Commentary

Reconstitution of the adult B cell repertoire after treatment with rituximab

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Abstract

B cells play diverse and fundamental roles in the pathogenesis of autoimmune diseases. Consequently, therapeutic targeting of B cells is gaining prominence in our clinical armamentarium for an ever expanding array of autoimmune and neoplastic disorders. Therefore, it is of great importance to understand the mechanism of action of B cell depletion. Given that the ideal consequence of B cell depletion would be the subsequent re-establishment of immunologic tolerance, a detailed analysis of the properties of the emerging repertoire will be required. The results presented by Rouzière and coworkers in their study of rheumatoid arthritis patients shed some light on this question and are discussed in this commentary.

As reflected in the work by Rouzière and coworkers [1], B cells have become a major therapeutic target for autoimmune diseases. This prominence stems from two convergent developments. One of these is the understanding that, in addition to conventional antibody dependent effects, B cells also play important regulatory and potentially pathogenic roles through antibody independent mechanisms, including antigen presentation, T cell activation and polarization, dendritic cell regulation, and cytokine and chemokine production [2]. Moreover, mounting clinical evidence strongly supports the therapeutic benefit of targeting B cells in an array of autoimmune conditions ranging from systemic lupus erhthematosus (SLE) to rheumatoid arthritis (RA) and Wegener's granulomatosis [3].

These findings raise a number of important questions that remain to be formally addressed. Such questions pertain to the specific pathogenic roles of B cells in different diseases, the different mechanisms whereby B cell depletion may improve disease, the relative sensitivity of different B cell subsets to depleting agents, and the kinetics, magnitude and quality of B cell repopulation. The latter issue is of central importance and is the focus of the article by Rouzière and coworkers [1]. Indeed, the breakdown of B cell tolerance for autoantigens may be at the core of the pathogenesis of SLE and RA and perhaps of other autoimmune diseases.

As is always the case with disease, the ultimate goal is to achieve cure and inevitably the elusive question of whether B cell tolerance can be restored must be asked. In order for this to be possible one must postulate that tolerance breakdown and the selection of a pathogenic repertoire is the result of environmental hits on a stochastically generated B cell repertoire in a genetically predisposed individual. On that basis, it is apparent that, given a second chance, the B cell repertoire could become a good citizen either by escaping harmful environmental influences and/or by sheer good luck in the stochastic generation of immunologic diversity. Furthermore, the prolonged 'absence' of B cells could also have important influences on the T cell repertoire either by decreasing T cell activation or by shifting T-helper cell polarization, presumably favoring a T-helper-1 profile [4,5].

Answering or even addressing this question in human studies is a tall order. In order to do so, formal testing of the antigenic reactivity of the emerging repertoire will ultimately be required. This approach will determine whether immature autoreactive B cells are appropriately purged or edited. Furthermore, given that even healthy individuals still bear a large load of autoreactive B cells in the mature compartment, it will also be important to achieve a phenotypic and functional definition of anergy to elucidate whether the newly developed mature B cells are being appropriately silenced [6,7].

Although the information obtained by Rouzière and coworkers [1] does not clarify these issues, it offers a molecular glimpse into the reconstitution of the heavy chain B cell repertoire in two patients with RA treated with rituximab, whose peripheral blood was analyzed before treatment and at different time

PCR = polymerase chain reaction; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus.

points after treatment. Some limitations of the work should be borne in mind, prominently the small number of patients studied and the nonquantitative nature of the bulk PCR approach employed by the investigators. Furthermore, for most of the study total B cells were studied without differentiating specific B cell subsets. This situation was corrected for by confirmation of somatic hypermutation in which single cell PCR analysis of VH genes was used and B cells were separated into a $CD19^+CD27^-$ fraction (which conventionally would include immature, transitional, and mature naïve B cells) and a $CD19^+CD27^+$ fraction (which would include both isotype switched and nonswitched memory B cells).

Unfortunately, the single cell PCR experiments did not discriminate between IgM and IgG sequences and suffered from a relatively small sample size. Nevertheless, the central finding that the 'early' (7 months) repopulating repertoire contained a substantial amount of somatic hypermutation that was significantly higher than before treatment only to decline again over time is tantalizing, and there are several potential explanations for this. As pointed out by the investigators, it seems likely that this finding may reflect initial expansion of residual memory cells. Whether this is indeed the case could be explored by analysis of residual B cells at earlier points after treatment. In fact, in SLE patients we showed that, even in those with 'complete' peripheral B cell depletion, it is possible to detect residual B cells that predominantly express a switched memory phenotype [8]. The expansion of residual B cells could be favored by a lymphopenic environment and lack of competition for survival factors such as BlyS. It would also be important to determine whether the surviving memory cells are enriched for autoreactivity.

A surprising and provocative aspect of the study by Rouzière and coworkers [1] is the finding that even $CD27^-$ B cells exhibited a level of somatic hypermutation that was substantially higher than expected for either naïve or immature/transitional B cells, and that was more in accord with the levels expressed by memory cells. An explanation for this could be that these cells represent a subset of memory cells lacking CD27, as suggested by the authors. Interestingly, however, published and unpublished data indicate that such a population may be greatly increased in patients with active SLE but not in patients with RA [8,9]. Furthermore, at least in SLE, such cells are highly sensitive to rituximab and do not appear to be preferentially expanded after treatment. An alternative, not mutually exclusive explanation is that the mutated $CD27^-$ B cells could represent an expansion of marginal zone B cells – a compartment whose expansion has been implicated in the pathogenesis of autoimmune diseases and that in humans predominantly contains $CD27^+$ memory cells. It has been shown that in children younger than 2 years marginal zone B cells undergo somatic hypermutation of their antibody genes early in ontogeny [10]. Although in that study the B cells analyzed expressed CD27, it is tempting to postulate that upon profound B cell depletion there could be a re-enactment of early B cell ontogeny and that the cells described in the report by Rouzière and coworkers [1] might represent mutated marginal zone B cell precursors that have not yet acquired CD27. Elucidation of this interesting question will undoubtedly require fine discrimination and separate analysis of B cell subsets in patients treated with rituximab.

Conclusion

It is safe to expect that even in patients with good clinical B cell depletion some B cells will survive and may experience preferential early expansion in a lymphopenic environment. Ultimately, the quality of the emerging repertoire will depend to a large extent on the interplay and competition between these cells and newly generated B cells. The growing availability of patients treated with B cell depletion should allow investigators to understand the determinants that underlie B cell reconstitution in different autoimmune diseases and in individual patients. The knowledge gained from such studies should greatly enhance our ability to treat these diseases and tailor therapy for individual cases. In addition, they should contribute to our understanding of basic aspects of B cell biology and homeostasis.

Competing interests

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