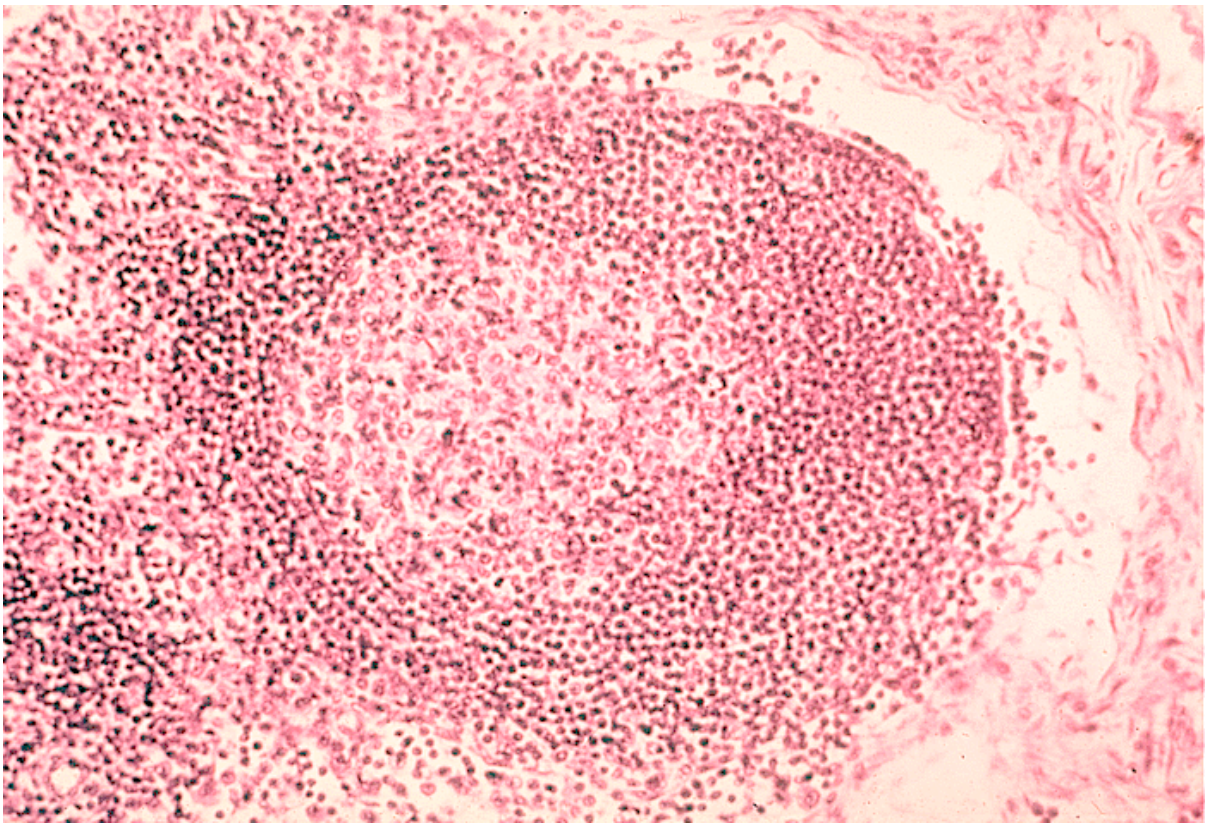
The role of T-cell B-cell interations in germinal center reactions in Rheumatoid Arthritis



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Master Thesis

The role of T-cell B-cell interactions in germinal center reactions in Rheumatoid Arthritis

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List of abbreviations

(A)Ab	(auto-)antibody
ACPA	anti-citrullinated protein antibody
Ag	antigen
AI	autoimmune
AID	activation-induced cytidine deaminase
AKA	anti-keratin antibody
Anti-CCP	anti-cyclic citrullinated protein
APC	antigen-presenting cell
APF	anti-perinuclear factor
BAFF	B-cell activating factor
BCA-1	B-cell chemoattractant-1
Bcl-6	B-cell lymphoma-6
CCL/CCR	CC-motif chemokine ligand/receptor
CDR	complementarity determining region
CIA	collagen-induced arthritis
CII	collagen type II
CTLA (-4)	cytotoxic T-lymphocyte activator (4)
CXCL/CXCR	CXC-motif chemokine ligand/receptor
DC	dendritic cell
FAIM	Fas-apoptosis inhibitory molecule
FDC	follicular dendritic cell
FLS	fibroblast-like synoviocyte
GC	germinal center
IC	immune complex
ICOS	inducible co-stimulatory molecule
IFN	interferon
Ig	immunoglobulin
IgV	immunoglobulin variable region
IRF (4)	Interferon-responsive factor (4)
LT	lymphotoxin
mAb	monoclonal antibody
MMP	matrix metalloprotease
OA	ostheoarthritis

OVA	ovalbumin
PB	peripheral blood
PD	Programmed Death
PSGL	P-selectin glycoprotein
RF	rheumatoid factor
SAP	SLAM-associated protein
SHM	somatic hypermutation
SLAM	signaling lymphocyte-activation molecule
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
TCR	T-cell receptor
TNF	tumour necrosis factor

Abstract

The systemic autoimmune disease Rheumatoid Arthritis (RA) is marked by chronic inflammation of the synovium and subsequent joint damage. Although most RA patients show seropositivity for RA-associated auto-antibodies, such as anti-citrullinated protein antibodies or rheumatoid factor, a great variability in auto-antibodies is observed and various mechanisms of disease have been suggested. Approximately 30% of RA patients show formation of ectopic lymphoid organs in inflamed synovia. A correlation between the phenotypical structure of these ectopic lymphoid organs and enhanced auto-antibody production as well as disease severity is suggested by several studies. Although the latter two might be directly related, it raises the question whether lymphoid neogenesis contributes directly to RA pathology or is just a side-effect of local, chronic inflammation. It is generally accepted that the development and maturation of long-lived antibody-producing B-cells is predominantly regulated in lymphoid organs within specialized micro-environments, called germinal centers (GC). Several studies have demonstrated an association between disease severity, incidence and size of germinal centers, and various signaling pathways involved in T-/B-cell interactions. A recently identified subset of $CD4^+$ helper T-cells, follicular helper T-cells (T_{FH}) are thought to be the predominant helper T-cell subset involved in these T-/B-cell interactions in germinal centers. Therefore, this thesis will discuss present evidence to elucidate whether and how T_{FH} B-cell help in germinal centers contributes to RA pathology.

1 Introduction

Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the synovium with systemic autoimmune features, resulting in joint damage. RA affects approximately 0.5 to 1.0 % of the Northern American and Northern European population, with annually 50 new cases per 100.000 inhabitants [reviewed by 1]. However, a great variation in prevalence is indicated based on RA occurrence amongst other populations [2]. Both genetic predisposition and environmental risk factors are involved in RA development, both of which are still under investigation [reviewed by 13, 4].

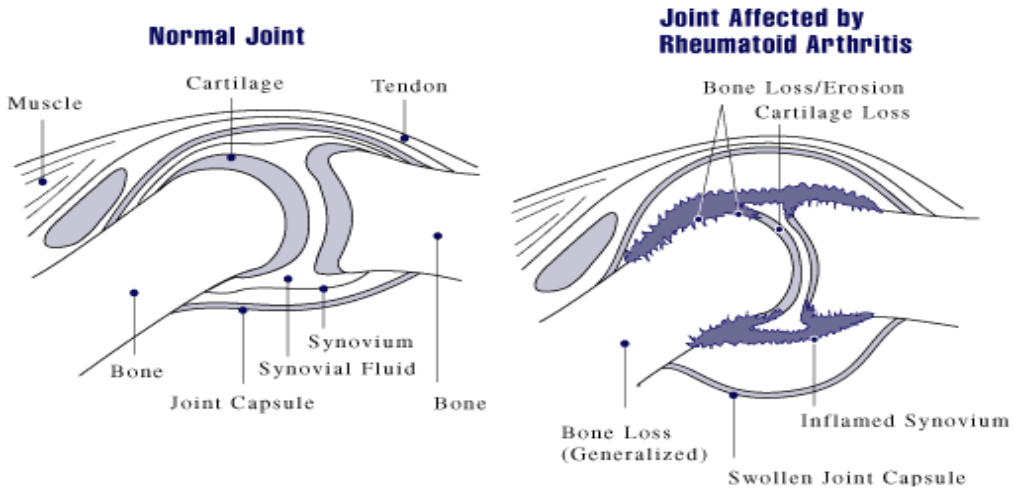

Figure 1; Schematic view of diarthridial joints in stage of health (left) and synovitis (right). *Source: www.faculty.washington.edu 10-01-2011*

Enhanced infiltration of the synovium with pro-inflammatory cells, such as monocytes and lymphocytes (synovitis, figure 1), results in pannus fomation [reviewed by 5, 6]. The pannus adheres to and invades the cartilage extracellular matrix of the joints, with matrix metalloproteinase (MMP) release being responsible for increasing degradation of the cartilage [7-9].

1.1 Auto-antibodies and enhanced B-cell maturation

An important feature of RA is the production of antibodies (Ab) directed against self-antigens. Various RA-associated Abs have been identified to date, although these are still under intensive study [reviewed by 10]. The first described RA-associated Abs are those directed against the body's own immunoglobulin: Rheumatoid Factor (RF). The Fab fragment (Fragment, antigen binding) of immunoglobulin (Ig) binds to the Fc fragment (Fragment, crystallizable) of IgG and together form immune complexes [11]. IgM RF are most detected in both serum and synovial fluid of RA patients, but also IgG and IgA RF have been observed to a lesser extent [reviewed by 10]. However, low-affinity RF is also produced by healthy individuals and by patients of other autoimmune diseases, which makes it an ambiguous marker for RA [11, 12]. Another, more specific set of auto-antibodies are those directed against citrullinated proteins. Citrullination is a common post-translational modification of peptides during apoptosis and inflammation, in which deamination of arginine creates a citrulline residue [13 reviewed by 14]. The common antigen recognized by all anti-citrullinated protein antibodies (ACPA) is citrulline and various ACPA have been identified in the last decade [15]. These include anti-fillagrin Ab (AFA), such as anti-keratin Ab (AKA) and anti-perinuclear factor (APF), but also anti-Sa Ab (directed against citrullinated vimentin) and anti-cyclic citrullinated protein (anti-CCP) Ab [reviewed by [10, 13]. Serum ACPA levels, especially anti-CCP Ab serum levels, are a relatively specific marker for RA, as compared to other RA-associated Abs [16].

Several studies suggest that synovial tissues are a direct source of auto-antibody production in RA, rather than a target of B-cell migration [17-19]. For example, IgG AFA levels are higher in synovial tissue than in serum or synovial fluid of RA patients, at various stages of disease [18, 20]. In addition, it has been indicated that auto-antibody production corresponds with disease severity [12, 16, 21, 22].
As most ACPAs are of the IgG subset, a T-cell mediated immune response, in which B-cell Ig-isotype switching is fascilitated, is suggested [15]. The chronic character of RA and the production of high-affinity auto-antibodies, suggests the involvement of specialized lymphoid structures. These structures, known as germinal centers (GC), are usually found within secondary lymphoid organs, such as the spleen and

lymph nodes, but have been observed in ectopic locations in RA and various other autoimmune diseases as well [reviewed by 23, 24]. These ectopic lymphoid organs are also called tertiary lymphoid organs and result from lymphoid neogenesis.

1.2 Lymphoid neogenesis in Rheumatoid Arthritis

In 25 to 30% of RA patients, the inflamed synovium shows lympoid organ neogenesis [23, 25]. Furthermore, the level of synovial infiltrate organization is suggested to be associated with disease severity [26]. Rheumatoid synovial tissues mainly present three distinctive structural organization patterns of lymphoid infiltrate, i.e. diffuse pattern, aggregate formation and follicle formation (including GC formation) [23]. Various studies have demonstrated that the level of tertiary lymphoid organ formation in rheumatoid synovia corresponds with auto-antibody production in RA patients [12, 17, 27]. However, a cohort study from Baeten et al. has shown no association between synovial lymphoid tissue organization and seropositivity of patients for ACPA or RF at all [25]. Interestingly, this study did show a strong association between ectopic lymphoid tissue organization and synovial inflammation. It suggests that lymphoid organization correlates with inflammatory degree, but not directly with Ab production. Lymphoid neogenesis without GC formation might contribute to enhanced synovial inflammation and with that, create an environment which promotes auto-antibody production by some other mechanism.

Production of some ACPA corresponds with the degree of B-cell differentiation and in addition with synovial lymphoid structure organization [20]. It is suggested that patients with synovial lymphoid aggregates have a bigger risk of developing a more severe form of RA than patients lacking aggregate formation (diffuse cell patterns), as the first group requires joint replacement. Organizational patterns of synovial lymphoid infiltrates are similar through the complete time course of disease, which suggests that the pattern of lymphoid structures characterizes distinct mechanisms of disease, rather than disease stages [23].

Together, these findings raise the questions whether and by which mechanisms lymphoid neogenesis contributes to ongoing auto-antibody production as observed in many RA patients.

It is well understood that the development of high-affinity Ab-producing B-cells is mainly regulated within GCs in lymphoid organs and that this requires help of $CD4^+$ T-cells [reviewed by 28-30]. A specific group of $CD4^+$ T-cells, called follicular helper T-cells (T_{FH}), are abundantly present within GCs and are now regarded to as being the main candidates for delivering B-cell help in GCs [reviewed by 5, 28, 31-34]. Thus, the formation of ectopic lymphoid organs and GCs in some RA patients raises the question to what extent T_{FH} B-cell help contributes to disease pathology. This thesis will discuss various studies concerning the mechanisms by which enhanced T_{FH} B-cell help might contribute to RA pathology.

2 Lymphoid tissues and structural organization

Antigen-induced immune reactions are mainly regulated within structurally organized lymphoid tissues, usually found within secondary lymphoid organs like the spleen, Peyer's patches, lymph nodes, tonsils and mucosal associated lymphoid tissue (MALT) [reviewed by 35]. However, formation of organized lymphoid structures in ectopic tissues in chronic inflammatory diseases is observed as well [reviewed by 36]. The morphological structure of lymphoid tissues optimizes the interactions between antigen-specific T- and B-cells, to regulate immune responses (figure 2). For this, B-cells and T-cells reside in separated areas, i.e. follicles and T-cell rich areas [reviewed by 30]. B- and T-cell homing to these specific areas is a result of local chemokine production by stromal cells, the accessory cells present in specific parts of the lymphoid tissue. The presence of antigen (Ag) induces structural reorganization of the lymphoid tissue and initiates GC formation. Within GCs, Ag-activated B-cells undergo differentiation, proliferation and Ig-affinity selection, eventually resulting in the production of high-affinity Ab production [reviewed by 30]. Various factors are involved in the formation and maintenance of GCs in secondary, as well as tertiary lymphoid tissues.

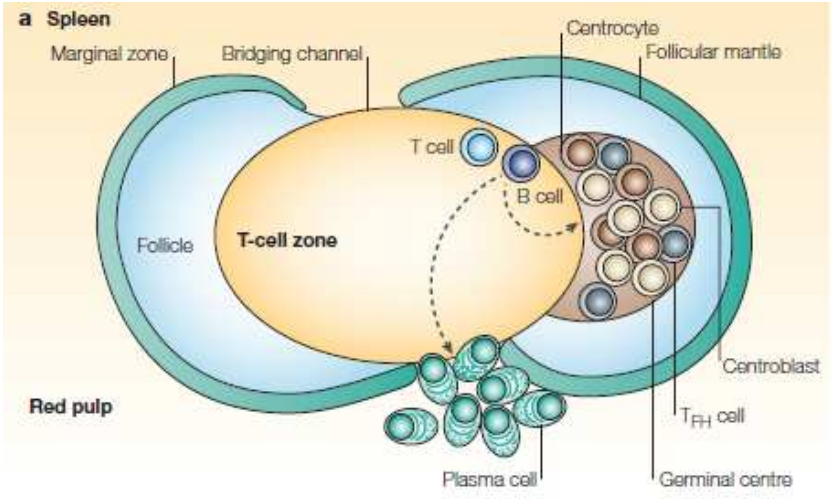

Figure 2; Schematic view of lymphoid structure organization in the spleen [34]

2.1 Homing chemokines in lymphoid tissue

The B-cell chemo attractant 1 (BCA-1), also called CXCL13, is predominantly produced in the B-cell follicle [37]. It is the ligand for CXC-chemokine receptor (CXCR) 5, which is highly expressed by follicular B cells [38]. In contrast, T-cell areas are rich in the CC-motif chemokines CCL19 and CCL21, responsible for the homing of naïve T-cells and dendritic cells (DC) which express high levels of the CC-chemokine-receptor CCR7, which specifically binds to CCL21 and CCL19 [38, 39]. Ag-activated B-cells within the follicle up-regulate their expression of CCR7, resulting in their migration towards the T-cell area, which is rich in CCL19 and CCL21 [40]. Chemotaxis has been identified as the driving force behind B-cell migration within follicles towards T-cell areas [41]. Ag-engaged B-cells migrate towards the T-cell area boundary by an increasing CCL21 gradient towards the T-cell area. Ag-engaged B-cells from $CCR7^{-}/^{-}$ mice move at similar speeds in random directions through the follicle, but wildtype Ag-engaged B-cells slow down upon antigen binding and then move primarily in the direction of the T-cell area [40, 41]. These findings indicate that up-regulated CCR7 expression on antigen-activated B-cells is obligatory for CCL21 gradient-directed B-cell movement in lympoid tissues towards the T-cell zone. In contrast, exact localization of B-cells along the T-cell boundary depends predominantly on CXCL13 [38]. Thus, whereas naïve follicular B-cells express high levels of CXCR5 and are therefore attracted to the CXCL13-rich follicle, Ag-activated B-cells up-regulate CCR7 expression, resulting in migration towards the T-cell area. However, some remaining expression of CXCR5 keeps activated B-cells from entering the T-cell area, though B-cells remain close to the boundary [40].

While Ag-activated B-cells up-regulate CCR7 expression, activation of $CD4^{+}$ T-cells by antigen-presenting cells (APCs) in the T-cell area leads to T-cell proliferation and subsequent up-regulation of CXCR5 expression [42]. Enhanced expression of CXCR5 on activated $CD4^{+}$ T-cells induces migration towards the B-cell follicle and this process is optimized by simultaneous down-regulation of $CD4^{+}$ T-cell CCR7 expression [32].

2.2 Germinal Centers

Although B-cells can differentiate into short-lived plasma cells outside GCs, plasma cells matured within GCs produce more Ab with higher Ag-affinity [43]. GC B-cells are generally divided into centroblasts and centrocytes, both residing in distinct areas (figure 3). The classical model describes that the dark zone exists of proliferating B-cells (i.e. centroblasts) which undergo somatic hypermutation (SHM) [44]. Expression of CXCR4 is specifically expressed by proliferating centroblasts [44]. Consequently, CXCR4 ligation with its ligand, CXCL12, is observed only in the GC dark zone [44]. Centrocytes which undergo Ig-isotype switching and Ab affinity maturation reside mainly in the GC light zone [45, 46].

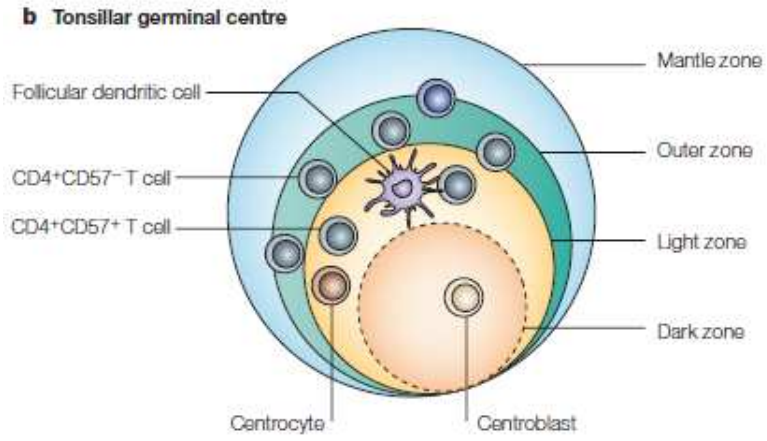

Figure 3; Schematic view of germinal center structure of the humane tonsil. *Note: CD57 expression has been identified in human GCs, whereas it is not expressed in murine GCs. Source:* [34].

In vivo imaging of GC B-cell movement has recently demonstrated that, in contrast to the classical model, GC B-cells continuously move between both zones and that the number of GC B-cells in various proliferating stages is similar in both zones [29]. However, as proliferating GC B-cells tend to accumulate only in the dark zone, it has been suggested that some mechanism selects B-cells to migrate out of or return to the GC. Ag-binding affinity is the selective marker for follicular Ag-engaged B-cells to join an already existing GC [47]. GC B-cells are selected by $CD4^+$ T-cells by Ig Ag-affinity and therefore B-cells compete for taking part in GC

reactions. Thus, Ag-activated B-cells are selected by $CD4^+$ T-cells in the GC environment for Ag-affinity and this selection procedure somehow results in enhanced Ab production by B-cells, together known as GC reactions.

2.3 Role of Follicular Dendritic Cells in germinal centers

The germinal center light zone, characterized by CXCR5 and CXCL13 expression, is rich in stromal cells called follicular dendritic cells (FDC) [33, 44]. B-cells depend on FDCs for their survival, for example by FDC-derived B-cell activating factor (BAFF) [48]. Also, FDCs can induce SHM in GC B-cells [43]. FDC networks play an essential role in maintaining GCs, as GC formation is abrogated in the absence of FDC networks [49]. It has been demonstrated that B-cell movement within GCs is highly dependent on both antigen and FDC networks [47]. In the absence of antigen, B-cells move through the FDC-rich light zone at a relatively constant speed. However, in the presence of antigen, activated B-cells slow down while repeatedly contacting FDCs during their way through the FDC network. It has been suggested that the decrease in velocity is caused by so called entrapment of B-cells in antigen-bound immune complexes on FDCs. Indeed, it has been observed that FDCs present immune complexes (IC) to B-cells in vitro as well as in vivo and that this process significantly enhances SHM and Ab production via cross-linking of multiple B-cell receptors [43, 50, 51]. In vitro co-cultures of GC B-cells and FDCs in the presence of ICs has shown that FDC-induced SHM results in mutations predominantly in complementarity determining regions (CDR) 1 and 2 [43, 51]. Interestingly, these mutations are associated with enhanced high-affinity IgG production [51].
Murine FDCs are also a source of interleukin (IL)-6, a cytokine which promotes B-cell maturation [51]. Treatment of FDC-positive mice with anti-IL-6 reduces Ab production in vivo by ~80% as compared to mice lacking FDCs. In addition, IL-6 production by FDCs is enhanced by IC-mediated activation, which specifically involves Fcγ receptor IIB (FcγRIIB) signaling [51]. Thus, FDCs are essential in regulating B-cell survival and maturation via various factors and immune complexes further enhance FDC-B-cell signaling.

Another factor involved in FDC-mediated signaling in vivo is Lymphotoxin β (LT-β). LT-β is a member of the TNF family and stromal cells, including FDCs, express high levels of receptors for both TNF-α (TNF-αR) and LT-β (LT-βR) [49]. LT-$\beta^{-/-}$ as well as TNF-$\alpha^{-/-}$ mice show impaired GC formation [49, 52]. It has been corroborated that stromal cell LT-βR ligation in secondary lymphoid tissues induces CXCL13 production in vitro, involving distinct NFκB signaling pathways [53]. In addition, LT-

βR mediated CXCL13 production is enhanced by additional ligation of TNF-αR [53]. Besides inducing the synthesis of follicular homing chemokines for T- and B-cells by FDCs, LT-β plays an essential role in regulating the homeostasis and proliferation of dendritic cells [39].

Murine knockout studies identified both T- and B-cells as being the main sources of LT-α/β expression, as both can induce CXCL13 production by stromal cells in vivo [52]. Moreover, CXCL13, CXCL12, CCL19 and CCL21 all enhance LT-β expression by B-cells in vitro [38]. It suggests that chemokine production and lymphocyte migration in lymphoid tissues is regulated by a positive feedback loop.

Conclusively, these results indicate that in lymphoid tissues, stromal accessory cells are highly involved in maintaining GC formation and contribute to GC reactions. Possibly, this regulatory mechanism involves both $CD4^+$ T-cells and FDCs, which simultaneously provide help for B-cell differentiation and survival in GCs. It suggests that B-cells circulate between the light and dark zone within the GC environment, while contacting both cell types for selection for Ig-affinity maturation and proliferation signals.

2.4 Altered somatic hypermutation in autoimmune diseases

CXCL13 attracts CXCR5-expressing B-cells toward FDC rich areas, where SHM and high-affinity Ab production by B-cells is enhanced. Analysis of various datasets on mutations in genes encoding the immunoglobulin-variable region (IgV) in various autoimmune (AI) diseases (multiple sclerosis, RA, Myastenia Gravis, and Sjörgen's syndrome) has revealed that AI B-cells possess more IgV mutations than healthy control B-cells [54]. However, the selection pressure is similar between ectopic GC B-cells and normal GC B-cells (based on normal selection procedure, including B-cell apoptosis). Therefore, it has been suggested that in AI GCs, B-cells accumulate IgV mutations in time, which would be consistent with disease chronicity. However, the mechanism underlying this process has not been completely identified yet. Interestingly, IgV mutations in RA GC B-cells are shown to be directed against only a small variety of antigens [55]. In addition, the mutational patterns and clonal relationship between RA GC B-cells has suggested that GC B-cells in RA re-enter GC reactions and SHM, resulting in the production of auto-antibodies with increasing Ag affinity. Together, these results indicate that GC formation contributes to RA pathology by supporting high-affinity Ab production by GC B-cells.

Autoimmune mice in the BXD2 model spontaneously develop a severe form of arthritis at a later age and this phenotype is accompanied by the production of multireactive auto-antibodies [56]. Consequently, GC B-cells of BXD2 mice express enhanced levels of the enzyme activation-induced cytidine deaminase (AID) as compared to wildtype littermates. AID induces SHM and Ig-isotype switching in vitro and AID expression is enhanced by T-/B-cell interactions via (T-cell) CD28 and (B-cell) CD86 [56, 57]. This is consistent with the observation that in vivo AID expression is higher in B-cells in T-cell dependent stages of development than in other B-cell types [56]. These results support the fact that AID plays a role in enhancing SHM and IgG production in autoimmunity as well and that AID expression is enhanced by $CD4^+$ T-cell help.

The need for T-cell help in the induction of AID in B-cells is supported by the fact that constitutive AID expression in transgenic B-cells does not affect B-cell development, nor do transgenic AID B-cells show enhanced SHM and Ig-isotype switching in the absence of T-cells [58]. Although these experiments were only performed in the absence of antigen, it does suggest that under normal conditions,

in vivo B-cell AID expression is negatively regulated by B-cells themselves and that an external signal is needed to enhance SHM, Ig-isotype switching and B-cell differentiation.

In rheumatoid synovial tissues AID expression is high, especially in tissues with FDC networks and lymphoid follicular organization [20]. In addition, it has been confirmed that AID expression in RA synovial tissue enhances B-cell Ig-isotype switching and ACPA production [20]. These findings suggest the involvement of enhanced signaling by other cells in RA tissue (either directly or indirectly), which induces B-cell AID expression, resulting in auto-antibody production.

2.5 Germinal center B-cells require CD4+ T-cell help

2.5.1 Contribution of follicular helper T-cells

Ab responses by GC B-cells are highly dependent on $CD4^+$ T-cell help, which regulate B-cell proliferation, apoptosis and differentiation into plasma cells [42]. Interestingly, rheumatoid synovial tissues with structurally organized lymphoid follicles show a higher degree of T-cell activation than those without a strict organizational pattern, consistent with the fact that lymphoid follicular structures fascilitate T-/B-cell interactions [26]. In addition, treatment of collagen-induced arthritis (CIA) rats with a CD4-mediated inhibitor of T-cell activation results in delayed onset of clinical athritic symptoms, but not in a reduction of symptom severity in already established RA [59]. Thus, $CD4^+$ T-cells play an important role in initiating and maintaining early B-cell mediated pathology of RA in vivo, although the mechanisms involved are not clear yet.

However, it has been suggested that GC B-cell selection and maturation specifically requires the help of T_{FH} and in the absence of T_{FH}, GC reactions are disrupted [28, 34, 60]. Mice in the BXD2 autoimmune model show increased production of auto-antibodies, which is associated with GC formation and enhanced B-cell proliferation, SHM, IgG isotype switching and IgG serum levels in vivo [56].

It has been suggested that T-/B-cell interactions at the follicular boundary determine whether activated B-cells migrate out of the follicle to become short-lived Ab-secreting cells, or start GC formation [61]. Within GCs, activated Ag-presenting B-cells form stable cognate interactions with one or more $CD4^+$ T-cells at the T-/B-cell boundary [41]. These stable conjugates have been observed to move around in the follicle, with a velocity and direction dependent on B-cell movement [41]. This is supported by in vivo imaging of GC reactions, showing that only a limited number of $CD4^+$ T-cells is involved in cognate T-/B-cell interactions and that the B-cell number highly exceeds the T-cell number [29]. These findings support the fact that B-cells compete for T-cell help in GCs in which Ag-affinity is the main selection factor. However, also IC-bearing FDCs have been suggested to play a role in the selection of GC B-cells by Ag-affinity and the exact cooperation between FDC- and $CD4^+$ T-cell-mediated B-cell selection still has to be elucidated [51].

A specific role of B-cell help by T_{FH} in autoimmunity has been indicated by studies on *Roquin* $^{san/san}$ mice. These mice are homozygous for a knockout in the *Roquin* (*Rc3h1*) allele, encoding a member of the RING-type ubiquitin ligase protein family, which is responsible for RNA translation and stability in $CD4^+$ T-cells [60]. *Roquin*$^{san/san}$ mice show spontaneaous GC formation with corresponding increased production of pathogenic auto-antibodies. Also, the mice have a phenotype like observed in systemic lupus erythematosus (SLE).

It is strongly suggested that auto-antibody production by *Roquin*$^{san/san}$ mice is the result of a defect in the selection process for autoreactive B-cells, by a specific group of $CD4^+$ T-cells, which express enhanced levels of CXCR5, PDCD-1 and CD200 [60]. Also an increase in IL-21 production by this subset has been observed. These characteristics are now known as specific markers for T_{FH} cells [reviewed by [62]].

In vivo treatment of arthritic mice with Abatacept, an antibody which reduces CD28 signaling, inhibits the generation of auto-antibodies via inhibition of T_{FH} development [63]. Abatacept inhibits both Ag-induced T-cell activation and proliferation and reduces T-cell migration towards B-cell follicles in lymph nodes of OVA-immunized RA mice [63]. These effects correspond with reduced Ag-induced expression of CXCR5, ICOS and PD-1, suggesting that Abatacept acts directly on T_{FH} cells in its inhibition of GC reactions and B-cell mediated auto-antibody production. It also suggests that $CD4^+$ T-cells play a role in AI pathology. Various molecules have been identified recently as characteristic of T_{FH} cells and the involvement of some of these molecules in autoimmunity has been suggested by several studies. These will be discussed in the next sections.

3 B-cell help by follicular helper T-cells involves various signaling molecules

3.1 Bcl-6 expression regulates T_{FH} cell differentiation

T_{FH} cells can be distinguished from other helper T-cell subsets by their high expression of the transcription factor B-cell lymphoma (Bcl) -6 [64]. In vivo studies have demonstrated that Bcl-6 expression by $CD4^+$ T-cells directly induces T_{FH} cell differentiation [64]. Master transcription factors have been identified for other T helper cell subsets as well, i.e. Tbet (T_H1), GATA-3 (T_H2), FoxP3 (T_{reg}) and RORγt (T_H17) (figure 4) [reviewed by 65]. Bcl-6 expression suppresses differentiation of the T-cell subsets T_H1 and T_H2 by directly suppressing expression of Tbet and GATA-3, respectively [64]. Also, T_{reg} cells and T_H17 cell differentiation is suppressed by Bcl-6, by Bcl-6-mediated suppression of FoxP3 and RORγt functionality [64]. These findings indicate that T_{FH} cell differentiation is favoured over differentiation into other T_H cell subsets in the presence of Bcl-6.

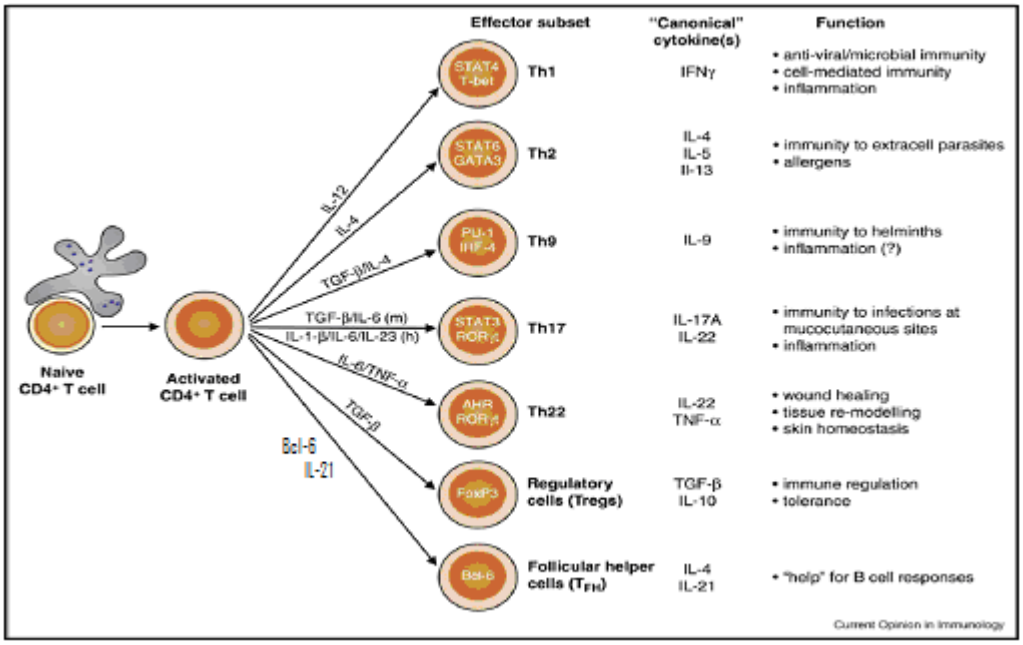

Figure 4; Overview of transcription factors and cytokines mediating differentiation into various $CD4^+$ effector T-cells *(adapted from [65])*

3.2 Co-stimulatory molecules enhance T-/B-cell interactions

3.2.1 ICOS / ICOSL signaling

It has been suggested that enhanced T_{FH} differentiation is the main contributor to the pathogenesis of *Roquin*$^{san/san}$ mice, with symptoms as described previously [60]. T_{FH} have the unique ability to drive GC formation in *Roquin*$^{san/san}$ mice, even in the absence of antigen [66]. In addition, it has been confirmed that the Roquin protein is involved in suppression of both survival and differentiation of T_{FH} cells [66]. Interestingly, both number and size of GCs in *Roquin*$^{san/san}$ mice correlate with enhanced expression of ICOS (Inducible COStimulatory molecule) on $CD4^+$ T-cells [66]. Also, enhanced ICOS expression corresponds with increased levels of B-cell activation. Accordingly, $ICOS^{-/-}$ mice show impaired GC formation and reduced numbers of T_{FH} cells in vivo [67]. The ligand for ICOS, B7RP-1, is expressed by various cells, such as resting B-cells, macrophages and dendritic cells, and ICOS-B7RP-1 signaling is essential for the formation of cognate T-/B-cell interactions as well as GC formation in vivo [49, 67, 68]. Autoimmune mice from models for both RA (CIA) and SLE (NZB/NZW F_1) produce high levels of IgG against self-antigens, but when treated with anti- B7RP-1, which blocks ICOS-B7RP-1 signaling, both T_{FH} and GC B-cell numbers are significantly reduced in vivo [69]. Consequently, anti-B7RP-1 treatment reduces CIA and SLE symptoms, auto-antibody production and disease onset in vivo. This strongly suggests that ICOS-B7RP-1 signaling is involved in mediating disease severity by regulating GC reactions in vivo. Interestingly, anti-B7RP-1 does not affect IgG production by isolated GC B-cells from CIA or SLE mice in vitro, which indicates that ICOS-B7RP-1 signaling does not directly affect the capacity of $CD4^+$ T-cells to help B-cells [69].

Mice deficient of ICOS are completely resistant to collagen-induced arthritis (CIA) (figure 6) [70]. $ICOS^{-/-}$ CIA mice show reduced IgG2a and IgM serum levels compared to wildtype controls, confirming the role of ICOS in the induction of Ab responses, Ab-affinity maturation and Ig-isotype switching. In vitro analysis of cytokine production by splenic T-cells of $ICOS^{-/-}$ and $ICOS^{+/+}$ mice has shown that IFN-γ and TNF-α levels are unaffected by the absence of ICOS expression. In contrast, IL-17 production by activated $ICOS^{-/-}$ T-cells is dramatically reduced in vitro [70]. It is

suggested that CIA-resistance of $ICOS^{-/-}$ mice is caused by reduced IL-17 production and/or T_H17 cell differentiation in vivo.

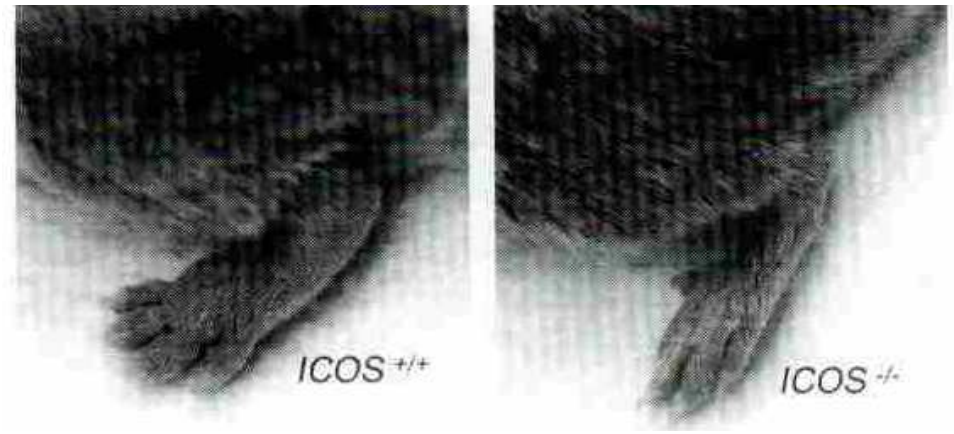

Figure 5; **Joint swelling in $ICOS^{+/+}$ and $ICOS^{-/-}$ DBA/1 mice in a CIA mouse model on day 58, resulting from 3 time point of immunization with Collagen type II (CII) in Complete Freud's Adjuvant (CFA)** [70].

Conclusively, ICOS-B7RP-1 interaction between antigen-specific T- and B-cells is required for the in vivo induction of GC B-cell responses, but other signaling pathways are involved in the eventual anti-body production.

3.2.2 CD40 / CD40L signaling

Another signaling pathway closely involved in T-/B-cell communication is the CD40/CD40L interaction. CD40 is constitutively expressed by B-cells, whereas CD40L expression by $CD4^+$ T-cells is quickly induced by TCR ligation, though only sustained by co-stimulation via either CD28 or ICOS signaling in vivo [71]. Interestingly, ICOS/ICOSL interaction induces CD40L expression more than CD28/CD80-CD86 interactions, suggesting a difference in T-cell activation pathways, depending on the expression of co-stimulatory molecules on the cell which contacts the T-cell [68]. CD40/CD40L interaction is essential in sustaining cognate interactions between T- and B-cells [49, 61]. Interuption of this signaling pathway has been shown to abrogate various T-cell mediated immune diseases reviewed by [72]. Interestingly, also inhibition of CD40/CD40L signaling leaves auto-antibody production by murine CIA or SLE B-cells unaffected in vitro, suggesting an important role for CD40/CD40L signaling in initiating GC reactions in vivo, but, like ICOS/ICOSL signaling, not directly in T-cell B-cell help in the production of antibodies [69].

In contrast, in vitro exposure to anti-CTLA4 (within a co-culture of T_{FH} and antigen-specific B-cells isolated from CIA and SLE mice) did suppress antibody production significantly [69]. CTLA-4 (Cytotoxic T-Lymhpocyte Activator 4) is expressed by activated $CD4^+$ T-cells and is a co-stimulatory molecule contributing to T-cell receptor (TCR) activation. Whereas co-stimulation of the TCR and CD28 predominantly induces IL-2 production and, therefore, proliferation, co-stimulation of the TCR and CTLA-4 results in apoptosis and inhibits IL-2 production by humane, activated $CD4^+$ T-cells in vitro [73]. Thus, the observation that CTLA-4 Ig treatment inhibits B-cell mediated auto-antibody production in mice in the previously mentioned autoimmune models suggests that enhanced T-cell survival is involved in auto-antibody production in vivo.

3.2.3 SAP signaling

Another molecule associated with germinal center formation is signaling lymphocyte activation molecule (SLAM)-associated protein (SAP, downstream of TCR signaling).

$SAP^{-}/^{-}$ mice show reduced and abnormal GC formation and, accordingly, impaired long-lasting but not acute humoral immunity [74]. Importantly, SAP-deficiency leads to reduced numbers of T_{FH} cells in non-immunized mice in vivo [66]. Also, the spontaneous formation of GC in *Roquin*$^{san/san}$ mice is abrogated in Roquin$^{san/san}$ SAP-deficient mice, corresponding with reduced auto-antibody production [66]. It indicates the involvement of SAP in GC formation, maintenance and auto-antibody production in vivo via inhibited T_{FH} function and, possibly, inhibited T_{FH} cell differentiation.

SAP expression by $CD4^{+}$ T-cells is required for the maintenance of specifically stable T-/B-cell interactions in lymphoid tissues [75]. SAP-deficient $CD4^{+}$ T-cells fail in maintaining contact with B-cells and this corresponds with decreased B-cell clonal expansion in GCs. Interestingly, SAP-deficient $CD4^{+}$ T-cells also migrate less towards B-cell follicles and, in addition, do not accumulate within the B-cell follicle along the GC border [75]. It has been recently shown that SAP-mediated T-/B-cell contact depends on both up-regulated expression of adhesion integrins LFA-1 (Lymphocyte Function-associated Antigen 1) and VLA-4 (Very Late Antigen 4) and by up-regulated expression of the SLAM family members CD84 and Ly108 by T-cells [76]. Interestingly, though $CD84^{-}/^{-}$ mice do show GC formation, GC T_{FH} cell numbers are significantly reduced. Conclusively, these findings suggest that SAP-mediated T_{FH} cell signaling up-regulates expression of some molecules involved in maintaining the structural organization of GCs, but that other molecules downstream of SAP contribute predominantly to T_{FH} cell differentiation and function in vivo.

3.2.4 PSGL-1 expression as a marker for T_{FH} cells

A specific $CD4^+$ T-cell subset has been identified in MRL mice (in the SLE autoimmune model) which migrate mainly to extra-follicular sites in the spleen and show decreased expression of P-selectin glycoprotein ligand 1 (PSGL-1) [31]. It has been suggested that this subset of $PSGL1^{low}$ T-cells must be related to T_{FH} cells, as they have T_{FH}-like abilities (such as IL-21-mediated induction of Ig-isotype switching and migration towards B-cell follicles upon Ag exposure). PSGL-1 has a high affinity for both CCL19 and CCL21 and down-regulation of PSGL-1 expression leads to migration of $CD4^+$ T-cells away from the T-cell area [31]. In addition, down-regulation of Ag-induced $CD4^+$ T-cell PSGL-1 expression results in enhanced expression of PD-1, CXCR5, Bcl-6, IL-21 and ICOS, suggesting that PSGL-1 is another characteristic of the T_{FH} cell subset in vivo [31]. Indeed, GC T-cells lack expression of PSGL-1 and it has been corroborated that down-regulation of PSGL-1 on T_{FH} cells is enhanced by Bcl-6 expression, but this process is independent of increased PD-1, IL-21 and CXCR5 expression [31]. It suggests that T_{FH} cell expression of PD-1, IL-21 and CXCR5 are related with down-regulation of PSGL-1, but that PD-1, IL-21 and CXCR5 are either downstream of PSGL-1 or part of a different signaling pathway as well. Indeed, it has been confirmed that Bcl-6 can directly suppress PSGL-1 expression and that T-/B-cell interactions are required to induce a robust T-cell Bcl-6 expression [31]. However, some Bcl-6 expression is observed in the absence of B-cells. T-/B-cell interactions are required for T-cell CXCR5 and PD-1 expression and it is suggested that down-regulated PSGL-1 expression fascilitates T-cell migration towards the B-cell area, where cognate T-/B-cell interactions result in up-regulation of CXCR5 and PD-1. It is hypothesized that $PSGL\text{-}1^{low}$ $CD4^+$ T-cell function in MRL mice is additionally regulated by P-selectin expression by endothelial cells [31]. Thus, down-regulation of PSGL-1 on activated $CD4^+$ T-cells by Bcl-6 or via interaction with endothelial cells, results in up-regulation of CXCR5 and PD-1 by T-cells, which simultaneously migrate away from the T-cell area via decreased responsiveness to CCL19 and CCL21.

3.3 T_{FH} cell-derived IL-21

Once fully differentiated, T_{FH} primarily produce IL-21, but also some IL-4 and IFN-γ [reviewed by [62]. The IL-21 receptor (IL-21R) is highly expressed by T_{FH} cells and, though in the absence of IL-21 some CXCR5 expression is induced via Ag-induced ICOS signaling, IL-21 is required for full CXCR5 expression by T-cells, resulting in complete T_{FH} differentiation [77]. Furthermore, IL-21 directly induces Bcl-6 expression in $CD4^+$ T-cells in vitro, resulting in increased T_{FH} differentiation [64]. This indicates that IL-21 is involved in T_{FH} differentiation in an autocrine manner. IL-21 reduces CCR7 expression by T-cells, which is required for T-cell migration away from the T-cell area and IL-21 therefore contributes to enhancing contact between T- and B-cells [77]. Coherently, ICOS-B7RP-1 signaling enhances T_{FH} IL-21 production, suggesting the need for T-/B-cell interactions in maintaining optimal T_{FH} differentiation and function in vivo [77].

A direct effect of IL-21 on B-cells has been demonstrated as well, as IL-21 induces Ig-isotype switching and Ab secretion in vitro and in vivo [77, 78]. IL-21 directly induces Bcl-6 expression in B-cells also and, consequently, Bcl-6 is primarily expressed in GC B-cells and not in extra-follicular B-cells [reviewed by 79]. In contrast, extra-follicular B-cells express high levels of Bcl-6 antagonist, Blimp-1, which is the reciprocal antagonist of Bcl-6 on expression level [80]. It is suggested that Bcl-6 expression reduces natural protection mechanisms against DNA damage in GC B-cells, which results in survival of GC B-cells while undergoing SHM [81, 82]. In addition, T-/B-cell interactions enhance Bcl-6 expression by CD40/CD40L signaling, which corresponds with reduced expression of DNA damage checkpoint proteins during GC reactions [83].

Conclusively, IL-21 is predominantly produced by T_{FH} cells and it directly induces Bcl-6 expression in both GC T- and B-cells. As Bcl-6 is the main regulator of T_{FH} differentiation and fascilitates B-cell survival during GC reactions, IL-21 contributes to GC reactions and, possibly, Ab production in vivo.

4 Lymphoid structures in rheumatoid synovial tissue

Rheumatoid synovial lymphoid tissue and secondary lymphoid tissues show similarities in phenotype as well as reactions. Rheumatoid ectopic GCs are marked by the presence of FDCs and diffuse lymphoid aggregates lack FDC networks [23]. It has been demonstrated that CXCL13 in rheumatoid synovial follicles is primarily produced by FDCs and that enhanced CXCL13 production correlates with increased B-cell numbers in the tissue [84]. Interestingly, two more sources of CXCL13 production have been identified in rheumatoid synovia, i.e. endothelial cells and synovial fibroblasts [23]. Furthermore, the presence of FDC networks correlates with AID expression in RA synovial tissue and both factors fascilitate SHM and Ig-isotype switching in rheumatoid B-cells in vivo [20].

Indeed, CXCL13 and CCL19 expression correlate with the degree of lymphoid organization in RA synovia [84]. Interestingly, CCL21 expression in synovial follicles shows only a small association with the incidence of lymphoid neogenesis [25]. However, the presence of GCs can only be predicted by CXCL13 and LT-β expression in RA synovia [26]. RA synovial GCs show a ten to twenty-fold higher production of the homing chemokines CXCL13 and CCL21, as compared to non-GC containing RA synovial lymphoid structures [84]. Together, these results point out the correlation between FDC networks, homing chemokine production and GC formation in rheumatoid synovial lymphoid tissue. Conclusively, it suggests that lymphoid neogenesis with GC formation contributes to B-cell maturation and Ab-production in rheumatoid synovial tissues.

4.1 Inflammatory environment in RA contributes to GC formation

Comparison of blood samples from patients with OA (ostheoarthritis), RA and SLE has shown that B-cell expression of CCR6, CCR7 and CXCR4 expression from RA patients are reduced compared to OA and healthy controls, suggesting the involvement of altered B-cell migration in RA [85]. Interestingly, RA serum B-cells express less CXCR5 than OA serum B-cells, which has been suggested to point out increased plasma cell differentiation. Furthermore, the decrease in CXCR5 expression corresponds with an increase in CXCR3 expression by RA serum B-cells [85]. CXCR3 specifically responds to inflammatory chemokines (such as CCL9 and CCL10) and is detected on RA synovial B cells as well [85, 86]. Also, CXCR3 blockade in RA patients reduces disease severity as well as disease onset [86]. These findings suggest that chronic inflammation in RA affects chemokine expression, as these effects are not observed in the non-inflammatory disease OA. However, it is not clear what mechanisms precede these effects.

Lymphoid organ neogenesis is dependent on various factors, including TNF-α and LT-β signaling [53]. Expression levels of LT-β differ between follicular GC B-cells, and it is suggested that LT-β expression is regulated by ICOS signaling [52]. GC B-cells from ICOS $^{-}/^{-}$ mice show significantly decreased LT-β expression compared to wildtype GC B-cells. In contrast, ICOS$^{-}/^{-}$ follicular B-cells express LT-β at the same level as wildtype controls [49]. It suggests that LT-β expression by GC B-cells is highly dependent on ICOS expression by $CD4^{+}$ T-cells.

Increased ICOS expression in RA synovia corresponds with increased T_{FH} cell numbers in organized lymphoid follicles [66, 70, 87]. Interestingly, TNF-α treatment reduces synovial inflammation and lymphoid neogenesis in already established RA, but the number of infiltrating B-cells seems unaffected by TNF-α [25]. This indicates that TNF-α plays a role in inflammation and (possibly) T-/B-cell signaling, which results in reduced pathology, rather than affecting B-cells directly.

Within GCs, SHM is typically involved in B-cell Ab responses and depends on expression of the enzyme AID. RA synovial tissue-derived cell lines, i.e. fibroblast-like synoviocytes (FLS), show enhanced AID expression compared to FLS derived from OA controls [88]. Moreover, RA-FLS AID expression is significantly enhanced by TNF-α and E2 (oestradiol) in vitro. In contrast, neither TNF-α nor E2 enhances AID

expression in OA-FLS. It suggests that RA-specific inflammatory factors are involved in the induction of AID expression in RA FLS rather than the effect being an inherent effect of inflammatory cytokines themselves [88]. The observation that E2 can enhance AID expression in RA-FLS supports a higher occurrence of RA in women than in men.

Autoimmune SKG mice spontaneously develop $CD4^+$ T-cell-mediated arthritis, via a defect in negative selection of autoimmune T-cells [89, 90]. In this model primarily T_H17 cells contribute to synovial inflammation, which is consistent with the humane RA synovial phenotype. It has been indicated that CCR6 expression is specifically expressed by T_H17 cells infiltrating the synovia of these mice and that CCR6 expression can distinguish infiltrating T_H17 cells from other infiltrating helper T-cell subsets [90]. Interestingly, SKG T_H17 cells show enhanced expression of IL-1R receptor 1 (IL-1R1), IL-17, IL-22, IL-21 as well as the chemokines CCR6 and CCL20 [90]. It has been demonstrated that RORγt expression is required for induction of CCR6 expression, whereas the cytokines TGF-β and IL-6 enhance CCR6-induced RORγt expression. However, TGF-β and IL-6 fail in inducing CCR6 expression by murine T_H17 cells directly [90]. Importantly, the ligand for CCR6, CCL20, is produced by FLS from arthritic joints of SKG mice in vitro. Furthermore, addition of IL-1β, TNF-α or IL-17 to the culture enhanced CCL20 production by FLS [90]. It suggests that inflammatory cytokines in RA synovia induce pro-inflammatory chemokine expression, which attracts T_H17 cells. Importantly, T_H17 cells themselves produce CCL20 as well, indicating an autocrine, positive feedback mechanism by CCR6/CCL20 interaction.

Conclusively, these findings indicate that the inflammatory environment of the rheumatoid synovium contributes to chemokine expression profiles and lymphocyte migration, and that further synovitis, in an autocrine way, fascilitates lymphoid neogenesis.

Various cytokines contribute to RA pathology, of which TNF-α, IL-1β and IL-17 contribute most robustly to chronic synovial inflammation as well as cartilage destruction [reviewed by 8, 91, 92]. Besides IL-1β, TNF-α and IL-17, enhanced levels of IL-6, IL-15, L-21 and IFN-γ have been observed in (isolated) RA synovial tissue [8, 9, 26, 93, 94]. Treatment of experimental CIA animals with TNF-α or IL-17A inhibitors reduces pathological symptoms significantly [91, 95]. Also, treatment with anti-IL-6 at the time of immunization reduces both incidence and disease activity scores in CIA mice, via reduced IL-17A production and T_H17 cell differentiation in vivo (but not

IFN-γ or IL-4 production) [96]. As T_H17-derived IL-17A promotes chronic inflammation and joint destruction, it suggests that blocking IL-6 levels is an interesting strategy for treatment of human RA by inhibiting T_H17 cell differentiation. Indeed, treatment of RA patients with tocilizumab, a humanized monoconal IL-6-receptor-blocking antibody, has shown positive results for reducing severity of already established RA [reviewed by 97]. Also, treatment of RA patients with biologicals like inhibitors for TNF-α and IL-1 reduces disease severity in a high proportion of patients, though not all patients respond well to one specific biological treatment [98].

In vivo T-cell depletion in RA SCID mice leads to decreased synovial macrophage numbers, resulting in reduced levels of IL-1β, TNF-α, IL-15, MMP-1 and MMP-2 [9]. It indicates that T-cells are involved in maintaining an environment that attracts macrophages to RA synovia. This is supported by the observed enhanced IFN-γ levels in RA synovia, which suggest enhanced synovial infiltration with macrophages. In addition, macrophages and fibroblasts, both present in RA synovia, produce the T-cell survival promoting cytokine IL-15 [99, 100]. Fibroblast-derived IL-15 has been shown to enhance $CD4^+$ T-cell survival in vitro [99]. Also IL-2 promotes T-cell survival within RA synovia, and synovia with highly organized lymphocyte patterns show higher levels of IL-2, IL-1β and IL-6 [26, 99]. Conclusively, these results suggest that $CD4^+$ T-cells play a prominent role in both development and chronicity of rheumatoid arthritis. There is no doubt, that the $CD4^+$ T-cell mediated inflammatory cytokine environment fascilitates synovitis and joint destruction. However, $CD4^+$ T-cells fascilitate B-cell mediated Ab production by direct cell-cell interactions as well, suggesting various mechanisms involved simultaneously in RA development.

4.2 RA synovia show enhanced lymphocyte survival

There is circumstantial evidence for reduced lymphocyte apoptosis in RA [56,99]. Although Rheumatoid synovial $CD4^+$ T-cells express high levels of apoptosis markers, such as Fas and Bcl-x_L, rheumatoid synovia show a reduction in T-cell apoptosis as compared to synovial tissue from healthy controls [99]. It is known, that GC T-/B-cell interactions require B-cell Fas expression in order to regulate

lymphocyte proliferation and differentiation accurately [101]. Transgenic mice with (only) B-cells deficient in Fas expression die of lymphoproliferative disorders, involving both enhanced T-cell and B-cell activation and proliferation [101]. Knockout of Fas expression on GC B-cells only, induces the same lymphoproliferative disorders in vivo, and T-cell depletion abrogates this effect. This supports the requirement of T-cells in the regulation of apoptotic pathways within GCs. Enhanced expression of Fas apoptosis inhibitory molecule, FAIM, reduces Fas-mediated B-cell apoptosis in vivo [102]. Whereas FAIM expression is observed primarily in GC B-cells, interferon-responsive factor 4 (IRF4) expression is characteristic of plasma cell differentiation, which is supported by the observed expansion of plasma cell number in vivo. It has recently been demonstrated that, in the presence of Ag, CD40 signaling enhances expression of FAIM by primary B-cells [102]. CD40-mediated induction of B-cell FAIM expression is regulated by NFκB signaling pathways and augments simultaneous CD40-mediated up-regulation of IRF4 and down-regulation of Bcl-6 in B-cells, in vitro [102]. Conclusively, this suggests that via CD40/CD40L signaling, T-/B-cell interactions in GCs suppress B-cell Bcl-6 expression and simultaneously induce IRF4 expression, resulting in increased plasma cell development. In addition, CD40/CD40L signaling reduces GC B-cell susceptibility to Fas-mediated apoptosis by induction of FAIM.

5 Evidence for enhanced T-cell help in RA

A correlation between organizational patterns of synovial lymphoid structures and T-cell activation levels has been shown in biopsies of rheumatoid knees [26]. Patients with highly organized mononuclear cell aggregates show a higher degree of local T-cell activation than patients with a diffuse infiltration pattern. Moreover, enhanced T-cell activation corresponds with IL-1β, IL-6 and IL-2 production in organized synovial infiltrates [26]. In these patients, increased IL-6 production has been associated with an increase in B-cell numbers, supportive of the positive effect of IL-6 on B-cell proliferation and maturation. Interestingly, TNF-α and IL-4 production do not correspond with synovial lymphoid tissue organization [26].

Activation of $CD4^+$ T-cells induces up-regulation of CD40L expression [94]. The fact that lymphoid tissue organization corresponds with T-cell activation therefore suggests enhanced CD40L expression and increased T-/B-cell interactions via CD40/CD40L signaling.

However, in vivo studies on mice have demonstrated that enhanced B-cell CD40 ligation by αCD40 monoclonal Ab (mAb) in combination with T-cell activation, favors extra-follicular B-cell differentiation over follicular B-cell differentiation in both lymph nodes and spleen [61]. Consequently, treatment with αCD40 mAb abrogates GC formation in spleen and lymph nodes and it has been corroborated that enhanced CD40 signaling via T-cells induces some effects like αCD40 mAb treatment. However, the effects were highly dependent on tissue type: T-cell dependent GC formation was abrogated by enhanced CD40 signaling in the spleen, but not in lymph nodes. It suggests that CD40 ligation during T-cell dependent Ag presentation affects the balance between follicular and extra-follicular B-cell differentiation pathways and, more importantly, that this balance is tissue specific. However, increased T-cell activation in rheumatoid synovial tissues suggests an increase in CD40L expression. Also, T-cell activation is suggested to correspond with increased GC reactions rather than decreased. This might be consistent with the pathways of antigen presentation involved in RA, such as B-cells, rather than other APC, activating $CD4^+$ T-cells in GCs, although this has to be investigated further.

Interestingly, a large cohort study, carried out in the UK, has revealed a strong association in single nucleotide polymorphisms (SNP) in the CD40 locus and RA

development [103]. Also, *CD40* and *CCL21* polymorphisms correlated with serum RF- and anti-CCP Ab levels, indicating the involvement of CD40 and CCL21 expression in the occurrence of auto-antibody production (RF and Anti-CCP) in RA. However, it has not been confirmed whether the auto-antibodies were produced during GC reactions. The maintenance of GC reactions is highly dependent on the duration of T-/B-cell interactions and therefore, on various signaling molecules. SAP and ICOS are essential in the formation of stable T-/B-cell conjugates in lymphoid tissue and both molecules show altered expression in RA patient samples [70,104,105].

Expression of SAP by peripheral blood (PB) T-cells from RA patients is significantly lower than SAP expression by T-cells from healthy controls [104]. This is consistent with the observation that also expression of an important signaling molecule downstream of SAP, SLAM, by PB T-cells from RA patients is low, although it does not differ from expression by healthy individuals [105]. In contrast, rheumatoid synovial T-cells show significantly higher SLAM expression than PB T-cells of the same RA patient, or synovial T-cells from healthy controls [105]. Interestingly, PB B-cells of RA patients show increased SLAM expression compared to healthy controls, which corresponds with increased SLAM expression by synovial fluid T-cells. It indicates that expression of these signaling molecules corresponds between RA T- and B-cells, which suggests that SAP/SLAM signaling is enhanced in RA synovia, prior to plasma cell differentiation in synovial lymphoid tissues.

6 Conclusion

Although clinical symptoms of rheumatoid arthritis do not necessarily correspond with patient seropositivity for auto-antibodies, an association between RA severity and auto-antibody production has been suggested by several studies. There is no doubt that lymphoid neogenesis in rheumatoid synovia contributes to the chronic character of RA by fascilitating B-cell proliferation, maturation and high-affinity antibody production. T-/B-cell interactions in lymphoid tissues robustly contribute to germinal center reactions and various signaling pathways are suggested to be involved in the initiation and maintenance of synovial lymphoid neogenesis and GC formation in RA. However, the exact mechanisms leading to GC formation in rheumatoid synovia still need to be elucidated.

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