Plan B (-cell) in atherosclerosis

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Abstract

Atherosclerosis is a leading cause of death worldwide. It is a complex chronic inflammatory disease involving interactions between vascular, circulating and immune cells. B cells play an important role in chronic inflammation producing antibodies and regulating T and natural killer (NKT) cell activation. The role of B cells in atherosclerosis is complex, with atherogenic and protective roles assigned for distinct B cell subsets. Drugs that deplete B cells or modulate their functions are now used in the treatment of various autoimmune diseases in humans. Here, we briefly review the roles of B cell subsets in atherogenesis, and emphasize the potential impact of B cell targeted therapies on the cardiovascular risk of treated patients. Developing more B cell subset-specific therapies would lead to more effective treatments with enhanced safety profile.

Atherosclerosis and immune response

Atherosclerotic artery disease is the leading cause of death and morbidity worldwide. Atherosclerosis is a complex inflammatory disease of the large and medium-sized blood vessels with multiple genetic and environmental risk factors. It is initiated in response to trapping of low-density lipoproteins (LDL) in the intima. LDL will then acquire immunogenic properties through both enzymatic and non-enzymatic modifications. The subsequent immune response involves interactions between many vascular (endothelial cells, smooth muscle cells, etc.) and immune cells (lymphocytes, myeloid cells, etc.), leading to a chronic inflammatory state that, in the absence of effective counter-regulatory mechanisms, will evolve to plaque disruption and vessel thrombosis ending in an acute ischemic (cardiac, cerebrovascular, etc.) event.

Nearly all of innate and adaptive immune cells have been implicated in atherogenesis. Murine and human atherosclerotic plaques contain macrophages, dendritic cells (DC), mast cells, T and B lymphocytes in the intima and adventitia (reviewed in (Hansson and Hermansson, 2011). Following intimal trapping of LDL and activation of the endothelial cell layer, resident and monocyte-derived macrophages will further promote atherosclerotic lesion development through foam cell formation and activation of the

inflammatory response. Dendritic cells and T cells become activated both locally and systemically in response to several atherosclerosis-related antigens, mainly derived from lipoproteins, and further contribute to the modulation of the immune response and the development of atherosclerosis. B cells accumulate only in small numbers in atherosclerotic plaques, while they are quite abundant in artery tertiary lymphoid organs (ATLO). ATLOs are complex and organized immune cell aggregates (T and B lymphocytes, and plasma cells) that infiltrate the adventitia at sites of advanced atherosclerotic plaques, and are capable of mounting local immune responses. Their exact role in atherosclerosis has not been deciphered yet, although some atheroprotective roles have been described (Yin et al., 2016). B cells in secondary lymphoid organs also contribute to the immune responses of atherosclerosis.

B cells and atherosclerosis

B cells play important roles in both innate and adaptive humoral and cellular immune responses. They are the only cells able to undergo hypersomatic mutations and become antibody-producing cells (germinal centre, plasmablasts and plasma cells). Antibodies are glycoproteins of the immunoglobulin family that are attached to the B cell membranes serving as B cell receptor (BCR) for antigens or can be secreted into the extracellular space and the circulation where they bind to auto- or foreign antigens. There are five main immunoglobulin classes (M, D, A, E and G) that are distinguishable by their different C-terminus region of the heavy chain (Fc). IgM antibodies are atheroprotective, while adaptive IgE and IgG antibodies are considered pro-atherogenic, even though contrasting data have been published for the latter (reviewed in (Tsiantoulas et al., 2014)). For many years, B cells were believed to participate exclusively in the humoral immune response of atherosclerosis, but we now know that they also have a prominent role in the cellular response by directly regulating T cell activation via antigen presentation, co-stimulation and cytokine production.

There are several B cell subsets. On one hand, B-1 cell subset comprises the main B cell population in the peritoneal and pleural cavities, while it is a relatively small population (expressing $CD43^{+}$) in the spleen. B-1 cells participate in the innate immune response and they are specialized in secreting high amount of natural IgM antibodies. On the other hand, B2 cells comprise the phenotypically distinct follicular (FO) B cells of the spleen and the lymph nodes, and the marginal zone (MZ) B cell population of the spleen. They contribute to both innate (MZ B cells) and adaptive (FO and MZ B cells) immune responses. Finally, new B cell activating factor (BAFF) receptor-dependent B cell subsets that share phenotypic characteristics of B-1 and B2 (mostly MZB) cells have been discovered and classified based on their cytokine production profile. Innate response activator (IRA) B cells secrete high

amounts of granulocyte-macrophage colony-stimulating factor (GM-CSF) (Rauch et al., 2012), and the regulatory B (Breg) cells that produce either interleukin (IL)-10 (B10) and/or IL-35 and dampen (auto)immune responses (Fillatreau, 2016) (Figure 1).

The implication of B cells in atherosclerosis was demonstrated 15 years ago, when two different groups demonstrated that atherosclerotic mice with complete or splenic-restricted B cell deficiency developed exacerbated atherosclerosis, suggesting that B cells were atheroprotective (Caligiuri et al., 2002; Major et al., 2002). Nevertheless, subsequent studies made by us and others targeting different B cell subsets challenged the overall atheroprotective concept of B cells, and B2 cells were assigned a detrimental role (Ait-Oufella et al., 2010; Kyaw et al., 2012, 2010; Sage et al., 2012) in contrast to B-1 cells which were shown to be atheroprotective (Kyaw et al., 2011). We have further refined this concept, and demonstrated that within the B2 cell population, a distinction should be made between atherogenic FOB cells and atheroprotective MZB cells (Nus et al., 2017). Moreover, an atherogenic role has been assigned to IRA-B cells (Hilgendorf et al., 2014), whereas the role of Bregs remains controversial (Sage et al., 2015; Strom et al., 2015). Thus, it becomes clear that B cell subsets have a differential impact in atheroma development that is also influenced by the environmental niche and stage of disease development.

Antibody production has been considered the hallmark of the protective effect of B cells in atherosclerosis. Thus, several translational studies have tried to find associations between atherosclerosis and circulating levels of 'atheroprotective' IgM and 'atherogenic' IgG antibody titres in humans. Reproducibly, low circulating IgM levels have been negatively correlated with the extent of atherosclerosis and CV events. However, studies on IgG antibody titres against OxLDL or MDA-LDL showed positive, negative or no correlation with atherosclerosis (reviewed in (Zhang et al., 2015)) urging for new studies to elucidate their roles in atherosclerosis. In contrast, very few clinical studies have addressed the association between B cells and atherogenesis beyond antibodies. One of the main reasons for this is the difficulty to identify B cell subsets in humans that are strictly equivalent to murine B cells, particularly B1 cells. In a genome wide association experiment using data from subjects included in the Framingham Study and comparing them to published human and murine GWAS studies, genes related to B cell activation were shown to be enriched in control vs coronary heart disease patients, suggesting that B cells may confer protection for CVD (Huan et al., 2013). In another association study, `pro-atherogenic` $CD19^{+}CD86^{+}$ B cells were positively correlated, while 'atheroprotective' $CD19^{+}CD40^{+}$ cells were negatively correlated with the risk of developing stroke, despite no association with carotid intima-media thickness (Mantani et al., 2014). In summary, experimental and human clinical data indicate that B cells play a very important role in atherosclerosis.

1. Follicular B cells

The majority of circulating B cells are derived from splenic FOB cells in both mice ($B220^{+}CD23^{hi}CD21^{lo}$) and humans ($B220^{+}CD27^{-}$) (Pillai and Cariappa, 2009). FO B cells originate from transitional B cells that have successfully escaped bone marrow selection. The major factor that determines the differentiation fate of transitional B cells towards FO B cells is the B cell receptor (BCR) signalling strength. Several studies have shown that FOB cells require strong self-antigen mediated BCR signalling as compared to MZ B cells for their development (Pillai and Cariappa, 2009). We have recently challenged this paradigm by showing that decreasing BCR signalling restricts MZB and concomitantly promotes FOB cell differentiation (Tsiantoulas et al., 2017b). To date, there are no experimental studies that have directly and specifically investigated the role of FOB cells in atherosclerosis.

Caligiuri *et al*. demonstrated that adoptive transfer of splenic B cells into splenectomised atherosclerosis-prone *ApoE*$^{-/-}$ mice reversed the splenectomy-induced acceleration of atherosclerosis (Caligiuri et al., 2002). In agreement with this, Doran *et al.*, reported decreased atherosclerosis in *μMT*/*ApoE*$^{-/-}$ mice upon adoptive transfer of splenic B-2 cells isolated from *ApoE*$^{-/-}$ donors (Doran et al., 2012). Furthermore, we have recently shown that atherosclerotic mice lacking secreted IgM (*sIgM*$^{-/-}$) develop increased atherosclerosis due to an accumulation of high IgE antibody levels. We also showed that these mice exhibit reduced expression of the low affinity receptor for IgE (CD23) on FOB cells, resulting in reduced clearance of IgE antibodies (Tsiantoulas et al., 2017a). Taken together and considering that FO B cells account for approximately 80% of total splenic B cells (Baumgarth, 2011; Tsiantoulas et al., 2014) one could hypothesize that FO B cells exhibit a protective effect in atherosclerosis. However, it has been reported that transfer of splenic B-2 cells into lymphocyte-deficient *Rag2*$^{-/-}$*γ-chain*$^{-/-}$*ApoE*$^{-/-}$ or *μMT/ApoE*$^{-/-}$ recipients enhanced atherosclerosis (Kyaw et al., 2010). Additional studies performed by us and others, which evaluated the effect of B2 cell subsets in atherosclerosis, also supported a proatherogenic role for FOB cells. Treatment of hypercholesterolemic *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice with anti-CD20 antibody, which preferentially depletes B-2 cells, led to decreased atherosclerosis (Ait-Oufella et al., 2010; Kyaw et al., 2010). Also, BAFFR deficiency in *ApoE*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, or B-2 cell depletion using anti-BAFFR blocking antibody, limit the development of atherosclerosis (Kyaw et al., 2013, 2012; Sage et al., 2012), further supporting an atherogenic role for FO B cells. The atheroprotective effect of B-2 cell depletion was suggested to be mediated via suppression of proatherogenic T cell responses (Ait-Oufella et al., 2010; Sage et al., 2012). However, B-2 cells and most likely FO B cells may also promote atherosclerosis in the absence of T cells, via secretion of proinflammatory cytokines (Kyaw et al., 2012; Tay et al., 2016).

Overall, the aforementioned studies - though indirectly - support a proatherogenic role for FOB cells. Nevertheless, the effect of FO B cells may be influenced by their "inflammatory experience" such as previous antigen encountering that could ultimately result in faster and more efficient response to a hyperlipidemic milieu. As mentioned above, in contrast to Kyaw et al. (Kyaw et al., 2012), Doran and colleagues reported decreased atherosclerosis upon transfer of splenic B-2 cells (Doran et al., 2012). In the former study, wild-type mice B-2 cells were transferred, while in the latter the injected B-2 cells were isolated from *ApoE*$^{-/-}$ donors. Therefore, the inflammatory milieu or the previous 'experience' of the B cells may be an important factor in determining the role of FO B cells in atherosclerosis. This is also corroborated in splenectomised *ApoE*$^{-/-}$ mice, where the protective effect of splenic B cell transfer was stronger when the donor cells were derived from old *ApoE*$^{-/-}$ compared to young *ApoE*$^{-/-}$ donors (Caligiuri et al., 2002). Another important issue may be related to the quality (FO B cells *versus* MZ B cells *versus* Bregs) and extent of B cell reconstitution in an immunodeficient environment, and to subtle but determinant changes in cytokines and growth factors. For example, B cell deficient mice show very high BAFF levels, which may differentially alter the properties of the transferred B cells. Indeed, we have recently shown that the pro-atherogenic effect of B cell reconstitution in *ApoE*$^{-/-}$/*Baffr*$^{-/-}$ mice (high BAFF environment) is lost when mice are infused with angiotensin II, due to a switch of the transferred B cells to a regulatory IL-10-producing phenotype, dependent on a synergistic effect of BAFF and angiotensin II (Ponnuswamy et al., 2017). Thus, FO B cells may exhibit different properties in atherosclerosis depending on the state of inflammation and the local microenvironment. B cells in SLE and RA, 2 diseases that predispose to premature atherosclerosis and increased CVD risk (Nurmohamed et al., 2015), may acquire a proinflammatory background that promotes the proatherogenic role of FO B cells. In conclusion, further studies are required to pinpoint the role of FO B cells in atherosclerosis.

2. Marginal zone B cells

MZ B cells are positioned at strategic sites in the outer white pulp of the spleen. Their development requires their migration to the marginal sinus where they become retained, pre-activated and where they acquire the ability to self-renew and survive for a whole life-span (Pillai et al., 2005). While they lack circulating properties, they can shuttle from the MZ to the follicle (Cinamon et al., 2008). Given their low threshold for activation, proliferation and differentiation into antibody-secreting cells, MZ B cells are vigorously engaged in early immune responses, for example by rapidly responding to encounter with blood borne pathogens (Cerutti et al., 2013). The first two studies indirectly suggesting a potential role of MZB in atherosclerosis were published 2 years ago. In one of those studies, MZB cell numbers were decreased in WT mice compared to aged *ApoE*$^{-/-}$ mice. The authors

hypothesized that in *ApoE*$^{-/-}$ mice, MZB cells apoptosis was dampened due to impaired iNKT activation (decreased IL4 and IFNγ) (Soh et al., 2016). However, a potential bias might be the possible contribution of ApoE itself to MZB function. Indeed, this gene is highly expressed in MZB cells (Nus et al., 2017) and the ApoE receptor is not only important for reverse cholesterol transport but also for mediating pathways of lipid antigen presentation (van den Elzen et al., 2005). In the second study, the authors assumed that MZB cells were most probably atheroprotective by secreting anti-oxidation specific epitope (OSE) antibodies of the IgM subtype in response to sterile inflammation induced by apoptotic cells. However, the authors could not exclude that B-1 cells were responsible for this phenotype (Grasset et al., 2015). Interestingly, they also found that apoptotic cell injections decreased cholesterol levels in a B cell dependent manner. MZB cells are able to take up OxLDL (unpublished data and Grasset et al. PNAS 2015) in a hypercholesterolemic environment, but the impact of this process is still unexplored. MZ B cells also express high levels of CD36 (Zhang et al., 2007) and up-regulate *CD36, Abcg1*, and *Abca1* in response to a high cholesterol diet (HCD) (Nus et al., 2017). Furthermore, BAFF overexpression, which notably leads to an expansion of MZ B cells (Mackay, JEM, 1999), results in strongly decreased plasma cholesterol levels accompanied by decreased lesion size in HCD-fed *Apoe*$^{-/-}$ mice (Jackson et al., 2016). It is then interesting to explore the potential role that MZB cells could play in cholesterol metabolism and whether this can be harnessed to develop new strategies to fight atherosclerosis.

We have now demonstrated using an atherogenic mouse model which specifically lacks MZB cells that this B-2 cell subset is atheroprotective, and that the atheroprotective effect is mediated through the control of T follicular helper cells (Tfh) (Nus et al., 2017) (a T cell subset previously suggested to be atherogenic (Clement et al., 2015)). In response to a HCD, MZB cells migrate into the T cell zone where they bind to pre-Tfh, through PDL1-PD1 interaction, impairing their motility and activation. This finding brings important conceptual changes to our understanding of the immune mechanisms of atherosclerosis, but also suggests that current strategies such as rituximab, belimumab and epratuzumab, that deplete mature B2 cells in patients with auto-immune diseases, should be evaluated for their impact on CV risk because they deplete both FO and MZ B cells.

In humans, a subset of human memory B cells resemble MZ-like cells ($CD27^+IgM^+IgD^+CD21^+Cd1d^+$), and MZ-like B cells have been detected in the spleen, tonsils, lymph nodes, Peyer´s patches, and the circulating blood (Weller et al., 2004). This offers a particularly interesting opportunity for investigating experimental data in the human disease setting. We have demonstrated that human splenic and circulating MZ-like cells express high levels of PDL1 (Nus et al., 2017), a potent immunoregulatory ligand in several disease settings, including atherosclerosis and cancer.

3. B-1 B cells

There is a growing consensus that B-1 cells exhibit an atheroprotective effect (Tsiantoulas et al., 2014). B-1 cells, which are divided into B-1a and B-1b subsets predominately localize in the peritoneum and pleural cavities. In contrast to B-2 cells, B-1 cells are derived from fetal liver and they maintain their numbers by self-replenishment. Peritoneal B-1 cells are responsible for the production of natural IgM antibodies in a T cell independent manner (Baumgarth, 2011), which increases greatly when these cells migrate to the spleen (Choi et al., 2012). Congenitally asplenic or splenectomised mice, which develop increased atherosclerosis (Caligiuri et al., 2002) have strongly reduced peritoneal B-1a cell numbers (Wardemann et al., 2002). Kyaw *et al*. showed that adoptive transfer of sIgM producing B-1a cells - but not *sIgM*$^{-/-}$ B-1a cells - into splenectomised *ApoE*$^{-/-}$ mice, reversed their accelerated atherosclerosis (Kyaw et al., 2011). These data suggest that B-1a cells exhibit their atheroprotective properties via production of natural IgM, which in naïve mice make up for approximately 80% of total IgM antibody levels. In agreement with this, *Ldlr*$^{-/-}$ *sIgM*$^{-/-}$ mice develop accelerated atherosclerosis (Lewis et al., 2009; Tsiantoulas et al., 2017a). Furthermore, we have recently shown that mice lacking sialic acid-binding immunoglobulin-like lectin G, which develop particularly high numbers of peritoneal B-1a cells and increased levels of total IgM antibodies (Hoffmann et al., 2007), are significantly protected against atherosclerosis (Gruber et al., 2016).

A large part of IgM antibodies (>35%) is directed against OSE which are present in apoptotic cells and cellular debris (Chou et al., 2009; Tsiantoulas et al., 2015). Notably, OSE are also present in OxLDL, which - similar to apoptotic debris - is recognized by different OSE-specific natural IgM antibodies such as T15/E06 (Tsiantoulas et al., 2012). In line with this, cholesterol-fed atherosclerosis-prone mice immunized with heat-killed *S. pneumoniae*, developed strongly increased T15/E06 antibodies and decreased atherosclerosis (Binder et al., 2003). However, it is likely that other OSE-specific IgM directed against PC epitopes antibodies may also contribute to the atheroprotective effect in the latter study (Centa et al., 2016). Similar to the protective capacity of B-1a cells, B-1b cell injection into *ApoE*$^{-/-}$ *Rag1*$^{-/-}$ mice resulted in decreased lesion formation compared to PBS injected controls (Rosenfeld et al., 2015). However, the underlying mechanism by which B-1b cells confer an atheroprotective effect remains elusive.

The studies above suggest that expansion of B-1 cells, which are also suggested to be present in humans and are defined as $CD20^{+}CD27^{+}CD43^{+}CD70^{-}$ (Griffin et al., 2011), may be a therapeutic approach against atherosclerosis. However, innate response activator (IRA) B cells which are derived from B-1a cells (Rauch et al., 2012) and are increased in the spleen

of patients with symptomatic CVD, have been shown to aggravate atherosclerosis (Hilgendorf et al., 2014). Therefore, some therapeutic strategies, which aim to expand B-1 cells may trigger unwanted effects that would confer a proatherogenic effect. For example, triggering TLR4 signalling, which facilitates the atheroprotective properties of B-1a cells (Hosseini et al., 2016) will also lead to expansion of the proatherogenic IRA B cells (Rauch et al., 2012). Therefore, the identification of molecular pathways that allow the selective expansion of B-1 cells would be of great interest. Interestingly, it has been recently demonstrated that expression of the transcription factor Bhlhe41 in B cells is specifically required for the development and self-renewal of B-1a cells (Browning, 2006). Further studies are needed to identify a precise approach to enhance the protective properties of B-1 cells in atherosclerosis.

4. B regulatory cells

Bregs exhibit immune-suppressive and regulatory functions triggered by an inflammatory stimulus. They are present in very low levels in naïve mice but their numbers are substantially increased in response to pro-inflammatory signals like IL6, IL1β, IL21, BAFF and GM-CSF (reviewed in (Rosser and Mauri, 2015)). They secrete high amounts of immunosupressive cytokines like IL10 and IL35, which favour an anti-inflammatory Treg response and limit pro-inflammatory Th1 and Th17 responses (reviewed in (Fillatreau, 2016)). It is believed that Bregs arise from different B cell subsets, and not from a common progenitor. However, a transcriptomic signature that determines Breg differentiation is yet to be identified. B10 are IL10-producing Bregs cells enriched in the $CD5^{hi}CD1d^{hi}$ B cell population but are also found in transitional 2-MZ progenitors (T2-MZP), $Tim1^{+}$ B cells, some $CD138^{+}$ plasma cells, and some plasmablasts. They play a protective role in several murine models of autoimmune diseases like experimental autoimmune encephalomyelitis (EAE), colitis and arthritis. Nevertheless, their role in atherosclerosis is still under debate. In hypercholesterolemic *ApoE*$^{-/-}$ mice, T2-MZP B10 cells are significantly increased compared to WT mice and their adoptive transfer in an arterial injury model in *ApoE*$^{-/-}$ mice reduced lesion size in a IL-10-dependent manner (Strom et al., 2015). On the other hand, experiments performed in our lab using a B cell specific IL10 deletion model in *Ldlr*$^{-/-}$ mice showed that B10 deletion had no effect on the development of atherosclerosis (Sage et al., 2015). These discrepancies could be due to different animal strains (*ApoE*$^{-/-}$ vs *Ldlr*$^{-/-}$) and experimental conditions (arterial injury vs. diet-induced atherosclerosis). Indeed, recent studies from our lab indicate that boosting of B10 cells in defined settings, such as a high BAFF/high angiotensin II environment, may endow the cells with atheroprotective properties (Ponnuswamy et al., 2017). Furthermore, Bregs may display IL10-independent

immunosuppressive properties through the expression of GITRL, PDL1, IL35, or FasL (reviewed in (Ray et al., 2015)), which is worth exploring in the context of atherosclerosis.
In humans, two Breg subsets have been identified, $CD19^{+}CD24^{hi}CD38^{hi}CD1^{hi}$ and $CD19^{+}CD24^{hi}CD27^{+}$ Bregs. Only one study examined the number and state of activation of $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs in the context of atherosclerosis. Patients with myocardial infarction or stable angina had significantly decreased $CD19^{+}CD24^{hi}CD38^{hi}$ cell numbers compared to healthy controls. Those patient 'Bregs' also had impaired IL10 production upon activation with CpG and CD40L (Dumitriu et al., 2017). These are interesting results. However, further validation experiments are needed before reaching any definitive conclusion.

Towards a translational strategy

Altered B cell immunity is a cardinal factor in the pathology of rheumatic diseases (Browning, 2006), such as SLE and RA (Nurmohamed et al., 2015). In 2011, FDA approved an anti-soluble BAFF blocking antibody (Belimumab) as a treatment in SLE patients. Although the effect of soluble BAFF in atherosclerosis is not known, one could hypothesize based on the atheroprotective effect of BAFFR deletion or blockage in mice (Kyaw et al., 2013, 2012; Sage et al., 2012) that Belimumab treatment may also reduce atherosclerosis via depletion of proatherogenic conventional B cells. However, as mentioned above atherosclerosis-prone mice that express particularly increased levels of circulating BAFF exhibit reduced plasma cholesterol levels (Jackson et al., 2016). Thus, BAFF neutralization may affect cholesterol metabolism in an unwanted manner.

Furthermore, considering the protective effect of a mouse anti-CD20 treatment in atherosclerotic mice, it would be particularly interesting to investigate the effect of Rituximab, widely used for the treatment of RA patients, on CVD risk. However, it is also important to mention that long-term B cell depletion could result in increased infection risk due to compromised humoral immunity. Therefore, more studies are needed in order to identify the mechanistic feature of the B cell machinery that affects atherosclerotic CVD. Finally, addressing the CVD risk associated with B cell targeting agents will not only improve our understanding of the role of B cell immunity in CVD but will also help the development of new, more precise and effective therapeutic options against this wide-spread disease.

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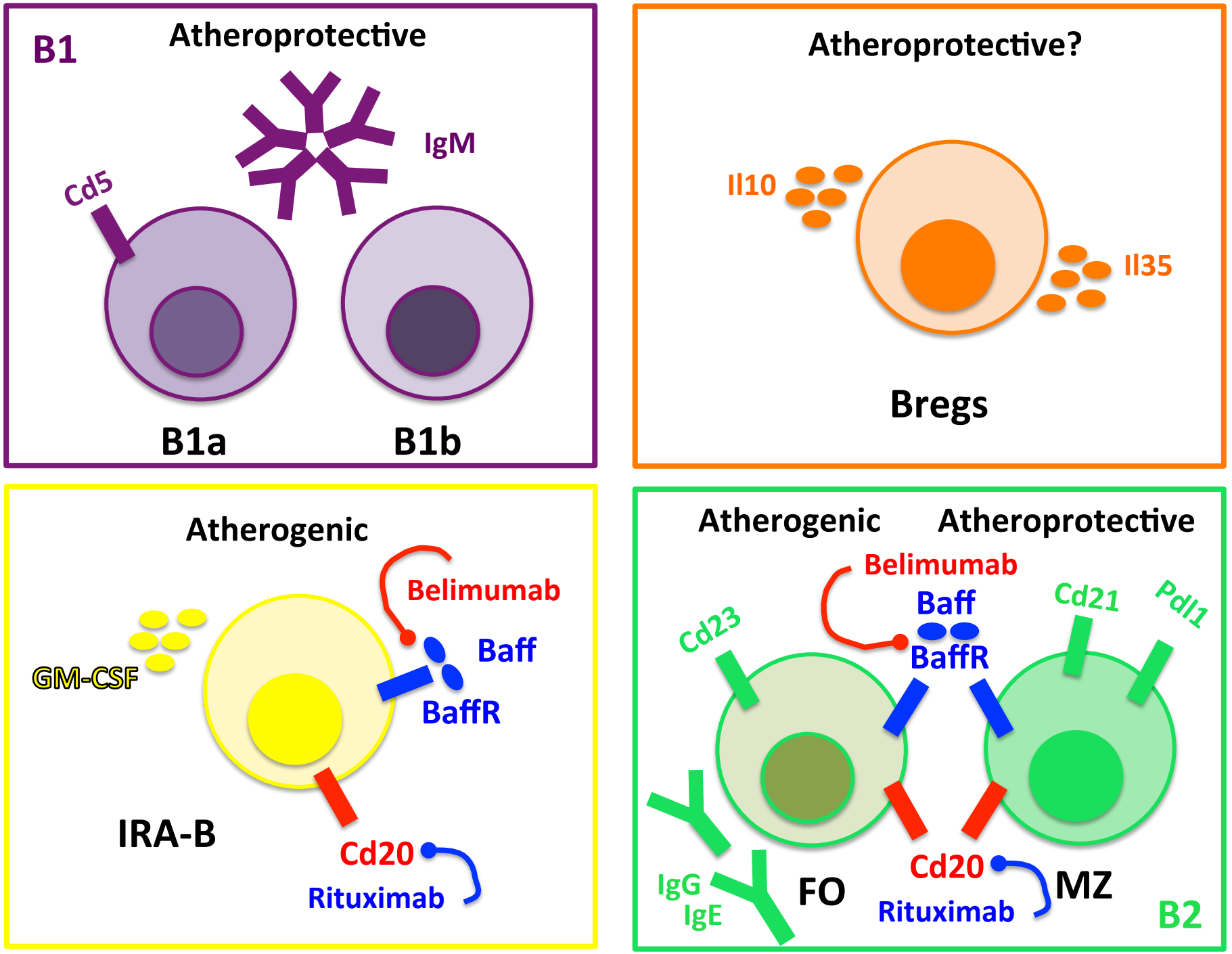
B1
Atheroprotective
IgM
Cd5
B1a
B1b
Atheroprotective?
Il10
Il35
Bregs
Atherogenic
Belimumab
Baff
BaffR
GM-CSF
IRA-B
Cd20
Rituximab
Atherogenic
Atheroprotective
Belimumab
Baff
BaffR
Cd23
Cd21
Pdl1
IgG
IgE
FO
Cd20
Rituximab
MZ
B2

Figure 1. B cell subsets that impact atherosclerosis. B cells are divided into two major subsets, the innate-like B-1 and conventional B-2 cells. B-1 cells consist of B-1a and B-1b subsets that are distinguished based on the surface receptor repertoire with the most prominent difference the presence of the CD5 receptor on B-1a cells. Both B-1a and B-1b cells confer an atheroprotective effect, which is likely mediated via natural IgM production. On the other hand, the B-1a derived IRA cells promote atherosclerosis via instructing proatherogenic Th1 responses. The role of Bregs, which are major producers of IL-10 and IL-35, in plaque formation remains under debate. B-2 cells, which account for the vast majority of mature B cells, consist of FO and MZ B cells that display distinct levels of surface receptors such as CD23 and CD21. MZ B cells exhibit atheroprotective properties via regulation of Tfh homeostasis in hyperlipidemic conditions. A specific depletion of B-2 cells via anti-CD20 antibody treatment results in reduced atherosclerosis suggesting that FO B cells (the larger subset of B-2 cells) display a proinflammatory role in atherosclerosis. Anti-BAFF antibody treatment (Belimumab), which similar to Rituximab (human anti-CD20) depletes conventional B-2 and IRA cells, is used in the clinic. However, the effect of belimumab in atherosclerosis remains elusive. IRA; innate response activator, Bregs: B regulatory cells, FO; follicular, MZ; marginal zone.