(Anti-)TNF alpha matters in rheumatoid arthritis
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Chapter 10

Synovial lymphoid neogenesis does not define a specific clinical rheumatoid arthritis phenotype.

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ABSTRACT

Objective.
To determine the relationship between lymphoid neogenesis in the synovium of patients with rheumatoid arthritis (RA) and characteristics of inflammation and disease severity.

Methods.
103 biological-naive patients with active RA (Disease Activity Score in 28 joints ≥ 3.2) underwent an arthroscopic synovial biopsy. Sections were stained and analyzed by digital image analysis. Lymphocyte aggregates were counted and graded for size (1-3). Synovial lymphoid neogenesis was defined as the presence of grade 2 or 3 aggregates and subdivided based on the presence of follicular dendritic cells (FDC).

Results.
Lymphoid neogenesis was present in 32% of the RA synovial tissues, whereas an additional 25% contained only grade 1 aggregates. FDC were present in 28% of samples with lymphoid neogenesis, corresponding to 8% of the total RA cohort. Histologically, synovia with lymphoid neogenesis showed increased infiltration by T and B lymphocytes, plasma cells, and macrophages, and increased expression of TNFα and LTβ compared with samples without lymphoid neogenesis. Patients with lymphoid neogenesis also had higher C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR) and elevated leukocyte and thrombocyte counts, but no increase in clinical signs and symptoms. Of importance, there was no relationship between the presence of lymphoid neogenesis and IgM rheumatoid factor or anti-citrullinated protein antibodies. The presence of lymphocyte aggregates with FDC did not define a specific clinical phenotype compared to lymphocyte aggregates without FDC.

Conclusion.
Synovial lymphoid neogenesis is associated with more severe synovial and systemic inflammation, but this is not confined to a specific clinical subset of RA.
Synovial lymphoid neogenesis does not define clinical RA subtype

1. The extent and pattern of lymphocyte infiltration in the inflamed synovial tissue is widely variable among different rheumatoid arthritis (RA) patients. In some tissues a diffuse or scarce infiltration of T cells is present, while in others B and T cells are organized in perivascular aggregates with surrounding fields of plasma cells [1-3]. Sometimes large aggregates are present containing clusters of follicular dendritic cells (FDC), exhibiting features normally present in germinal centers of lymphoid tissue [1, 3, 4]. This suggests lymphoid neogenesis occurs in rheumatoid synovial tissue.

2. It has been suggested that synovial tissues with diffuse infiltration and tissues with lymphoid neogenesis represent different pathophysiological subtypes of RA [5]. A further subdivision has been suggested into highly organized ‘germinal center-like’ lymphoid neogenesis and a less organized form of lymphoid neogenesis in which T and B cells form aggregates, but exhibit no other ‘germinal center-like’ features [5]. In contrast, other studies have shown that lymphoid neogenesis in rheumatoid synovial tissue is a more continuous spectrum, with lymphocyte aggregates exhibiting different stages of lymphoid neogenesis present in the same patient [3]. Indications of a stable presence of synovial lymphoid neogenesis and a relationship with a distinct clinical phenotype have been suggested in some studies. First, it was shown that lymphoid neogenesis can already be found at the onset of clinical arthritis [2]. In line with this, it has been suggested to be a ‘fixed’ feature of synovial inflammation within different joints and patients [5]. Furthermore, synovial tissues containing lymphocyte aggregates are associated with increased expression of cytokines and adhesion molecules locally in the joint as well as in peripheral blood [6-8]. Finally, histologic subtypes of lymphocyte infiltration appeared to be correlated with the presence of rheumatoid factor, rheumatoid nodules, and joint erosiveness in small RA cohorts [1, 6, 9].

3. Since previous studies were small and mainly based on synovial tissue retrieved during joint surgery from patients with end-stage, destructive disease, it is still unclear whether lymphoid neogenesis defines a specific subset of RA and whether this is related to the presence of circulating autoantibodies. Therefore, we analyzed synovial tissue samples from a large cohort of patients with active RA for the presence of lymphoid neogenesis in relationship to clinical features and autoantibody status.

METHODS

Patients.

Synovial tissue was obtained from 103 RA patients fulfilling the American Rheumatism Association 1987 revised criteria [10]. All patients used methotrexate (5-30 mg/week), were naive to biologicals, and had active disease defined as a disease activity score in 28 joints (DAS28) ≥ 3.2 at the time of biopsy [11]. Oral corticosteroids (≤10 mg/day) and non-steroidal anti-inflammatory
drug (NSAIDs) were also allowed. Disease characteristics were assessed, including disease duration, number of prior disease modifying antirheumatic drugs (DMARDs), presence of IgM rheumatoid factor (IgM-RF) and anti-citrullinated protein antibodies (ACPA) as measured by the anti-CCP2 ELISA (Immunoscan RA, Mark 2, Euro Diagnostica No. RA-96RT, Arnhem, the Netherlands), and radiographic damage as evaluated by the Sharp van de Heijde score [12]. Disease activity was assessed by the DAS28, the tender and swollen joint count in 28 joints, the patients’ assessment of global disease activity (VAS gda), the erythrocyte sedimentation rate (ESR), C-reactive protein levels (CRP), hemoglobin levels, and thrombocyte and leukocyte counts. The study was conducted in compliance with the Helsinki Declaration, and the Medical Ethics Committee of the Academic Medical Center, University of Amsterdam approved the protocol. All patients gave written informed consent.

Arthroscopy and synovial biopsy.

A mini-arthroscopy was performed under local anesthesia in all patients to obtain synovial tissue samples from an actively inflamed knee, ankle or wrist joint [13]. Biopsies were taken with a 2-mm grasping forceps (Storz, Tuttlingen, Germany) from 6 or more sites within the joint to minimize sampling error. Previous work has shown that for T cell infiltration and expression of activation antigens in RA synovium, a variance of less than 10% can be reached when at least six biopsy specimens are examined [14], suggesting that representative data can be obtained when a limited number of biopsy samples from different areas within one joint are investigated. Consistent with these data, it has been demonstrated that using about six tissue samples allows for the detection of twofold differences in gene expression by quantitative polymerase chain reaction (PCR [15]). Thus, as T cells form an important cell population within the lymphocyte aggregates, we extrapolated these findings to the present study and decided to examine at least 6 biopsy samples per patient. The synovial biopsy samples were snap frozen en bloc in Tissue Tek OCT (Miles, Elkhart, IN) immediately after collection. Sections of 5 μm were cut and mounted on Star Frost adhesive glass slides (Knittelgläser, Braunschweig, Germany). Sealed slides were stored at -80°C.

Immunohistochemical analysis.

Synovial tissue sections were stained using the following monoclonal antibodies: anti-CD55 (67:Serotec, Oxford, UK) to detect fibroblast-like synoviocytes (FLS), anti-CD68 (EBM11: DAKO, Glostrup, Denmark) to detect macrophages, anti-CD3 (SK7, Becton Dickinson, San Jose, CA) for T cells, anti-CD22 (CLB-B-ly/1,6B11, the Netherlands) for B cells, and anti-CD38 (HB7, Becton Dickinson, San Jose, CA) for plasma cells. For detection of follicular dendritic cells (FDC) anti-CD21 long isoform (anti-CD21L; a kind gift from dr. Y.J. Liu) was used [16]. The following markers were used for detection of cytokines: anti-human TNFα (52B83; Monosan, Uden, Belgium) and anti-LTβ (c0404; Santa Cruz Biotechnology Inc, Santa Cruz, CA). Staining of cellular markers was performed using a 3-step immunoperoxidase method as described.
Synovial lymphoid neogenesis does not define clinical RA subtype

Previously [17]. For staining of cytokines, biotinylated tyramine was used as amplification, as described previously [18]. As negative control irrelevant immunoglobulins were applied to the sections instead of the primary antibody, or the primary antibody was omitted.

Assessment of lymphocyte aggregates.

The presence of lymphocyte aggregates was assessed on anti-CD3 stained sections. Aggregates were counted and graded by size according to the method described by Manzo et al. [3] with a slight modification: the aggregate size was assessed by counting the number of cells in the radius starting from an imaginary center of the aggregate. Aggregate size was classified into grade 1 (1-5 cells radius), grade 2 (5-10 cells radius) or grade 3 (more than 10 cells radius).

Tissue sections with no lymphocyte aggregates at all were graded as 0.

The presence of FDC, as stained by CD21L, was assessed in lymphocyte aggregates that were graded for size on 3 different tissue levels; T-B cell segregation was assessed at 2 different tissue levels at least 50 μm apart on sequential sections stained with CD3 and CD22. Thus, multiple sections representing different levels of a tissue block consisting of at least 6 biopsy samples were examined to minimize sampling error.

Digital image analysis.

All sections were analyzed at random by trained analysts who were blinded for clinical characteristics. The analysis was done by a computer-assisted image analysis algorithm as previously described in detail [19]. Images were acquired and analyzed using a Syndia algorithm on a Qwin-based analysis system (Leica, Cambridge, UK). Positive staining of cellular markers was expressed as positive cells/ mm² (counts/mm²), and positive staining of cytokines was expressed as integrated optical density/mm² (IOD/mm²). CD68+ macrophages, anti-LTβ and TNFα expression were analyzed separately in the intimal lining layer and the synovial sublining.

Statistical analysis.

Independent t-tests or Mann Whitney U tests were used to compare synovial (CD68, CD3, CD22 and CD38 positive cells), serological (ESR, CRP, leukocyte and thrombocyte counts), clinical inflammatory parameters (DAS28), and Sharp van de Heijde scores. The Chi-square test was employed to evaluate specific features of ACPA and IgM-RF positive disease. The same test was performed to compare these parameters between FDC positive and FDC negative aggregate synovitis. SPSS 12.0.2 for Windows (SPSS, Chicago, IL) was used.
RESULTS

Patient characteristics.

103 patients were analyzed. Demographic and clinical features are shown in Table 1. All patients used methotrexate. Oral low-dose corticosteroids were used by 25% of the patients. On average, patients failed treatment with 2.1 DMARDs prior to inclusion in the study. Data on rheumatoid nodules was available for 58 patients, of which 22 patients (38%) had nodular RA.

Frequencies of lymphocyte aggregates and FDC.

A mixture of lymphocyte aggregates of different sizes was present in the synovial tissue of 58 patients: in 16 patients (16%) aggregates up to grade 3 were present. In another 16 patients (16%) aggregates up to grade 2 were found and 26 patients (25%) had only small peri-vascular infiltrates (grade 1 aggregates). In the remaining 45 patients (44%) no lymphocyte aggregates were detectable (Figure 1).

Of the 103 synovial samples, 95 were analyzable for FDC staining. Eight of 16 samples with grade 3 lymphocyte aggregates showed CD21L positive staining (50% of tissues containing grade 3 aggregates and 8% of all tissues). FDC containing aggregates were found next to aggregates without FDC (Figure 2). Separate clusters of T cells and B cells were found in 7 out of 16 biopsies with grade 3 aggregates and did not occur in grade 1 or 2 aggregates.

<table>
<thead>
<tr>
<th>Table 1. Baseline patient characteristics.</th>
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<tbody>
<tr>
<td>N=103</td>
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<tr>
<td><strong>Demographics</strong></td>
</tr>
<tr>
<td>Age, mean (SD) years</td>
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<tr>
<td>Female, no (%)</td>
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<tr>
<td><strong>Disease status</strong></td>
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<tr>
<td>Duration, mean (SD) months</td>
</tr>
<tr>
<td>Erosive disease, no (%)</td>
</tr>
<tr>
<td>Rheumatoid factor positive, no (%)</td>
</tr>
<tr>
<td>ACPA positive, no (%)</td>
</tr>
<tr>
<td>DAS28, mean (SD)</td>
</tr>
<tr>
<td>ESR, median (IQR) mm/hour</td>
</tr>
<tr>
<td>CRP, median (IQR) mg/dL</td>
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<tr>
<td><strong>Drug treatments</strong></td>
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<tr>
<td>Previous DMARDs, median (IQR)</td>
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<tr>
<td>Methotrexate, median (IQR) mg/wk</td>
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<tr>
<td>Receiving corticosteroids, no (%)</td>
</tr>
<tr>
<td>Receiving NSAIDs, no (%)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD, median (interquartile range) or percentages, as appropriate. *IgM-RF = IgM rheumatoid factor; IQR = interquartile range; ACPA = anti–cyclic citrullinated peptide; DAS28 = Disease Activity Score 28-joint assessment; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DMARDs = disease-modifying antirheumatic drugs.
Synovial lymphoid neogenesis does not define clinical RA subtype

1. The presence of lymphoid neogenesis is associated with synovial inflammatory cell infiltration.

To study the relationship between synovial lymphoid neogenesis and other inflammatory features we divided the tissue into 2 groups: those with grade 2 or 3 aggregates (n = 32) and those with no aggregates or only grade 1 aggregates (n = 71). Synovial lymphoid neogenesis was defined as the presence of grade 2 or 3 aggregates, since aggregates of these sizes frequently exhibit characteristics of lymphoid neogenesis, such as T-B cell separation and CXCL13 and CCL21 expression [3, 20]; the remaining tissue was defined as diffuse synovitis. For both analyses, there were no significant differences in potentially confounding factors such as gender, age, disease duration, number of failed DMARDs, as well as use and dosage of prednisone or methotrexate between these 2 groups.

Figure 1. Different patterns of lymphocyte infiltration can be detected in the synovial tissue. In a proportion of patients a mixed infiltration of aggregates of T and B cells is present (A and B), together with a high number of infiltrating macrophages (C). Both TNFα (D) and LTβ (E) are abundantly expressed. In other patients a diffuse or scarce infiltrate of CD3+ T cells is found (F), and little or no B cells (G), while macrophages are the dominant infiltrating cell population (H). TNFα and LTβ are expressed at low levels in these patients (I,J). (Original magnification × 20.) See color figure page 220.

Figure 2. In 8% of the synovial tissue samples, clusters of follicular dendritic cells, expressing the CD21 long isoform (A), could be detected in lymphocyte aggregates enriched with CD22 positive B cells (B). (Original magnification × 20, inset × 40.) See color figures page 220
In tissue with lymphoid neogenesis the number of CD3+ T cells as well as CD22+ B cells and CD38+ plasma cells was higher than in diffuse synovitis \((P < 0.001\) for all; Table 2). Furthermore, lymphoid neogenesis concurred with higher numbers of CD68+ macrophages in both the intimal lining layer \((P = 0.002)\) and synovial sublining \((P < 0.001)\). Also, the pro-inflammatory cytokine TNFα in the sublining was expressed at higher levels in tissue containing lymphoid neogenesis \((P = 0.018)\). In line with previous data, LTβ, a cytokine involved in lymphoid neogenesis, was more abundantly expressed in the synovial sublining of these tissues \((P < 0.001)\).

Lymphoid neogenesis is associated with biomarkers of systemic inflammation, but not with clinical characteristics of disease severity.

Subsequently, we investigated the relationship between lymphoid neogenesis, systemic inflammatory features, and disease severity (Table 2). Compared to patients with diffuse synovitis, patients with lymphoid neogenesis had higher ESR \((P = 0.031)\) and serum CRP levels \((P = 0.034)\), as well as leukocyte \((P = 0.002)\) and thrombocyte counts \((P = 0.001)\). In contrast, no difference was found for disease activity as measured by the DAS28, the swollen and tender joint count, or the global disease activity (VAS).

Bone and cartilage damage was assessed by radiology in 76 patients. Patients with lymphoid neogenesis tended to have less erosions and a lower overall Sharp van de Heijde score \((P = 0.085\) and \(P = 0.100\), respectively). However, when analyzing only the ACPA positive patients, this trend towards inverse correlation between lymphoid neogenesis and joint destruction was no longer found \((P = 0.178\) and \(P = 0.332\), respectively). There was also no relationship between lymphoid neogenesis and the presence of rheumatoid nodules (Table 2).

Finally, lymphoid neogenesis was not associated with IgM-RF positivity. Surprisingly, patients with lymphoid neogenesis were significantly less often ACPA positive \((18\) of \(31\) patients with lymphoid neogenesis versus \(58\) of \(72\) in patients with diffuse synovitis; \(P = 0.018)\).

Since the relatively high number of tissues containing only grade 2 aggregates in the subset of tissues categorized in the group of lymphoid neogenesis might theoretically contribute to underestimate the potential differences between the diffuse group and the subset of RA patients with more advanced features of secondary lymphoid organs, we performed a sub-analysis comparing disease severity parameters and autoantibody status between patients with diffuse synovitis \((n = 71)\) versus those with grade 3 aggregates \((n = 16)\). Similar results were found compared to our previous analysis described above. In tissues with grade 3 aggregates significantly more T cells, B cells, plasma cells and macrophages were found, while expression of LTβ and TNFα was significantly increased. There was no significant difference in disease activity, autoantibody status, or joint destruction between these 2 groups (data not shown).
Synovial lymphoid neogenesis does not define clinical RA subtype

1. **Table 2. Associations of clinical, serological and synovial parameters with lymphoid neogenesis.**

<table>
<thead>
<tr>
<th></th>
<th>Diffuse synovitis</th>
<th>Lymphoid neogenesis</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>N= 71</td>
<td>N= 32</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DAS28</td>
<td>5.9 ± 1.1</td>
<td>6.1 ± 1.1</td>
<td>0.330</td>
</tr>
<tr>
<td>Sharp vd Heijde</td>
<td>66 (9-161)</td>
<td>30 (1-77)</td>
<td>0.100†</td>
</tr>
<tr>
<td>Nodules, no (%)</td>
<td>18 (41)</td>
<td>4 (28)</td>
<td>0.308</td>
</tr>
<tr>
<td><strong>Sero logical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR (mm/hour)</td>
<td>30 (15-41)</td>
<td>36 (23-70)</td>
<td>0.031</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>11 (4-27)</td>
<td>16 (10-44)</td>
<td>0.034</td>
</tr>
<tr>
<td>Haemoglobin (mmol/L)</td>
<td>7.8 (7.2-8.4)</td>
<td>7.8 (6.6-8.5)</td>
<td>0.307</td>
</tr>
<tr>
<td>Leucocytes (10⁹/L)</td>
<td>7.2 (5.9-8.6)</td>
<td>9.3 (6.8-11.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Thrombocytes (10⁹/L)</td>
<td>292 (240-364)</td>
<td>350 (302-403)</td>
<td>0.001</td>
</tr>
<tr>
<td>IgM-RF, no (%)</td>
<td>54 (75)</td>
<td>22 (71)</td>
<td>0.422</td>
</tr>
<tr>
<td>ACPA, no (%)</td>
<td>58 (81)</td>
<td>18 (58)</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Cytokines (IOD/mm² )</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα lining</td>
<td>46734 (28456-68777)</td>
<td>46099 (36778-96977)</td>
<td>0.134</td>
</tr>
<tr>
<td>TNFα sublining</td>
<td>63948 (31986-97594)</td>
<td>99811 (52118-134603)</td>
<td>0.018</td>
</tr>
<tr>
<td>LTB4 sublining (counts/mm²)</td>
<td>30 (11-71)</td>
<td>151 (51-611)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Cellular markers (Counts/mm² )</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD55</td>
<td>5345 (402-934)</td>
<td>569 (303-1066)</td>
<td>0.933</td>
</tr>
<tr>
<td>CD3</td>
<td>72 (37-149)</td>
<td>381 (220-678)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD22</td>
<td>0 (0-15)</td>
<td>88 (35-164)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD38</td>
<td>30 (0-144)</td>
<td>631 (253-1089)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD68 lining</td>
<td>261 (177-414)</td>
<td>431 (300-573)</td>
<td>0.002</td>
</tr>
<tr>
<td>CD68 sublining</td>
<td>296 (166-633)</td>
<td>755 (407-1081)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD or median (interquartile range) whichever appropriate. The t-value is derived from the independent samples t-test. The Z-value is derived from the Mann Whitney U test. P values < 0.05 (two-sided) are significant and shown in Italic. DAS28 = Disease Activity Score 28-joint assessment; IgM-RF = IgM rheumatoid factor; ACPA = anti–cyclic citrullinated peptide antibodies; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein. † When analyzing only the ACPA positive patients, the trend towards inverse correlation between lymphoid neogenesis and the Sharp van de Heijde score is no longer present (P = 0.332).

FDC positive lymphoid neogenesis is not related to increased inflammation.

Although previous work has shown the sensitivity of immunohistochemistry to detect CD21L positive cells compared to PCR [4, 21] we performed PCR to detect CD21L mRNA in a subset of 12 patients. In 2/12 samples CD21L mRNA was detected. In both of these samples we also detected CD21L protein (data not shown). One sample tested positive for CD21L protein, but not for CD21L mRNA (it should be noted that immunohistochemistry allows the detection of scarce, isolated cells). Thus, these results suggest that we do not appear to underestimate the presence of FDCs in our study.

In a previous study it was suggested that a subdivision exists between highly organized, FDC containing, ‘germinal center-like’ lymphoid neogenesis and a less organized form of lymphoid neogenesis in which T and B cells form aggregates, but no FDC or other ‘germinal center-like’ features are present [5]. Therefore, we performed a sub-analysis comparing patients with FDC
positive (n = 8) versus negative (n = 21) lymphoid neogenesis. We only found a significantly higher number of CD22+ B cells and CD38+ plasma cells to be present in FDC positive compared to FDC negative lymphoid neogenesis (median 157 B cells/mm², IQR 90-347 and median 66, IQR 31-160, respectively, \( P = 0.047 \); median 858 plasma cells/mm², IQR 637-1370 and median 593, IQR 210-902, respectively, \( P = 0.041 \)). However, all other tissue and clinical parameters did not differ statistically between these forms of lymphoid neogenesis.

**DISCUSSION**

The current study was performed to investigate whether lymphoid neogenesis in RA synovium is related to specific clinical or immunological features. Previous data suggesting that RA characterized by lymphocyte aggregates or ectopic germinal centers represents a specific subset were obtained in small cohorts of patients with end-stage, destructive disease [1, 6, 9]. In contrast, the current study was performed in a large and well characterized cohort of RA patients with active disease despite methotrexate treatment.

In comparison with previous data, we found lymphoid neogenesis in a relatively low number of patients (32% vs respectively 44-90%) [2-4]. We also found lower frequency of FDC and T-B cell separation. The differences might be related to differences in antirheumatic treatments or selection of end-stage, destructive RA in previous studies. In addition, differences may be explained in part by the specific definitions of lymphocyte aggregates adopted in previous studies. We performed a detailed analysis of lymphocyte aggregates as originally proposed by C. Pitzalis [3], and made a subdivision based on aggregate size.

We found that lymphoid neogenesis in rheumatoid synovial tissue coincided with inflammatory features such as increased numbers of infiltrating macrophages and expression of TNFα at the site of inflammation and increased leukocyte and thrombocyte counts, ESR and CRP titers in peripheral blood. These findings do not explain whether the presence of lymphoid neogenesis in rheumatoid synovial tissue is a cause or a consequence of inflammation in RA. It has been suggested that synovial lymphoid neogenesis contributes to ectopic maturation of B cell responses, since clonal expansion, somatic hypermutation, and diversification of B cells have been described in RA synovial tissue [22]. Otherwise, to date no direct proof of functionality of lymphoid neogenesis has been shown. Since lymphoid neogenesis also occurs in non-autoantibody associated forms of arthritis such as psoriatic arthritis and osteoarthritis [18, 23], lymphoid neogenesis could arise as a result of non-specific inflammation.

We found no indication that synovial tissue containing FDC positive aggregates represents a ‘germinal-center like’ subset of lymphoid neogenesis, but we could confirm previous
observations showing that lymphocyte aggregates are present in a heterogeneous mix of
different numbers and sizes in synovial tissue [3]. FDC positive aggregates were detected next
to aggregates without FDC or other germinal center-like features such as T-B cell separation.
Additionally, we observed no increase in macrophage infiltration or cytokine expression in
synovial tissue containing FDC positive aggregates. These data suggest that the presence of
FDC in the synovium may be a phenomenon secondary to inflammation rather than a primary
phenomenon.

Lymphoid neogenesis was related to biomarkers of inflammation, but in contrast not to
increased clinical signs and symptoms. This suggests that the extent to which synovial tissue
lymphoid neogenesis relates to systemic inflammation is not such that this translates into the
clinical expression of the disease. This could be, because the link between lymphoid neogenesis
and local synovial inflammation is not pivotal or because synovial inflammation and concurrent
lymphoid neogenesis might vary between joints, although previous work indicated that
there is little variation in synovial inflammation between different joints of the same patient
[13]. Otherwise, clinical measures of disease activity may also be influenced by factors other
than synovial inflammation including joint destruction. In addition, there was no indication
that lymphoid neogenesis is related to more erosive or nodular disease, as has been suggested
previously in a small patient cohort [9]. This also indicates that either the link between lymphoid
neogenesis and synovial inflammation is not dominant or that the presence of lymphoid
neogenesis and concurrent synovial inflammation may vary between joints and over time.

Previous work has indicated that the rheumatoid synovium is a potent autoantibody-producing
organ; the antibodies may form immune complexes in the joint leading to complement fixation
and macrophage activation [24]. Synovial plasma cells may synthesize and secrete rheumatoid
factors, ACPA, and other autoantibodies [25, 26]. In keeping with these findings, the levels of
rheumatoid factors and ACPA are higher in synovial fluid than in peripheral blood [27, 28]. We
demonstrate here that the presence of circulating autoantibodies is not related to lymphoid
neogenesis. We cannot completely exclude the possibility that lymphocyte aggregates were
not detected in some autoantibody positive, lymphocyte aggregate negative RA patients, but
we did minimize sampling error by analysis of 2 levels of a tissue block representing at least 6
synovial tissue samples. In fact, circulating ACPA were detected even less frequently in patients
with lymphoid neogenesis. Thus, our data do not support a critical role for lymphoid neogen-
esis in the synovium for the production of rheumatoid factor and/or ACPA.

In conclusion, the data presented here show that lymphoid neogenesis in the synovium does
not define a clinically or immunologically defined subtype of RA. The presence of lymphocyte
aggregates and germinal center-like structures may be a secondary phenomenon due to
chronic inflammation.
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