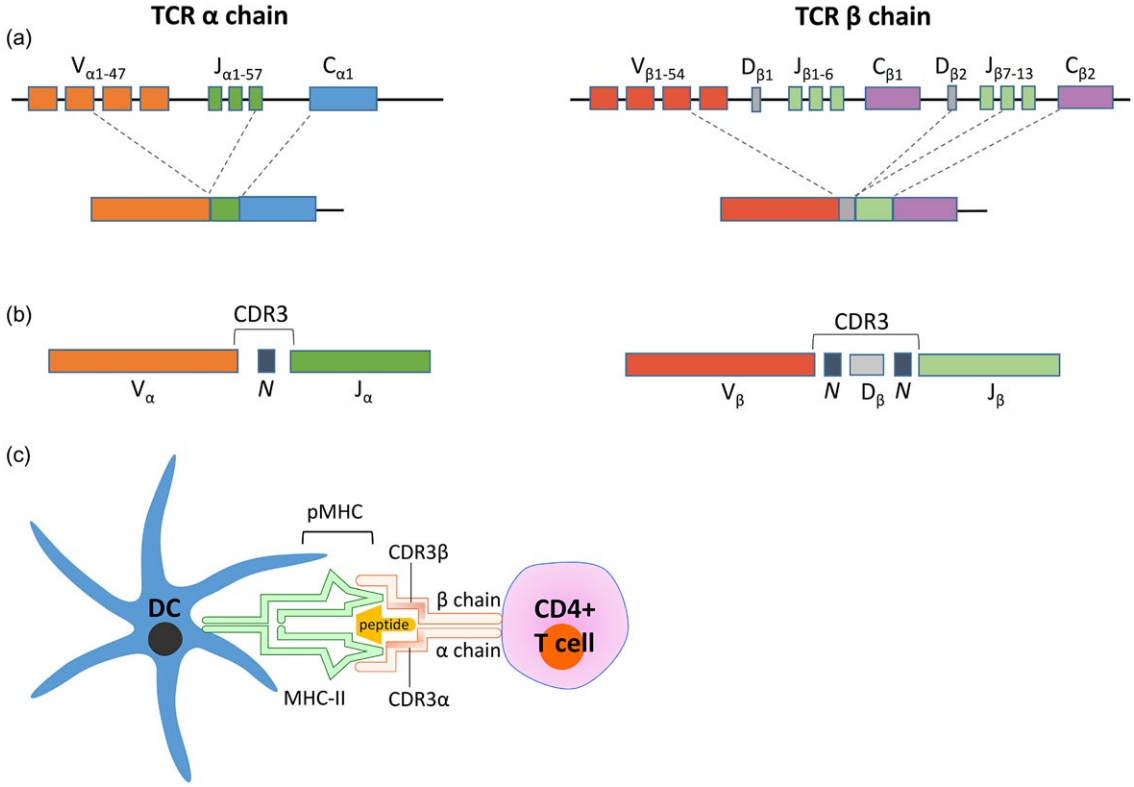

Fig. 4. Schematic demonstrating the generation of the T cell receptor, the resultant complementarity-determining region 3 (CDR3) of both α and β chains and T cell receptor–peptide–major histocompatibility complex (MHC) interaction. (a) Functional T cell receptors (TCRs) comprise an α and a β chain formed by somatic recombination of variable (V), diversity (D; β chain only) and junctional (J) gene segments. Recombined gene segments are then spliced together with the constant region (C) to form the functional TCR-αβ. (b) CDR3 is formed at the V, (D), J junction, where imprecise V, (D), J joining and random addition of non-template encoded nucleotides (N) makes this region the most diverse component of the TCR. (c) The heterodimeric TCR on a $CD4^+$ T cell engages peptide displayed in the major histocompatibility complex class II (MHC-II) on dendritic cells (DC). The CDR3 region is the main TCR domain in contact with the antigenic peptide. Figure (c) was created with the help of Motfolio PPT Drawing Toolkits (www.motfolio.com), and figures adapted from [74,150].

TCR repertoire analysis: evidence for autoantigen-specific CD4$^+$ T cell involvement

While several lines of evidence suggest that self-antigen-specific CD4$^+$ T cells are involved in RA pathogenesis, their identification in inflamed RA synovial tissue has been a challenge. Characterization of CD4$^+$ T cells and determining their antigen-specificity is crucial to understanding their role in autoimmune inflammation, and to develop T cell-directed immunotherapies.

Elucidation of the TCR repertoire makes it possible to infer which T cells, if any, are expanding in response to potentially autoantigenic stimuli in RA. TCR repertoire biases may already manifest in the naive pool. Proposed mechanisms include convergent recombination, whereby certain TCR sequences are produced at high frequency [80,81]. Furthermore, TCRs with a selective advantage, such as structural features that are optimal for peptide recognition within the context of particular MHC molecules, may provoke emergence of a biased repertoire [82]. Preferential (clonal) expansion of particular precursor T cells from the naive repertoire in response to antigen priming can further skew the usage of particular TCRs. This is because expanding daughter T cell clonotypes use an identical nucleotide sequence in the CDR3 region, in addition to identical V, (D) and J genes in TCRα and β chains [74,83–86].

TCR bias, oligoclonal T cell expansion in RA patients

In an attempt to unravel the role of antigen-specific T cells in RA, numerous studies have examined the TCR repertoire in the synovial compartment and PB of RA patients, aiming to identify biased TCRα and/or β usage that would suggest oligoclonal expansions. However, no consensus has emerged (Table 1). There are many possible reasons why these studies failed to elucidate a common TCR clonal signature, including non-homogeneous patient cohorts comprising individuals of different HLA haplotypes, patients treated with various immunosuppressive therapies, differences in disease stage of recruited patients and dilution of clonal bias by multiple autoantigen specificities in RA.

By application of powerful technologies in more homogeneous groups of RA patients, the TCR repertoire diversity in PB, particularly within the memory CD4$^+$ T cell compartment, was shown to be significantly reduced in SE$^+$ RA compared with SE$^-$ RA patients and healthy controls [87]. This negative correlation was dose-dependent, where the lowest TCR diversity occurred in RA patients carrying two SE alleles. These findings complement those of Scally *et al.* [33], wherein the SE was shown to permit the binding and presentation of RA-associated peptides, associated with enrichment in peripheral blood of memory CD4$^+$ T cells recognizing autoantigenic peptides presented in the SE. Expansion of such clonotypes contributes to reduced TCR diversity observed in SE$^+$ RA patients [34,87].

At the target site of autoimmune inflammation, TCR repertoire profiling in the synovial tissue (ST) of recent-onset RA patients revealed several highly expanded clones [88]. Furthermore, the degree of clonal dominance (exclusive expansion of a few clones) observed in recently diagnosed RA patients was significantly greater than in established RA patients [88]. These findings suggest the contribution of a limited number of expanded T cell clones to early RA pathogenesis, and highlight that clonotypical analysis of ST harvested from patients with preclinical RA may be fruitful. Of the oligoclonal expansions found in a recent study of RA ST, the same expanded TCRβ clones dominated at different locations within the single inflamed joint as well as in different joints when compared to the paired PB repertoire [89]. These findings suggest that there is widespread TCR recognition of the same antigens presented by APCs in ST.

In this regard, a small population of 'circulating pathogenic-like lymphocytes' (CPLs) has been identified in blood of patients affected by RA, juvenile idiopathic arthritis (JIA) and healthy individuals [90,91]. CPLs

Table 1. Summary of studies of TCR αβ V gene usage in rheumatoid arthritis (RA) patients

Authors	Source	TCRα/β V gene usage
[137]	PB, ST and SF	all Vβ genes in SF and ST relative to PB
[138]	PB and SM	Vβ3, Vβ17, Vβ22 and Vβ4 in SM relative to PB
[139]	PB, ST and SF	Vβ6, Vβ15 in ST; Vβ14 in SF; Vβ1, Vβ4, Vβ5.1, Vβ10, Vβ16, Vβ19 in ST, relative to PB
[140]	PB and SF	Vβ14 in SF compared to PB
[141]	PB and SF	Vβ17 in PB and SF of RA patients compared to healthy controls
[142]	PB and SF	Vβ2, Vβ6; Vβ13.1 and Vβ13.2 in SF compared to PB
[143]	PB and SF	Vβ7 in SF compared to PB
[144]	PB and SF	Vα10, Vα15, Vα18; Vβ4, Vβ5, Vβ13 in SF compared to PB
[145]	PB, ST and SF	Vβ14 and Vβ16 in ST; Vβ16 in SF, relative to PB
[146]	SF	Vα17

PB = peripheral blood; ST = synovial tissue; SF = synovial fluid.

express an activated, antigen-experienced T cell signature, are capable of recirculating through inflamed sites, retain proinflammatory capacity and correlate with disease activity in both JIA and adult RA. Notably, CPLs have reduced TCR diversity and share a high fraction of CDR3 sequences with synovial T cells, consistent with antigen-specific expansion *in situ*. Furthermore, a subset of synovial inflammation-associated (ia) suppressive T_{reg} cells, which appear to recirculate through inflamed sites of patients with JIA and adult RA, expand during inflammation and exhibit lower TCR diversity than other T_{reg}, demonstrating an antigen-driven response [92]. While iaT_{reg} are ontogenically related to the T_{reg} cell lineage, they share substantial TCR repertoire overlap with pathogenic synovial effector T cells (T_{eff}) and blood CPLs [92]. These findings imply that iaT_{reg} selectively expand in active disease from T_{eff}, consistent with the origin of T_{reg} type 1 (Tr1) cells [93]. iaT_{reg} may be unsuccessful at restraining these effector responses *in vivo* due to T_{eff} resistance to T_{reg}-mediated suppression – such as protein kinase B hyperactivation in JIA and secretion of TNF-related apoptosis-inducing ligand (TRAIL) in RA by effector cells – in combination with the proinflammatory milieu [94–97].

The deep sequencing technologies used for TCR repertoire profiling in these studies are advantageous in that these methods do not rely on prior (auto)antigen knowledge, which poses a major hurdle in autoimmune diseases where multiple putative autoantigens exist or where autoantigens are still yet to be defined [98,99]. Furthermore, the low natural frequency of antigen-specific T cells can add serious constraints on the number of cells analysed, limiting depth of repertoire analysis. However, as perturbations of the TCR repertoire often occur as a result of transient or chronic infection, a greater understanding of the fine epitope-specificity of the oligoclonally expanded T cell clones in the aforementioned studies is needed to determine their contribution to autoimmune pathogenesis [100].

Autoantigen-specific $CD4^+$ T cells in RA

In this regard, MHC class II multimers have emerged as a valuable tool for identifying and characterizing $CD4^+$ T cells of a particular specificity, albeit a smaller, predefined subset of the repertoire. In the RA context, tetramers comprise fluorescently labelled multivalent complexes of HLA-DR susceptibility molecules loaded with putative autoantigenic peptides (pMHCII) which bind the appropriate antigen-specific $CD4^+$ T cell receptor (Fig. 4a). As the pMHCII multimers are fluorescently labelled, $CD4^+$ T cells specific for the (auto)antigen can be detected and quantified by flow cytometry [101,102] (Fig. 4b).

Tetramer staining identified vimentin, cit-vimentin and cit-aggrecan-reactive $CD4^+$ T cells in the PB of RA patients, $ACPA^-$ first-degree relatives (FDR) and also healthy controls, all HLA-matched with RA susceptibility alleles [33,34,103]. While $tetramer^+$ $CD4^+$ T cells from RA patients and FDR displayed an effector-memory phenotype, those from healthy controls were more likely to be naive or $Foxp3^+$. This suggests that while self-antigen-specific $CD4^+$ T cells circulate in healthy individuals not suffering from autoimmune disease, there is a T cell regulatory capacity to control these autoreactive $CD4^+$ T cells in healthy controls that is deficient in RA patients [33]. Single-cell sorting and TCR sequencing of $tetramer^+$ $CD4^+$ T cells identified repeated CDR3α and CDR3β sequences, indicating antigen-driven oligoclonal expansion. In Indigenous North American subjects at high risk of RA, oligoclonal expansion, including public clonotypes, were observed both in RA patients and $ACPA^-$ FDR, suggesting that self-peptide-specific T cell expansion occurs in individuals with high-risk HLA during the preclinical period prior to the development of ACPA [34] (Fig. 5).

Antigen-specific T cells in mouse models of RA

There is evidence in mouse models that an adaptive immune response commences prior to disease onset and then continues. Transgenic HLA-DR1 humanized mouse models with collagen-induced arthritis (CIA) indicate that, at disease onset, autoantigen-specific effector $CD4^+$ T cells preferentially use TCRβ chain variable genes (TRBV) 31 and 13, are highly clonal based on CDR3 length polymorphism analysis and migrate to arthritic joints [104]. Selective use of TRBV genes results from clonal expansion due to recognition by the preferentially used TCRαβ of the dominant type II $collagen_{257–274}$ epitope presented by HLA-DR1, rather than predisposition to autoreactivity due to selection of a pathogenic naive repertoire by the DR1 transgene [104]. However, it is unclear how autoantigen-specific $CD4^+$ T cells contribute to arthritis pathogenesis in the predisease period prior to the development of autoantibodies.

Conversely, the arthritogenic potential of antibodies in the effector phase has been demonstrated in animal models: transfer of autoantibodies present in serum are sufficient to induce arthritic disease [105–107]. Downstream antibody-driven innate mechanisms, including immune complexes which trigger vascular leak and Fc-dependent mast cell activation, as well as macrophage and complement activation, together have been shown to provoke inflammatory arthritis in mouse models [108–110].

A novel murine transgenic (Tg) antigen-induced arthritis model, in which ovalbumin (OVA)-specific $CD4^+$ TCR transgenic cells are adoptively transferred, provides insight into the contribution of antigen-specific T cells to

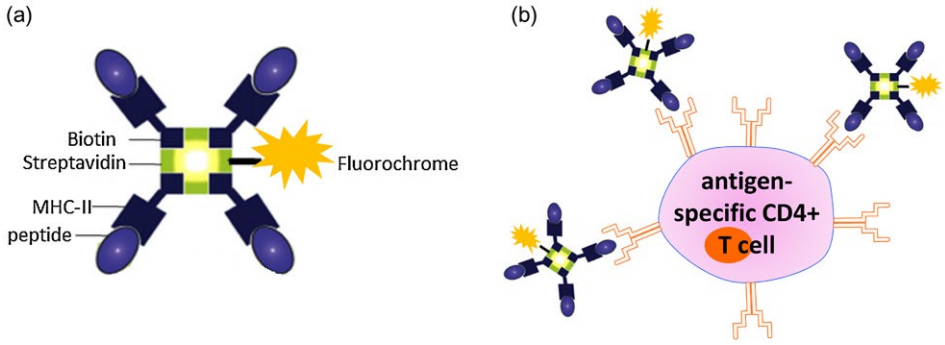

Fig. 5. Class II tetramers as tools for antigen-specific CD4⁺ T cell detection. (a) Schematic depicting the structure of a major histocompatibility complex (MHC) class II tetramer. Four identical biotinylated MHC class II molecules loaded with a candidate (auto)antigenic peptide are linked to a central streptavidin molecule. The central streptavidin is conjugated to a fluorochrome of interest. (b) Binding of fluorescently labelled class II tetramer molecules to the antigen-specific CD4⁺ T cell via the T cell receptor (TCR) enables detection of antigen-specific T cells by flow cytometry. Figure (b) was created with the help of Motfolio PPT Drawing Toolkits (www.motfolio.com), and figures adapted from [151] and https://www.intechopen.com/books/allergen/strategies-to-study-t-cells-and-t-cell-targets-in-allergic-disease, chapter 7, page 128, figure 1.

experimental arthritis. After immunization with OVA, heat-aggregated ovalbumin (HAO) is injected into the footpad. Th1 OVA-specific T cells recruited to the footpad are capable of breaking self-tolerance to joint-specific antigens and inducing mild arthritis, associated with the production of antibodies against CII, demonstrating bystander self-antigen activation during an immune response to foreign antigen [111]. HAO challenge, in the presence or absence of non-specific inflammatory LPS, increased CII-specific T cell proliferative autoreactivity and anti-CII, anti-ssDNA and rheumatoid factor (RF) autoantibody titres [112]. Challenge with non-specific inflammatory lipopolysaccharide (LPS) alone was insufficient to break self-tolerance. The authors concluded that non-self-antigen-specific T cell responses (such as might occur during infection) when recruited into the joint, and when boosted by pathogen-associated molecules, may disrupt local immunoregulation [112]. Bacterial DNA and peptidoglycans are detectable in synovial tissue of RA patients, which could stimulate both innate and adaptive immune responses [113]. In an inflammatory setting, breakdown of tolerance to self-antigen may occur when infectious antigen-specific T cells provide help to DCs cross-presenting both self- and foreign antigens. Autoreactive Tfh may in turn provide help to autoreactive B cell clones to stimulate their differentiation. In addition, inflammation driven by innate or T cell-driven mechanisms may promote cartilage destruction, resulting in the uptake of neoepitopes by DCs, triggering epitope spreading to other T cell specificities [112]. Interestingly, in this model, transferred OVA-specific T cells could drive activation of autoreactive B cells and production of autoantibodies, thus contradicting the requirement for cognate T cell–B cell interactions to break tolerance. Autoreactive B cells may elicit help from activated OVA-specific T cells in a bystander manner, or autoreactive B cells may co-capture and present HAO and self-antigen liberated in the inflamed joint to mount an autoantibody response dependent on OVA-specific T cells, as has been recently reported in other mouse models of autoimmune disease [114].

Abatacept is a soluble fusion protein comprising the extracellular domain of cytotoxic T lymphocyte 4 (CTLA-4) linked to the Fc domain of human IgG (CTLA-4-Ig) used as a biological DMARD in RA. Abatacept binds to CD80/86 expressed by mature DCs with higher affinity than CD28, thereby interfering with co-stimulation obligatory for full T cell activation [115,116]. CTLA-4-Ig prevents CIA, experimental autoimmune encephalomyelitis and murine lupus *in vivo* [117–119]. Platt *et al.* [120] demonstrated that abatacept inhibits T cell activation, proliferation and inflammatory mediator production [IFN-γ, IL-17, macrophage inflammatory protein (MIP)-1α, IL-13] by cells in the joint and draining lymph nodes. Abatacept also suppressed the acquisition of a Tfh phenotype and reduced the proportion of antigen-specific T cells migrating into B cell follicles: the few antigen-specific T cells were restricted to the paracortical T cell zone, due probably to their inability to migrate to CXCL13 via CXCR5. Consistent with the expression of CD80/CD86 by activated B cells, abatacept also inhibited the endogenous breach of B cell self-tolerance, with reduction in GC formation and autoantibody tires [120].

In this model, the majority of activated T cells infiltrating the inflamed joint were not OVA antigen-specific [121]. However, the adoptively transferred, OVA antigen-specific population and a subpopulation of endogenous T cells interacted for prolonged periods with synovial

DCs consistent with cognate antigen recognition. The articular T cells used a diverse range of TRBV genes and only one or two CDR3 clones were represented per TRBV, suggesting that T cell activation and clonal expansion occurred in secondary lymphoid organs prior to the migration and selective accumulation of particular clones in the joint. While abatacept seemed to interfere with priming of potentially autoreactive (Tfh) T cells in joint dLNs, it did not alter endogenous T cell–DC interactions in the inflamed joint.

Quality of DC–T cell interaction

As mentioned earlier, citrullination of self-antigens modulates the quality of MHC binding and TCR signalling. For example, citrullinated aggrecan reduced T cell proliferation but enhanced Th17 differentiation, which was promoted by low-strength TCR signalling [122]. TCR signalling strength can also determine the severity of arthritis in murine models of RA [123,124]. In recent-onset HLA-DR SE^+ RA patients, T cells were more likely to produce IL-6 in response to cit-aggrecan than cit-vimentin or cit-fibrinogen epitopes [101]. Hyperactivity of the rat sarcoma extracellular signal-regulated kinase (Ras-Erk) pathway was shown to prevent negative regulation of TCR signalling by the phosphatase SHP-1, leading to sustained TCR signalling in RA patients [125]. Other genetic polymorphisms associated with RA may impact the quality of TCR signalling and immune synapse formation. RA-associated polymorphisms in CD28, CTLA4, protein tyrosine phosphatase, non-receptor type 22 (PTPN22), protein kinase C theta (PRKCQ) and CD247 (TCRζ) all impact TCR signalling [124,126]. Perturbations to the function of these molecules may impact TCR signalling thresholds and therefore the ensuing immune response. Conversely, RA-associated polymorphisms in MHC class II, *REL*, *CD40*, peptidyl arginine deiminase type IV (*PADI4*), *IL12B*, TNF-α-induced protein 3 (*TNFAIP3*), *CD83*, *CCL21*, IL-1 receptor-associated kinase 1 (*IRAK1*) and NFKB Inhibitor Epsilon (*NFKBIE*) may impact DC activation and signal transmission to T cells. Modulating the quality of interactions between DCs and T cells is therefore an attractive therapeutic target for RA.

Antigen-specific immunotherapy targeting the DC–T cell interaction

Despite considerable scientific and therapeutic advances, currently available treatment agents – although effective in reducing inflammation – do not address the upstream immune dysfunctions initiating the pathogenic processes. The collaborative interaction between specific autoantigen presentation by DCs to autoreactive T cells is a major untapped target in RA, which has been exploited in several recent and ongoing clinical trials to restore immune tolerance. If effective, tolerance-inducing antigen-specific immunotherapy would have lower toxicity and would represent a longer-term solution to controlling or preventing autoimmune disease. Such strategies would specifically target the autoimmune disease process involving RA-specific antigens presented by specific high-risk HLA-DR SE molecules and autoimmunity based on autoantibody positivity.

As discussed, DCs present RA autoantigens to T cells, and DCs with regulatory properties can promote the induction of pT_{reg}. An important body of work demonstrated that certain subsets of DCs *in vivo*, such as $CD103^+$ intestinal DCs, promote pT_{reg} [127]. Furthermore, after exposure to antigen, RelB-deficient myeloid DCs or DCs generated in the presence of various NF-κB suppressive drugs ('tolerogenic DCs') could restore antigen-specific tolerance in primed mice or mice with antigen-induced inflammatory arthritis [128–130]. In the first Phase I proof-of-concept clinical trial of antigen-specific DC immunotherapy, HLA-DR SE^+ $ACPA^+$ RA patients were treated with autologous PB DCs modified with NF-κB inhibitor, BAY11-7082, and exposed to four citrullinated peptide autoantigens. Treatment was safe, and reduced both inflammation and $CD4^+$ effector T cells and increased the T_{reg} to effector T cell ratio. In treated subjects, *ex-vivo* IL-6 production to cit-vimentin$_{447–455}$ was suppressed [131]. A second trial of tolerogenic DCs exposed to antigens in autologous synovial fluid also demonstrated safety and feasibility [132], and further DC trials are ongoing (see clinicaltrials.gov). These trials begin to build a mechanistic understanding around the potential for DC-based antigen-specific immunotherapies to rebalance antigen-specific regulatory to effector T cells, and highlight the need for sensitive, standardized and clinically qualified immunological assays, such as tetramers, to determine the pharmacodynamic immunological effects of antigen-specific therapies so that effects can be evaluated and compared in early-stage trials [102].

What might the future hold for patients with and/or at risk of RA? Murine proof-of-concept studies show that DCs or T cells may be targeted *in situ* for induction of tolerance. In mice, liposomes encapsulating mBSA antigen and an NF-κB inhibitor (curcumin, quercetin or BAY11-7082) suppressed mBSA antigen-induced inflammatory arthritis in an antigen-specific manner. The liposomes suppressed antigen-specific T cells and induced antigen-specific pT_{reg} [133]. A Phase I clinical trial is in progress in patients with HLA-DRB1*04:01 and $01:01^+$ RA to ascertain safety and immunomodulatory effects of liposomes encapsulating the collagen $II_{259–273}$ epitope (restricted by these HLA-DR allomorphs) and NF-κB

inhibitor 1α,25-dihydroxyvitamin D3 (calcitriol) (see anzctr.org.au). Mouse models demonstrate other potential uses for immune tolerance in RA: PLGA nanoparticles encapsulating rapamycin delivered with adalimumab suppressed the development of anti-drug antibodies and improved drug efficacy in inflammatory arthritis [134,135]. In an alternative approach, iron nanoparticles coated with peptide-MHC molecules directly targeted autoreactive TCR to induce antigen-specific T_{reg} and suppress inflammatory arthritis [136]. Of interest, these studies show that just as bystander activation can be sufficient to activate autoreactive T cells, Tr1 cells of a single autoantigen specificity are sufficient to regulate autoreactive T cells of multiple specificities.

Thus, basic and translational studies indicate that DC antigen presentation to T cells is a ripe area for future drug development in RA. The field is moving increasingly towards precise definition of target populations, more sophisticated immunophenotypical characterization of patients prior to treatment and more consistent application of immunomonitoring to clinical trials. If successful, immunotherapies targeting the DC-antigen–T cell interaction should deliver improved safety, specificity and durability of disease control.

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Disclosures

R. T. has filed provisional patents surrounding technology for targeting DCs for antigen-specific tolerance, and is a director of the spin-off company, Dendright, which is commercializing immunotherapy to target DCs to suppress rheumatoid arthritis in collaboration with Janssen Biotech Inc. R. T. has also received speaker fees and/or consulting fees from Janssen and Abbvie.

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