Metadata of the chapter that will be visualized in SpringerLink

Book Title		
Series Title		
Chapter Title	Tertiary Lymphoid Organs in Rheumatoid Arthritis	
Copyright Year	2020	
Copyright HolderName	Springer Nature Switzerland AG	
Author	Family Name	**Rivellese**
	Particle	
	Given Name	**Felice**
	Prefix	
	Suffix	
	Role	
	Division	Barts and the London School of Medicine & Dentistry
	Organization	Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, John Vane Science Centre
	Address	London, UK
	Email	
Author	Family Name	**Pontarini**
	Particle	
	Given Name	**Elena**
	Prefix	
	Suffix	
	Role	
	Division	Barts and the London School of Medicine & Dentistry
	Organization	Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, John Vane Science Centre
	Address	London, UK
	Email	
Corresponding Author	Family Name	**Pitzalis**
	Particle	
	Given Name	**Costantino**
	Prefix	
	Suffix	
	Role	
	Division	Barts and the London School of Medicine & Dentistry
	Organization	Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, John Vane Science Centre
	Address	London, UK
	Email	c.pitzalis@qmul.ac.uk

Abstract

Rheumatoid Arthritis (RA) is a chronic systemic autoimmune disease. RA mainly affects the joints, with inflammation of the synovial membrane, characterized by hyperplasia, neo-angiogenesis, and immune cell infiltration that drives local inflammation and, if untreated, can lead to joint destruction and disability. In

parallel to the well-known clinical heterogeneity, the underlying synovitis can also be significantly heterogeneous. In particular, in about 40% of patients with RA, synovitis is characterized by a dense lymphocytic infiltrate that can acquire the features of fully functional tertiary lymphoid organs (TLO). These structures amplify autoimmunity and inflammation locally associated with worse prognosis and potential implications for treatment response. Here, we will review the current knowledge on TLO in RA, with a focus on their pathogenetic and clinical relevance.

Tertiary Lymphoid Organs in Rheumatoid Arthritis

Felice Rivellese, Elena Pontarini, and Costantino Pitzalis

Contents

Abstract Rheumatoid Arthritis (RA) is a chronic systemic autoimmune disease. RA mainly affects the joints, with inflammation of the synovial membrane, characterized by hyperplasia, neo-angiogenesis, and immune cell infiltration that drives local inflammation and, if untreated, can lead to joint destruction and disability. In parallel to the well-known clinical heterogeneity, the underlying synovitis can also be significantly heterogeneous. In particular, in about 40% of patients with RA, synovitis is characterized by a dense lymphocytic infiltrate that can acquire the features of fully functional tertiary lymphoid organs (TLO). These structures amplify autoimmunity and inflammation locally associated with worse prognosis and potential implications for treatment response. Here, we will review the current knowledge on TLO in RA, with a focus on their pathogenetic and clinical relevance.

AQ1

F. Rivellese · E. Pontarini · C. Pitzalis (✉)
Barts and the London School of Medicine & Dentistry, Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, John Vane Science Centre, London, UK
e-mail: c.pitzalis@qmul.ac.uk

Current Topics in Microbiology and Immunology
https://doi.org/10.1007/82_2020_216

1 Introduction

Rheumatoid Arthritis (RA) is the most common autoimmune disease, affecting up to 1% of the population worldwide (Smolen et al. 2016). Although RA is well recognized as a systemic disease, its main feature is the chronic inflammation of the synovial membrane, which is characterized by infiltration of immune cells, cellular hyperplasia, and neo-angiogenesis (McInnes and Schett 2011, 2017). Ongoing synovitis and its corresponding clinical features of joint pain and swelling are the main causes of functional disability in patients with RA. Despite the availability of effective medications, in a large proportion of patients, the treatments fail to control the inflammatory response. When un-optimally controlled, synovial inflammation can progress and ultimately lead to joint destruction and permanent disability. Such inconsistent response to treatment has been attributed at least in part to the clinical and physiopathological heterogeneity of RA. In fact, similar to other autoimmune diseases, under the umbrella of RA, we are grouping a diverse spectrum of patients with different clinical features, which are mirrored by significant differences in terms of pathogenesis and, therefore, variable response to targeted treatments. For example, it is well recognized that the positivity for anti-citrullinated protein antibodies (ACPA) identifies a group of patients—around 70%- with a clinical phenotype of highly aggressive and destructive disease (Willemze et al. 2012). In line with its marked clinical heterogeneity, a variable degree of immune cell infiltration has been described in the synovia of RA patients and has been recently linked to distinct clinical features, including disease severity, progression, and treatment response.

2 The Synovial Membrane as Site of Inflammation in RA

The main physiopathological feature of RA is the inflammation of the synovial membrane (SM). In physiological condition, the SM is a composed by an intimal layer formed of synoviocytes, also known as fibroblast-like synoviocytes (FLS), which are specialized fibroblast-like cells with the main function of producing the synovial fluid that lubricates and nourish the avascular articular surfaces. Below the thin layer of FLS, there is a sub-intimal layer composed by connective tissues, scattered infiltrating macrophage-like cells, and blood vessels. During RA, the synovial membrane undergoes the following changes: (i) infiltration of immune cells, including cells of innate (e.g., macrophages, natural killer [NK] cells, innate lymphoid cells, dendritic cells, mast cells) and adaptive immunity (e.g., B and T lymphocytes, plasma cells); (ii) proliferation of FLS, leading to the thickening of the intimal layer, and (iii) growth of new blood vessels (neo-angiogenesis) which

further sustains the infiltration of immune cells, thus facilitating the perpetuation of the inflammatory response. Despite the enormous advancements in our understanding of the pathogenesis of RA, leading us to recognize a number of genetic and environmental factors contributing to its pathogenesis, the initial trigger of synovial inflammation is currently unknown. Also, we do not know whether the first hit happens directly in the joints or somewhere else, such as the lungs or other organs. However, once the inflammatory response is triggered and gets perpetuated, synovitis represents the main feature of RA, thus the study of synovial inflammation is of utmost importance to improve our understanding of RA (Pitzalis et al. 2013).

2.1 *Histological Patterns of Synovial Inflammation*

The infiltration of immune cells is one of the main features of RA synovitis. In line with the clinical heterogeneity of the disease, a variable degree of immune cell infiltration in synovia has been described. Despite the complexity and partial overlap of immune cell infiltration, the parallel study of large numbers of synovial samples from patients with early untreated RA (Humby et al. 2019) has allowed to describe three distinct groups based on the patterns of immune cell infiltration in synovia: (1) lympho-myeloid, dominated by lymphoid lineage infiltration (T cells, B cells, plasma cells) in addition to myeloid cells; (2) diffuse-myeloid, with myeloid lineage predominance but poor in B cells/plasma cells and (3) pauci-immune, characterized by scanty immune cells and prevalent stromal cells. Within the lympho-myeloid group, the infiltrating B cells, T cells, and plasma cells often organize into aggregates that resemble the lymphoid follicles of secondary lymphoid organs, acquiring features such as segregation of T cells and B cells, the presence of high endothelial venules (HEVs), and follicular dendritic cells (FDCs) networks. Although TLO can also be detected at extra-articular sites, including the lungs (Barone et al. 2015) and bone marrow (Bugatti et al. 2005) of RA patients, they mainly form within the sublining of the synovial tissue, where they have been described in about 40% of patients with early untreated RA (Pitzalis et al. 2013). A representative example of TLO is offered in Fig. 1a–c, including a schematic representation of their organization in Fig. 1d, with additional details in Fig. 2. In the next paragraphs, we will describe the ontogeny of tertiary lymphoid organs in RA, their functions, and their correlation with clinical features and disease prognosis, including response to treatment.

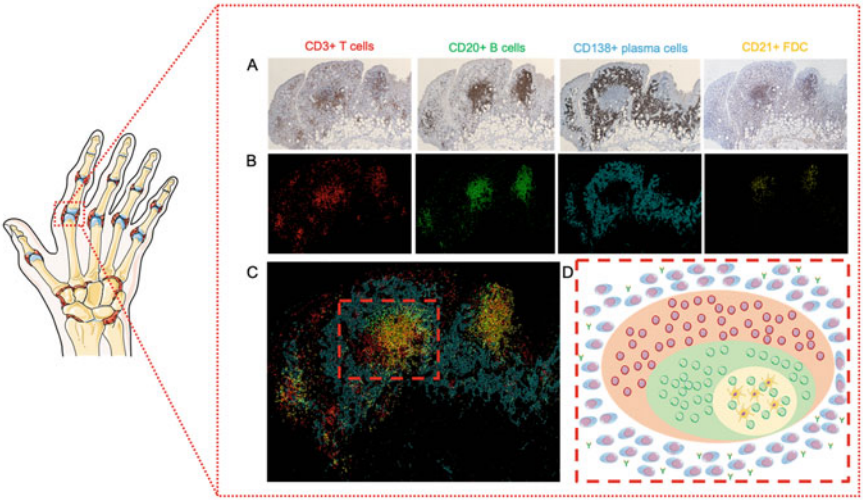

Fig. 1 Tertiary lymphoid organs in synovia. **a** Immunohistochemical staining of synovial membrane, **b** color deconvolution of the images in (**a**), **c** overlap of the above images, and **d** schematic representation of the organization of TLO in synovia, with FDC in yellow, B cells in green, T cells in red, and plasma cells in blue

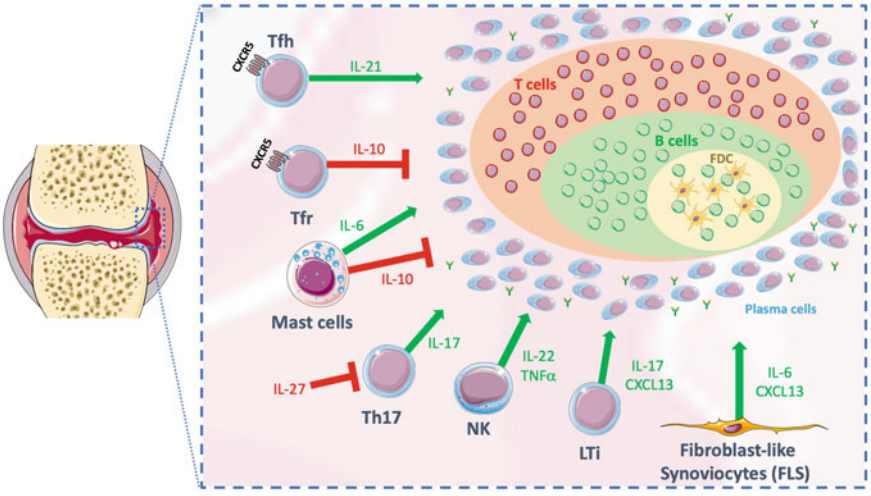

Fig. 2 Schematic representation of synovial TLOs and immune cells contributing to their development. Tfh = T follicular helper cells; Tfr = T follicular regulatory cells, Th17 = T helper cells 17, NK = Natural killer cells; LTi = Lymphoid tissue inducer

3 Synovial Tertiary Lymphoid Organs in RA

3.1 The Development and Regulation of Synovial Tertiary Lymphoid Organs in RA

3.1.1 Chemokine and Lymphotoxin Beta

One of the initial steps in the formation of TLO is the infiltration of lymphoid cells into the synovia, which is driven by the inflammatory milieu produced by FLS and innate immunity cells. As the inflammatory process becomes chronic, however, a number of specific mediators are required for the formation of TLO, such as lymphotoxin-β (LTβ), CXCL13, CCL19, and CCL21 (Corsiero et al. 2012). The development of TLO largely mirrors the ontogeny of secondary lymphoid organs, thus most of our knowledge on TLO development is derived from the study of secondary lymphoid organs, where animal models have identified a number of stimuli which are essential for the development of secondary lymphoid organs (Randall et al. 2008; Drayton et al. 2006). Among these stimuli, the *primum movens* has been recognized to be the production of lymphotoxin-β from so-called lymphoid tissue inducer (LTi) cells (Bar-Ephraim and Mebius 2016), which in turns leads to the production of lymphoid chemokines (CXCL13, CCL19, and CCL21) from lymphoid tissue organizers and mesenchymal cells. Although the presence of many of these lymphogenic stimuli has been confirmed in TLO in rheumatoid synovium (Bugatti et al. 2014; Manzo et al. 2007), the initial trigger of TLO formation in RA has not been identified. Several immune cells have been shown to be a source of lymphoid chemokines, and some of these key cells are represented in Fig. 2b. Among the various mediators, CXCL13 produced by follicular dendritic cells (Takemura et al. 2001) and other immune cells plays a pivotal role in determining the spatial organization of TLO, inducing the segregation of B cells within the germinal center, which is an essential drive for affinity maturation and antigenic selection (De Silva and Klein 2015). In line with its pivotal role, serum levels of CXCL13 have been associated with the presence of synovial TLO in patients with RA (Bugatti et al. 2014; Dennis et al. 2014).

3.1.2 T Follicular Helper Cells

In recent years, a specialized class of T helper cells, named T follicular helper cells (Tfh), has been recognized for their central role in sustaining B cell activation and differentiation in the germinal center (GC) reactions in secondary lymphoid organs. Tfh cells are specialized T helper cells that upon priming by antigen presenting cells (APCs) acquire the expression of CXCR5, the receptor for CXCL13, enabling them to migrate into the B cell area of GC.

The ectopic expression of CXCL13 has been described in RA synovium (Manzo et al. 2005, 2008) and has been shown to induce TLO formation and recruits B cells

to non-lymphoid tissues in mice (Luther et al. 2000). In fully formed GC, Tfh cells support somatic hypermutation of auto-reactive B cells and plasmablast generation directly in the diseased tissues mainly through the production of IL-21. The latter is Tfh signature cytokine, known to be a potent cofactor for B cell survival, proliferation and plasma cell differentiation, in particular in the context of CD40 co-stimulation and in synergy with B cell activating factor (Karnell and Ettinger 2012; Liu et al. 2015).

Importantly, because of the role of IL-21 and Tfh cells in supporting GC response, they have been implicated in the development of TLO in rheumatic autoimmune diseases, including RA, as represented in Fig. 2.

Data in animal models of arthritis identified a number of Tfh-associated markers during the development of inflammatory arthritis. In particular, CXCR5 has been shown to be an essential factor for the development of inflammatory arthritis: CXCR5-deficient animals or lacking CXCR5 on T cells are resistant to RA, showing impaired GC response (Moschovakis et al. 2017). Also, selective deficiency in T helper cells of SLAM-associated protein (SAP), required for the B/T cell interaction, thus essential for Tfh differentiation, protects mice from RA, further supporting the pathogenic role of ectopic GC formation (McCausland et al. 2014).

In parallel, IL-21 and its receptor are highly expressed in synovial tissue of patients with RA (Jüngel et al. 2004; Kwok et al. 2012), and increased IL-21 expression is associated with synovial TLO (Jones et al. 2015). IL-21R up-regulation is mainly described on macrophages and fibroblast with an activated phenotype (Jüngel et al. 2004), and IL-21 has been involved in the development of articular damage by promoting both osteoclastogenesis (Kwok et al. 2012) and metalloproteinase release by fibroblast-like synoviocytes (Xing et al. 2016). Finally, Tfh cells are also enriched in the synovia of patients with RA, while almost absent in osteoarthritis and normal synovium (Penatti et al. 2017; Chu et al. 2014).

In addition to conventional CXCR5+ Tfh cells, a population of T helper cells lacking CXCR5 expression and producing CXCL13 has been also described in the synovia of RA patients (Manzo et al. 2008). A recent breakthrough publication has shed new light on these cells, which have been re-named as PD1+ CXCR5—T peripheral helper cells (Tph), since they have been found in the synovia but also in the peripheral blood of patients with RA and their ability to induce the activation of B cells has been confirmed in vitro (Rao et al. 2017). Similar to GC-Tfh, these cells are an important source of CXCL13, support synovial B cell proliferation and activation through IL-21 production and SLAMF5 receptor ligation, and co-localize with B cells in synovial TLO (Rao et al. 2017). Although Tfh and Tph cells share the main markers, the tissue localization, and the ability to support B cell activation, it is unclear whether Tph in RA are Tfh cells with impaired CXCR5 expression, or a more distantly related cell type. Despite the evidence of Tfh and Tph contribution to the pathogenesis of RA, the functional link between these cells and TLO formation remains to be elucidated, as well as their contribution to the local production of autoantibodies within TLO.

Additionally, although the enrichment of Tfh in RA synovium has been well described, there are conflicting data regarding circulating Tfh cell frequency [comprehensively reviewed in (Gensous et al. 2018)]. Some authors reported IL-21 directly correlating with the frequency of Tfh-like cells, with IL-21 level and number of Tfh-like cells associated with higher titer of anti-CCP antibodies and disease activity score in RA (Ma et al. 2012). The circulating counterpart shares phenotypic and functional features with tissue Tfh cells, except for the expression of prototypical Tfh transcription factor B cell lymphoma protein 6 (Bcl-6), but their biology is still poorly defined. Data from SAP-deficient mice show how these cells are committed to Tfh lineage and are generated prior the GC response (He et al. 2013; Tsai and Yu 2014). Moreover, it is still unclear if circulating Tfh can reflect an ongoing humoral activity.

3.1.3 Other Pro-inflammatory Cytokines and Cells

It is now clear that a number of other pro-inflammatory cytokines, such as IL-17, IL-21, IL-22, IL-23, and TNFα, are also critical for lymphoid neogenesis in autoimmune diseases (Jones and Jones 2016).

The IL-23–IL-17 pathway has been involved in the initiation and perpetuation of TLO, and several cells of the innate and adaptive immunity are able to produce IL-17. In particular, a subset of adult innate lymphoid cells [type-3 innate lymphoid cells (ILC3 cells)] can produce IL-17 in the initial phases of TLO formation (Sawa et al. 2010). Accordingly, IL-17 positive cells are observed in the proximity of TLO in RA synovia (Chabaud et al. 1999), and the activation of the IL-23–IL-17 pathway correlates with the presence of synovial TLO (Cañete et al. 2011).

Another important aspect is the potential plasticity between other T helper subsets and the Tfh. In fact, several other subsets, including Th17 cells, Th1 and Th2, have been described to acquire Tfh-like phenotype (Ueno et al. 2015). For example, Tfh2 and Tfh17, but not Tfh1, are able to secrete IL-21 and induce naïve B cells to secrete class-switched immunoglobulin (Ig) (Morita et al. 2011).

Within RA synovium, proliferation of fibroblast-like synoviocytes is sustained by IL-22, a cytokine required for the development and maintenance of TLO. IL-22 role in ectopic lymphoneogenesis comes from data in experimental models of inducible TLO in salivary glands, mimicking TLO formation in Sjogren's syndrome salivary glands. In this animal model, IL-22 is able to directly induce CXCL13 production in a subset of GP38+ stromal cells through phosphorylation of signal transduced and activator of transcription 3 (STAT3) (Barone et al. 2015). Once lymphocytes are recruited, IL-22, together with LTα1β2, supports also proliferation of a population of podoplanin (pdpn)-positive stromal cells, over-expressing IL21R, into a network of immunofibroblasts that are able to support the earliest phases of TLS establishment (Nayar et al. 2019) in the same model. In RA synovium, IL-22 expression and IL-22 receptor on fibroblast-like synoviocytes have been reported (Ikeuchi et al. 2005), suggesting its contribution to the maintenance of TLO. In particular, IL-22 expression is increased in cells expressing

the long isoform of complement receptor type 2 (Cr2, also known as CD21) (Cañete et al. 2011), usually present in networks of stromal-derived follicular dendritic cells (FDCs), that contribute to the presentation of immune complexes necessary to generate activated B cells, in TLO. In synovial tissue, IL-22 is also produced by NK cells (Zhu et al. 2015). NK cells are innate immune lymphocytes with cytolytic and immune-regulatory activities representing a significant proportion (8–25%) of immune infiltration in synovial fluid of RA patients, identified in the joints in the early stage of RA development (Tak et al. 1994). Initially, NK cells were described in RA pathogenesis for their production of cytotoxic serin protease granzyme-A and B and pro-inflammatory cytokines, such as IL-1 and TNFα as dominant mediators of proliferative synovitis in RA (Klimiuk et al. 1997), supporting ocleoclastogenesis and thus involved in the development of articular damage (Kotake et al. 2001). In fact, increased production of IFNγ and TNFα characterizes synovial fluid NK cells of erosive RA patients with joint damage in comparison with non-erosive RA (Yamin et al. 2019). Recent evidence suggests that NK cells may support TLO maintenance within RA synovium as a subset of NK cells expressing a natural cytotoxicity receptor NKp44 which is able to produce IL-22 (Zhu et al. 2015). NKp44+ NK cells are enriched in both peripheral blood and synovium of RA patients secreting IL-22 and TNFα, which in vitro studies showed to support RA FLS proliferation (Ren et al. 2011), through the activation of STAT3 pathway (Zhu et al. 2015). IL-22 induced proliferation of synovial fibroblast, an effect that was inhibited by neutralizing antibodies targeting IL-22 and TNFα (Ren et al. 2011). Thus, NK cells may participate in TLO organization supporting the proliferation of synovial fibroblasts responsible for the local secretion of chemoattractant molecules and, as consequence, lymphocytes recruitment.

In addition to cells of the adaptive immunity, many other innate immunity cells and the stromal compartment have been shown to contribute to the development of synovial TLO (Barone et al. 2016).

Fibroblast-like synoviocytes (FLS), for example, have been shown to produce the T cell/dendritic cell chemoattractant CCL21 (Manzo et al. 2007) and express CXCL12 and IL-7, involved in immune cell retention and lymphoid-like microanatomical organization (Timmer et al. 2007; Bradfield et al. 2003).

Recently, we have also shown a strong association between synovial mast cells (MCs) and the presence of TLO in a large cohort of patients with early RA (Rivellese et al. 2018). MCs were also found to induce B cell activation and differentiation in vitro, including the production of ACPA autoantibodies. Finally, in animal models of inducible TLO (IL27R knockout), we confirmed the association of MCs with TLO. Overall, this points out to the relevance of MCs as potential contributors to the formation of TLO, although additional studies are needed to confirm their functional relevance (Rivellese et al. 2017, 2019b).

3.1.4 Negative Regulators of TLO Including Tfr

In addition to the mediators and pathways acting as positive regulators of TLO, several cells and cytokines have been characterized as negative regulators of TLO development.

For example, IL-27, an heterodimeric cytokines part of the IL-12 family (Yoshida and Hunter 2015), has been recently identified as a negative regulator of TLO. In fact, animals with knockout of the IL27Rα develop a severe form of antigen-induced arthritis, including the development of synovial TLO (Jones et al. 2015). Importantly, synovial TLO are not normally produced in animal models of arthritis; thus, the identification of these structures in IL-27Rα knockout animals points to the relevance of IL-27 as a regulator of TLO development. Accordingly, in patients with RA, IL-27 was found to be inversely correlated with TLO and with TLO-related gene signatures. Finally, both in clinical and experimental arthritis, synovial TLO coincided with an increased local expression of cytokines and transcription factors of the Th17 and T follicular helper (Tfh) cell lineages, where IL-27 is able to inhibit the differentiation of Th17 cells, in line with previous evidence (Stumhofer et al. 2006).

As local counterpart of the circulating T regulatory cells, T follicular regulatory cells (Tfr) have been recently described within GCs, including GCs in TLO. Tfr cells are able to prevent the differentiation of auto-reactive B cells (Wu et al. 2016; Botta et al. 2017), by regulating Tfh cells, but also by directly inhibiting B cell activation (Wing et al. 2014).

Although the relevance of Tfr cells in the regulation of GCs in animal models is well established (Linterman et al. 2011), the involvement of Tfr cells in human autoimmune disease, including RA, is still unclear.

Several studies have reported decreased levels of Tfr in patients with active RA and, accordingly, negative correlations with autoantibodies and disease activity (Romão et al. 2018; Niu et al. 2018). On the other hand, increased levels of Tfr were found in patients who were in remission (Liu et al. 2018). Using animal models of autoimmunity with spontaneous development of GCs, IL-21 was shown to induce an unbalance between Tfh and Tfr, increasing the formation of GCs, while administration of Tfr was able to restore Tfh:Tfr ration and suppress GC responses (Ding et al. 2014).

Another group found that the resolution of collagen-induced arthritis following administration of intravenous immunoglobulins was accompanied by an increase of Tfr cells (Lee et al. 2014). Taken together, this suggests that the reduction of circulating Tfr cells is associated to the development of RA and that restoration of Tfr cells could potentially improve autoimmune responses.

In line with this, monitoring the ratio between Tfr and Tfr could be useful in patients with RA, as confirmed by a several observations (Niu et al. 2018; Wang et al. 2019).

As for the function of Tfr in RA, these cells have been shown to have suppressive effects in vitro, which were enhanced in patients in remission (Liu et al. 2018). However, it has also been speculated that Tfr in autoimmune diseases might be functionally deficient (Fonseca et al. 2017).

3.2 *The Function of Tertiary Lymphoid Organs in RA*

As TLO mirrors secondary lymphoid organs in their ontogeny and maturation, it is expected that they also recapitulate the main functions of secondary lymphoid organs, which is supporting germinal centers (GC) reaction toward maturation of B cells and antibody production.

Within a considerable proportion of TLO forming in rheumatoid synovium, ectopic GC reactions take place similar to secondary lymphoid organs (Bombardieri et al. 2017). Many of RA-associated autoantibodies are high affinity IgG (e.g., ACPA) (van Delft and Huizinga 2020), and B cells forming TLO are auto-reactive and somatically mutated (Humby et al. 2009), indicating the involvement of a GC response in RA progression. Indeed, TLO in RA synovium can display functional features of germinal centers, like the expression of the enzyme activation-induced cytidine deaminase (AID) involved in in situ B cell affinity maturation and clonal selection (Humby et al. 2009).

Accordingly, the analysis of B cells isolated from the synovia of patients with RA has confirmed the generation of synovial plasma cells from locally activated B cells (Scheel et al. 2011), and the local production of class-switched autoantibodies in rheumatoid synovium has been demonstrated (Humby et al. 2009). Also, we have recently demonstrated that the presence of synovial TLO in early untreated RA is associated with autoantibody positivity (Humby et al. 2019). Interestingly, this is in contrast with previous data that failed to show an association between TLO and autoantibody positivity (Thurlings et al. 2008). Recently, comparing two large cohorts of patients with early and established RA, we were able to confirm the strong association between TLO and autoantibody positivity in early RA that could not be observed in established RA, thus explaining the previous findings, possibly because of treatment effect or other biases from long-standing diseases (Rivellese et al. 2019a).

Importantly, the initiation of a germinal center reaction requires antigen presentation to B cells. In RA, the aberrant immune response against citrullinated proteins culminating in the production of anti-citrullinated protein antibodies (ACPA) is well recognized as a key pathogenetic feature (Derksen et al. 2017).

Accordingly, citrullinated proteins have been described in the synovia of RA patients (Baeten et al. 2001) together with PAD enzymes, which are responsible for citrullination (De Rycke et al. 2005). The specificity of synovial citrullinated protein has been challenged (Vossenaar et al. 2004), but this does not come as a surprise since citrullination and other post-translational modifications of proteins are recognized as physiological processes (Trouw et al. 2017). On the contrary, the

aberrant immune response to modified proteins represents the hallmark of RA, and accordingly, the local production of ACPA in synovia has been confirmed (Humby et al. 2009; Amara et al. 2013; Masson-Bessière et al. 2000). Finally, several groups have been able to isolate ACPA-producing B cell clones from the synovia and synovial fluid of patients with RA (Germar et al. 2019; Corsiero et al. 2016, 2018).

3.3 The Clinical Relevance of Tertiary Lymphoid Organs in RA

3.3.1 TLO and Disease Severity

Early studies on the analysis of synovial membrane relied on the use arthroscopy to obtain synovial samples. These analyses pointed out a marked heterogeneity in terms of synovial inflammation, particularly in the degree of immune cell infiltration, with the description of aggregates of lymphoid cells in a proportion of patients. However, when looking for an association with clinical features, these studies yielded contradictory results: some found an association of lymphoid aggregates with disease severity and autoantibody positivity (Humby et al. 2019; Bugatti et al. 2014; Orr et al. 2017) and others did not (Thurlings et al. 2008; Cantaert et al. 2008; Van De Sande et al. 2011) (Table 1). These inconsistencies could be explained by a number of biases: (i) the exclusive analysis of large joints, in which there can be commonly overlapping osteoarthritis and are not the most representative of the inflammatory process in RA (Linn-Rasker et al. 2007) (ii) the inclusion of patients with long-standing disease, with the obvious bias of treatment and disease duration, and (iii) the lack of a gold standard for the histological assessment of immune cell infiltration (Humby et al. 2016).

The development of minimally invasive techniques such as ultrasound-guided synovial biopsies has overcome most of these limitations, as it made possible to obtain synovial tissues from small joints of a large cohort of patients with early RA and, very importantly, prior to treatment star. Thus, it is not surprising that the recently published analyses on this cohort highlighted a strong association with disease severity and autoantibody positivity (Humby et al. 2019). Interestingly, a direct comparison of early and established RA, using a validated semi-quantitative score for the assessment of B cells, showed that while in early RA the presence of B cell-rich synovitis was associated with disease severity, this was not the case in established RA, possibly explaining the discrepancies from previous studies analyzing patient with different disease duration Rivellese et al. (2019a).

When analyzing exclusively patients with early untreated RA, our group has recently shown that patients with a synovial lympho-myeloid pathotype, characterized by the presence of B and T cell aggregates, have significantly higher disease severity, autoantibody positivity, and baseline erosive load (Humby et al. 2019). Furthermore, molecular analyses showed that myeloid- and lymphoid-associated

Layout: T1 Standard Series ID: 82 MS No.: CTMIKenji Kahashima_Costantino

Table 1 Association of TLO with disease severity and clinical phenotype in RA

Author and year	References	Population	Joints biopsied and procedure	Treatment (if any)	Time points	Analyses	Results
Van Oosterhout 2008	Van Oosterhout et al. (2008)	57 RA	Knee arthroscopy	N.a.	Biopsy at time 0	IHC	ACPA + patients showed higher mean number of infiltrating lymphocytes and higher rate of local joint destruction
Van de Sande 2011	Van De Sande et al. (2011)	93 (24 RA)	Knee arthroscopy	sDMARDs	Biopsy at 0 (93) and 6 months (17)	IHC	Lymphoid neogenesis present in 36% of all patients, associated with the degree of synovial inflammation, but not specific of RA. No relationship between the presence of lymphocyte aggregates at baseline and definitive diagnosis or clinical outcome after follow-up
De Hair 2013	De Hair et al. (2013)	55 seropositive individuals without clinical evidence of arthritis	Knee arthroscopy	N.a.	Biopsy at time 0	IHC	CD3 T cell numbers in the biopsy tissue showed a borderline association with subsequent development of clinically manifest arthritis. CD8 T cells were associated with ACPA positivity
Gómez-Puerta 2013	Gómez-Puerta et al. (2013)	83 RA	Knee arthroscopy	N.a.	Biopsy at time 0	IHC	No significant differences in clinical variables, acute phase reactants, synovial cell infiltrate or lymphoid neogenesis (LN) between ACPA positive and negative patients
Orr 2017	Orr et al. (2017)	123 RA	Knee arthroscopy	sDMARDs and bDMARDs	Biopsy at time 0	IHC	ACPA + RA patients were characterized by significantly higher levels of CD19+ B cells and CD3+ and CD8+ T cells. Levels of lymphoid aggregates of CD19+ B cells and serum CXCL13 levels were significantly

(continued)

Layout: T1 Standard
Series ID: 82
MS No.: CTMIKenji Kahashima_Costantino

Table 1 (continued)

Author and year	References	Population	Joints biopsied and procedure	Treatment (if any)	Time points	Analyses	Results
							higher in ACPA + patients. EULAR response was significantly associated with the level of CD3+ T cell infiltrates, while CD68+ macrophage and CD8+ T cell levels were predictive of the response to tumor necrosis factor inhibitors
Humby 2019	Humby et al. (2019)	144 early (<1 year) treatment naïve RA	US-guided synovial biopsy	sDMARDs	Biopsy at time 0	IHC and nanostring	Patients with a lympho-myeloid pathotype have significantly higher disease severity, autoantibody positivity, and baseline erosive load. Myeloid- and lymphoid-associated gene expression strongly correlated with disease activity and acute phase reactants
Lliso-Ribera 2019	Lliso-Ribera et al. (2019)	200 early patients with inflammatory arthritis	US-guided synovial biopsy	sDMARDs	Biopsy at time 0	IHC	Patients fulfilling the 1987 RA criteria had significantly higher levels of disease activity, histological synovitis, degree of immune cell infiltration, and differential upregulation of genes involved in B and T cell activation/function compared with RA 2010 criteria or UA, which shared similar clinical and pathobiological feature

genes strongly correlate with disease activity and acute phase reactants. Another more recent publication in early RA has further highlighted the value of synovial tissue analyses in refining the diagnosis of RA vs undifferentiated arthritis.

Moreover, deep phenotyping of synovial tissue by molecular analyses has identified specific gene signatures associated with clinical phenotype. In particular, for example, peripheral blood interferon response genes were associated with the lymhpo-myeloid pathotype, while synovial plasma cell signature was associated with progression of structural damage (Lewis et al. 2019). Additional analyses from the Accelerating Medicine Partners group, by integrating single cell RNA sequencing and mass cytometry, have recently identified unique cell population expanded in RA synovia that allow to distinguish the degree of synovial inflammation (Rao et al. 2017; Zhang et al. 2019). Specific cell populations included HY1 (CD90) +HLA-DRAhi sublining fibroblasts, IL1B+ pro-inflammatory monocytes, ITGAX + TBX21 + autoimmune-associated B cells, and PDCD1+ peripheral helper T (TPH) cells and follicular helper T (TFH) cells. The latter, in particular, are essential for the formation of TLO and have been already discussed in the previous paragraph. However, to date, little is known about the association of these cell types with disease features, such as disease severity, progression, and response to treatment. In the near future, it will be of utmost importance to confirm the relevance of these immune populations, by studying their association with clinical phenotype in larger cohorts of patients with RA.

3.3.2 TLO as Direct Therapeutic Targets

Because of their well-established relevance in driving the pathogenesis of RA and their association with worse disease outcomes, several strategies aiming at targeting TLO in RA have also been tested.

A number of studies have attempted to target mediators that are relevant in the formation or maintenance of TLO. The modulation of the IL-21/IL-21R pathway as a treatment strategy was first tested in experimental models of RA. IL-21R deficiency in the K/BxN mouse model of inflammatory arthritis (Kim et al. 2009) and antigen-induced arthritis (Roeleveld et al. 2017) is sufficient to block RA initiation, while the blockade of the IL-21/IL-21R pathway ameliorates disease in collagen-induced arthritis models treated with murine IL-21R Fc fusion protein (Young et al. 2007). However, there are still no data in patients with RA on the blockade of IL-21/IL-21R.

Some other molecules have been already tested in patients, but results have not been particularly striking, as in the case of inhibiting LTβ, which did not show clinical efficacy (Bienkowska et al. 2014). Similarly, drugs inhibiting IL-17 and IL-12/IL-23 showed little or no differences compared with placebo in RA (Kerschbaumer et al. 2019). This is in contrast with data on seronegative arthritis, where inhibition of IL-17 and its axis proved to be extremely effective, although it has been suggested that the analysis of targeted expression of these molecules could potentially help in predicting treatment response (Boutet et al. 2018).

Importantly, none of the above studies targeting mediators involved in TLO formation or maintenance in RA has stratified patients on the basis of TLO presence, which could have helped in selecting patients with higher chances of response.

3.3.3 TLO as Predictors of Treatment Response

As highlighted in the previous paragraphs, the presence of TLO is able to identify a subset of RA patients with a specific disease phenotype, specifically higher disease activity and higher prevalence of autoantibodies. Therefore, it is plausible to hypothesize that the presence of TLO could help to predict treatment response. A number of studies have explored the analyses of synovial tissues to predict treatment response. However, relatively few included the systematic analysis of TLO. Futhermore, because of the relatively small number of patients, the inconsistency in the definition of TLO, and the use of different time points for repeated biopsy, most of the results are fragmented and difficult to interpret.

Canete et al., for example, demonstrated significantly lower response in patients who were TLO positive despite a significantly higher use of anti-TNFa agents. (Cañete et al. 2009) By linear regression, TLO positive were found to predict lack of response to anti-TNFa. In this study, however, patients started sequential treatment with escalation to anti-TNFa in non-responders, and therefore, there could have been a selection of TLO + patients as the most severe, thus non-responders.

On the contrary, Klaasen et al., by analyzing synovial samples obtained before and after standardized treatment with infliximab in a cohort of 97 patients, found that the presence of TLO at baseline was a highly significant predictor of the clinical response to anti-TNF treatment (Klaasen et al. 2009).

More recently, Dennis et al. provided the molecular confirmation of the histological pathotypes previously described by histology. In addition, by analyzing the data from a previous cohort undergoing treatment with infliximab, they were able to identify TLO signature as predictor of response to TNFi (Dennis et al. 2014). The limitation of this manuscript consisted in the analysis of synovial samples obtained from arthroplasty, thus without standardization of treatment.

The observations published from our early RA cohort allowed to overcome such limitations and have shown a reduction of lymphoid-associated genes in EULAR good responders to csDMARDs (Humby et al. 2019). Similarly, molecular analyses by RNAseq identified a number of cell modules, including B cells, in association with B response to csDMARDs (Lewis et al. 2019). Importantly, these data come from the analysis of synovial tissue obtained by US-guided synovial biopsies in untreated patients with early Rheumatoid Arthritis, thus eliminating the bias of long-standing disease, treatment or the exclusive inclusion of large joints in studies based on arthroscopy.

In recent years, continuing on the same line, two international consortia have driven the delivery of the first two large-scale biopsy-driven RCTs in Rheumatoid Arthritis. As part of a study funded by the UK National Institute of Health AQ2

Research, a randomized, open labeled study in anti-TNFa inadequate responders to investigate the mechanisms for Response—resistance to rituximab versus tocilizumab in RA (R4-RA), a total of 165 patients failing treatment with TNFi have been recruited. Promising preliminary results were presented at the ACR 2019, while the trial is currently being analyzed and final results will be soon published. Similarly, as part of the MRC and versus arthritis-funded consortium maximizing therapeutic utility in RA (MATURA), the stratifying therapies for rheumatoid arthritis by pathobiology (STRAP) RCT has enrolled a total of 226 patients who failed csDMARDs and is due to being completed in the last quarter of 2020.

These studies have been appropriately powered and thus will hopefully give clear answers on the utility of synovial biopsy analysis in predicting treatment response in RA. Specifically, the studies aimed at understanding if patients lacking synovial B cells have a lower response to B cell targeted treatment (Rituximab) as opposed to other treatments. At the same time, the studies will provide invaluable information to answer additional research questions, including the association of TLO with disease severity, progression, and treatment response.

4 Conclusions

Here, we offered a comprehensive review on the relevance of synovial TLO in RA. The data presented indicate that the ontogeny of TLO resembles the development of secondary lymphoid organs, since many of the mediators known to be involved in lymphoneogenesis have been identified in the synotia of RA patients. Importantly, these structures are fully functional, as they induce the local maturation of B cells toward the production of autoantibodies. Their presence has been described in about 40% of patients with RA from early disease stages and has been strongly associated with disease severity and progression. Despite the availability of several drug treatments that can directly or indirectly target TLO and their components, a stratified medicine approach is needed to fully appreciate the potential effect of such treatments.

Bibliography

Amara K, Steen J, Murray F, Morbach H, Fernandez-Rodriguez BM, Joshua V et al (2013) Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. J Exp Med. https://doi.org/10.1084/jem.20121486

Baeten D, Peene I, Union A, Meheus L, Sebbag M, Serre G et al (2001) Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium: Relevance to antifilaggrin autoantibodies. Arthritis Rheum. https://doi.org/10.1002/1529-0131(200110)44:10%3c2255:aid-art388%3e3.0.co;2-%23

513 Bar-Ephraim YE, Mebius RE (2016) Innate lymphoid cells in secondary lymphoid organs.
514 Immunol Rev 185–99. https://doi.org/10.1111/imr.12407
515 Barone F, Nayar S, Campos J, Cloake T, Withers DR, Toellner K-M et al (2015) IL-22 regulates
516 lymphoid chemokine production and assembly of tertiary lymphoid organs. Proc Natl Acad Sci
517 112:11024–11029. https://doi.org/10.1073/pnas.1503315112
518 Barone F, Gardner DH, Nayar S, Steinthal N, Buckley CD, Luther SA (2016) Stromal fibroblasts
519 in tertiary lymphoid structures: a novel target in chronic inflammation. Front Immunol 7:477.
520 https://doi.org/10.3389/fimmu.2016.00477
521 Bienkowska J, Norm A, Thai A, Goyal J, Plavina T, Nirula A et al (2014). Lymphotoxin-LIGHT
522 pathway regulates the interferon signature in rheumatoid arthritis. PLoS One 9. https://doi.org/
523 10.1371/journal.pone.0112545
524 Bombardieri M, Lewis M, Pitzalis C (2017) Ectopic lymphoid neogenesis in rheumatic
525 autoimmune diseases. Nat Rev Rheumatol 13:141–154. https://doi.org/10.1038/nrrheum.
526 2016.217
527 Botta D, Fuller MJ, Marquez-Lago TT, Bachus H, Bradley JE, Weinmann AS et al (2017)
528 Dynamic regulation of T follicular regulatory cell responses by interleukin 2 during influenza
529 infection. Nat Immunol. https://doi.org/10.1038/ni.3837
530 Boutet MA, Nerviani A, Gallo Afflitto G, Pitzalis C (2018) Role of the IL-23/IL-17 axis in
531 psoriasis and psoriatic arthritis: The clinical importance of its divergence in skin and joints.
532 Int J Mol Sci 19. https://doi.org/10.3390/ijms19020530
533 Bradfield PF, Amft N, Vernon-Wilson E, Exley AE, Parsonage G, Rainger GE et al (2003)
534 Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor
535 1 (CXCL12), which supports distinct patterns and rates of $CD4^+$ and $CD8^+$ T cell migration
536 within synovial tissue. Arthritis Rheum 48:2472–2482. https://doi.org/10.1002/art.11219
537 Bugatti S, Caporali R, Manzo A, Vitolo B, Pitzalis C, Montecucco C (2005) Involvement of
538 subchondral bone marrow in rheumatoid arthritis: lymphoid neogenesis and in situ relationship
539 to subchondral bone marrow osteoclast recruitment. Arthritis Rheum 52:3448–3459
540 Bugatti S, Manzo A, Vitolo B, Benaglio F, Binda E, Scarabelli M et al (2014) High expression
541 levels of the B cell chemoattractant CXCL13 in rheumatoid synovium are a marker of severe
542 disease. Rheumatol (United Kingdom) 53:1886–1895. https://doi.org/10.1093/rheumatology/
543 keu163
544 Cañete JD, Celis R, Moll C, Izquierdo E, Marsal S, Sanmartí R et al (2009) Clinical significance of
545 synovial lymphoid neogenesis and its reversal after anti-tumour necrosis factor alpha therapy in
546 rheumatoid arthritis. Ann Rheum Dis 68:751–756
547 Cañete JD, Celis R, Yeremenko N, Sanmartí R, Van Duivenvoorde L, Ramírez J et al (2011)
548 Ectopic lymphoid neogenesis is strongly associated with activation of the IL-23 pathway in
549 rheumatoid synovitis. https://doi.org/10.1186/s13075-015-0688-0
550 Cantaert T, Kolln J, Timmer T, van der Pouw Kraan TC, Vandooren B, Thurlings RM et al (2008)
551 B lymphocyte autoimmunity in rheumatoid synovitis is independent of ectopic lymphoid
552 neogenesis. J Immunol 181:785–794. https://doi.org/10.4049/jimmunol.181.1.785
553 Chabaud M, Durand JM, Buchs N, Fossiez F, Page G, Frappart L et al (1999) Human
554 interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid
555 synovium. Arthritis Rheum 42:963–970. https://doi.org/10.1002/1529-0131(199905)42:5%
556 3c963:AID-ANR15%3e3.0.CO;2-E
557 Chu Y, Wang F, Zhou M, Chen L, Lu Y (2014) A preliminary study on the characterization of
558 follicular helper T (Tfh) cells in rheumatoid arthritis synovium. Acta Histochem 116:539–543.
559 https://doi.org/10.1016/j.acthis.2013.10.009
560 Corsiero E, Bombardieri M, Manzo A, Bugatti S, Uguccioni M, Pitzalis C (2012) Role of
561 lymphoid chemokines in the development of functional ectopic lymphoid structures in
562 rheumatic autoimmune diseases. Immunol Lett 145:62–67. https://doi.org/10.1016/j.imlet.
563 2012.04.013
564 Corsiero E, Bombardieri M, Carlotti E, Pratesi F, Robinson W, Migliorini P et al (2016) Single cell
565 cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal

frequent targeting of citrullinated histones of NETs. Ann Rheum Dis 75:1866–1875. https://doi.org/10.1136/annrheumdis-2015-208356

Corsiero E, Jagemann L, Perretti M, Pitzalis C, Bombardieri M (2018) Characterization of a synovial B cell-derived recombinant monoclonal antibody targeting stromal calreticulin in the rheumatoid joints. J Immunol 201:1373–1381. https://doi.org/10.4049/jimmunol.1800346

de Hair MJH, van de Sande MGH, Ramwadhdoebe TH, Hansson M, Landewé R, van der Leij C et al (2013) Features of the synovium of individuals at risk of developing rheumatoid arthritis: implications for understanding preclinical rheumatoid arthritis. Arthritis Rheum 66:513–522. https://doi.org/10.1002/art.38273

De Rycke L, Nicholas AP, Cantaert T, Kruithof E, Echols JD, Vandekerckhove B et al (2005) Synovial intracellular citrullinated proteins colocalizing with peptidyl arginine deiminase as pathophysiologically relevant antigenic determinants of rheumatoid arthritis-specific humoral autoimmunity. Arthritis Rheum. https://doi.org/10.1002/art.21220

De Silva NS, Klein U (2015) Dynamics of B cells in germinal centres. Nat Rev Immunol 15:137–148. https://doi.org/10.1038/nri3804

Dennis G, Holweg CTJ, Kummerfeld SK, Choy DF, Setiadi AF, Hackney JA et al (2014) Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. Arthritis Res Ther 16:R90. https://doi.org/10.1186/ar4555

Derksen VFAM, Huizinga TWJ, van der Woude D (2017) The role of autoantibodies in the pathophysiology of rheumatoid arthritis. Semin Immunopathol 39:437–446. https://doi.org/10.1007/s00281-017-0627-z

Ding Y, Li J, Yang PA, Luo B, Wu Q, Zajac AJ et al (2014) Interleukin-21 promotes germinal center reaction by skewing the follicular regulatory T cell to follicular helper T cell balance in autoimmune BXD2 mice. Arthritis Rheumatol. https://doi.org/10.1002/art.38735

Drayton DL, Liao S, Mounzer RH, Ruddle NH (2006) Lymphoid organ development: from ontogeny to neogenesis. Nat Immunol 7:344–353. https://doi.org/10.1038/ni1330

Fonseca VR, Agua-Doce A, Maceiras AR, Pierson W, Ribeiro F, Romão VC et al (2017) Human blood Tfr cells are indicators of ongoing humoral activity not fully licensed with suppressive function. Sci Immunol. https://doi.org/10.1126/sciimmunol.aan1487

Gensous N, Charrier M, Duluc D, Contin-Bordes C, Truchetet ME, Lazaro E et al (2018) T follicular helper cells in autoimmune disorders. Front Immunol 9:1637. https://doi.org/10.3389/fimmu.2018.01637

Germar K, Fehres CM, Scherer HU, van Uden N, Pollastro S, Yeremenko N et al (2019) Generation and characterization of anti-citrullinated protein antibody-producing B cell clones from rheumatoid arthritis patients. Arthritis Rheum. https://doi.org/10.1002/art.40739

Gómez-Puerta JA, Celis R, Hernández MV, Ruiz-Esquide V, Ramírez J, Haro I et al (2013) Differences in synovial fluid cytokine levels but not in synovial tissue cell infiltrate between anti-citrullinated peptide/protein antibody-positive and -negative rheumatoid arthritis patients. Arthritis Res Ther 15:R182. https://doi.org/10.1186/ar4372

He J, Tsai LM, Leong YA, Hu X, Ma CS, Chevalier N et al (2013) Circulating precursor CCR7loPD-1hi CXCR5^{+} CD4^{+} T cells indicate Tfh cell activity and promote antibody responses upon antigen reexposure. Immunity 39:770–781. https://doi.org/10.1016/j.immuni.2013.09.007

Humby F, Bombardieri M, Manzo A, Kelly S, Blades MC, Kirkham B et al (2009) Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. PLoS Med 6:e1. https://doi.org/10.1371/journal.pmed.0060001

Humby F, Kelly S, Bugatti S, Manzo A, Filer A, Mahto A et al (2016) Evaluation of minimally invasive, ultrasound-guided synovial biopsy techniques by the OMERACT filter—determining validation requirements. J Rheumatol 43:208–213. https://doi.org/10.3899/jrheum.141199

Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M et al (2019) Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis 78:761–772. https://doi.org/10.1136/annrheumdis-2018-214539

AQ3

Ikeuchi H, Kuroiwa T, Hiramatsu N, Kaneko Y, Hiromura K, Ueki K et al (2005) Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum 52:1037–1046. https://doi.org/10.1002/art.20965

Jones GW, Jones SA (2016) Ectopic lymphoid follicles: inducible centres for generating antigen-specific immune responses within tissues. Immunology 147:141–151. https://doi.org/10.1111/imm.12554

Jones GW, Bombardieri M, Greenhill CJ, McLeod L, Nerviani A, Rocher-Ros V et al (2015) Interleukin-27 inhibits ectopic lymphoid-like structure development in early inflammatory arthritis. J Exp Med 212:1793–1802. https://doi.org/10.1084/jem.20132307

Jüngel A, Distler JHW, Kurowska-Stolarska M, Seemayer CA, Seibl R, Forster A et al (2004) Expression of interleukin-21 receptor, but not interleukin-21, in synovial fibroblasts and synovial macrophages of patients with rheumatoid arthritis. Arthritis Rheum 50:1468–1476. https://doi.org/10.1002/art.20218

Karnell JL, Ettinger R (2012) The interplay of IL-21 and BAFF in the formation and maintenance of human B cell memory. Front Immunol 3. https://doi.org/10.3389/fimmu.2012.00002

Kerschbaumer A, Sepriano A, Smolen JS, van der Heijde D, Dougados M, van Vollenhoven R et al (2019) Efficacy of pharmacological treatment in rheumatoid arthritis: a systematic literature research informing the, update of the EULAR recommendations for management of rheumatoid arthritis. Ann Rheum Dis 2020:1–15. https://doi.org/10.1136/annrheumdis-2019-216656

Kim JM, Youn J, Jang E, Cho S-H, Park H, Paik D-J (2009) K/BxN mice development of arthritis in autoimmune cell expansion is essential for the T−CD25+ provoked by homeostatic CD4 a positive feedback loop of IL-21 signaling. J Immunol Ref 182:4649–4656. https://doi.org/10.4049/jimmunol.0804350

Klaasen R, Thurlings RM, Wijbrandts CA, van Kuijk AW, Baeten D, Gerlag DM et al (2009) The relationship between synovial lymphocyte aggregates and the clinical response to infliximab in rheumatoid arthritis: a prospective study. Arthritis Rheum 60:3217–3224

Klimiuk PA, Goronzy JJ, Björnsson J, Beckenbaugh RD, Weyand CM (1997) Tissue cytokine patterns distinguish variants of rheumatoid synovitis. Am J Pathol 151:1311–1319

Kotake S, Udagawa N, Hakoda M, Mogi M, Yano K, Tsuda E et al (2001) Activated human T cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone destruction in rheumatoid arthritis patients. Arthritis Rheum 44:1003–1012. https://doi.org/10.1002/1529-0131(200105)44:5%3c1003:aid-anr179%3e3.0.co;2-%23

Kwok SK, La Cho M, Park MK, Oh HJ, Park JS, Her YM et al (2012) Interleukin-21 promotes osteoclastogenesis in humans with rheumatoid arthritis and in mice with collagen-induced arthritis. Arthritis Rheum 64:740–751. https://doi.org/10.1002/art.33390

Lee SY, Jung YO, Ryu JG, Kang CM, Kim EK, Son HJ et al (2014) Intravenous immunoglobulin attenuates experimental autoimmune arthritis by inducing reciprocal regulation of th17 and treg cells in an interleukin-10- dependent manner. Arthritis Rheum. https://doi.org/10.1002/art.38627

Lewis MJ, Barnes MR, Blighe K, Goldmann K, Rana S, Hackney JA et al (2019) Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. Cell Rep 28(2455–2470):e5. https://doi.org/10.1016/j.celrep.2019.07.091

Linn-Rasker SP, Van Der Helm-Van Mil a. HM, Breedveld FC, Huizinga TWJ (2007) Arthritis of the large joints—in particular, the knee—at first presentation is predictive for a high level of radiological destruction of the small joints in rheumatoid arthritis. Ann Rheum Dis 66:646–650. https://doi.org/10.1136/ard.2006.066704

Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF et al (2011) Foxp3 + follicular regulatory T cells control the germinal center response. Nat Med 17:975–982. https://doi.org/10.1038/nm.2425

Liu D, Xu H, Shih C, Wan Z, Ma X, Ma W et al (2015) T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. Nature 517:214–218. https://doi.org/10.1038/nature13803

672 Liu C, Wang D, Lu S, Xu Q, Zhao L, Zhao J et al (2018) Increased circulating follicular Treg cells
673 are associated with lower levels of auto antibodies in patients with rheumatoid arthritis in stable
674 remission. Arthritis Rheum. https://doi.org/10.1002/art.40430
675 Lliso-Ribera G, Humby F, Lewis M (n.d.) Synovial tissue signatures enhance clinical classification
676 and prognostic/treatment response algorithms in early inflammatory arthritis and predict
677 requirement for subsequent biologic therapy: results from the Pathobiology of Early Arthritis
678 Cohort (PEAC). Ann Rheum Dis https://doi.org/10.1136/annrheumdis-2019-215751, https://
679 doi.org/10.1136/annrheumdis-2019-215751
680 Lliso-Ribera G, Humby F, Lewis M, Nerviani A, Mauro D, Rivellese F et al (2019) Synovial tissue
681 signatures enhance clinical classification and prognostic/treatment response algorithms in early
682 inflammatory arthritis and predict requirement for subsequent biological therapy: results from
683 the pathobiology of early arthritis cohort (PEA. Ann Rheum Dis 78:1642–1652. https://doi.org/
684 10.1136/annrheumdis-2019-215751
685 Luther SA, Lopez T, Bai W, Hanahan D, Cyster JG (2000) BLC expression in pancreatic islets
686 causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. Immunity
687 12:471–481. https://doi.org/10.1016/s1074-7613(00)80199-5
688 Ma J, Zhu C, Ma B, Tian J, Baidoo SE, Mao C et al (2012) Increased frequency of circulating
689 follicular helper T cells in patients with rheumatoid arthritis. Clin Dev Immunol 2012:827480.
690 https://doi.org/10.1155/2012/827480
691 Manzo A, Paoletti S, Carulli M, Blades MC, Barone F, Yanni G et al (2005) Systematic
692 microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid
693 organization in rheumatoid synovitis. Eur J Immunol 35:1347–1359. https://doi.org/10.1002/
694 eji.200425830
695 Manzo A, Bugatti S, Caporali R, Prevo R, Jackson DG, Uguccioni M et al (2007) CCL21
696 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory
697 lesions with lymphoid neogenesis. Am J Pathol. https://doi.org/10.2353/ajpath.2007.061275
698 Manzo A, Vitolo B, Humby F, Caporali R, Jarrossay D, Dell'accio F et al (2008) Mature
699 antigen-experienced T helper cells synthesize and secrete the B cell chemoattractant CXCL13
700 in the inflammatory environment of the rheumatoid joint. Arthritis Rheum 58:3377–3387.
701 https://doi.org/10.1002/art.23966
702 Masson-Bessière C, Sebbag M, Durieux JJ, Nogueira L, Vincent C, Girbal-Neuhauser E et al
703 (2000) In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma
704 cells and constitute a higher proportion of IgG than in synovial fluid and serum. Clin Exp
705 Immunol. https://doi.org/10.1046/j.1365-2249.2000.01171.x
706 McCausland MM, Yusuf I, Tran H, Ono N, Yanagi Y, Crotty S (2014) SAP regulation of follicular
707 helper CD4 T cell development and humoral immunity is independent of SLAM and Fyn
708 Kinase. J Immunol 178:817–828. https://doi.org/10.4049/jimmunol.178.2.817
709 McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. N Engl J Med 365:2205–
710 2219. https://doi.org/10.7748/phc2011.11.21.9.29.c8797
711 McInnes IB, Schett G (2017) Pathogenetic insights from the treatment of rheumatoid arthritis.
712 Lancet 389:2328–2337. https://doi.org/10.1016/S0140-6736(17)31472-1
713 Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G et al (2011) Human
714 blood CXCR5+ CD4+ T cells are counterparts of T follicular cells and contain specific subsets
715 that differentially support antibody secretion. Immunity 34:108–121. https://doi.org/10.1016/j.
716 immuni.2010.12.012
717 Moschovakis GL, Bubke A, Friedrichsen M, Falk CS, Feederle R, Förster R (2017) T cell specific
718 Cxcr5 deficiency prevents rheumatoid arthritis. Sci Rep 7. https://doi.org/10.1038/s41598-017-
719 08935-6
720 Nayar S, Campos J, Smith CG, Iannizzotto V, Gardner DH, Mourcin F et al (2019)
721 Immunofibroblasts are pivotal drivers of tertiary lymphoid structure formation and local
722 pathology. Proc Natl Acad Sci 116:13490–13497. https://doi.org/10.1073/pnas.1905301116
723 Níu Q, Huang Z, Wu X, Jin Y, An Y, Li Y et al (2018) Enhanced IL-6/phosphorylated STAT3
724 signaling is related to the imbalance of circulating T follicular helper/T follicular regulatory

cells in patients with rheumatoid arthritis. Arthritis Res Ther. https://doi.org/10.1186/s13075-018-1690-0

Orr C, Najm A, Biniecka M, McGarry T, Ng C-T, Young F et al (2017) Synovial immunophenotype and anti-citrullinated peptide antibodies in rheumatoid arthritis patients: relationship to treatment response and radiologic prognosis. Arthritis Rheum (Hoboken, NJ) 69:2114–2123. https://doi.org/10.1002/art.40218

Penatti A, Facciotti F, De Matteis R, Larghi P, Paroni M, Murgo A et al (2017) Differences in serum and synovial CD4+ T cells and cytokine profiles to stratify patients with inflammatory osteoarthritis and rheumatoid arthritis. Arthritis Res Ther 19:103. https://doi.org/10.1186/s13075-017-1305-1

Pitzalis C, Kelly S, Humby F (2013) New learnings on the pathophysiology of RA from synovial biopsies. Curr Opin Rheumatol 25:334–344. https://doi.org/10.1097/BOR.0b013e32835fd8eb

Randall TD, Carragher DM, Rangel-Moreno J (2008) Development of Secondary lymphoid organs. Annu Rev Immunol 26:627–650. https://doi.org/10.1146/annurev.immunol.26.021607.090257

Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y et al (2017) Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nat Publ Gr 542:110–114. https://doi.org/10.1038/nature20810

Ren J, Feng Z, Lv Z, Chen X, Li J (2011) Natural killer-22 cells in the synovial fluid of patients with rheumatoid arthritis are an innate source of interleukin 22 and tumor necrosis factor-α. J Rheumatol 38:2112–2118. https://doi.org/10.3899/jrheum.101377

Rivellese F, Nerviani A, Rossi FW, Marone G, Matucci-Cerinic M, de Paulis A et al (2017) Mast cells in rheumatoid arthritis: friends or foes? Autoimmun Rev 16:557–563. https://doi.org/10.1016/j.autrev.2017.04.001

Rivellese F, Mauro D, Nerviani A, Pagani S, Fossati-Jimack L, Messemaker T et al (2018) Mast cells in early rheumatoid arthritis associate with disease severity and support B cell autoantibody production. Ann Rheum Dis 77:1773–1781

Rivellese F, Humby F, Bugatti S, Fossati-Jimack L, Rizvi H, Lucchesi D et al (2019a) B cell synovitis and clinical phenotypes in rheumatoid arthritis: relationship to disease stages and drug exposure. Arthritis Rheum 4–11. https://doi.org/10.1002/art.41184

Rivellese F, Rossi FW, Galdiero MR, Pitzalis C, de Paulis A (2019b) Mast cells in early rheumatoid arthritis. Int J Mol Sci 20:1–13. https://doi.org/10.3390/ijms20082040

Roeleveld DM, Marijnissen RJ, Walgreen B, Helsen MM, Van Den Bersselaar L, Van De Loo FA et al (2017) Higher efficacy of anti-IL-6/IL-21 combination therapy compared to monotherapy in the induction phase of Th17-driven experimental arthritis 2017. https://doi.org/10.1371/journal.pone.0171757

Romão VC, Fonseca JE, Agua-Doce A, Graca L (2018) T follicular regulatory cells are decreased in patients with established treated rheumatoid arthritis with active disease: comment on the article by Liu et al. Arthritis Rheum. https://doi.org/10.1002/art.40586

Ronday HK, Van Der Laan WH, Tak PP, De Roos JADM, Bank RA, Tekoppele JM et al (n.d.) Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis

Sawa S, Cherrier M, Lochner M, Satoh-Takayama N, Fehling HJ, Langa F et al (2010) Lineage relationship analysis of RORγt+ innate lymphoid cells. Science (80-) 330:665–669. https://doi.org/10.1126/science.1194597

Scheel T, Gursche A, Zacher J, Berek C (2011) V-region gene analysis of locally defined synovial B and plasma cells reveals selected B cell expansion and accumulation of plasma cell clones in rheumatoid arthritis. Arthritis Rheum. https://doi.org/10.1002/art.27767

Smolen JS, Aletaha D, McInnes IB (2016) Rheumatoid arthritis. Lancet 388:2023–2038. https://doi.org/10.1016/S0140-6736(16)30173-8

Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM et al (2006) Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. Nat Immunol. https://doi.org/10.1038/ni1376

Tak PP, Kummer JA, Hack CE, Daha MR, Smeets TJM, Erkelens GW et al (1994) Granzyme-positive cytotoxic cells are specifically increased in early rheumatoid synovial tissue. Arthritis Rheum 37:1735–1743. https://doi.org/10.1002/art.1780371205

Takemura S, Braun A, Crowson C, Kurtin PJ, Cofield RH, O'Fallon WM et al (2001) Lymphoid neogenesis in rheumatoid synovitis. J Immunol. https://doi.org/10.4049/jimmunol.167.2.1072

Thurlings RM, Wijbrandts CA, Mebius RE, Cantaert T, Dinant HJ, Van Der Pouw-Kraan TCTMTM et al (2008) Synovial lymphoid neogenesis does not define a specific clinical rheumatoid arthritis phenotype. Arthritis Rheum 58:1582–1589. https://doi.org/10.1002/art.23505

Timmer TCG, Baltus B, Vondenhoff M, Huizinga TWJ, Tak PP, Verweij CL et al (2007) Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected by genomics technology: identification of the interleukin-7 signaling pathway in tissues with lymphoid neogenesis. Arthritis Rheum 56:2492–2502. https://doi.org/10.1002/art.22748

Trouw LA, Rispens T, Toes REM (2017) Beyond citrullination: other post-translational protein modifications in rheumatoid arthritis. Nat Rev Rheumatol. https://doi.org/10.1038/nrrheum.2017.15

Tsai LM, Yu D (2014) Follicular helper T-cell memory: establishing new frontiers during antibody response. Immunol Cell Biol 92:57–63. https://doi.org/10.1038/icb.2013.68

Ueno H, Banchereau J, Vinuesa CG (2015) Pathophysiology of T follicular helper cells in humans and mice. Nat Immunol 16:142–152. https://doi.org/10.1038/ni.3054

Van De Sande MGHH, Thurlings RM, Boumans MJHH, Wijbrandts CA, Modesti MG, Gerlag DM et al (2011) Presence of lymphocyte aggregates in the synovium of patients with early arthritis in relationship to diagnosis and outcome: is it a constant feature over time? Ann Rheum Dis 70:700–703. https://doi.org/10.1136/ard.2010.139287

van Delft MAM, Huizinga TWJ (2020) An overview of autoantibodies in rheumatoid arthritis. J Autoimmun. https://doi.org/10.1016/j.jaut.2019.102392

Van Oosterhout M, Bajema I, Levarht EWN, Toes REM, Huizinga TWJ, Van Laar JM (2008) Differences in synovial tissue infiltrates between anti-cyclic citrullinated peptide-positive rheumatoid arthritis and anti-cyclic citrullinated peptide-negative rheumatoid arthritis. Arthritis Rheum 58:53–60. https://doi.org/10.1002/art.23148

Vossenaar ER, Smeets TJM, Kraan MC, Raats JM, Van Venrooij WJ, Tak PP (2004) The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. Arthritis Rheum. https://doi.org/10.1002/art.20584

Wang X, Yang C, Xu F, Qi L, Wang J, Yang P (2019) Imbalance of circulating Tfr/Tfh ratio in patients with rheumatoid arthritis. Clin Exp Med. https://doi.org/10.1007/s10238-018-0530-5

Willemze A, Trouw LA, Toes REM, Huizinga TWJ (2012) The influence of ACPA status and characteristics on the course of RA. Nat Rev Rheumatol 8:144–152. https://doi.org/10.1038/nrrheum.2011.204

Wing JB, Ise W, Kurosaki T, Sakaguchi S (2014) Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4. Immunity. https://doi.org/10.1016/j.immuni.2014.12.006

Wu H, Chen Y, Liu H, Xu LL, Teuscher P, Wang S et al (2016) Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice. Eur J Immunol. https://doi.org/10.1002/eji.201546094

Xing R, Jin Y, Sun L, Yang L, Li C, Li Z et al (2016) Interleukin-21 induces migration and invasion of fibroblast-like synoviocytes from patients with rheumatoid arthritis. Clin Exp Immunol 184:147–158. https://doi.org/10.1111/cei.12751

Yamin R, Berhani O, Peleg H, Aamar S, Stein N, Gamliel M et al (2019) High percentages and activity of synovial fluid NK cells present in patients with advanced stage active rheumatoid arthritis. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-018-37448-z

Yoshida H, Hunter CA (2015) The immunobiology of interleukin-27. Annu Rev Immunol. https://doi.org/10.1146/annurev-immunol-032414-112134

Young DA, Hegen M, Ma HLR, Whitters MJ, Albert LM, Lowe L et al (2007) Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis. Arthritis Rheum 56:1152–1163. https://doi.org/10.1002/art.22452

Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S et al (2019) Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol. https://doi.org/10.1038/s41590-019-0378-1

Zhu J, Jia E, Zhou Y, Xu J, Feng Z, Wang H et al (2015) Interleukin-22 secreted by NKp44 + natural killer cells promotes proliferation of fibroblast-like synoviocytes in rheumatoid arthritis. Medicine (Baltimore) 94:e2137. https://doi.org/10.1097/MD.0000000000002137

Author Query Form

Book ID :

Chapter No : **216**

Please ensure you fill out your response to the queries raised below and return this form along with your corrections.

Dear Author,
During the process of typesetting your chapter, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query Refs.	Details Required	Author's Response
AQ1	Please confirm if the inserted city and country name is correct. Amend if necessary.	
AQ2	Please check whether the edit made in the sentence 'As part of a study … have been recruited.' conveys the intended meaning.	
AQ3	Kindly note that the references Humby et al. (2019), Manzo et al. (2008), Rivellese et al. (2019) have been repeated twice in a list. Hence, duplicate references are deleted and reordered accordingly.	

MARKED PROOF

Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

Instruction to printer	*Textual mark*	*Marginal mark*
Leave unchanged	··· under matter to remain	New matter followed by
Insert in text the matter indicated in the margin		or (x2)
Delete	through single character, rule or underline or through all characters to be deleted	or (x2)
Substitute character or substitute part of one or more word(s)	through letter or through characters	new character or new characters
Change to italics	under matter to be changed	
Change to capitals	under matter to be changed	
Change to small capitals	under matter to be changed	
Change to bold type	under matter to be changed	
Change to bold italic	under matter to be changed	
Change to lower case	Encircle matter to be changed	
Change italic to upright type	(As above)	
Change bold to non-bold type	(As above)	
Insert 'superior' character	through character or where required	or under character e.g. or
Insert 'inferior' character	(As above)	over character e.g.
Insert full stop	(As above)	
Insert comma	(As above)	
Insert single quotation marks	(As above)	or and/or or
Insert double quotation marks	(As above)	or and/or or
Insert hyphen	(As above)	
Start new paragraph		
No new paragraph		
Transpose		
Close up	linking characters	
Insert or substitute space between characters or words	through character or where required	
Reduce space between characters or words	between characters or words affected	