REPORT

B cell signalling as therapeutic target

R H Carter

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Biological therapy holds the promise of specificity of intervention. Recent progress in development of such therapies has proved that rational targeting can lead to clinical benefit. B cells pose an attractive target in autoimmunity. Approaches currently being tested lead to depletion of B cells or at least circulating B cells.^{1–3} Such therapies appear remarkably benign, but the effects of repeated depletion, which might be required to fight autoimmune disease, have yet to be defined. Fundamental studies have uncovered pathways of B cell activation. This work opens the possibility of targeting activated B cells. This review asks how we might approach therapeutic manipulation of activated B cells. The underlying assumption is that it is the activated B cells that are important in pathophysiology of autoimmune disease. The activation pathways lead to the differentiation of self-reactive B cells to plasmablasts that produce the autoantibodies, which may be directly pathogenic.⁴ In addition, activation of B cells leads to the expression of ligands on B cells that bind costimulatory receptors on T cells.⁵ Along with presentation of self-derived peptides on surface major histocompatibility complex class II molecules, this drives activation of autoimmune T cells.⁶ Targeting activated B cells would eliminate both contributions of B cells to disease without depletion of resting memory B cells acquired from previous infection or immunisation.

The white pulp in the spleen is organised around the terminal branches of the arterioles. The periarteriolar lymphoid sheath (PALS), which surrounds the arteriole, is rich in T cells. Budding eccentrically off the PALS are collections of B cells, which form the follicular mantle. This is bounded by the marginal sinuses, around which are abundant macrophages. The marginal zone is a band of B cells and macrophages lying between the marginal sinuses and the red pulp. These structures are maintained by signals from chemokine receptors and expression of specific adhesion molecules.^{7–8}

There are multiple pathways of B cell activation in the spleen.^{9–11} The first response is the rapid mobilisation of marginal zone B cells to produce a predominantly immunoglobulin (Ig)M response. The germinal centre reaction requires days to develop but leads to a higher affinity IgG response. The germinal centre also leads to the development of memory B cells, which have switched from IgM to other classes of Ig.

When pathogens reach the blood, they pass through the marginal sinuses, where they are captured by pathogen recognition receptors on macrophages.^{12–13} The marginal zone B cells, in close proximity to the macrophages, both recognise specific antigen by their surface Ig and danger signals through recognition of pathogen associated molecular patterns by toll-like receptors (TLRs). These signals lead to the rapid migration of activated cells out of the marginal zone, through the follicular mantle, and into the T cell zone, based on changes in chemokine signals and altered expression of adhesion molecules.^{14–15} The B cells upregulate expression of ligands for T cell costimulatory receptors and are very efficient antigen presenting cells, even though activation of

marginal zone B cells does not require T cell help. The activated B cells continue to migrate into channels linking the PALS with the red pulp. In these bridging channels, the B cells encounter dendritic cells that have trapped antigen and that can drive further B cell differentiation. The B cells upregulate Blimp-1, a transcription factor that regulates genes involved in terminal differentiation into antibody secreting plasmablasts.

Shlomchik's lab has demonstrated that autoimmune cells can develop in the red pulp in disease models of lupus.¹⁶ Thus, activation of marginal zone B cells to become antibody secreting cells in the red pulp may provide precursors for such cells. The activation of these cells is very dependent on B lymphocyte stimulator (BLyS).¹⁵ BLyS can also drive class switching in the activated cells.¹⁷ With the development of antibodies and receptor fusion constructs that bind and neutralise BLyS, we have an opportunity to prevent the production of autoantibodies that derive from the marginal zone B cell pathway. Although chronic BLyS blockade depletes most mature B cells, in the short term one can block the marginal zone response without depleting non-responding B cells. Our analysis in disease models demonstrates that BLyS blockade significantly reduces autoantibodies but had minimal effects on total serum IgG in normal mice.¹⁸

Signals from the antigen receptor, TLR, and BLyS receptors are likely involved in the response of the marginal zone B cells. The antigen receptor signals through the formation of the cytoplasmic "signalosome", a complex of adapters (BLNK), kinases (Bruton's tyrosine kinase (Btk)), phospholipase C (PLC), and other proteins (Vav).¹⁹ These lead to the generation of a variety of chemical changes including "second messenger" intermediates, such as calcium, and phosphorylation of other proteins and lipids. These lead, in turn, to activation of downstream cascades, which activate mitogen activated protein kinases (MAPKs) such as extracellular regulating kinase (ERK), c-Jun-N-terminal kinase (JNK) and p38, nuclear factor of activated T cells (NFAT), and nuclear factor (NF)- κ B, which results in altered gene transcription in the nucleus. The BLyS receptors and TLR signal through associated cytoplasmic molecules but also lead to activation of NF- κ B (although different dimers) and MAPKs.^{20–21} The induction of these pathways provides a rationale for the use of inhibitors of the signalling intermediates. Analysis of these signalling pathways reveals that specific upstream adapters link to common downstream factors. The good news is that inhibition of NF- κ B and MAPKs could inhibit activation or survival of marginal zone B cells, but the bad news is that these proteins serve diverse functions.²²

The germinal centre response grows as antibody production by the marginal zone B cell response wanes. While the

Abbreviations: BLyS, B lymphocyte stimulator; Btk, Bruton's tyrosine kinase; FDC, follicular dendritic cell; MAPK, mitogen activated protein kinase; (NF)AT, (nuclear factor) of activated T cells; PALS, periarteriolar lymphoid sheath; PLC, phospholipase C; TLR, toll-like receptor

marginal zone provides a rapid, first line response, the germinal centre is slower but entails selection for higher affinity antibodies. The germinal centre includes follicular dendritic cells (FDCs), activated B cells, and T cells,^{23–24} and it can be divided into different poles.⁹ FDCs are found in the pole of the germinal centre away from the PALS, extending in the follicular mantle up to the marginal sinuses. In mouse spleens, proliferating cells are found in both poles of the germinal centre. The pole of the germinal centre proximal to the PALS and the T cell zone contains only switched cells, while the cells that express IgM are found in association with the FDCs (Y Wang and R H Carter, manuscript in preparation). These data suggest that initial activation and switching occurs in the FDC pole of the germinal centre.

Unlike the marginal zone B cell response, the germinal centre, beyond the initial stages, is dependent on T cell help and is inhibited by blocking of costimulatory molecules of either the tumour necrosis factor (TNF) receptor family (CD40) or the CD28 family (inducible costimulator (ICOS)).^{25–26} Thus, the signals that drive the germinal centre B cells, derived from the TNF receptor family and the antigen receptor, are, like those in marginal zone B cells, driven by relatively specific upstream adapters leading to common downstream pathways, including MAPK, NFAT, and NF- κ B. These drive transcription of specific genes. The specificity may derive in part from the availability of individual components of the transcription machinery and differentiation specific chromatin changes.

Our recent data suggest that specific signals regulate different stages of the germinal centre response (manuscript submitted for publication). CD19 is a B cell coreceptor that is non-essential for development of mature B cells but is required for both normal differentiation into marginal zone cells and for production of T dependent antibody responses, even in the presence of adjuvant. Germinal centre form in mice with defective CD19, but these centres are smaller than in normal mice. CD19 interacts with the lipid kinase PI3K, and the interaction is essential for CD19 function in vivo. Antibody responses are blocked in mice that express mutated CD19 that cannot bind PI3K. Again, germinal centres form but are not able to support production of specific antibodies.²⁷ We have found that the differentiation and proliferation of B cells in the FDC pole of the germinal centre is defective, while proliferation in the FDC-free zone is normal (Y Wang and R H Carter, manuscript in preparation). Thus, different specific signals could be targeted that block specific aspects of the germinal centre response. However, specific protein–protein interactions appear to be difficult targets to inhibit. Kinase and NF- κ B inhibitors will reach trials more quickly.²⁸ The brief review of signalling suggests these could suppress the germinal centre response, although effects on non-targeted tissues are likely.

The final level of activation of B cells is that of switched memory B cells. Except in germinal centres, the switched B cells are relatively rare in the white pulp of the spleen. Thus, the switched, postgerminal centre memory B cells appear to leave the spleen and likely reside in the bone marrow. These cells become antibody producing cells in response to non-specific stimuli.²⁹ Such stimuli, including cytokines, T cell help, and TLR ligands, are likely to be relatively abundant in autoimmunity, providing a mechanism for chronic production of autoantibodies, if autoimmune memory B cells are developed to self antigens.

In summary, different B cell responses derive from different subsets of B cells and are triggered by specific receptors. These receptors signal by binding relatively specific cytoplasmic adapters. These activate relatively generalised

downstream pathways. Agents in development that target downstream pathways could suppress B cell responses but are likely to have other effects. It is more difficult to develop inhibitors of more specific upstream adapters, but this holds the promise of more specific therapies to modulate B cell activation clinically.

Correspondence to: R H Carter, University of Alabama at Birmingham, Birmingham, AL, USA; rcarter@uab.edu

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