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Review

Mechanisms underlying DMARD inefficacy in difficult-to-treat rheumatoid arthritis: a narrative review with systematic literature search

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Abstract

Management of RA patients has significantly improved over the past decades. However, a substantial proportion of patients is difficult-to-treat (D2T), remaining symptomatic after failing biological and/or targeted synthetic DMARDs. Multiple factors can contribute to D2T RA, including treatment non-adherence, comorbidities and co-existing mimicking diseases (e.g. fibromyalgia). Additionally, currently available biological and/or targeted synthetic DMARDs may be truly ineffective ('true' refractory RA) and/or lead to unacceptable side effects. In this narrative review based on a systematic literature search, an overview of underlying (immune) mechanisms is presented. Potential scenarios are discussed including the influence of different levels of gene expression and clinical characteristics. Although the exact underlying mechanisms remain largely unknown, the heterogeneity between individual patients supports the assumption that D2T RA is a syndrome involving different pathogenic mechanisms.

Key words: RA, difficult-to-treat, immune mechanisms, review

REVIEW

Rheumatology key messages

- Difficult-to-treat rheumatoid arthritis (D2T RA) is a heterogeneous disease state, probably involving individual differences in the pathogenesis in patients with 'true' refractory RA.
- Different immune mechanisms can underlie DMARD inefficacy, influenced by differences in gene expression regulation and clinical characteristics.
- 'True' refractory RA represents a subgroup of D2T RA.

Introduction

With the introduction of biological and targeted synthetic DMARDs (b/tsDMARDs), outcomes of RA have significantly improved [1]. However, a substantial proportion of patients can be categorized as having 'difficult-to-treat' (D2T) RA. As per consensus, D2T RA remain symptomatic after failing at least two b/tsDMARD with different mechanisms of action (Table 1) [2]. The unmet need for these patients has been recognized by rheumatologists across Europe [3].

The term 'refractory' RA is frequently used to describe D2T RA patients and may be incorrect in some cases [2, 4]. Multiple factors may contribute to the persistence of symptoms and/or signs in D2T RA patients (Fig. 1) [4–7]. Only if all currently available (b/ts)DMARDs are

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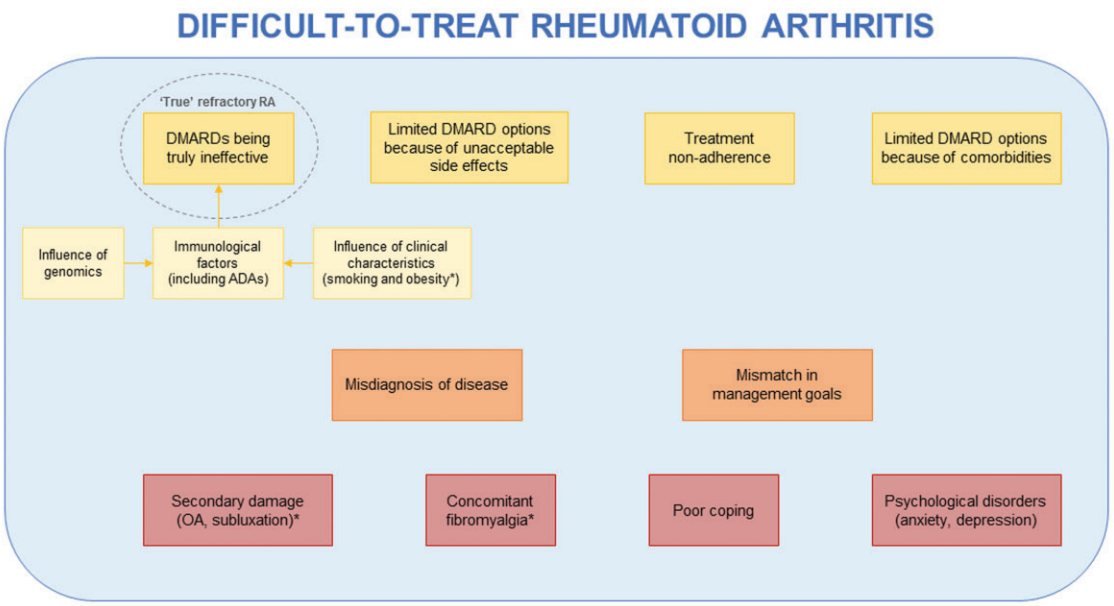
Table 1 EULAR definition of difficult-to-treat RA [2]

1. Treatment according to EULAR recommendations and failure of ≥2 b/tsDMARDs (with different mechanisms of action)[a] after failing csDMARD therapy (unless contraindicated).[b]
2. Signs suggestive of active/progressive disease, defined as ≥1 of:
   a. at least moderate disease activity (according to validated composite measures including joint counts e.g. DAS28-ESR >3.2 or CDAI >10);
   b. signs (including acute phase reactants and imaging) and/or symptoms suggestive of active disease (joint related or other);
   c. inability to taper glucocorticoid treatment (below 7.5 mg/day prednisone or equivalent);
   d. rapid radiographic progression (with or without signs of active disease)[c]; and
   e. well-controlled disease according to above standards, but still having RA symptoms that are causing a reduction in quality of life.
3. The management of signs and/or symptoms is perceived as problematic by the rheumatologist and/or the patient.

All three criteria need to be present in D2T RA. b: biological; CDAI: clinical disease activity index; cs: conventional synthetic; DAS28-ESR: disease activity score assessing 28 joints using erythrocyte sedimentation rate; DMARD: disease-modifying antirheumatic drug; EULAR: European League Against Rheumatism; mg: milligram; RA: rheumatoid arthritis; ts: targeted synthetic. [a]Unless restricted by access to treatment due to socioeconomic factors. [b]If csDMARD treatment is contraindicated, failure of ≥2 b/tsDMARDs with different mechanisms of action is sufficient. [c]Rapid radiographic progression: change in van der Heijde-modified Sharp score ≥5 points at 1 year.

Fig. 1 Factors contributing to difficult-to-treat RA



Multiple factors that potentially contribute to difficult-to-treat RA are presented [5, 7]. All contributing factors, except for misdiagnosis of disease, could coexist. 'true' refractory RA is only present if all currently available DMARDs are truly ineffective. Factors in yellow may result in persistent inflammation (factors in light yellow may influence these factors); factors in orange may result in non-inflammatory symptoms and/or persistent inflammation; factors in red may result in non-inflammatory symptoms. *These factors may additionally hamper proper grading of inflammatory disease activity. ADAs: anti-drug antibodies.

truly ineffective, 'true' refractory RA is present. Mechanisms underlying DMARD inefficacy as well as (unacceptable) side effects in D2T RA are largely unknown [4–7]. To optimize treatment strategies, including the discovery of new therapeutic drug targets, more insight is needed into these underlying mechanisms.

The aim of this narrative review based on a systematic literature search was to explore if 'true' refractory RA exists and, if so, how prevalent it is, and to explore and summarize potential (immune) mechanisms underlying DMARD inefficacy as well as (unacceptable) side effects in D2T RA patients.

Methods

Research questions

The systematic literature search was conducted following the EULAR Standardized Operating Procedures [8]. The research questions were formulated and approved by the Task Force on management recommendations for D2T RA [9]. The questions focussed on (i) the frequency of 'true' refractory RA and (ii) reasons for DMARDs being ineffective or toxic (including side effects) in D2T RA (Supplementary Data S1, available at *Rheumatology* online) [10].

Search strategy

The databases of PubMed and Embase were searched for papers in English until November 2020. Additionally, conference abstracts of EULAR and ACR from 2017 to 2020 were screened. In addition to terms for RA, terms for potential underlying mechanisms, outcome measures and DMARDs were included (Supplementary Data S1, available at *Rheumatology* online). A search limit was set to the last seven years. This cut-off was chosen because D2T RA is a new concept.

Selection of studies, data extraction and analyses

First, titles and abstracts were screened by N.M.T.R. and P.M.J.W. according to a set list of selection criteria (Supplementary Data S1, available at *Rheumatology* online). Articles regarding RA patients who had been treated with at least one b/tsDMARD were eligible for inclusion, as evidence in D2T RA specifically was expected to be scarce. Articles were selected if they reported the frequency of refractory RA and/or an association between an underlying mechanism and DMARD inefficacy or (unacceptable) side effects. Second, full-text versions were screened. Articles were screened in duplicate, until the percentage of conflicts was below 5%. Disagreements were discussed until consensus was reached. Information was extracted from the included articles using a predetermined format.

As many different underlying mechanisms exist and can be studied, results are summarized descriptively and additional context is narratively added.

Results

Study characteristics

The systematic literature search resulted in 3801 unique papers. After title, abstract and full text screening, 115 papers were selected (Fig. 2) [4, 11–124].

Frequency of 'true' refractory RA

The frequency of refractory RA was only reported in two studies, which used different definitions and follow-up periods (Table 2) [4, 11]. Both studies defined refractory RA as failure of at least two bDMARDs. Only the cohort study explicitly reported that these should have different mechanisms of action [11], as in the D2T RA definition [2]. In this cohort study, 6% started a third bDMARD with a different mechanism of action after a median of 8 years. In the narrative review, it was estimated that 20% of patients progress to a third bDMARD during their disease course, which was based on data from RCTs into the efficacy of different bDMARDs [4]. Although the methodology used in these studies (i.e. a cohort study; and estimations based on RCT data in the narrative review) and reported incidence measures are different [i.e. different definitions of refractory RA (using bDMARDs with another mechanism of action in the cohort study; and using a low hurdle response criterion in the narrative review)], results may indicate that over lifetime a considerable part of RA patients become refractory to multiple ($\geq$2) bDMARDs.

Nevertheless, not all currently available b/tsDMARDs have been tried in these patients nor have other factors potentially contributing to D2T RA (Fig. 1) been excluded in these studies and, therefore, it remains formally unknown whether 'true' refractory RA really exists. The frequency of 'true' refractory RA may be much lower than estimated above, as 'true' refractory RA only represents a subgroup of these patients.

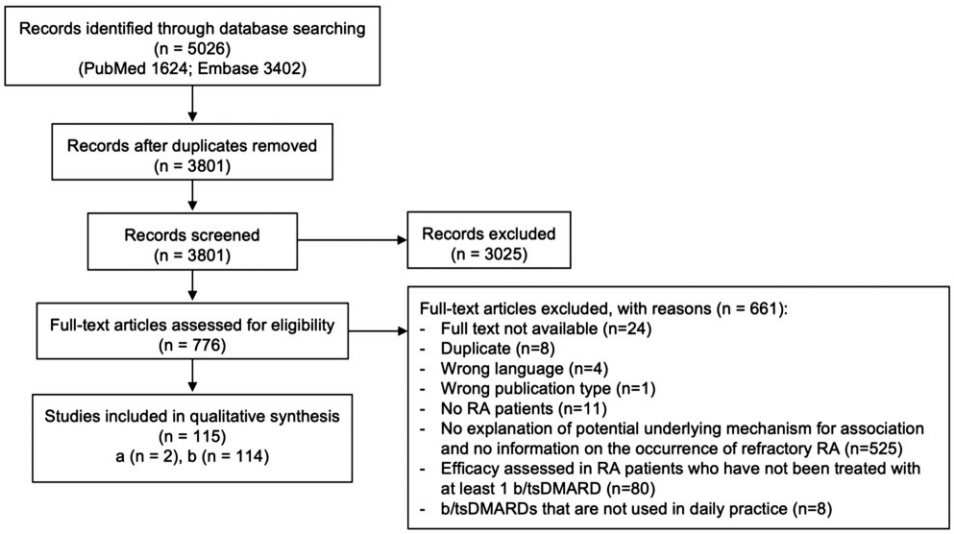
Immune mechanisms underlying inefficacy

Numerous studies have been performed on associations between treatment response to specific drugs and mechanisms underlying inefficacy. These do not only highlight the mechanism of action of the drug, but also reveal potential pathogenic pathways in non-responders *vs* responders. Although no studies have been performed in D2T RA patients (with 'true' refractory RA) specifically, the identified studies may aid in unravelling mechanisms underlying DMARD inefficacy in 'true' refractory RA. Below, we will discuss some examples as identified in the literature search (see also Fig. 3).

The innate and the adaptive immune system

Differences in the role of the innate *vs* the adaptive immune system have been suggested to explain differences in DMARD (in-)efficacy [4]. In patients treated with TNFi, genes associated with innate immune cells were expressed at higher levels at baseline in good responders, while genes associated with adaptive immune cells were expressed at higher levels in non-responders [43]. Similarly, a pre-treatment myeloid phenotype (macrophages and NF-κB expression) was associated with good response to TNFi in a study using synovial biopsies [21]. In another study, higher baseline levels of TNFR1-expressing monocytes were associated with better response to etanercept [79]. In contrast, response to tocilizumab [an interleukin-6 receptor (IL-6R) antagonist] was associated with the adaptive immune system. In the aforementioned synovial biopsy study, a pre-treatment lymphoid phenotype (B-cell- and plasmablast-dominated) was associated with good response to tocilizumab [38].

Fig. 2 Flow chart of search and selection of papers



The questions focussed on (i) the frequency of 'true' refractory RA and (ii) reasons for DMARDs being ineffective or toxic in D2T RA. b/tsDMARD: biological/targeted synthetic DMARD; *n*: number of studies.

Table 2 Papers on the frequency of 'true' refractory RA

Paper	Design	Description of population	Description of refractory RA	Frequency of 'true' refractory RA
Buch, 2018 [4]	Narrative review with an estimation based on RCT data (with 'low hurdle response endpoints', e.g. ACR20 response)	NA	Failure of ≥2 bDMARDs	Almost 20% progress to a 3rd bDMARD (estimation)
Kearsley-Fleet, 2018 [11]	Cohort (BSRBR-RA)	Patients with RA starting first-line TNFi from 2001 to 2014 ($n = 13\,502$; 111 034 person years)	Starting 3rd class of bDMARD (with different mechanisms of action)	6% ($n = 867$) developed refractory RA (median duration 8 years)

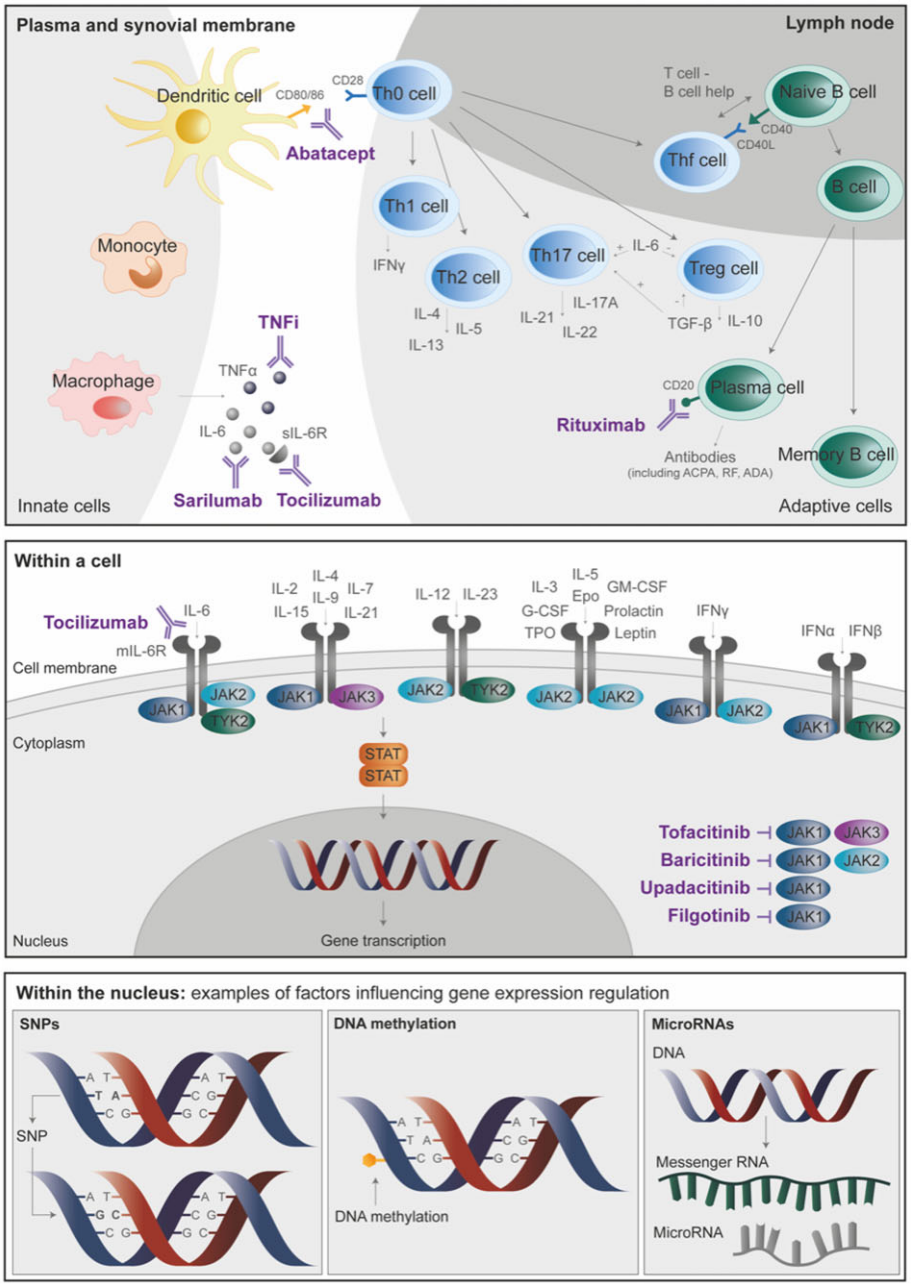
BSRBR-RA: British Society for Rheumatology Biologics Register for RA; n: number; NA: not applicable; RCT: randomized controlled trial; TNFi: TNF inhibitor.

However, the association between the innate or adaptive immune system and treatment response is not always as clear-cut as described above. For instance, higher baseline levels of innate cells [i.e. CD3-CD56+ natural killer (NK) cells] have also been associated with good response to tocilizumab [34]. The innate and adaptive immune systems are interacting on various levels and can therefore not be seen as distinct. Cytokines, such as TNFα and IL-6, are crucial in this interaction, which also explains why both drugs can influence innate and adaptive immune cells [78]. The importance of TNFα and IL-6 in the pathogenesis of RA is underlined by the success of bDMARDs targeting these cytokines (i.e. TNFi, tocilizumab and sarilumab) [14, 41, 85]. However, in D2T RA patients who fail both drugs due to true inefficacy, other immune factors may account for persistent inflammation.

T cells

Decrease of self-tolerance by reduced frequencies of regulatory T cells (Tregs) is thought to play a major role in the pathogenesis of RA and has also been related to DMARD (in-)efficacy. In patients treated with tocilizumab, an increase in Tregs was associated with achieving remission [68]. IL-6 decreases the differentiation of Tregs by inhibiting the expression of a specific transcriptional factor (FoxP3). Tocilizumab prevents this by blocking IL-6R, resulting in an increase in Tregs. This was also reflected in another study, in which increased Helios expression (a transcription factor selectively induced in FoxP3+ Tregs and inhibited by IL-6) in CD4+ Tregs after treatment with tocilizumab was associated with good response [115]. A link between Tregs and treatment response was also found in patients treated with abatacept (which inhibits T-cell co-stimulation). In

Fig. 3 A simplified overview of innate and adaptive immune processes and intracellular signalling pathways in RA as well as potential factors influencing gene expression regulation



In the plasma and synovial membrane (white and light grey) and lymph node (dark grey) a simplified overview of different innate and adaptive immune cells is shown as well as their interactions that lead to a multitude of proinflammatory mediators (including cytokines) that play a key role. Within the cell, different signalling pathways (e.g. JAK/STAT) that contribute to cell activation are shown. Biological and targeted synthetic disease-modifying antirheumatic drugs approved for the treatment of RA are shown in purple as well as the mechanisms/mediators they target. Within the nucleus, examples of factors influencing gene expression regulation are presented. SNPs in the genetic code can enhance or repress gene transcription and affect the function of genes, if the SNP results in an amino acid change. Epigenetic modification can render the DNA more or less accessible to transcription factors. For instance, DNA methylation at CpG sites can repress genes. Additionally, microRNAs are another mode of gene expression regulation. MicroRNAs bind to messenger RNA products of target genes and block gene translation into proteins. ADA: anti-drug antibodies; DNA: deoxyribonucleic acid; EPO: erythropoietin; JAK: Janus kinase; G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; mIL-6R: membrane-bound IL-6 receptor; RNA: ribonucleic acid; sIL-6R: soluble IL-6 receptor; SNP; single-nucleotide polymorphisms; STAT: signal transducer and activator of transcription; Th cell: T helper cell; Thf cell: follicular T helper cell; TNFi: TNF inhibitor; Treg cell: regulatory T cell; TPO: thrombopoietin.

responders to abatacept, increased activity of Tregs after treatment was found [84]. Abatacept may increase the number of Tregs by enhancing early growth response gene 2 (EGR2) expression, which is responsible for negative regulation of T-cell activation [125]. Although these studies emphasize the heterogeneity in the underlying mechanisms between responders and non-responders, unique pathways operative in non-responders were not identified.

Furthermore, the decreased self-tolerance by reduced frequencies of Tregs contributes to an expansion of Th1 and Th17 cells because the differentiation of Th17 cells is stimulated by the same signals that inhibit Treg differentiation (e.g. IL-6 and TGF-β) [126]. In a study in patients treated with TNFi, high baseline levels of IL-17A-producing Th17 cells were associated with non-response [29]. Targeting these Th17 cells may therefore be a potential drug target in 'true' refractory RA. However, clinical trials in RA patients with drugs targeting IL-17A showed insufficient benefit [127], although this may be due to patient selection and does not directly mean that these drugs are not beneficial in 'true' refractory RA patients. Nevertheless, IL-17A serves as an amplifier of inflammation [128], so it might be hypothesized that IL-17A-targeting drugs need to be combined with a TNFi or another cytokine-targeted therapy to be effective [129]. Whether such combination therapy is useful in 'true' refractory RA remains to be demonstrated.

B cells

Higher baseline levels of (synovial) B cells resulted in a more favourable response to rituximab [14]. Yet higher baseline levels of activated peripheral memory B cells (CD95+ Ki67) was associated with non-response to rituximab [37]. Whether other b/tsDMARDs may influence these cells is unknown. Presence of activated memory B cells may therefore underlie DMARD inefficacy in 'true' refractory RA.

Furthermore, the presence of synovial lymphoid aggregates (containing B and T cells) has been associated with worse response to TNFi [130]. These lymphoid aggregates produce TNFα [131] which may at least partly explain this association. Additionally, the presence of these lymphoid aggregates has been associated with more refractory disease, potentially suggesting that these can also underlie DMARD inefficacy in 'true' refractory RA.

Anti-drug antibodies

The formation of ADAs can lead to neutralization of bDMARDs and subtherapeutic serum drug levels (i.e. immunogenicity) [4, 80, 86, 94, 101, 103, 121]. ADAs are mainly IgM and IgG antibodies although IgE antibodies may also occur [23, 86]. ADAs are more frequently found during treatment with non-fully-human-derived antibodies: the chimeric TNFi (infliximab) and humanized antibodies (TNFis: adalimumab, certolizumab pegol and golimumab) [23, 80, 121]. However, ADAs have also been found in patients treated with etanercept, abatacept, rituximab and tocilizumab [23, 132, 133]. Figures up to 53% for infliximab and 30% for adalimumab have been reported [23, 134]. For adalimumab, certolizumab pegol, golimumab and infliximab, ADAs mostly target the Fab part, directly neutralizing the drug [86]. ADAs can also target the Fc part, which does not neutralize the drug, but can result in deceased circulating levels due to enhanced pharmacokinetic clearance of these immune complexes [86].

Subcutaneous and intramuscular administration may result in a higher occurrence of ADAs due to differences in antigen presentation by dendritic cells (DCs) [25, 80, 94]. Additionally, a longer dose interval has been linked to a higher occurrence of ADAs, as this could trigger a secondary immune response [19, 80, 94]. Patients with higher disease activity may also be more prone to ADA formation via enhanced interaction between DCs and T cells. This may even trigger ADAs for bDMARDs that are less immunogenic [135]. Genetic factors, e.g. IL-10 polymorphisms, have also been associated with ADAs [101, 136].

Addition of methotrexate to bDMARDs reduces the formation of ADAs and is associated with better response [26, 46, 101]. This may be explained by the suppression of early T- and B-cell expansion resulting in reduced ADA formation, but could also be due to the additional anti-inflammatory effect of methotrexate itself.

Autoantibodies

Inefficacy of bDMARDs has also been linked to seropositivity for ACPA and/or RF. Presence of these autoantibodies has been related to differences in synovial tissue phenotypes, B-cell-rich synovitis being associated with higher titres of autoantibodies [137]. Seropositive patients were found to have a more favourable response to rituximab and abatacept compared with seronegative patients [45, 48, 95]. This may be explained by the influence of these treatments on autoantibody producing B cells, contrary to cytokine-targeted therapies, such as TNFi and tocilizumab [48, 138].

Intracellular signalling pathways

The JAK/STAT signalling pathway has also been related to bDMARD (in-)efficacy. For example, higher baseline levels of STAT-1 and -3 phosphorylation after *ex vivo* cytokine stimulation of leukocytes [with interferon-γ (IFNγ), IL-10, IL-4 and IL-2] have been associated with better clinical response to tocilizumab [89]. STAT-1 and -3 regulate Th17 differentiation and may become exhausted after persistent activation by high IL-6 signals through IL-6R [89]. By blocking IL-6R with tocilizumab, these STATs become available again for other cytokines (e.g. IFNγ) resulting in restored T-cell balance. In another study in differently treated patients (TNFi, tocilizumab, rituximab), this was confirmed for STAT1 and also found for STAT6 [72]. STAT6 promotes expression of several Th2-specific transcription factors resulting in production of Th2 cytokines (e.g. IL-4, IL-5, IL-13) and Treg cell response [139]. Again, the pathways that are still active in non-responders remain unknown.

Heterogeneity in underlying immunology

In recent studies in early RA, three pre-treatment synovial pathotypes were identified: lympho-myeloid [i.e. B cells and myeloid cells (innate and adaptive cells)], diffuse-myeloid [i.e. myeloid lineage predominance (innate cells)] and pauci-immune (i.e. stromal cells, few immune cells) [140]. Patients with a pauci-immune synovial pathotype responded less to bDMARDs, yet those with a lympho-myeloid phenotype had poorer prognosis: worse radiographic outcomes and a higher proportion of patients requiring bDMARDs at 12-month follow-up. This phenotype may thus be an early indicator of 'true' refractory RA.

In the R4RA trial, patients were treated with tocilizumab and rituximab, stratified using synovial biopsies at baseline in B-cell-poor and B-cell-rich patients [141]. In patients histologically classified as B-cell poor and in those classified as B-cell rich, no difference in responsiveness to tocilizumab or rituximab was found. However, when patients were classified as B-cell poor based on RNA sequencing, significant higher response rates at 16 weeks were found for tocilizumab compared with rituximab. RNA sequencing could be more sensitive than histology to assess the underlying immunology and may have a role in selecting the appropriate (b/ts)DMARD strategy, this also further highlights the heterogeneity in the underlying immunology in RA. An important limitation is the difference in treatment history of included patients, which may have confounded the differences in histopathology.

Influence of (epi-)genetics

Epigenetic and genetic heterogeneity strongly affect gene expression, which can contribute to DMARD inefficacy as another independent layer (Fig. 3). Although the majority of the findings is not (well) validated, the data reveal influences of gene expression regulation at multiple levels of genetic organization (see also Supplementary Table S1, available at *Rheumatology* online).

Single-nucleotide polymorphisms

Single-nucleotide polymorphisms (SNPs) in the genetic code may be related to differences in DMARD (in-)efficacy. These SNPs can either be located in regulatory or coding regions of genes.

Alterations in the regulatory region can enhance or repress gene transcription. For example, a more favourable response to TNFi treatment was found in carriers of the rs28362491-94ins/del ATTG polymorphism in the NF-kB1 promoter, while homozygous carriers of the T-allele of the rs187084-1486T>C SNP in the toll-like receptor (TLR)9 promoter showed a less favourable response [51]. TLRs are part of the innate immune response and promote an increase in inflammatory chemokines, cytokines and cell adhesion molecules via NF-kB [142].

Variations in the coding regions of genes may lead to amino-acid substitution in the corresponding proteins and affect their function. The Fcγ receptor regulates immune responses through the interaction with antibodies (including bDMARDs) and is expressed on all immune cells. In FCGR3A, within the gene for the Fcγ receptor 3B, the rs396991 SNP 596 T > G nucleotide substitution causes an amino acid change of phenylalanine into valine. The valine isoform is considered to have a higher affinity to IgG than the phenylalanine isoform and correlates with a stronger immunological response [62, 91]. Carriers of one or more of the valine alleles had a better treatment response to rituximab [62, 91], while carriers of the FCGR3A 596 T-genotype (lower affinity genotype) had a better response to tocilizumab [62].

Epigenetics

Epigenetic modification can render the DNA more or less accessible to transcription factors. For instance, DNA methylation at CpG sites represses genes, while hypomethylation results in a permissive chromatin structure. For etanercept, five differentially methylated DNA positions at baseline have been identified to differ between responders and non-responders, although this could not be confirmed in another study [143, 144].

MicroRNAs are another mode of gene expression regulation [145]. These non-coding short single-stranded RNA molecules act by base pairing to messenger RNA products of target genes. The resulting double-stranded RNA molecules are recognized by the cell's machinery and targeted for degradation. Depending on the mode of action of the target gene, microRNAs could enhance or inhibit certain pathways. The microRNA expression profile of patients could therefore constitute another level at which differences in the DNA sequence can account for altered DMARD efficacy.

The expression of several microRNAs in RA patients who failed multiple bDMARDs has been compared with RA patients who failed csDMARDs, patients having low disease activity and healthy controls [47]. A cluster of microRNA-23a, -24–2 and -27a was significantly reduced in patients who failed multiple bDMARDs and in those who failed csDMARDs. Interestingly, microRNA-23a and -27a were found to mediate the regulatory loop of IL-6 and their expression was found to be repressed by cytokines of the JAK/STAT signalling pathway (e.g. IFN-γ and GM-CSF). Additionally, cells lacking microRNA-23a and -27a expressed higher levels of pro-inflammatory cytokines (e.g. TNFα and IL-6). Whether these patients failed bDMARDs interfering with these cytokines was not described.

Influence of clinical characteristics

Smoking

Smoking has been associated with more severe disease and reduced responsiveness to (b)DMARDs, notably TNFi [31, 73, 146]. Smoking has been associated with higher levels of (IgA) RF and ACPA, a poor prognostic factor for response, by promoting the citrullination of proteins [1, 147]. Furthermore, smoking has been associated with higher levels of inflammatory cytokines:

higher ratios of TNFα/soluble TNF receptor and higher levels of serum soluble IL-2R [148]. Additionally, a higher basal metabolism resulting in increased drug metabolism has been found among smokers [149]. Higher expression levels of SEMA6B [a protein resulting in signal transduction of fibroblast growth factor-receptor-1 and -2 (FGFR1-2) and vascular endothelial growth factor-receptor 2 (VEGFR2)] and GPR15 (a chemo attractant for T cells on the cell surface of monocytes and neutrophils) are increased in smokers, potentially increasing T-cell involvement and synovial vascular proliferation [32]. In another study, the T allele of NLRP3(rs4612666) was associated with non-response to TNFi in current smokers. Smoking may result in the production of reactive oxygen species leading to increased expression and activation of the NLRP3 inflammasome, an intracellular innate immune sensor, which releases strong pro-inflammatory cytokines (IL-1β and IL-18) [107].

Obesity

Obese patients may respond less favourably to TNFi [13, 123]. A first explanation may be the higher distribution volume and different pharmacokinetics in obese patients [13, 123]. However, the same association has been found for infliximab, which is dosed based on body weight. In early RA, the association between inefficacy and obesity was even found with infliximab doses up to 10 milligrams per kilogram [150]. The increased level of adipocytes as a result of more fatty tissue may be another explanation [13]. Adipocytes release adipokines, including leptin that is known to induce the expression of pro-inflammatory cytokines, such as TNFα and IL-6. The impact of obesity on synovial tissue has recently been shown [151]. Even in obese RA patients who achieved remission after TNFi treatment, a higher degree of residual synovitis (CD68+ macrophages, CD20+ B cells, CD3+ T cells) was found compared with normal weight patients.

Furthermore, the higher level of Fc-receptors in omental adipocytes may play a role [152]. Infliximab binds to these receptors, which may also explain why the association between obesity and inefficacy of infliximab is even stronger than with certolizumab pegol that lacks the Fc fragment [123].

Immune mechanisms underlying (unacceptable) side effects

Also for mechanisms underlying (unacceptable) side effects, no studies have been reported in D2T RA patients specifically. In studies in RA patients who failed b/tsDMARDs, mechanisms were generally found to be directly related to the mechanism of action of DMARDs and general immune mechanisms were not found (Supplementary Table S2, available at *Rheumatology* online) [15, 17, 23, 27, 53–55, 59, 67, 71, 80, 81, 93, 96, 97, 100, 102, 113, 116]. As for mechanisms underlying inefficacy, immune mechanisms underlying side effects were also found to be influenced by (epi)genetics and clinical characteristics.

Future perspective

Despite insights from studies on immune mechanisms underlying (b/ts)DMARD (in-)efficacy, studies assessing the mechanisms that are still active in non-responders are scarce and studies in D2T RA patients who failed all currently available b/tsDMARDs are lacking. Therefore, it remains unknown which exact mechanisms are responsible for the persistence of inflammatory disease activity in 'true' refractory RA, although some hints were found for a role of Th17 cells, activated memory B cells (CD95+ Ki67) and synovial lymphoid aggregates.

In addition to individual differences in DMARD (in-)efficacy that underline the heterogeneity in the pathogenesis of (D2T) RA, individual differences in (epi-)genetics and clinical characteristics add an additional layer of complexity to this heterogeneity. Future studies assessing mechanisms underlying DMARD inefficacy in 'true' refractory RA patients are needed, comparing D2T and non-D2T RA patients by combining analyses of blood and synovial tissue, molecular profiling using different technological platforms (e.g. proteomics, transcriptomics, metabolomics) and (epi-)genetic analyses.

Before these analyses are conducted, the origin of the signs and symptoms of D2T RA patients should be ascertained (Fig. 1). Recently, three subgroups of established RA patients (although not fulfilling the D2T RA definition) [2] were identified using synovial histologic features and RNA sequencing data: high inflammatory, mixed and low inflammatory [153]. The low inflammatory subgroup had high pain scores suggesting a non-inflammatory origin of the symptoms. Therefore, careful assessment of inflammation is important to select the appropriate ('true' refractory RA) patients, as non-inflammatory factors may confound findings.

In addition to a comparison of mechanisms in D2T and in non-D2T RA, a deeper understanding of the pathogenesis of RA might also help in unravelling mechanisms that are still active in 'true' refractory RA. For example, deep single-cell profiling of the synovium has shown that key cell lineages, such as synovial macrophages, are represented in functionally distinct populations, with levels of MerTK+ macrophage populations that express negative regulators of inflammation inversely correlating with the risk of clinical flare, suggesting a role for these cells [154]. Additionally, the Accelerating Medicine Partnership—a public-private partnership established to identify and validate promising targets for therapeutics—has identified 18 synovial key cells involved in the pathogenesis of RA: innate cells (e.g. IL-1β pro-inflammatory monocytes), adaptive cells (e.g. PDCD1+ peripheral and follicular Th cells) and THY1(CD90)+HLA-DRA^{hi} sublining fibroblasts [155]. Studies on associations with treatment response and clinical phenotypes may provide useful insights.

Furthermore, studies on mechanisms underlying (in-)efficacy of newer treatment options may aid in increasing our understanding of mechanisms underlying failure of multiple b/tsDMARDs. For instance, cellular therapies

(such as tolerogenic DC transfer) and therapies selectively stimulating Tregs (e.g. using low-dose IL-2 therapy), which aim to restore immune tolerance, are currently under evaluation and may be promising in 'true' refractory RA [156, 157]. Additionally, vagus nerve stimulation has recently been introduced as a therapeutic option for patients who failed at least two b/tsDMARDs with different mechanisms of action [158]. Vagus nerve stimulation activates the inflammatory reflex, which plays a role in the regulation of innate and adaptive immunity through the activation of the cholinergic anti-inflammatory pathway. When conducting such pharmacological studies, the heterogeneity in the pathogenesis of RA patients should be considered. As these new drugs may only be beneficial for a subgroup of RA patients, this heterogeneity may confound efficacy outcomes and could result in the incorrect conclusion that these new drugs are not beneficial. Therefore, selecting the most appropriate patient population (i.e. 'true' refractory RA patients) may help to discover novel pharmacological strategies.

Conclusions

The presented heterogeneity in immune mechanisms substantiates the assumption that RA is a heterogeneous disease, in which the pathogenesis differs between individuals. (Epi-)genetic predisposition and clinical characteristics further contribute to this complex interplay. D2T RA should specifically be seen as heterogeneous: not only different immune mechanisms may underlie DMARD inefficacy, but other (additional) contributing factors may also result in the persistence of signs and symptoms suggestive of active disease.

Few studies were found in RA patients who failed multiple b/tsDMARDs and no studies in patients with 'true' refractory RA, a subgroup of D2T RA patients in whom DMARDs are truly ineffective. Therefore, the exact immune mechanisms that are still active and can potentially be targeted in 'true' refractory RA, a subgroup of D2T RA patients in whom DMARDs are truly ineffective, as well as whether 'true' refractory RA really exists remain unknown. Future studies will be needed to increase our understanding and ultimately improve outcomes of D2T RA patients.

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Data availability statement

Data are available from the corresponding author upon reasonable request.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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