

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 Rivellesse, Elena Pontarini, and Costantino Pitzalis

Contents

1	Introduction.....	2
2	The Synovial Membrane as Site of Inflammation in RA	2
2.1	Histological Patterns of Synovial Inflammation	3
3	Synovial Tertiary Lymphoid Organs in RA.....	5
3.1	The Development and Regulation of Synovial Tertiary Lymphoid Organs in RA	5
3.2	The Function of Tertiary Lymphoid Organs in RA.....	10
3.3	The Clinical Relevance of Tertiary Lymphoid Organs in RA.....	11
4	Conclusions.....	16
Bibliography.....		16

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.

F. Rivellesse · 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

© Springer Nature Switzerland AG 2020



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 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

73 further sustains the infiltration of immune cells, thus facilitating the perpetuation of
74 the inflammatory response. Despite the enormous advancements in our under-
75 standing of the pathogenesis of RA, leading us to recognize a number of genetic
76 and environmental factors contributing to its pathogenesis, the initial trigger of
77 synovial inflammation is currently unknown. Also, we do not know whether the
78 first hit happens directly in the joints or somewhere else, such as the lungs or other
79 organs. However, once the inflammatory response is triggered and gets perpetuated,
80 synovitis represents the main feature of RA, thus the study of synovial inflamma-
81 tion is of utmost importance to improve our understanding of RA (Pitzalis et al.
82 2013).

83 **2.1 Histological Patterns of Synovial Inflammation**

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

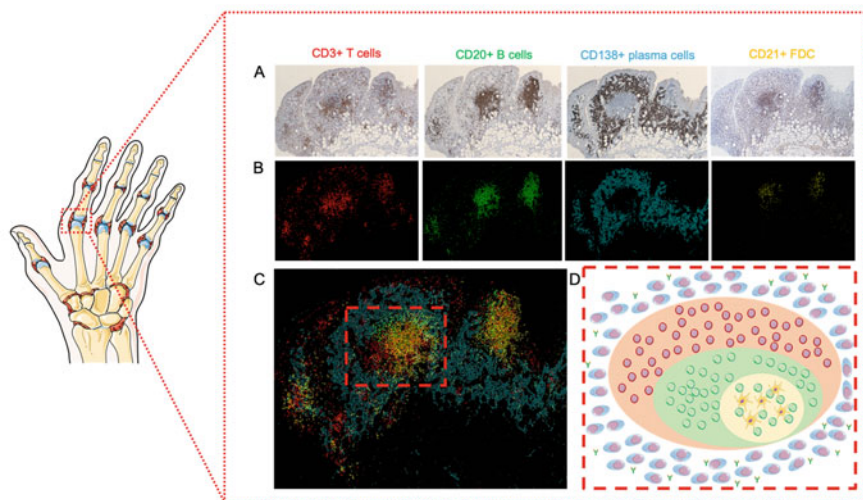


Fig. 1 Tertiary lymphoid organs in synovia. **a** Immunohistochemical staining of synovial membrane, **b** color deconvolution of the images in **(a)**, **c** overlap of the above images, and **d** schematic representation of the organization of TLO in synovia, with FDC in yellow, B cells in green, T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

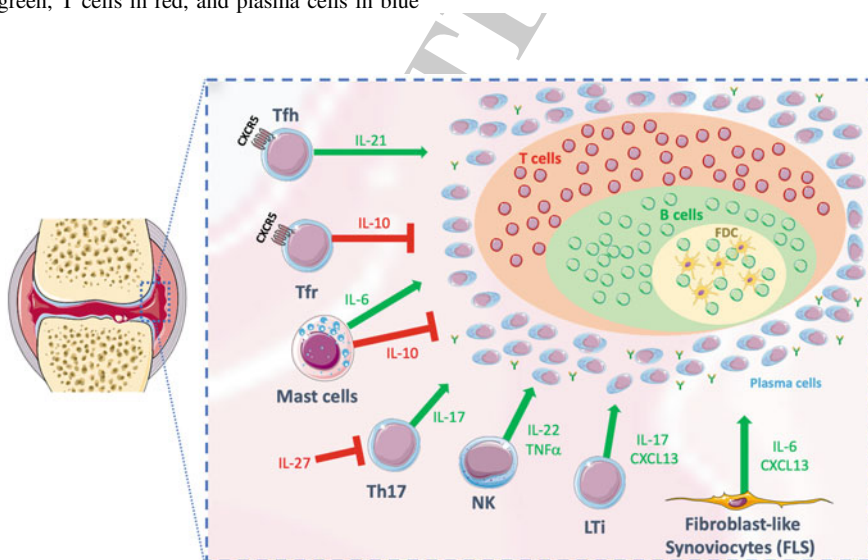


Fig. 2 Schematic representation of synovial TLOs and immune cells contributing to their development. Tfh = T follicular helper cells; Tfr = T follicular regulatory cells, Th17 = T helper cells 17, NK = Natural killer cells; LTi = Lymphoid tissue inducer

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 (LT β), 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

145 to non-lymphoid tissues in mice (Luther et al. 2000). In fully formed GC, Tfh cells
146 support somatic hypermutation of auto-reactive B cells and plasmablast generation
147 directly in the diseased tissues mainly through the production of IL-21. The latter is
148 Tfh signature cytokine, known to be a potent cofactor for B cell survival, prolifer-
149 eration and plasma cell differentiation, in particular in the context of CD40
150 co-stimulation and in synergy with B cell activating factor (Karnell and Ettinger
151 2012; Liu et al. 2015).

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

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

163 In parallel, IL-21 and its receptor are highly expressed in synovial tissue of
164 patients with RA (Jüngel et al. 2004; Kwok et al. 2012), and increased IL-21
165 expression is associated with synovial TLO (Jones et al. 2015). IL-21R
166 up-regulation is mainly described on macrophages and fibroblast with an acti-
167 vated phenotype (Jüngel et al. 2004), and IL-21 has been involved in the devel-
168 opment of articular damage by promoting both osteoclastogenesis (Kwok et al.
169 2012) and metalloproteinase release by fibroblast-like synoviocytes (Xing et al.
170 2016). Finally, Tfh cells are also enriched in the synovia of patients with RA, while
171 almost absent in osteoarthritis and normal synovium (Penatti et al. 2017; Chu et al.
172 2014).

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

189 Additionally, although the enrichment of Tfh in RA synovium has been well
190 described, there are conflicting data regarding circulating Tfh cell frequency
191 [comprehensively reviewed in (Gensous et al. 2018)]. Some authors reported IL-21
192 directly correlating with the frequency of Tfh-like cells, with IL-21 level and
193 number of Tfh-like cells associated with higher titer of anti-CCP antibodies and
194 disease activity score in RA (Ma et al. 2012). The circulating counterpart shares
195 phenotypic and functional features with tissue Tfh cells, except for the expression
196 of prototypical Tfh transcription factor B cell lymphoma protein 6 (Bcl-6), but their
197 biology is still poorly defined. Data from SAP-deficient mice show how these cells
198 are committed to Tfh lineage and are generated prior the GC response (He et al.
199 2013; Tsai and Yu 2014). Moreover, it is still unclear if circulating Tfh can reflect
200 an ongoing humoral activity.

201 3.1.3 Other Pro-inflammatory Cytokines and Cells

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

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

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

217 Within RA synovium, proliferation of fibroblast-like synoviocytes is sustained
218 by IL-22, a cytokine required for the development and maintenance of TLO. IL-22
219 role in ectopic lymphoneogenesis comes from data in experimental models of
220 inducible TLO in salivary glands, mimicking TLO formation in Sjogren's syn-
221 drome salivary glands. In this animal model, IL-22 is able to directly induce
222 CXCL13 production in a subset of GP38+ stromal cells through phosphorylation of
223 signal transduced and activator of transcription 3 (STAT3) (Barone et al. 2015).
224 Once lymphocytes are recruited, IL-22, together with LT α 1 β 2, supports also pro-
225 liferation of a population of podoplanin (pdpn)-positive stromal cells,
226 over-expressing IL21R, into a network of immunofibroblasts that are able to sup-
227 port the earliest phases of TLS establishment (Nayar et al. 2019) in the same model.
228 In RA synovium, IL-22 expression and IL-22 receptor on fibroblast-like synovi-
229 cytes have been reported (Ikeuchi et al. 2005), suggesting its contribution to the
230 maintenance of TLO. In particular, IL-22 expression is increased in cells expressing

231 the long isoform of complement receptor type 2 (Cr2, also known as CD21) (Cañete
232 et al. 2011), usually present in networks of stromal-derived follicular dendritic cells
233 (FDCs), that contribute to the presentation of immune complexes necessary to
234 generate activated B cells, in TLO. In synovial tissue, IL-22 is also produced by NK
235 cells (Zhu et al. 2015). NK cells are innate immune lymphocytes with cytolytic and
236 immune-regulatory activities representing a significant proportion (8–25%) of
237 immune infiltration in synovial fluid of RA patients, identified in the joints in the
238 early stage of RA development (Tak et al. 1994). Initially, NK cells were described
239 in RA pathogenesis for their production of cytotoxic serin protease granzyme-A and
240 B and pro-inflammatory cytokines, such as IL-1 and TNF α as dominant mediators
241 of proliferative synovitis in RA (Klimiuk et al. 1997), supporting osteoclastogen-
242 esis and thus involved in the development of articular damage (Kotake et al. 2001).
243 In fact, increased production of IFN γ and TNF α characterizes synovial fluid NK
244 cells of erosive RA patients with joint damage in comparison with non-erosive RA
245 (Yamin et al. 2019). Recent evidence suggests that NK cells may support TLO
246 maintenance within RA synovium as a subset of NK cells expressing a natural
247 cytotoxicity receptor Nkp44 which is able to produce IL-22 (Zhu et al. 2015).
248 Nkp44+ NK cells are enriched in both peripheral blood and synovium of RA
249 patients secreting IL-22 and TNF α , which in vitro studies showed to support
250 RA FLS proliferation (Ren et al. 2011), through the activation of STAT3 pathway
251 (Zhu et al. 2015). IL-22 induced proliferation of synovial fibroblast, an effect that
252 was inhibited by neutralizing antibodies targeting IL-22 and TNF α (Ren et al.
253 2011). Thus, NK cells may participate in TLO organization supporting the prolifer-
254 ation of synovial fibroblasts responsible for the local secretion of chemoattractant
255 molecules and, as consequence, lymphocytes recruitment.

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

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

263 Recently, we have also shown a strong association between synovial mast cells
264 (MCs) and the presence of TLO in a large cohort of patients with early RA
265 (Rivellese et al. 2018). MCs were also found to induce B cell activation and
266 differentiation in vitro, including the production of ACPA autoantibodies. Finally,
267 in animal models of inducible TLO (IL27R knockout), we confirmed the associa-
268 tion of MCs with TLO. Overall, this points out to the relevance of MCs as potential
269 contributors to the formation of TLO, although additional studies are needed to
270 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 IL27R α 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-27R α 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 imbalance 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 Tfh and Tfr could be useful in patients with RA, as confirmed by a several observations (Niu et al. 2018; Wang et al. 2019).



312 As for the function of Tfr in RA, these cells have been shown to have sup-
313 pressive effects in vitro, which were enhanced in patients in remission (Liu et al.
314 2018). However, it has also been speculated that Tfr in autoimmune diseases might
315 be functionally deficient (Fonseca et al. 2017).

316 **3.2 The Function of Tertiary Lymphoid Organs in RA**

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

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

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

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

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



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

358 **3.3 The Clinical Relevance of Tertiary Lymphoid Organs** 359 **in RA**

360 **3.3.1 TLO and Disease Severity**

361 Early studies on the analysis of synovial membrane relied on the use arthroscopy to
362 obtain synovial samples. These analyses pointed out a marked heterogeneity in
363 terms of synovial inflammation, particularly in the degree of immune cell infiltra-
364 tion, with the description of aggregates of lymphoid cells in a proportion of patients.
365 However, when looking for an association with clinical features, these studies
366 yielded contradictory results: some found an association of lymphoid aggregates
367 with disease severity and autoantibody positivity (Humby et al. 2019; Bugatti et al.
368 2014; Orr et al. 2017) and others did not (Thurlings et al. 2008; Cantaert et al. 2008;
369 Van De Sande et al. 2011) (Table 1). These inconsistencies could be explained by a
370 number of biases: (i) the exclusive analysis of large joints, in which there can be
371 commonly overlapping osteoarthritis and are not the most representative of the
372 inflammatory process in RA (Linn-Rasker et al. 2007) (ii) the inclusion of patients
373 with long-standing disease, with the obvious bias of treatment and disease duration,
374 and (iii) the lack of a gold standard for the histological assessment of immune cell
375 infiltration (Humby et al. 2016).

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

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

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)

Table 1 (continued)

Author and year	References	Population	Joints biopsied and procedure	Treatment (if any)	Time points	Analyses	Results
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	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 Patients with a lympho-myeloid phenotype 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



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

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

413 3.3.2 TLO as Direct Therapeutic Targets

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

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

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

434 Importantly, none of the above studies targeting mediators involved in TLO
435 formation or maintenance in RA has stratified patients on the basis of TLO pres-
436 ence, which could have helped in selecting patients with higher chances of
437 response.

438 3.3.3 TLO as Predictors of Treatment Response

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

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

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

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

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

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



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

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

492 4 Conclusions

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

504 Bibliography

- 505 Amara K, Steen J, Murray F, Morbach H, Fernandez-Rodriguez BM, Joshua V et al (2013)
506 Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong
507 bias toward citrullinated autoantigen recognition. *J Exp Med.* <https://doi.org/10.1084/jem.20121486>
- 508
509 Baeten D, Peene I, Union A, Meheus L, Sebbag M, Serre G et al (2001) Specific presence of
510 intracellular citrullinated proteins in rheumatoid arthritis synovium: Relevance to antiflaggrin
511 autoantibodies. *Arthritis Rheum.* [https://doi.org/10.1002/1529-0131\(200110\)44:10%3c2255:aid-art388%3e3.0.co;2-%23](https://doi.org/10.1002/1529-0131(200110)44:10%3c2255:aid-art388%3e3.0.co;2-%23)
- 512



- 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, Steintal 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/10.1371/journal.pone.0112545>
- 523 Bombardieri M, Lewis M, Pitzalis C (2017) Ectopic lymphoid neogenesis in rheumatic
524 autoimmune diseases. *Nat Rev Rheumatol* 13:141–154. <https://doi.org/10.1038/nrrheum.2016.217>
- 527 Botta D, Fuller MJ, Marquez-Lago TT, Bachus H, Bradley JE, Weimann 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 Affitto 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/keu163>
- 543 Cañete JD, Celis R, Moll C, Izquierdo E, Marsal S, Sanmartí R et al (2009) Clinical significance of
544 synovial lymphoid neogenesis and its reversal after anti-tumour necrosis factor alpha therapy in
545 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%3c963::AID-ANR15%3e3.0.CO;2-E](https://doi.org/10.1002/1529-0131(199905)42:5%3c963::AID-ANR15%3e3.0.CO;2-E)
- 556
557 Chu Y, Wang F, Zhou M, Chen L, Lu Y (2014) A preliminary study on the characterization of
558 follicular helper T (T_{fh}) 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.2012.04.013>
- 563
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, Ramwadhoebe 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>
- Fonsous 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, Yermenko 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>



- 619 Ikeuchi H, Kuroiwa T, Hiramatsu N, Kaneko Y, Hiromura K, Ueki K et al (2005) Expression of
620 interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. *Arthritis*
621 *Rheum* 52:1037–1046. <https://doi.org/10.1002/art.20965>
- 622 Jones GW, Jones SA (2016) Ectopic lymphoid follicles: inducible centres for generating
623 antigen-specific immune responses within tissues. *Immunology* 147:141–151. <https://doi.org/10.1111/imm.12554>
- 624
625 Jones GW, Bombardieri M, Greenhill CJ, McLeod L, Nerviani A, Rocher-Ros V et al (2015)
626 Interleukin-27 inhibits ectopic lymphoid-like structure development in early inflammatory
627 arthritis. *J Exp Med* 212:1793–1802. <https://doi.org/10.1084/jem.20132307>
- 628 Jüngel A, Distler JHW, Kurowska-Stolarska M, Seemayer CA, Seibl R, Forster A et al (2004)
629 Expression of interleukin-21 receptor, but not interleukin-21, in synovial fibroblasts and
630 synovial macrophages of patients with rheumatoid arthritis. *Arthritis Rheum* 50:1468–1476.
631 <https://doi.org/10.1002/art.20218>
- 632 Karnell JL, Ettinger R (2012) The interplay of IL-21 and BAFF in the formation and maintenance
633 of human B cell memory. *Front Immunol* 3. <https://doi.org/10.3389/fimmu.2012.00002>
- 634 Kerschbaumer A, Sepriano A, Smolen JS, van der Heijde D, Dougados M, van Vollenhoven R
635 et al (2019) Efficacy of pharmacological treatment in rheumatoid arthritis: a systematic
636 literature research informing the, update of the EULAR recommendations for management of
637 rheumatoid arthritis. *Ann Rheum Dis* 2020:1–15. <https://doi.org/10.1136/annrheumdis-2019-216656>
- 638
639 Kim JM, Youn J, Jang E, Cho S-H, Park H, Paik D-J (2009) K/BxN mice development of arthritis
640 in autoimmune cell expansion is essential for the T-CD25+ provoked by homeostatic CD4 a
641 positive feedback loop of IL-21 signaling. *J Immunol* 182:4649–4656. <https://doi.org/10.4049/jimmunol.0804350>
- 642
643 Klaasen R, Thurlings RM, Wijbrandts CA, van Kuijk AW, Baeten D, Gerlag DM et al (2009) The
644 relationship between synovial lymphocyte aggregates and the clinical response to infliximab in
645 rheumatoid arthritis: a prospective study. *Arthritis Rheum* 60:3217–3224
- 646 Klimiuk PA, Goronzy JJ, Björnsson J, Beckenbaugh RD, Weyand CM (1997) Tissue cytokine
647 patterns distinguish variants of rheumatoid synovitis. *Am J Pathol* 151:1311–1319
- 648 Kotake S, Udagawa N, Hakoda M, Mogi M, Yano K, Tsuda E et al (2001) Activated human T
649 cells directly induce osteoclastogenesis from human monocytes: possible role of T cells in bone
650 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](https://doi.org/10.1002/1529-0131(200105)44:5%3c1003::aid-anr179%3e3.0.co;2-%23)
- 651
652 Kwok SK, La Cho M, Park MK, Oh HJ, Park JS, Her YM et al (2012) Interleukin-21 promotes
653 osteoclastogenesis in humans with rheumatoid arthritis and in mice with collagen-induced
654 arthritis. *Arthritis Rheum* 64:740–751. <https://doi.org/10.1002/art.33390>
- 655
656 Lee SY, Jung YO, Ryu JG, Kang CM, Kim EK, Son HJ et al (2014) Intravenous immunoglobulin
657 attenuates experimental autoimmune arthritis by inducing reciprocal regulation of th17 and treg
658 cells in an interleukin-10- dependent manner. *Arthritis Rheum*. <https://doi.org/10.1002/art.38627>
- 659
660 Lewis MJ, Barnes MR, Blighe K, Goldmann K, Rana S, Hackney JA et al (2019) Molecular
661 portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell*
662 *Rep* 28(2455–2470):e5. <https://doi.org/10.1016/j.celrep.2019.07.091>
- 663
664 Linn-Rasker SP, Van Der Helm-Van Mil a. HM, Breedveld FC, Huizinga TWJ (2007) Arthritis of
665 the large joints—in particular, the knee—at first presentation is predictive for a high level of
666 radiological destruction of the small joints in rheumatoid arthritis. *Ann Rheum Dis* 66:646–
667 650. <https://doi.org/10.1136/ard.2006.066704>
- 668
669 Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF et al (2011) Foxp3
670 + follicular regulatory T cells control the germinal center response. *Nat Med* 17:975–982.
671 <https://doi.org/10.1038/nm.2425>
- 672
673 Liu D, Xu H, Shih C, Wan Z, Ma X, Ma W et al (2015) T-B-cell entanglement and ICOSL-driven
674 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://doi.org/10.1136/annrheumdis-2019-215751>
- 680 Lliso-Ribera G, Humby F, Lewis M, Nerviani A, Mauro D, Rivellesse 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/10.1136/annrheumdis-2019-215751>
- 684 Luther SA, Lopez T, Bai W, Hanahan D, Cyster JG (2000) BLC expression in pancreatic islets
685 causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. *Immunity*
686 12:471–481. [https://doi.org/10.1016/s1074-7613\(00\)80199-5](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/](https://doi.org/10.1002/eji.200425830)
694 [eji.200425830](https://doi.org/10.1002/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-Neuhausser 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](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.](https://doi.org/10.1016/j.immuni.2010.12.012)
716 [immuni.2010.12.012](https://doi.org/10.1016/j.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-](https://doi.org/10.1038/s41598-017-08935-6)
719 [08935-6](https://doi.org/10.1038/s41598-017-08935-6)
- 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 Niu 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



- 725 cells in patients with rheumatoid arthritis. *Arthritis Res Ther*. [https://doi.org/10.1186/s13075-](https://doi.org/10.1186/s13075-018-1690-0)
726 [018-1690-0](https://doi.org/10.1186/s13075-018-1690-0)
- 727 Orr C, Najm A, Biniecka M, McGarry T, Ng C-T, Young F et al (2017) Synovial
728 immunophenotype and anti-citrullinated peptide antibodies in rheumatoid arthritis patients:
729 relationship to treatment response and radiologic prognosis. *Arthritis Rheum* (Hoboken, NJ)
730 69:2114–2123. <https://doi.org/10.1002/art.40218>
- 731 Penatti A, Facciotti F, De Matteis R, Larghi P, Paroni M, Murgo A et al (2017) Differences in
732 serum and synovial CD4+ T cells and cytokine profiles to stratify patients with inflammatory
733 osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther* 19:103. [https://doi.org/10.1186/](https://doi.org/10.1186/s13075-017-1305-1)
734 [s13075-017-1305-1](https://doi.org/10.1186/s13075-017-1305-1)
- 735 Pitzalis C, Kelly S, Humby F (2013) New learnings on the pathophysiology of RA from synovial
736 biopsies. *Curr Opin Rheumatol* 25:334–344. <https://doi.org/10.1097/BOR.0b013e32835fd8eb>
- 737 Randall TD, Carragher DM, Rangel-Moreno J (2008) Development of Secondary lymphoid
738 organs. *Annu Rev Immunol* 26:627–650. [https://doi.org/10.1146/annurev.immunol.26.021607.](https://doi.org/10.1146/annurev.immunol.26.021607.090257)
739 [090257](https://doi.org/10.1146/annurev.immunol.26.021607.090257)
- 740 Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y et al (2017) Pathologically
741 expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nat Publ Gr*
742 542:110–114. <https://doi.org/10.1038/nature20810>
- 743 Ren J, Feng Z, Lv Z, Chen X, Li J (2011) Natural killer-22 cells in the synovial fluid of patients
744 with rheumatoid arthritis are an innate source of interleukin 22 and tumor necrosis factor- α .
745 *J Rheumatol* 38:2112–2118. <https://doi.org/10.3899/jrheum.101377>
- 746 Rivellese F, Nerviani A, Rossi FW, Marone G, Matucci-Cerinic M, de Paulis A et al (2017) Mast
747 cells in rheumatoid arthritis: friends or foes? *Autoimmun Rev* 16:557–563. [https://doi.org/10.](https://doi.org/10.1016/j.autrev.2017.04.001)
748 [1016/j.autrev.2017.04.001](https://doi.org/10.1016/j.autrev.2017.04.001)
- 749 Rivellese F, Mauro D, Nerviani A, Pagani S, Fossati-Jimack L, Messemaker T et al (2018) Mast
750 cells in early rheumatoid arthritis associate with disease severity and support B cell
751 autoantibody production. *Ann Rheum Dis* 77:1773–1781
- 752 Rivellese F, Humby F, Bugatti S, Fossati-Jimack L, Rizvi H, Lucchesi D et al (2019a) B cell
753 synovitis and clinical phenotypes in rheumatoid arthritis: relationship to disease stages and
754 drug exposure. *Arthritis Rheum* 4–11. <https://doi.org/10.1002/art.41184>
- 755 Rivellese F, Rossi FW, Galdiero MR, Pitzalis C, de Paulis A (2019b) Mast cells in early
756 rheumatoid arthritis. *Int J Mol Sci* 20:1–13. <https://doi.org/10.3390/ijms20082040>
- 757 Roeleveld DM, Marijnissen RJ, Walgreen B, Helsen MM, Van Den Berselaar L, Van De Loo FA
758 et al (2017) Higher efficacy of anti-IL-6/IL-21 combination therapy compared to monotherapy
759 in the induction phase of Th17-driven experimental arthritis 2017. [https://doi.org/10.1371/](https://doi.org/10.1371/journal.pone.0171757)
760 [journal.pone.0171757](https://doi.org/10.1371/journal.pone.0171757)
- 761 Romão VC, Fonseca JE, Agua-Doce A, Graca L (2018) T follicular regulatory cells are decreased
762 in patients with established treated rheumatoid arthritis with active disease: comment on the
763 article by Liu et al. *Arthritis Rheum*. <https://doi.org/10.1002/art.40586>
- 764 Ronday HK, Van Der Laan WH, Tak PP, De Roos JADM, Bank RA, Tekoppele JM et al (n.d.)
765 Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the
766 invasive front of the synovium in rheumatoid arthritis
- 767 Sawa S, Cherrier M, Lochner M, Satoh-Takayama N, Fehling HJ, Langa F et al (2010) Lineage
768 relationship analysis of ROR γ t+ innate lymphoid cells. *Science* (80-) 330:665–669. [https://doi.](https://doi.org/10.1126/science.1194597)
769 [org/10.1126/science.1194597](https://doi.org/10.1126/science.1194597)
- 770 Scheel T, Gursche A, Zacher J, Berek C (2011) V-region gene analysis of locally defined synovial
771 B and plasma cells reveals selected B cell expansion and accumulation of plasma cell clones in
772 rheumatoid arthritis. *Arthritis Rheum*. <https://doi.org/10.1002/art.27767>
- 773 Smolen JS, Aletaha D, McInnes IB (2016) Rheumatoid arthritis. *Lancet* 388:2023–2038. [https://](https://doi.org/10.1016/S0140-6736(16)30173-8)
774 [doi.org/10.1016/S0140-6736\(16\)30173-8](https://doi.org/10.1016/S0140-6736(16)30173-8)
- 775 Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM et al (2006) Interleukin
776 27 negatively regulates the development of interleukin 17-producing T helper cells during
777 chronic inflammation of the central nervous system. *Nat Immunol*. [https://doi.org/10.1038/](https://doi.org/10.1038/ni1376)
778 [ni1376](https://doi.org/10.1038/ni1376)



- 779 Tak PP, Kummer JA, Hack CE, Daha MR, Smeets TJM, Erkelens GW et al (1994)
780 Granzyme-positive cytotoxic cells are specifically increased in early rheumatoid synovial
781 tissue. *Arthritis Rheum* 37:1735–1743. <https://doi.org/10.1002/art.1780371205>
- 782 Takemura S, Braun A, Crowson C, Kurtin PJ, Cofield RH, O’Fallon WM et al (2001) Lymphoid
783 neogenesis in rheumatoid synovitis. *J Immunol*. <https://doi.org/10.4049/jimmunol.167.2.1072>
- 784 Thurlings RM, Wijbrandts CA, Mebius RE, Cantaert T, Dinant HJ, Van Der Pouw-Kraan
785 TCTMTM et al (2008) Synovial lymphoid neogenesis does not define a specific clinical
786 rheumatoid arthritis phenotype. *Arthritis Rheum* 58:1582–1589. <https://doi.org/10.1002/art.23505>
- 787
- 788 Timmer TCG, Baltus B, Vondenhoff M, Huizinga TWJ, Tak PP, Verweij CL et al (2007)
789 Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected
790 by genomics technology: identification of the interleukin-7 signaling pathway in tissues with
791 lymphoid neogenesis. *Arthritis Rheum* 56:2492–2502. <https://doi.org/10.1002/art.22748>
- 792 Trouw LA, Rispens T, Toes REM (2017) Beyond citrullination: other post-translational protein
793 modifications in rheumatoid arthritis. *Nat Rev Rheumatol*. <https://doi.org/10.1038/nrrheum.2017.15>
- 794
- 795 Tsai LM, Yu D (2014) Follicular helper T-cell memory: establishing new frontiers during antibody
796 response. *Immunol Cell Biol* 92:57–63. <https://doi.org/10.1038/icb.2013.68>
- 797 Ueno H, Bancheau J, Vinuesa CG (2015) Pathophysiology of T follicular helper cells in humans
798 and mice. *Nat Immunol* 16:142–152. <https://doi.org/10.1038/ni.3054>
- 799 Van De Sande MGH, Thurlings RM, Boumans MJHH, Wijbrandts CA, Modesti MG,
800 Gerlag DM et al (2011) Presence of lymphocyte aggregates in the synovium of patients with
801 early arthritis in relationship to diagnosis and outcome: is it a constant feature over time? *Ann*
802 *Rheum Dis* 70:700–703. <https://doi.org/10.1136/ard.2010.139287>
- 803 van Delft MAM, Huizinga TWJ (2020) An overview of autoantibodies in rheumatoid arthritis.
804 *J Autoimmun*. <https://doi.org/10.1016/j.jaut.2019.102392>
- 805 Van Oosterhout M, Bajema I, Levarht EWN, Toes REM, Huizinga TWJ, Van Laar JM (2008)
806 Differences in synovial tissue infiltrates between anti-cyclic citrullinated peptide-positive
807 rheumatoid arthritis and anti-cyclic citrullinated peptide-negative rheumatoid arthritis. *Arthritis*
808 *Rheum* 58:53–60. <https://doi.org/10.1002/art.23148>
- 809 Vossenaar ER, Smeets TJM, Kraan MC, Raats JM, Van Venrooij WJ, Tak PP (2004) The presence
810 of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis Rheum*. <https://doi.org/10.1002/art.20584>
- 811
- 812 Wang X, Yang C, Xu F, Qi L, Wang J, Yang P (2019) Imbalance of circulating Tfr/Tfh ratio in
813 patients with rheumatoid arthritis. *Clin Exp Med*. <https://doi.org/10.1007/s10238-018-0530-5>
- 814 Willemze A, Trouw LA, Toes REM, Huizinga TWJ (2012) The influence of ACPA status and
815 characteristics on the course of RA. *Nat Rev Rheumatol* 8:144–152. <https://doi.org/10.1038/nrrheum.2011.204>
- 816
- 817 Wing JB, Ise W, Kurosaki T, Sakaguchi S (2014) Regulatory T cells control antigen-specific
818 expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4.
819 *Immunity*. <https://doi.org/10.1016/j.immuni.2014.12.006>
- 820 Wu H, Chen Y, Liu H, Xu LL, Teuscher P, Wang S et al (2016) Follicular regulatory T cells
821 repress cytokine production by follicular helper T cells and optimize IgG responses in mice.
822 *Eur J Immunol*. <https://doi.org/10.1002/eji.201546094>
- 823 Xing R, Jin Y, Sun L, Yang L, Li C, Li Z et al (2016) Interleukin-21 induces migration and
824 invasion of fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Clin Exp*
825 *Immunol* 184:147–158. <https://doi.org/10.1111/cei.12751>
- 826 Yamin R, Berhani O, Peleg H, Aamar S, Stein N, Gamliel M et al (2019) High percentages and
827 activity of synovial fluid NK cells present in patients with advanced stage active rheumatoid
828 arthritis. *Sci Rep* 9:1–12. <https://doi.org/10.1038/s41598-018-37448-z>
- 829 Yoshida H, Hunter CA (2015) The immunobiology of interleukin-27. *Annu Rev Immunol*. <https://doi.org/10.1146/annurev-immunol-032414-112134>
- 830



- 831 Young DA, Hegen M, Ma HLR, Whitters MJ, Albert LM, Lowe L et al (2007) Blockade of the
832 interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of
833 rheumatoid arthritis. *Arthritis Rheum* 56:1152–1163. <https://doi.org/10.1002/art.22452>
- 834 Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S et al (2019) Defining
835 inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell
836 transcriptomics and mass cytometry. *Nat Immunol*. <https://doi.org/10.1038/s41590-019-0378-1>
- 837 Zhu J, Jia E, Zhou Y, Xu J, Feng Z, Wang H et al (2015) Interleukin-22 secreted by NKp44
838 + natural killer cells promotes proliferation of fibroblast-like synoviocytes in rheumatoid
839 arthritis. *Medicine (Baltimore)* 94:e2137. <https://doi.org/10.1097/MD.0000000000002137>

UNCORRECTED PROOF

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.

<i>Instruction to printer</i>	<i>Textual mark</i>	<i>Marginal mark</i>
Leave unchanged	... under matter to remain	Ⓟ
Insert in text the matter indicated in the margin	∧	New matter followed by ∧ or ∧ [Ⓢ]
Delete	/ through single character, rule or underline or ┌───┐ through all characters to be deleted	Ⓞ or Ⓞ [Ⓢ]
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	Υ or Υ under character e.g. Υ or Υ
Insert 'inferior' character	(As above)	∧ over character e.g. ∧
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	Ƴ or ƴ and/or ƶ or Ʒ
Insert double quotation marks	(As above)	ƶ or Ʒ and/or ƶ or Ʒ
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		⸵