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It is widely accepted that B cells play an essential role in the pathogenesis of rheumatoid arthritis (RA). B cell involvement is well documented in the presence of detectable autoantibodies in the majority of patients, in particular, rheumatoid factor (RF) and antibodies against citrullinated proteins (ACPA), which can be detected many years before development of clinical disease, indicating that autoreactive B cell clones are involved in disease induction. B cell depletion therapy with rituximab (anti-CD20) is effective in treating RA, proving an essential role for B cells in disease persistence. Although B cell depletion therapy can induce long-term responses in a small number of patients, almost all patients eventually relapse.

There is therefore much interest in finding out whether analysis of B cell-related factors can guide the development of treatment strategies and/or prediction of disease flare. The cytokine B cell activating factor (BAFF; also known as BLyS) plays an essential role in B cell survival and homeostasis. BAFF binds to three different receptors: BAFF-R, TACI, and BCMA.

Expression of the different BAFF-binding receptors varies in distinct subsets of B cells, and their expression is coordinated and intimately related to maturation and activation status.

In the study by Moura, et al in this issue of The Journal, gene expression of molecules related to B cell survival and differentiation were studied in peripheral blood mononuclear cells (PBMC) in a small group of patients with very early RA (VERA; i.e., < 6 weeks of symptoms) and compared with other patients with RA at different stages [early RA (ERA) > 6 weeks and < 1 year duration; and established RA > 1 year duration], with patients with other forms of early arthritis (EA), and with healthy controls (HC). Patients with established RA were all taking methotrexate, and none were taking biologics. Samples for other groups were collected prior to treatment. The authors looked at gene expression of molecules involved in B cell homeostasis and survival (BAFF, BAFF-R, TACI, BCMA), class-switching (AID), chemotaxis (CXCR5), B cell commitment and plasma cell differentiation (PAX5 and BLIMP-1), immune system activation (β2-microglobulin), and apoptosis (Bcl-2). The goals of the study were to provide insight into RA pathogenesis and identify potential biomarkers of disease progression.

The study found differences in gene expression of BAFF, BAFF-binding receptors (BAFF-R and TACI), and other B cell-related genes between patients with early and with established RA and HC. BAFF-R gene expression was increased in patients with ERA and in established RA when compared with HC, but not in VERA or ERA. In addition, patients with established RA also had higher levels of BAFF-R gene expression compared to patients with VERA, suggesting that BAFF-R gene expression increases with disease progression. TACI gene expression was higher in all RA patient groups compared to HC, whereas no differences were seen between EA and HC. BAFF gene expression was only higher than in HC in patients with VERA. CXCR5 expression was higher only in patients with established RA. PAX5 gene expression was elevated in all RA groups in comparison with HC but especially in established RA. β2-microglobulin expression was higher in the groups with very early or early disease compared to those with established RA and HC. No differences were seen in BCMA, AID, BLIMP-1, and Bcl-2 gene expression. The authors report that no significant differences were seen between seropositive and seronegative patients, and also no correlations were found with measures of disease activity, that is, Disease Activity Score 28 or erythrocyte sedimentation rate.

There is much interest in the role of BAFF and its receptors in the pathogenesis of autoimmune diseases in terms of autoreactive B cell survival and function. The authors hypothesize that TACI gene expression is disturbed in RA from the onset of clinical disease and that BAFF-R expression increases with disease progression. Results were interpreted on the basis that these variations represented differences in expression of these receptors with functional consequences. They hypothesize that these changes may
occur in response to increased availability of serum BAFF and could be a protective response against excessive BAFF stimulation of autoreactive B cells through BAFF-R.

However, to our knowledge, there is no evidence that this system is disturbed in RA, and changes described in the study by Moura, et al could equally reflect relative changes in B cell subpopulations associated with the disease. This does not diminish the interest of this finding as it can help us develop hypotheses on the role that B cells and, in particular, different B cell subpopulations may play in the pathogenesis of RA. The problem is that in the absence of detailed data on protein expression and on relative proportions of B cells and B cell subpopulations in the PBMC samples, interpretation of the data is limited. Unfortunately, studies including all the necessary data for a detailed analysis are difficult to do, in particular because of costs and ethical limitations on the amount of blood needed from patients.

Therefore an alternative explanation is that the changes reflect differences in total numbers of circulating B cells and relative proportions of B cell subpopulations that are responding appropriately to the conditions present in RA. Some differences could reflect wider activation of other inflammatory cells able to express BAFF and β₂-microglobulin very early in the disease. In established RA the differences found may be associated with increased proportions of activated circulating naïve B cells reflected in increased TACI, PAX5, and possibly CXCR5 gene expression. As discussed later, the constant expression fairly uniform and similar between mature naïve B cells in peripheral blood3,5. In healthy individuals, BAFF-R is almost always occupied by BAFF in all CD19+ cells in peripheral blood3,5. In healthy individuals, BAFF-R is almost always occupied by BAFF in B cells outside the germinal center, in which supply is limited to favor T cell-driven antigen-specific responses3,6. Also, when activated B cells undergo differentiation into immunoglobulin-secreting cells, BAFF-R expression decreases significantly. It is unlikely that increased BAFF-R gene expression could be explained by a relatively higher absolute number of circulating B cells within PBMC because frequencies and total B cells also tend to be slightly, but not significantly, lower in RA compared with HC7. Our group has indeed observed a tendency toward lower expression of BAFF-R in established RA compared with HC5. Increased BAFF-R gene expression in patients with established RA could thus be interpreted as representing increased turnover of BAFF-R due to an increased proportion of activated B cells as disease progresses. This would also explain the higher gene expression of CXCR5 in established RA, which could reflect recirculation from inflamed synovium9.

TACI gene expression was found to be increased in all RA groups. As shown by Darce, et al, all CD27+ memory B cells express TACI. It is unlikely that the increased TACI gene expression found was associated with increased numbers of memory B cells, as the opposite was described in VERA patients by the same group10. Darce, et al have also shown that a small, variable proportion of circulating naïve (CD27−) B cells express TACI, which have an activated phenotype as defined by coexpression of CD25 and CD80. Some or all of this population may be analogous to the activated B cells described by Rudnicka, et al11, which were found to show evidence of prior Toll-like receptor-9 (TLR) activation. Normal human naïve B cells express only low levels of most TLR12, supporting the view that an inappropriate “early” activation of B cells exiting the bone marrow occurs in RA11.

BAFF gene expression was increased in VERA patients when compared to HC and established RA. However, BAFF serum levels, despite being clearly statistically significantly higher in patients with early RA, were still very low and probably within the normal range for the assay; for example, only 1 patient with VERA had levels higher than controls or 1 ng/ml. It is possible that increased expression of BAFF gene was associated with membrane BAFF, but not soluble BAFF, either by B cells13 or by other types of mononuclear cells in the sample. The significance of BAFF in RA has also been based on the finding of BAFF and BAFF-R gene expression in RA synovial tissue14. However, because BAFF-R is expressed on all B cells, this is to be expected because RA biopsy samples can include significant numbers of B cells and a number of infiltrating and somatic cells that produce BAFF. It is therefore difficult to ascribe any “control” of specific responses by the BAFF-R/BAFF system in such an inflammatory environment.

PAX5 expression is known to be higher in the naïve B cell subpopulation15. Here, it was higher than in HC in all RA groups, especially in established RA. Increased PAX5 gene expression could therefore also be linked to increased proportions of naïve B cells in the samples, which may also underly apparent overexpression of certain B cell-associated genes.

Studies have shown that BAFF-R signaling in humans seems to be mainly a survival signal, with surface expression fairly uniform and similar between mature naïve and memory B cells (BAFF-R is expressed on virtually 100% of CD19+ cells in peripheral blood3,5. In healthy individuals, BAFF-R is almost always occupied by BAFF in B cells outside the germinal center, in which supply is limited to favor T cell-driven antigen-specific responses3,6. Also, when activated B cells undergo differentiation into immunoglobulin-secreting cells, BAFF-R expression decreases significantly. It is unlikely that increased BAFF-R gene expression could be explained by a relatively higher absolute number of circulating B cells within PBMC because frequencies and total B cells also tend to be slightly, but not significantly, lower in RA compared with HC7. Our group has indeed observed a tendency toward lower expression of BAFF-R in established RA compared with HC5. Increased BAFF-R gene expression in patients with established RA could thus be interpreted as representing increased turnover of BAFF-R due to an increased proportion of activated B cells as disease progresses. This would also explain the higher gene expression of CXCR5 in
naive B cells. Although serum levels of autoantibodies can remain elevated throughout clinical remission, expansion or differentiation of cells into memory B cells and subsequently into autoantibody-producing plasmablasts is also associated with relapse\textsuperscript{18,19}. Our hypothesis is that clinical relapse is driven by naive autoreactive B cells, contributing directly to pathogenic autoantibody production or by reengaging perifollicular T cell help and memory B cell expansion\textsuperscript{20}. Such an hypothesis would therefore suggest the continuation of an acquired loss of central tolerance in patients with RA. This process can only be transiently disrupted by rituximab, which serves to prevent egress of “new” autoreactive B cells, but autoreactivity is hard-wired into the system. In patients where disease does not resume on repopulation, we would suggest that a critical mass of autoreactive B cell expansion needs to be achieved. Therefore, in RA, acquired defects in central tolerance may be important, but with additional roles also for expansion of autoreactive processes in the periphery.

Disturbances of central tolerance in patients with RA have been elegantly demonstrated by Samuels and colleagues\textsuperscript{21}. Immunoglobulin transcripts with increased polyreactive and novel ACPA binding (not present in healthy controls) were shown to be produced from early emigrant B cells (CD10\textsuperscript{+}IgD\textsuperscript{+}) from patients with RA. These autoreactive B cells were also found to survive and mature into naive B cells (CD10\textsuperscript{–}IgD\textsuperscript{+}) in the periphery. Further evidence for the possibility that the autoimmune response is constantly being generated in these patients comes from observations that IgM-class ACPA are being recruited into the peripheral ACPA repertoire, thereby reflecting continuous reactivation of preswitched autoreactive B cells\textsuperscript{22}. It is not known what initiates RA, and therefore the study of patients with very early disease may provide clues as to what drives the immune process into full clinical expression. The results reported by Moura, et al\textsuperscript{4} represent an important contribution to our knowledge regarding expression of B cell-related factors in patients with the earliest signs of RA compared with those with established disease. The results could be interpreted to show that B cell homeostasis is indeed disrupted, but equally perhaps to suggest the presence of a naive activated B cell phenotype, detectable from the earliest stages of the disease. This information, combined with the unique opportunity to investigate the B cell biology underlying relapse following B cell-targeting therapies, gives us a realistic opportunity to inform the development of more targeted therapies capable of inducing longterm remission.

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