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Interaction between Synovial Inflammatory Tissue and Bone Marrow in Rheumatoid Arthritis

Esther Jimenez-Boj,* Kurt Redlich,* Birgit Türk,* Beatrice Hanslik-Schnabel,†
Axel Wanivenhaus,‡ Andreas Chott,‡ Josef S. Smolen,* and Georg Schett2*

Rheumatoid arthritis (RA) is due to a chronic inflammatory process of the synovial membrane. This membrane, the innermost part of the joint capsule, is normally a fine strand of only a few cell layers. It bridges the two neighboring bones and inserts at periosteal regions close to the articular cartilage. This insertion site, where articular cartilage, periosteum, and synovial membrane come into close contact is also called the junction zone. In RA, the synovial membrane is transformed into a hypertrophic inflammatory tissue. This is based on an influx of inflammatory cells, such as monocytes, T cells, and B cells, from the blood stream as well as hyperplasia of resident synovial cells.

An important feature of synovial inflammatory tissue is its capacity to invade neighboring structures, such as cartilage and bone (1–3). This invasive synovial tissue is also termed “pannus.” This property makes RA the most disabling joint disease, since it leads to structural damage of the joint, which finally leads to loss of function and disability. Mineralized tissue, such as mineralized cartilage and bone, is considered as primary target of synovial inflammatory tissue, since at the aforementioned junction zone, it is directly located underneath the inflammatory tissue of the inserting synovial membrane and the periosteum (2). Resorption of subchondral bone, which appears in radiography, is a criterion for the classification of RA and assessment of radiographic bone damage has become an important tool for monitoring RA patients in clinical studies and daily practice (4, 5).

The ability of synovial tissue to invade bone is closely linked to the generation of osteoclasts (6–8). Differentiation of osteoclasts appears to be enhanced in the RA synovial membrane, since there is an increased influx of mononuclear cells serving as osteoclast precursors and a plethora of signals, such as TNF and receptor activator of NF-κB ligand, which stimulate osteoclast differentiation (9, 10). In animal models, osteoclasts are essential for arthritic bone resorption and blockade of osteoclasts has emerged as a powerful tool to interfere with structural bone damage in arthritis (11–13). Thus, osteoclasts are the tools of the inflamed synovial membrane to invade bone. Whether cortical bone can actually be completely penetrated by the inflamed synovial tissue, and what the consequences of such penetration into the marrow space might be, is less clear.

The cortical bone layer, which separates bone marrow from synovial tissue, is comparatively thin, suggesting that an attack by osteoclasts from the outside could penetrate cortical bone and open the marrow space for synovial tissue. Such interaction could entail profound changes of cellularity of synovial inflammatory tissue, since the marrow space harbors vast amounts of leukocytes and their precursors, and vice versa. To test this hypothesis, we studied specimens from RA patients subjected to joint replacement surgery, which contained not only synovial inflammatory tissue but also neighboring cartilage, bone, and bone marrow. We performed a histochemical and immunohistochemical study to search for and define the nature of penetration of synovial inflammatory tissue into the marrow space in human RA patients.

Materials and Methods

Patients and tissue specimens

Twelve patients, fulfilling the American College of Radiology criteria for diagnosis of RA were analyzed in this study (4). All 12 patients were routinely scheduled for joint replacement surgery because of refractory RA in the affected joint. All joints showed clinical signs for active synovitis (pain, swelling, and stiffness). Age, sex, and the years of disease duration were recorded from all subjects. In addition, the number of tender and
swollen joints, the presence of radiographic bone erosions in the replaced joint, and the levels of C-reactive protein (mg/dl), erythrocyte sedimentation rate (ESR) (mm/h), and rheumatoid factor (U/L) were assessed in these patients. Moreover use of steroids and disease-modifying antirheumatic drugs (DMARDs) including biological agents was recorded. Resected material consisted of juxtaarticular bone, articular cartilage and synovial tissue attached to bone and cartilage in all 12 patients. In addition, four joints from healthy patients receiving amputation surgery of the lower limb after traumatic injury were collected. All participants gave written informed consent. After explanation, the material was immediately placed into 0.9% NaCl and fixed within 1 h in 4.0% formalin. Fixation lasted for at least 18 h.

Preparation of tissue sections

After fixation, joints were longitudinally cut into two equally sized pieces. One part was used to prepare decalciﬁed parafﬁn-embedded tissue sections. Material was ﬁxed in 4.0% formalin overnight and then decalciﬁed in 14% EDTA (Sigma-Aldrich) at 4°C (pH adjusted to 7.2 by addition of ammonium hydroxide) until the bones were pliable. Ten paraffin sections (2 μm) were cut sequentially throughout the joint and used for the histochemical analyses. Twenty additional serial sections were cut for immunohistochemical analysis (see below). The other part was used for preparation of undecalciﬁed plastic-embedded tissue sections. Material was ﬁxed in 70% ethanol, dehydrated in 100% methanol, and embedded in methacrylate (K-Plast; MDS GmbH), as previously described (14, 15). Ten sequential sections 3-μm thick throughout this part of the joint were made on a Jung microtome (Jung).

Antibodies

Monoclonal Abs against the following Ags were used: CD3 (1:50, clone PS1; Novocastra), CD5 (1:20, clone 4C7; Novocastra), CD20 (1:200, clone L26; DakoCytomation), CD21 (1:20, clone 1F8; DakoCytomation), CD23 (1:40, clone 1B12; Novocastra), CD27 (1:80, clone 137B4; Novocastra), CD45RA (1:100, clone 4K8; DakoCytomation), CD68 (1:100, clone PG-M1; DakoCytomation), CD79a (1:25, clone JCB117; DakoCytomation), CD138 (=Syndecan-1, 1:40, clone B-B4; Serotec), CXCL13 (=B-CA-1, 1:30, clone 53610; R&D Systems), Ki-67 (1:50, clone MIB-1; DakoCytomation), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (1:20, clone 355G8; Zymed Laboratories). Rabbit polyclonal Abs against the following Ags were used: IgD (1:100; DakoCytomation), IgM (1:100; DakoCytomation), κ light chain (1:1200; DakoCytomation), λ light chain (1:1200; DakoCytomation), and myeloperoxidase (1:1200; DakoCytomation). Goat polyclonal Abs were used for labeling BAFF (1:30, clone 53610; R&D Systems) and CCL21 (1:6Kine; 1:20; R&D Systems).

Immunohistochemistry

All joint specimens were assessed by immunohistochemistry. For Ag retrieval, sections subjected to microwave in citrate buffer at 600 W for 12 min (CD3, CD5, CD23) or treated with 500 W (CD5, CD20, CD21, CD79a, CD138, CCL21, IgD, IgM, M1; DakoCytomation), and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (1:20, clone 355G8; Zymed Laboratories). Rabbit polyclonal Abs against the following Ags were used: IgD (1:100; DakoCytomation), IgM (1:100; DakoCytomation), κ light chain (1:1200; DakoCytomation), λ light chain (1:1200; DakoCytomation), and myeloperoxidase (1:1200; DakoCytomation). Goat polyclonal Abs were used for labeling BAFF (1:30, clone 53610; R&D Systems) and CCL21 (1:6Kine; 1:20; R&D Systems).

Histochemistry

Parafﬁn-embedded tissue sections were stained by H&E staining, tartrate-resistant acid phosphatase (TRAP) staining (leukocyte acid phosphatase kit; Sigma-Aldrich) for identiﬁcation of osteoclasts and toulidine blue for detecting cartilage proteoglycan content. Plastic embedded tissue sections were stained by Goldner trichrome, von Kossa and Movat pentachrome, according to standard protocols (15, 16).

Histological assessments

Number and size of subcortical bone marrow cell aggregates associated with penetrated synovial tissue were determined from 10 sequential decalciﬁed parafﬁn sections and 10 sequential plastic-embedded undecalciﬁed sections. For assessing the size of these subcortical bone marrow cell aggregates, area of each single aggregate was measured by histomorphometry using OsteoMeasure system (OsteoMetrics) and results were summarized to deﬁne the total area of bone marrow affected by aggregates. For control purposes, number and size of bone marrow cell aggregates, which were not in association with synovial tissue penetration and were not directly localized subcortically, were assessed. Width of cortical defects at sites of synovial tissue penetration into the marrow space was also measured. All measurements were done by histomorphometry using OsteoMeasure system. Immunohistochemistry was done on 20 serial parafﬁn-embedded sections from each specimen. For each cell surface marker and each aggregate, the fraction of positively labeled cells was assessed and a mean ± SEM was calculated for all patients. These analyses were performed in subcortical bone marrow aggregates and for control purposes in cell aggregates within synovial tissue as well as in the inﬂamed synovial membrane. Osteoid deposits in the subcortical region were assessed on Movat-labeled sections of undecalciﬁed tissue at two different sites, at the bone surface next to subcortical bone marrow aggregates and at a site devoid of such aggregates. The fraction of surface covered by osteoid from total bone surface was measured and calculated in both compartments by using histomorphometry. Finally, standard osteomorphometry measures were applied for assessing juxtaarticular trabecular bone in healthy normal subjects and RA patients. The following parameters were measured: the fraction of bone volume of total volume (BV/TV), trabecular thickness (Tb.Th), number (Tb.N), and separation (Tb.S), number of osteoblasts and osteoblasts per bone perimeter (N.Oc/B.Pm, N.Ob/B.Pm), and the fraction of bone surface covered by osteoblasts and osteoblasts (OcS/BS, ObS/BS).

Statistical analysis

Data are shown as means ± SEM. Number and size of subcortical bone marrow aggregates, size of cortical penetration, cortical width, and bone surface covered by osteoid were compared by Mann-Whitney U test. For correlating the size of subcortical bone marrow aggregates to markers of disease activity, Spearman’s correlation coefﬁcients and Bonferroni correction were calculated. A value of p < 0.05 was regarded as statistically signiﬁcant.

Results

Patients characteristics

Of the 12 patients studied, eight were female and four were male (Table I). Mean (+SEM) age was 60 (+3) years, and mean disease duration was 18.9 (+2.8) years, indicating long-standing RA. Most patients had active disease as revealed by the number of tender joints (mean ± SEM number of tender joints: 6.8 ± 2.9) and swollen joints (4.4 ± 1.3) and/or elevated acute phase response (C-reactive protein, 4.8 ± 2.7 mg/dl; ESR, 51 ± 10 mm/h). Eleven patients were rheumatoid factor positive and its mean (+SEM) level was 286 (+127) U/L. Ten patients received DMARD therapy with methotrexate, ﬁve of them as a monotherapy, one in combination with leﬂunomide, two with anakinra, a rIL-1 receptor antagonist, and two with TNF blockers (infliximab and etanercept). The remaining two patients received monotherapy with anakinra or leﬂunomide. Low dose oral glucocorticoids were taken by eight of 12 patients. Material originated from joint replacement surgery of the metacarpophalangeal and proximal interphalangeal joint joints in ﬁve patients, the metatarsophalangeal joints in another ﬁve patients, and the knee as well as the wrist joint in each patient.

Cortical penetration leads to mononuclear cell aggregates in the adjacent bone marrow

To search for a possible cortical penetration of synovial inﬂammatory tissue into the bone marrow, we generated axial sections of the joints to allow a simultaneous assessment of cartilage, subchondral bone and bone marrow. Cortical penetration was found in nine of 12 patients and originated from subchondral bone erosions (Fig. 1). Areas with cortical penetration by the pannus were associated with a widespread resorption of subchondral mineralized cartilage and bone, separating unmineralized articular cartilage from underlying bone and characterized by small regions of bone marrow invasion (Fig. 1A). Articular cartilage covering these areas

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Table I. Characteristics of rheumatoid arthritis patients

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*DD, Disease duration (years); SJC, swollen joint count (n from 28 joints); TJC, tender joint count (n from 28 joints); RF, rheumatoid factor (U/L); GC, glucocorticoids; MTX, methotrexate; Lef, leflunomide; BIOL, biological drug; ANA, anakinra; IFX, infliximab; ETA, etanercept; MTP, metatarsophalangeal joint; MCP, metacarpophalangeal joint; ND, not determined; PIP, proximal interphalangeal joint.

Subcortical bone marrow aggregates in RA are linked to penetration of cortical bone

To characterize bone marrow changes in RA in more detail, we quantitatively assessed the number and size of subcortical bone marrow aggregates. An average number of three (mean ± SEM 3.0 ± 0.9) sites of cortical penetration with consecutive bone marrow aggregates were observed in each joint (Fig. 2A). In healthy joints, no such lesions were found. The average size of subcortical bone marrow aggregates was 0.06 mm², whereas no region of the subcortical bone marrow was covered by such aggregates in normal joints (Fig. 2B). The mean diameter of the cortical destruction sites was 236 ± 27 µm, which is big enough to allow a meaningful interaction between cells from synovial tissue and bone marrow (Fig. 2C). Moreover, cortical width was significantly diminished in the vicinity of cortical penetration sites (mean ± SEM, 99 ± 5 µm) compared with normal cortical width (162 ± 13 µm), suggesting that bone resorption had significantly weakened the cortical barrier (Fig. 2D).

Interestingly, distant from the site of penetration, bone marrow cell aggregates, when found, were of much smaller size than those at the penetration site.

Subcortical bone marrow aggregates are associated with high disease activity

To clarify whether clinical features of RA influence cortical bone penetration and formation of bone marrow aggregates, we next correlated clinical parameters of RA with the area of subcortical penetration. For this purpose, we assessed the number and size of bone marrow aggregates in RA patients with respect to clinical disease features. In a group of 28 RA patients, we did not observe any significant correlation between the area of subcortical penetration and formation of bone marrow aggregates and disease activity parameters (Table I). However, subcortical bone marrow aggregates were significantly larger and more numerous in RA patients with high disease activity (mean ± SEM 0.5 ± 0.3 mm² and 1.0 ± 0.3 sites of penetration per joint, respectively) compared with patients with low disease activity (mean ± SEM 0.1 ± 0.1 mm² and 0.2 ± 0.1 sites of penetration per joint, respectively).

FIGURE 1. Cortical penetration and bone marrow invasion in RA. Histological sections from metacarpal heads of patients with RA. A, H&E stained section showing invasion of synovial inflammatory tissue into the subchondral space previously filled with mineralized cartilage (black arrowhead). Unmineralized cartilage appears on the top as a pink band; cortical bone barrier appears at the bottom as dark pink band, which is penetrated by synovial tissue leading to formation of a mononuclear aggregate in the bone marrow (black arrow). B, Toluidine blue staining showing extensive proteoglycan loss in articular cartilage. C, Close-up view of the interface of synovial inflammatory tissue (top) and bone marrow aggregate (bottom) at site of cortical penetration (black arrow). D, Weakened but intact cortical barrier with no changes of the underlying bone marrow (H&E stain). E, The same regions stained by TRAP showing osteoclasts (purple staining, black arrow) resorbing cortical bone from the outside. F, Close-up view of osteoclasts stained for TRAP (black arrow). A, B, D, and E, Original magnification, ×50; C and F, original magnification, ×400.
bone marrow aggregates. For comparative purposes clinical parameters were also related to the area of small bone marrow aggregates, which were distant from cortical bone and thus not associated to synovial inflammatory tissue. Subcortical infiltrates were more prominent in RA than healthy individuals and patients with RA. All measures were performed by histomorphometry of H&E stained sections. Data are mean ± SEM from four healthy joints and 12 joints affected by RA. *p < 0.05.

Table II. Correlation of subcortical bone marrow aggregates with clinical parameters of disease

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a Area of small bone marrow aggregates linked to penetration of cortical bone and attached to synovial inflammatory tissue.

B lymphocytes dominate subcortical bone marrow aggregates

Next, we turned to characterize cell-specific surface marker expression in subcortical bone marrow aggregates by immunohistochemical analysis. Interestingly, B cells, as detected by CD20 expression, were by far the most frequent cell type (mean ± SEM: 55 ± 8%) (Fig. 4, A and E, and Fig. 5A). This was confirmed by concomitant expression of other pan-B cell markers, such as CD45RA (Figs. 4F and 5A) and CD79a (Fig. 5A), both of which were found in a similar frequency in subcortical bone marrow aggregates. T cells, as detected by positive labeling for CD3 (35 ± 4%) and CD5 (37 ± 3%) were found in considerable lower frequency and only few macrophages were present in subcortical bone marrow aggregates (8 ± 1%) (Figs. 4, B, C, F, and G, and 5A). Follicular dendritic cells, as detected by CD21 expression (3 ± 1%) and neutrophils, as detected by labeling for myeloperoxidase (0.8 ± 0.6%), were almost completely absent (Figs. 4K and 5A). This pattern of cellular composition of subcortical aggregates was very similar among all patients investigated and was also not different among the various DMARD therapies.

A more detailed analysis of B cell markers revealed abundance of CD27, indicating that the main proportion of B cells are mature B cells (53 ± 2%) (Figs. 4J and 5A). In contrast, expression of IgM (18 ± 4%), representing immature and mature naive B cells, as well as IgD (5 ± 1%), representing mature naive B cells was much less frequent (Fig. 5A). CD23 expression was virtually absent (1 ± 0.5%) in subcortical bone marrow aggregates. Plasma cells, as detected by CD138 labeling were especially found at the periphery of subcortical bone marrow aggregates and were usually found in between aggregate and synovial inflammatory tissue (Fig. 4, D and H). Plasma cell attributed to 8 ± 4% of total cells in aggregates (Fig. 5A). Less than 5% of cells showed signs of proliferation, as detected by expression Ki-67 Ag (Fig. 5A).

Compared with subcortical bone marrow aggregates, synovial inflammatory tissue showed a different cellular composition (Fig. 5B). The proportion of B cells was significantly smaller and B cells only attributed to up to 10% of synovial cells. The number of T cells in synovial tissue was comparable to subcortical bone marrow aggregates (24 ± 6%), whereas macrophages (27 ± 4%) were much more frequent. Plasma cell content was very scarce (1 ± 1%). In seven patients, mononuclear aggregates were present within synovial tissue, whereas no such aggregates were found in the remaining five patients. All of these aggregates were localized in the synovial tissue of the joint space and were distant from subchondral bone erosions and sites of cortical penetration. This cellular composition showed some similarities with subcortical bone erosions, although their proportion of B cells (20 ± 4%) and plasma cells (2 ± 2%) was lower (Fig. 5C). Composition of B cells was very similar with a majority of mature B cells. T cells were found in a similar frequency, whereas the fraction of macrophages was more prominent in synovial aggregates than subcortical bone marrow aggregates.

Expression of molecules for B cell chemotaxis, homing, and activation in the vicinity of subcortical bone marrow aggregates

Hypothesizing that invading synovial inflammatory tissue expresses molecules, which facilitate accumulation of B cells in the neighboring bone marrow, we next stained for molecules responsible for B cell chemotaxis, homing, and activation (Fig. 6). Chemotactants for B cells, such as CXCL-13, also termed B cell...
Chemoattractant protein-1, as well as CCL-21 were found expressed at the interface of synovial inflammatory and subcortical bone marrow aggregates (Fig. 6, A and B). Moreover, numerous MAdCAM-1-positive blood vessels, resembling high endothelial venules were found within these aggregates (Fig. 6C). BAFF, also termed BlyS, a molecule important for B cell survival, was also expressed within inflammatory tissue next to bone marrow aggregates, suggesting that accumulation of B cells is triggered by local expression of molecules involved in B cell migration, homing, and survival (Fig. 6D).

**FIGURE 3.** Subcortical bone marrow aggregates are associated with surrogate markers for disease activity. The area covered by subcortical bone marrow aggregates linked to cortical penetration of synovial inflammatory tissue (A, C, E) as well as the area covered by small aggregates in the bone marrow, which are not linked to cortical penetration (B, D, F) was correlated to the number of swollen joints (A, B), the serum level of C-reactive protein (C, D) and rheumatoid factor (E, F).

**FIGURE 4.** Immunophenotypical characterization of subcortical bone marrow aggregates. Serial histological section from the specimen shown in Fig. 1, A and B, stained by Abs against CD20 for detection of B cells (A and E), CD3 for T cells (B and F), CD68 for macrophages (C and G) and CD138 for plasma cells (D and H). The images depicted in E–H show close-up views of the respective images depicted in A–D. I. Staining by Abs against CD45RO as a pan-B cell marker; J, CD27 for mature B cells; and K, CD21 for follicular dendritic cells. L. Control staining with normal mouse Ig. Note abundance of CD20 expression in subcortical bone marrow aggregates as well as the focused expression of CD138 at the interface of synovial inflammatory tissue and aggregates. A–D and I–L, original magnification ×100; E–H, original magnification ×400.
Bone marrow penetration and formation of subcortical bone marrow aggregates leads to endosteal bone formation

To address whether cortical bone reacts upon penetration and aggregate formation in the bone marrow, we investigated undecalcified plastic sections of the same joints (Fig. 7, A–H). Interestingly, the inner endosteal surface of cortical bone revealed wide areas covered by osteoblasts (Fig. 7, E–G), whereas osteoclasts were absent. Moreover, underneath these osteoblast-covered areas, new bone formation, as visualized by the presence of osteoid seams could be detected (Fig. 7, A, C–G). These areas were only found in the vicinity of subcortical bone marrow aggregates, whereas the endosteal surface distant from such sites was largely unaffected (Fig. 7, B and D). Quantification of these areas revealed that <5% of bone surface at sites without subcortical bone marrow aggregates was covered by osteoid seams, whereas up to 40% of endosteal bone surface was covered at sites close to the aggregates (Fig. 7H).

Severe loss of periarticular bone in RA is associated with high bone turnover

Considering the radiographic signs of periarticular bone loss in RA, we also investigated the structure of periarticular bone in this group of RA patients and for comparative purpose also in healthy controls. Histomorphometric analysis of undecalcified sections revealed massive loss of juxtaarticular trabecular bone as evident from a 4-fold decrease of bone volume in RA patients compared with normal controls (Fig. 8A). Bone loss was based on a more

FIGURE 5. Differences in expression pattern of surface molecules upon subcortical bone marrow aggregates, synovial inflammatory tissue, and synovial tissue aggregates. Subcortical bone marrow aggregates (A), synovial inflammatory tissue (B), and synovial tissue aggregates (C) were analyzed for the following B cell markers: CD20, CD23, CD27, CD45RO, CD79a, CD138, IgD, and IgM. In addition, markers for T cells (CD3, CD5), macrophage (CD68), granulocytes (myeloperoxidase, MPO), and follicular dendritic cells (CD21) were analyzed. Also cell proliferation was assessed by staining for Ki-67. Note the preponderance of B cells in subcortical bone marrow aggregates, of macrophages, granulocytes, and T cells in synovial inflammatory tissue and T cells, B cells, and macrophages in synovial tissue aggregates.

FIGURE 6. Expression of molecules for B cell migration, homing, and activation. Histological sections of the interface between synovial inflammatory tissue and subcortical bone marrow aggregates stained for CXCL-13 (also termed BCA-1) (A) and CCL21 (also termed 6cKine) (B). C, Staining for MadCAM-1 showing a transverse (black arrow) and longitudinal section (black arrowhead) through high endothelial venules within subcortical bone marrow aggregates. D, Expression of BAFF (also termed BlyS) at the interface between synovial inflammatory tissue and subcortical bone marrow aggregate. Control staining with normal mouse (E) and goat (F) Ig, as first Abs. A–F, original magnification ×400.
than 2-fold decrease of the trabecular thickness leading to an almost 3-fold increased trabecular separation (Fig. 8, B–D). Whereas dynamic histomorphometry revealed only very low bone turnover in periarticular bone of normal individuals, as evident from the low signal intensity on T1-weighted images, are present in patients with RA (21, 22). Extending these observations, our study provides direct histological evidence for cortical penetration and bone marrow changes in RA.

We found localized mononuclear cell aggregates in the bone marrow at sites of inflammatory tissue invasion. Surprisingly, B cells were the leading cell type in these lesions. T cells were far less frequent, and macrophages, granulocytes, and follicular dendritic cells were almost completely absent in these lesions. Thus, these subcortical bone marrow aggregates constitute a subcompartment with a unique cellular composition. Inflamed synovial tissue, for example, has a completely distinct cellular composition with abundant macrophages but only a small fraction of B cells. Even mononuclear cell aggregates within synovial inflammatory tissue were different in cellular composition, showing a lower proportion of B cells but more macrophages than bone marrow aggregates. The majority of cells of the latter were mature B cells expressing markers such as CD20, CD27, CD45RA, and CD79.

Although it has been known for many years that synovial tissue of RA patients harbors B cells, the observation that the juxtaarticular bone marrow serves as a pool of B cells in the direct neighborhood of arthritis is interesting. It is known that B cells in the
such as IL-4 and IL-10 (29, 30). In support T cell activation and are producers of regulatory cytokines cells are not only a source of rheumatoid factor production but also synovial membrane of RA patients undergo affinity maturation, somatic hypermutation, and terminal differentiation (23–28). B cells are not only a source of rheumatoid factor production but also support T cell activation and are producers of regulatory cytokines such as IL-4 and IL-10 (29, 30). In ~60% of RA patients, aggregates consisting of T and B cells are a major source of B cells in the synovial tissue (23, 30). However, such aggregates are not completely specific for RA; they also are found in ankylosing spondylitis and even sometimes in osteoarthritis (31, 32). Synovial aggregates develop early in RA and increase in frequency with disease duration (33–35). Although, there are clear similarities between bone marrow aggregates and synovial aggregates, the former do not represent classical germinal centers, as synovial aggregates in less than one third of RA patients do (30, 36, 37). Importantly, bone marrow aggregates did not contain a significant number of follicular dendritic cells, which are a typical feature of germinal centers.

At the interface between synovial inflammatory tissue and subcortical bone marrow aggregates, plasma cell concentrations were maximal. In contrast, only very limited numbers of plasma cells were present in other compartments of the synovial membrane. Plasma cells have been described to accumulate around germinal center-like aggregates. This reflects the distribution as found in subcortical aggregates, when synovial tissue comes in contact with B cell rich bone marrow aggregates (24). As suggested by previous experimental data, synovial tissue and neighboring mononuclear aggregates appear to have an intensive cross talk. Thus, synovial fibroblasts can support B cell survival by molecules such as CXCL-12 (SDF-1) and VCAM-1, which is termed pseudoperi-polesis (38–42). Other chemokines expressed by synoviocytes promote B cell recruitment, such as CXCL-13 (also termed BCA-1) and CCL-21 (also termed 6Ckine) (30, 43–45). In fact, expression of the two latter chemokines could be detected at the front of synovial tissue invasion in the vicinity of subcortical aggregates, and numerous MadCAM-1-positive high endothelial venules, which are important for B cell homing, were found within aggregates (46). Moreover, synovial tissue is also a source of B cell survival factors, such as BAFF (also termed BlyS), expression of which was also found in synovial tissue close to aggregates (47). These observations suggest that synovial tissue provide signals of B cell chemotaxis, homing and activation, which allow the formation of bone marrow aggregates. Interestingly, as previously observed in synovial B cells (48), B cells of subcortical bone marrow aggregates were EBV-negative (data not shown).

Compelling evidence for new bone formation was found at sites of bone marrow aggregates. Osteoblasts accumulated and covered large areas of the endosteal bone with osteoid deposits. In contrast, bone distant from these lesions showed only few scattered bone formation sites. The exact same pattern of skeletal effects has been observed in human TNF transgenic mice (15). There were no osteoclasts attached to the endosteum; however, numerous osteoclasts were embedded in synovial tissue attacking bone from the subchondral side of compact bone. This observation indicates that the synovial and subchondral side of the cortical bone is affected by resorption, whereas formation, as an attempt to repair bone comes from the inner endosteal area of bone. This observation also supports the hypothesis that bone erosion starts from the outside due to synovial inflammation rather than from the bone marrow.

The B cell-rich bone marrow aggregates, which occur exclusively at sites of pannus penetration through cortical bone into the bone marrow space, appear to “shield” bone marrow from the invading synovial tissue by forming a physical barrier and attempting to elicit new bone formation.

Currently targeted therapy of B cells has become a promising new tool for the treatment of RA. Rituximab, an Ab directed against CD20 leads to depletion of B cells and has shown efficacy in inhibiting signs and symptoms of RA (49, 50). Due to the striking accumulation of CD20-positive B cells, the subcortical bone marrow aggregates will represent a target of rituximab therapy. Although rituximab could thus eliminate a presumably protective process, direct or systemic synovial effects of anti-CD20 may counterbalance and exceed such potentially negative aspects of

**FIGURE 8.** Histomorphometric evidence for bone loss and high bone turnover in periarticular bone. Periarticular trabecular bone was assessed by histomorphometry in a specimen from healthy normal individuals (normal, left bars) and patients with RA (right bars). The following parameters were measured: percentage of bone volume of total volume (BV/TV) (A), trabecular thickness (Tb.Th) (B), trabecular number (Tb.N) (C), trabecular separation (Tb.S) (D), percentage of osteoclast covered surface of bone surface (Oc.S/BS) (E), percentage of osteoblast covered surface of bone surface (Ob.S/BS) (F), number of osteoclasts per bone perimeter (N.Oc/B.Pm) (G), and number of osteoblasts per bone perimeter (N.Ob/B.Pm) (H). Values are means ± SEM; *, significant difference (*p < 0.05).
that this therapy, it will be interesting to learn about the effects of rituximab on structural damage.

In summary, bone marrow can be regarded as a compartment, which is actively involved in the disease process of RA. It harbors cell aggregates if the cortical barrier is disrupted and is a source for bone repair.

Disclosures

The authors have no financial conflict of interest.

References


