B cells and B cell directed therapies in rheumatoid arthritis: towards personalized medicine
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CD22 IS NOT EXPRESSED MERELY ON B CELLS: COMMENT ON THE ARTICLE BY VOS ET AL
We read with interest the recent report by Vos and colleagues on the early depleting effects of rituximab in the synovial tissue of patients with rheumatoid arthritis (RA), showing that the depletion of CD22+ cells was incomplete. Recently, we reported the depleting effects of rituximab in paired samples of peripheral blood, bone marrow, and synovium. Our study revealed a complete depletion of the CD20+ subset of B cells in synovium, as shown by staining the cytoplasmic tail of the CD20 membrane protein. Our results and those of Vos et al are seemingly contradictory, and the existence of a CD22+,CD20- B cell subset may be relevant to the pathogenic mechanisms of RA. Therefore, we investigated the specificity and sensitivity of CD22, CD19, and CD20 as markers of B cells.

Briefly, peripheral blood mononuclear cells from 5 healthy volunteers were obtained through isolation over a Ficoll and freshly stained with the following markers: fluorescein isothiocyanate (FITC)–conjugated anti-CD20 (clone 2H7); phycoerythrin-conjugated anti-CD19 (clone HIB19); allophycocyanin (APC)–conjugated anti-CD22 (clone S-HCL-1); APC-conjugated anti-CD3 (UCHT1); and FITC-conjugated anti-CD3 (clone 5K7) (all from BD Biosciences, San Jose, CA). After incubation for 30 minutes in the dark, cells were washed and read on a FACSCalibur flow cytometer (BD Biosciences) and analyzed with the FlowJo software program (Tree Star, Ashland, OR). We observed that 97.6% of CD19+ cells and 96.7% of CD20+ cells were CD22 positive. However, only 78.1% and 75.2% of CD22+ cells were positive for CD19 and CD20, respectively (P = 0.04, by Mann-Whitney U test) (Table 1).

In conclusion, these data indicated that CD22 is not a specific marker for B cells, raising the possibility that in the study by Vos et al the residual CD22 positivity after rituximab treatment can be explained by the presence of cell types not belonging to the B cell lineage. In this context, Han et al previously reported that in healthy persons basophils can be isolated with a purity of 99.4% by sorting CD22+,CD19- lymphocytes. Mast cells (tissue-infiltrating basophils) do not seem to express CD22 on their membrane but do show intracellular messenger RNA expression of CD22. Therefore, we believe further research into the residual CD22+ expressing cells is warranted, and that the results of CD22 single-staining should be interpreted with caution when used in the context of B cell depletion with rituximab.

### Table No.1

<table>
<thead>
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<th>% POSITIVE CELLS</th>
<th>CD22</th>
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<td>GATED POPULATION</td>
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<tr>
<td>CD22+cells</td>
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</tr>
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<tr>
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REFERENCES


REPLY

To the Editor

We appreciate the interest of Dr. Teng and his colleagues in our report. Based on their analysis of peripheral blood mononuclear cells from 5 healthy donors, they suggest that the residual CD20+ positivity in rheumatoid synovial tissue observed in a subset of the patients in our study after rituximab treatment does not necessarily represent persistent B cell infiltration but could be explained by expression of CD22 by basophils or perhaps mast cells in the synovium. We need to reject this hypothesis for the following reasons. 1) Previous work has shown that basophil granulocytes are absent in the synovium of patients with rheumatoid arthritis (RA) (2). Mast cells do not express CD22 at the protein level (7). We have confirmed our previous results by demonstrating CD19+ cells in the synovium after rituximab treatment, with results similar to those observed with CD22 staining (Figure 1).

Recently, we also showed a variable tissue response 16 weeks after rituximab treatment (3). Three other groups of investigators have confirmed our results using CD19 and CD20 as markers (8 weeks and 24 weeks after the initiation of treatment) (4–6). Taken together, the evidence is clear that B cells may persist in the synovium in some patients, although on average there is a marked reduction in the number of such cells, as shown in our study.

How could we explain the complete depletion of CD20+ B cells in the synovium in the study by Teng and colleagues, when 4 other groups of investigators observed a variable tissue response? First, we need to consider false-negative results due to technical reasons. Teng et al used an antibody directed against the cytoplasmic tail of CD20 to detect synovial B cells after treatment with rituximab. When rituximab binds to CD20, this may induce redistribution of the CD20 molecule into lipid rafts, which induces proximity with molecules involved in signal transduction.

In addition, oligomers with other CD20 molecules may form. Because redistribution of CD20 requires the cytoplasmic tail, it is conceivable that this process may interfere with the sensitivity of the immunohistologic method. Thus, the results reported by Teng et al need to be confirmed by staining for CD19 or CD22. Second, we need to take into account the difference in sensitivity of the method used to quantify the stained tissue sections. Whereas Teng et al evaluated the sections by semiquantitative analysis using a 5-point scale, we quantified the results by fully quantitative digital image analysis, which is a more sensitive method. An alternative explanation for the discrepancy between the results described by Teng et al and those observed by other groups of investigators is the use of high-dose corticosteroids, which may have biased the results: the patients received 100 mg of methylprednisolone twice, and they were allowed to receive up to 20 mg of oral prednisolone daily and also 80 mg of intraarticular prednisolone after the first arthroscopy (which could have an effect on the contralateral joints as well). Thus, complete depletion of CD20+ B cells in the tissue might be explained in part by use of high-dose corticosteroids rather than by the effect of rituximab alone. As we have shown previously, prednisolone treatment has marked effects on synovial B cells. Consequently, the changes in the synovium and bone marrow shown by Teng and colleagues are not necessarily specific for rituximab treatment but may be related to combination therapy with corticosteroids.

In conclusion, ample evidence suggests that B cell infiltration in the synovium may be persistent in a subset of patients with RA after rituximab treatment. It is tempting to speculate that the recently described persistence of synovial plasma cells in nonresponders to rituximab treatment is related to the persistence of these synovial B cells, which are thought to serve as their precursors.
FIGURE No.1

Figure 1. Change in the number of CD19+ B cells in synovial tissue 4 weeks after rituximab treatment. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers. * P<0.05