# A Chronological Journey of Breg Subsets: Implications in Health and Disease

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**Abstract**

B cells play a multidimensional role in host immunity. Regulatory B (Breg) cells are a class of B lymphocytes with immunomodulatory properties that play an important role in maintaining immunological tolerance along with dampening harmful immune responses. Bregs suppress various immune pathologies through the production of interleukin (IL)-10, IL-35, and transforming growth factor-β (TGF-β). They act by inhibition of T helper 1 (Th1) and Th17 cells proliferation, suppression of dendritic cell (DC), differentiation and simultaneous enhancement of the expression and differentiation of fork head transcription factor P3-positive regulatory T cells (FoxP3$^+$ Tregs). In this chapter, we discuss the induction, function, and phenotypes of the various Breg cell subsets defined in both mice and humans along with their proposed mechanism of action in various immune responses.

**Keywords**

Regulatory B cells (Bregs) · Plasma Bregs · BR2 Bregs · B10 Bregs · T2-MZP Bregs · TIM1$^+$ Bregs · B1 B cells · Br1 Bregs · Plasmablast · iBregs · IgA$^+$ Bregs · GrB$^+$Bregs

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## Abbreviations

| | |
|---|---|
| Bregs | B regulatory cells |
| BCR | B cell receptor |
| TLR | Toll-like receptor |
| PAMPs | pathogen-associated molecular patterns |
| EAE | experimental autoimmune encephalomyelitis |
| IL | interleukin |
| LPS | lipopolysaccharide |
| TGF-β | transforming growth factor Beta |
| Tregs | T regulatory cells |
| MHC | major histocompatibility complex |
| AIA | antigen-induced arthritis |
| Th | T helper cells |
| STAT | Signal Transducer and Activator of Transcription |
| IFN-γ | interferon gamma |
| TNF-α | tumor necrosis factor alpha |
| mAbs | monoclonal antibodies |
| T2-MZP | transitional *2* marginal-zone precursor |
| TIM-1 | T-cell Ig mucin domain-1 |
| CTLA-4 | cytotoxic T lymphocyte-associated protein 4 |
| iBreg | induced B regulatory cells |
| IDO | indoleamine 2,3-dioxygenase |
| MS | multiple sclerosis |
| SLE | systemic lupus erythematosus |
| RA | rheumatoid arthritis |
| NOD | non-obese diabetic |
| RANKL | receptor activator of nuclear factor-κB ligand |
| OPG | osteoprotegerin |
| T1D | Type 1 diabetes |
| Tr1 | T regulatory type 1 |

## 5.1 Discovery of Breg Cells

The concept of B cells regulating immune responses dates back to 1974, when the suppressive nature of B cells in modulating delayed type hypersensitivity in guinea pigs was described [1]. Wolf et al. suggested a regulatory subset of B cells (Bregs) exhibiting immunomodulatory properties in an experimental autoimmune encephalomyelitis (EAE) model of mice in 1996 [2]. From 2002 to 2003, Fillatreau et al., Mizoguchi et al., and Mauri et al. through independent studies demonstrated that B cells produce IL-10 and suppress inflammatory conditions such as EAE, inflammatory bowel disease and collagen-induced arthritis respectively [3–5]. Further, Parekh et al. were the first to show a IL-10-independent mechanism of action in 2003, demonstrating TGF-β-dependent B cell–mediated regulation of $CD8^+$ T cell

responses, though they did not name these as Bregs at the time [6]. It was only after 3 years that Mizoguchi and Bhan proposed the concept of Bregs while studying their role in colitis, demonstrating that B cell–deficient mice experienced higher severity of colitis than normal [7]. Moreover, Mizoguchi et al. also established that a specific B cell subset induced in gut-associated lymphoid tissue was secreting higher levels of IL-10 and had increased CD1d expression during intestinal inflammatory condition [4]. Till date, numerous studies have been carried out to illustrate the role of various Breg subsets via IL-10-dependent or IL-10-independent manner in modulating host immunity. In 2008, Yanaba et al. also showed the role of $CD1d^{hi}CD5^{+}$ cells in negatively regulating T-cell responses through IL-10 in contact hypersensitivity model [8]. Dittel et al. observed that mice with B cell deficiency have reduced numbers of both $Foxp3^{+}$ regulatory T cells (Tregs) and IL-10 levels in EAE and demonstrated a novel IL-10, B7, and MHC class II-independent regulatory role for B cells in suppressing autoimmunity by the maintenance of Tregs via glucocorticoid-induced TNFR family–related gene ligands [9, 10]. In 2010, Amu et al. reported that helminths-induced Bregs were responsible for Treg induction that could suppress allergic airway inflammation (AAI) in the murine model [11]. Carter et al. demonstrated the unique ability of Bregs in inhibiting Th1/Th17 cells during arthritic conditions in mice [12]. Strikingly, the regulatory function of B cells is mediated by the production of various regulatory cytokines such as IL-10, IL-35, and TGF-β1, which are responsible for suppressing autoreactive B cells and pathogenic T cells in a cytokine or cell-cell contact-dependent manner [7, 13]. Another mechanism of immune regulation by B cells involve expression of FAS ligand on $CD5^{+}$ B cells, known as killer B cells that regulate effector immune responses by inducing cell death [14]. Kaku et al. showed a population of B cells that express both CD73 and CD39, ectoenzymes responsible for the production of adenosine, which inhibited the severity of colitis [15]. Khan et al. described additional phenotype of Bregs, $PD\text{-}L1^{hi}$ B cells, which regulate humoral immunity through their interaction with $CD4^{+}CXCR5^{+}PD\text{-}1^{+}$ follicular helper T cells and ameliorate EAE [16]. Recently, Oleinika et al. reported a novel role of $CD1d^{+}$ T2-MZP Bregs in the induction of immunosuppressive iNKT cells that downregulate excessive Th1/Th17 responses partially via secreting IFN-γ and limit inflammation in experimental arthritis [17]. Together, these studies indicate that Bregs suppress inflammation by inhibiting the differentiation of pro-inflammatory cells and inducing a population of immunosuppressive cells. In addition, studies on exacerbation of colitis and development of psoriasis in patients treated with anti-CD20 mAb (rituximab) suggest the regulatory function of B cells in human subjects [18, 19]. Bregs constitute fewer than 10% of immature B cells in healthy individuals and play an important role in functioning of the immune system by maintaining tolerance and immune homeostasis [20]. Over the last decade, numerous studies in both mice and human have extensively shown the importance of Bregs in regulating various diseases, including inflammatory disorders, autoimmunity, and cancer [21, 22] Bregs with their wide range of immunomodulatory functions can thus be exploited for therapy in various B cell–mediated diseases. Thus, it is important to exhaustively consider the known Breg cell phenotypes, their induction, and function in a chronological manner (Fig. 5.1 and Table 5.1).

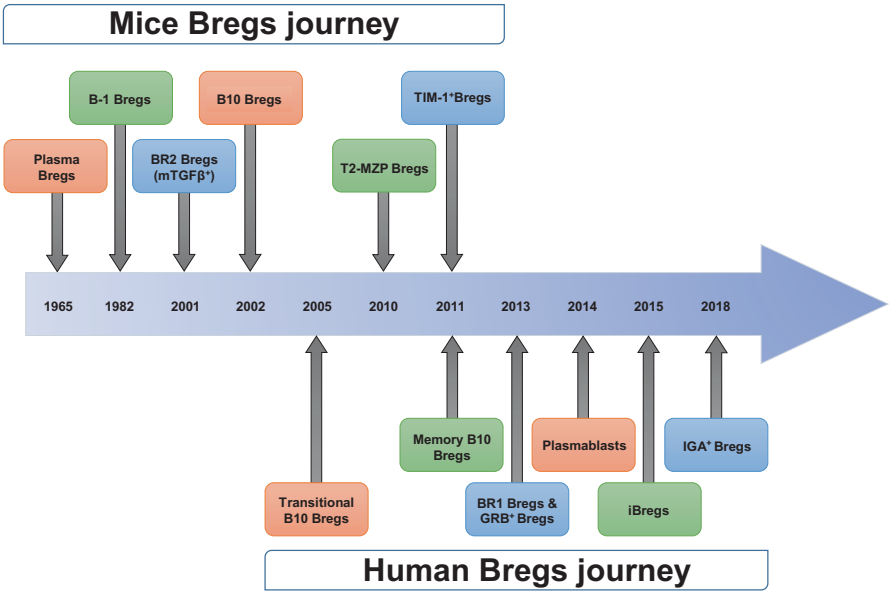


**Fig. 5.1** **Chronological journey of Bregs**. This timeline represents the important events in the journey of Bregs discovery, establishing them as a functionally and developmentally distinct cell lineage

## 5.2 Identification and Phenotypes of Breg Cells

B cell subsets with strong immunomodulatory functions have been reported both in vitro and in vivo (Figs. 5.2 and 5.3) (Table 5.1). Phenotypic identification of Breg cells using the immunomodulatory cytokine IL-10 continues to be a matter of debate due to difficulties in assessing the functionality of Bregs, because IL-10 detection requires intracellular staining. Therefore, other surrogate markers have been employed to identify various Breg subsets. Different overlapping markers are presently being used to describe these cells. Here we discuss both murine and human Breg subsets under separate heads for clarity and distinction among these subsets.

### 5.2.1 Mouse Breg Subsets

In mice, Plasma B cells, B-1 cells, $CD5^{+}CD1d^{hi}$ B10 B cells, $CD21^{hi}CD23^{hi}CD24^{hi}$ transitional type 2 marginal zone precursors (T2-MZP) Breg cells, and TIM-$1^{+}$ B cells have been proposed with regulatory functions in a variety of infections, in autoimmune and transplantation settings [21, 23]. IL-$10^{+}$ Bregs have also been observed to inhibit IFN-γ production in hepatitis B virus (HBV) infection by modulating $CD8^{+}$ T cell responses [24, 25]. Furthermore, IL-$10^{+}$ Bregs inhibit TNF-α production by activated monocytes following stimulation with LPS and bacterial

**Table 5.1** Breg cell subsets in mice and humans

| S. no. | Types of Breg subset | Phenotype | Year of discovery | Mechanism of action | Refs. |
|---|---|---|---|---|---|
| Mice Breg subsets | | | | | |
| 1. | Plasma Bregs | $CD138^{+}MHC\text{-}11^{lo}\ B220^{+}$ | 1965 | Found in bone marrow, spleen, mucosa-associated lymphoid tissues (MALT), or lymph nodes. They secrete IL-10 and IL-35, and play a key role in host defense against infection | [28, 29] |
| 2. | B-1 Bregs | $CD5^{+}$ | 1982 | Found in bone marrow, lymph node, spleen, and blood, leading to innate adaptive regulation via IL-10 production | [28–30, 33] |
| 3. | BR2 (mTGFβ+Bregs) | $CD40^{+}TGF\beta1$ | 2001 | Found in spleen, lymph node, and blood, express membrane TGF-β1, and cause anergy and hyporesponsiveness in $CD8^{+}$ T cells | [6] |
| 4. | B10 Bregs | $CD19^{hi}CD1d^{hi}CD5^{+}$ | 2002 | Found in spleen and blood, produce IL-10, and inhibit expression of effector $CD4^{+}$ T cells, DCs, and monocytes | [4] |
| | | $TIM\text{-}1^{+}CD19^{+}$ | 2011 | Found in the spleen and suppress the expression of effector $CD4^{+}$ T cells through IL-10 production | [5, 10] |
| 5. | T2-MZP Bregs | $CD19^{+}CD21^{hi}CD23^{hi}CD24^{hi}$ | 2010 | Found in the spleen, produce IL-10, enhance Treg cells, and inhibit the expression of effector $CD4^{+}$ and $CD8^{+}$ T cells | [65] |
| Human Breg subsets | | | | | |
| 1. | $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs | Transitional B10 cells | 2005 | Found in blood and support the development of Tregs through IL-10 and TGFβ production | [144] |
| 2. | $CD19^{+}CD24^{hi}CD27^{+}$ Bregs | Memory B10 cells | 2011 | Inhibit the proliferation of TNF-α and IFNγ producing $CD4^{+}$ T cells, DCs, and monocytes via IL-10-dependent and -independent pathways | [22] |
| 3. | Br1 Bregs | $CD19^{+}CD25^{+}CD71^{+}CD73^{-}$ | 2013 | Found in blood and produce IL-10 | [72] |
| 4. | $GrB^{+}$ Bregs | $CD19^{+}CD38^{+}CD1d^{+}IgM^{+}CD147^{+}$ | 2013 | Inhibit the proliferation of T cells through the expression of Granzyme B | [88] |
| 5. | Plasmablasts | $CD19^{+}CD27^{int}CD38^{+}$ | 2014 | Found in draining lymph nodes in mice and in blood from humans and inhibit DCs and effector T cells through IL-10 expression | [73] |
| 6. | iBregs | IDO, TGFβ | 2015 | Through IDO and TGFβ production, they induce natural Tregs as well as TGFβ and IL-10–producing Tregs | [92] |
| 7. | $IgA^{+}$ Bregs | $IgA^{+}$ | 2018 | Induce the differentiation of T cells more toward a regulatory phenotype through the expression of IL-10 and PD-L1 | [93] |

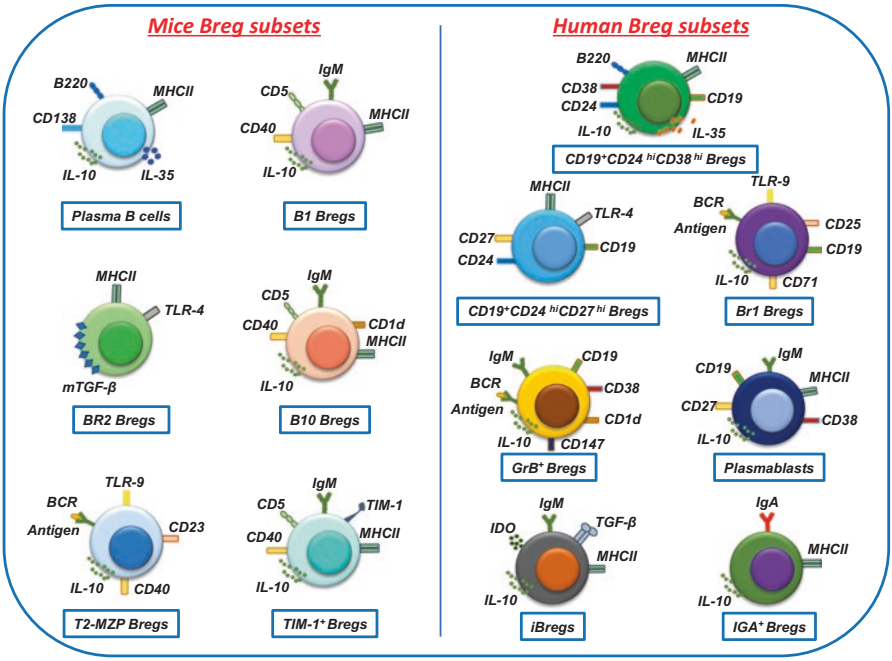


**Fig. 5.2 Breg subsets in mice and humans**. Mice have a total of five defined Breg subsets: Plasma B cells (CD138$^{+}$MHC-11$^{lo}$ B220$^{+}$), B1 Bregs (CD5$^{+}$), BR2 Bregs (CD40$^{+}$TGFβ1), B10 Bregs (CD19$^{hi}$CD1d$^{hi}$CD5$^{+}$), and T2-MZP Bregs (CD19$^{+}$CD21$^{hi}$CD23$^{hi}$CD24$^{hi}$). Humans, on the contrary, have seven defined human Breg subsets: Br1 Bregs (CD19$^{+}$CD25$^{+}$CD71$^{+}$ CD73$^{-}$), CD19$^{+}$CD24$^{hi}$CD38$^{hi}$ Bregs, CD19$^{+}$CD24$^{hi}$CD27$^{hi}$ Bregs, Plasmablasts (CD19$^{+}$ CD27$^{int}$CD38$^{+}$), iBregs (IDO, TGFβ), GrB$^{+}$Bregs (CD19 $^{+}$CD38$^{+}$CD1d$^{+}$IgM$^{+}$CD147$^{+}$), and IGA$^{+}$Bregs (IgA$^{+}$)

CpG DNA [9, 22]. Bacterial components such as LPS and CpG are known to induce the expansion, differentiation, and activation of murine Bregs through TLR signaling in vitro [26, 27]. Furthermore, mice harboring TLR2- or TLR4-deficient B cells fail to recover from EAE. Alltogether these studies clearly indicate that inflammation acts as stimuli for the activation and differentiation of Bregs.

### 5.2.1.1 Plasma Bregs

Plasma B cells are representative antibody-secreting cells (ASCs) [28] present in all lymphoid organs. Plasma cells have also been found to occur in significant numbers in the bone marrow compared to their lower numbers in the spleen. Indeed, the bone marrow is primarily responsible for the long-term maintenance of plasma cells arising from immunization [29]. Recently, Lino et al. described a subset of resident Plasma B cells specialized for producing IL-10 upon TLR stimulation and are found to occur naturally, i.e., prior to antigenic challenge [30]. Genome-wide approaches have shown that this Breg lineage is triple-positive for the following markers: IL-10$^{+}$LAG-3$^{+}$CD138$^{hi}$. The lymphocyte activation gene 3 (LAG-3$^{+}$) helps in

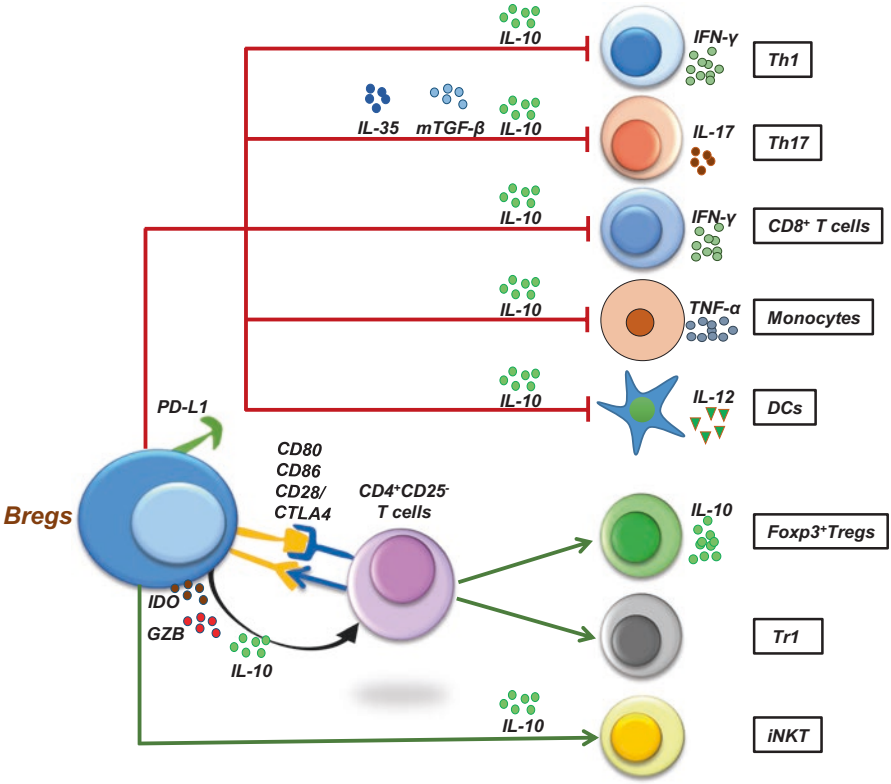


**Fig. 5.3 Regulatory mechanisms of Bregs in various immune responses**. Bregs lead to the suppression and inhibition of pro-inflammatory lymphocytes such as Th1, Th17, cytotoxic $CD8^+$ T cells, monocytes, and IL-12-producing dendritic cells through the production of various factors like IL-10, IL-35, TGF-β, IDO, GZB, and so on. IL-10 production by Bregs is primarily responsible for restoring the Th1/Th2 balance, where it is shifted toward Th2. One more mechanism of inhibiting inflammatory cascades is via tweaking the Treg/Th17 balance, leading to suppression of Th17 cells. The Breg population is reportedly responsible for enhancing the differentiation of $Foxp3^+$Treg cells and helps in the maintenance of iNKT cells

regulating humoral immunity and in maintaining immunological tolerance toward endogenous T-independent type 2 antigens, which are normally not detected by $CD4^+Foxp3^+$ T regulatory cells. Unlike conventional plasma cell differentiation, which requires several days for proliferation, the detection of $IL\text{-}10^+LAG\text{-}3^+CD138^{hi}$ plasma cells at day one post-infection with *Salmonella typhimurium* in the spleen of mice, confirmed that this subset is derived from already existing cells $LAG\text{-}3^+CD138^{hi}$ cells. These $LAG\text{-}3^+CD138^{hi}$ cells are likely induced by self-antigen and remain in a quiescent state. Further, genome-wide methylome, transcriptome, and gene-set enrichment analysis of $LAG\text{-}3^+CD138^{hi}$ cells in naïve mice and at day one post-*Salmonella* infection showed that after antigenic challenge, $LAG\text{-}3^+CD138^{hi}$ cells express IL-10 and become $IL\text{-}10^+LAG\text{-}3^+CD138^{hi}$ plasma Bregs [30].

Thus, these results indicate that plasma Bregs provide a first layer of immune regulation in response to stimuli. In contrast, Matsumoto et al. showed that mice lacking genes such as Prdm1 and IRF4, which are required for plasma cell differentiation, develop a severe form of EAE compared to control mice. This study suggested that Bregs are inducible in nature. Thus, these studies clearly establish both the innate and inducible nature of Bregs. During EAE, plasma B cells are known to be the main source of IL-35 and facilitate recovery from EAE. IL-35 secreted by plasma Bregs exhibits anti-inflammatory properties by expanding the immunosuppressive $CD4^+CD25^+$ Tregs population which inhibits $CD4^+CD25^-$ T effector cell proliferation when cultured in vitro [31]. IL-35 also inhibits the differentiation of inflammatory Th17 cells. Recent studies have indicated the role of BATF/IRF-4/IRF-8 axis in regulating IL-35 and IL-10 expression in activated B cells [32]. IL-35 cytokine can act as a potential target in the treatment of both autoimmune and inflammatory conditions. Interestingly, declined populations of $LAG\text{-}3^+CD138^{hi}$ cells have been reported in mice deficient in CD19 or Bruton's tyrosine kinase [33], further establishing that differentiation of $LAG\text{-}3^+CD138^{hi}$ cells to plasma cells is under the control of BCR. Taken together, these studies establish that B cell differentiation into $LAG\text{-}3^+CD138^{hi}$ cells is a steady-state process driven primarily by BCR signaling rather than TLR-mediated signaling or T cells.

#### 5.2.1.2 B1 Bregs

B-1 cells represent a class of innate immune cells that are responsible for higher antibody production, especially IgMs for mounting rapid immune responses against pathogens [34]. This subset of $CD5^+$ B cells was initially identified in the early 90s in mice, as a set of distinctive fetal B cells to differentiate them from B-2 cells that usually develop in the adult bone marrow [35, 36]. B-1 cells represent a population of B cells found predominantly in the pleural and peritoneal cavities (35–70%). A smaller number of B-1 cells are also found in the spleen [37], bone marrow, mucosal sites, lymph nodes, and blood [38]. Despite their very low frequency in lymphoid tissues, B-1 cells are important regulators of immune defense and tissue homeostasis. B-1 B cells are chiefly produced in the absence of any antigen exposure [39, 40] and are a major source (>80%) of naturally occurring antibodies [41]. Higher levels of natural IgMs are produced by B-1 cells residing in the spleen and bone marrow [38]. These polyreactive [42, 43] antibodies help in recognizing self as well as foreign antigens [44, 45], act as the first line of defense, and are analogously linked to innate immune responses. B-1 cells are categorized into different functioning subsubsets based on the relative CD5 expression. B-1a represents a class of $CD5^+$(Ly-1) B-1 cells that chiefly express IL-10 upon innate activation [46] whereas B-1b represents a class of $CD5^-$ B-1 cells [34, 45]. B-1a cells are major producers of B-cell-derived IL-10 [46], and their activation and expansion are regulated by cross-regulatory cytokines such as IL-12 and IFN-γ [47]. Using Schistosomal infection model, Vellupillai P et al. demonstrated that the outgrowth of IL-10 producing B-1 after infection is genetically restricted and regulated by polylactosamine sugars. Interestingly, it has also been shown that B-cell defect in BALB.Xid mice impart susceptibility to develop filariosis and is associated with lack of antibody

production and IL-10 production in response to dominant surface molecule of invading pathogen [48]. B-1a cells were shown to inhibit TLR-mediated excessive inflammation in neonatal mice in an IL-10-dependent manner [49]. Another subset of B-1a, FAS ligand expressing B-1a cells also known as killer B cells, has been shown to mediate T cell apoptosis during schistosomal infection and prevent granulomatous inflammation [14]. Interestingly, the regulatory role of IgM-producing B-1a cells has also been associated with the suppression of colitis in mice that were kept in conventional facility as compared to mice kept under specific pathogen free facility [50]. Thus, B-1a cells play an important role in immune regulation and tissue homeostasis.

#### 5.2.1.3 BR2 (mTGFβ$^+$) Bregs

Here, we propose a novel subset of Bregs called "BR2" Bregs. These Bregs were first reported and studied by Parekh et al. in 2003. They found that B cells activated via T-independent mechanisms such as LPS showed membrane expression of TGFβ1, leading to CD8$^+$ T cell anergy. These Bregs thus have the unique phenotype of mTGFβ$^+$ Bregs. This manner of B cell activation is a major factor influencing CD8$^+$ T cell responses as T-dependent activated B cells provide higher stimulatory properties to CD8$^+$ T cells [6]. Membrane expression of TGFβ1 was found to be solely responsible for conferring these B cells with regulatory properties, thus influencing CD8$^+$ T cell responses. Thus, we now name these Bregs as BR2 (mTGFβ$^+$Bregs), with regulatory properties governed by membrane TGFβ expression. These findings provide insights into the immune evasion strategies adopted by retroviruses and gram-negative bacteria that target toll-like receptor-4 (TLR-4) signaling in B cells. Recent reports have also shown that Bregs producing TGF-β induce Tregs for promoting transplantation tolerance [51]. These results illuminate the importance of novel modes of B-cell activation in the development of therapeutic strategies to modulate the balance between active immunity and tolerance [6].

#### 5.2.1.4 B10 Bregs

B10 cells are defined by their ability to express IL-10 following ex vivo stimulation with PMA and ionomycin and are enriched within CD1d$^{hi}$CD5$^+$ B cell subset [8]. Mouse B10 cells represent around 1–3% of cells in the spleen. Other tissues like the lymph nodes, central nervous system, Peyer's patches, and intestinal tissues comprise a very small number of B10 cells. Their presence in peritoneal cavity is also prominent [29, 52, 53]. Mouse B10 cells have a typical phenotype as IgD$^{lo}$IgM$^{hi}$ cells, although a very small number of B10 cells are also reported to co-express IgA or IgG [54]. B10 cells secrete polyreactive or Ag-specific IgMs and IgGs upon differentiation [53, 54]. T-cell Ig mucin domain-1 (TIM-1) is a type of transmembrane glycoprotein responsible for immunomodulatory responses [55], and its expression was found to be important for the induction and maintenance of IL-10-producing B cells, whereas a defect in TIM-1 expression leads to increased production of proinflammatory cytokines such as IL-1 and IL-6 [56]. During allotransplantation, TIM-1 is particularly responsible for Breg stimulation to prolong allograft survival. TIM-1$^+$ B cells usually express IL-4 and IL-10 and promote Th2 responses with subsequent

allograft tolerance [57]. Numerous studies have shown the potential of B10 cells in inhibiting disease initiation and subsequent pathology after their adoptive transfer in models of contact hypersensitivity [8], EAE [3, 52, 58], lupus [59], IBD [53, 60], and graft-versus-host disease [61]. Mauri et al. were the first to elucidate the therapeutic potential of B cells using agonistic CD40 mAbs for treating mice with collagen-induced arthritis [5, 62]. Depletion of B10 cells can have either therapeutic or detrimental effects in the course of various human pathological mouse models. Depletion of IL-10-producing B cells is known to enhance the innate, humoral, and cellular immune responses in mice [62, 63]. This intensifies the severity of disease-related symptoms in various autoimmune diseases in mice such as EAE, skin transplant rejection, and contact hypersensitivity [27, 58, 64].

#### 5.2.1.5 T2-MZP Bregs

The T2-MZP Breg cell subset was discovered by Evans et al. in 2007 [65]. T2-MZP Bregs are immature transitional B cells found in the spleen with a $CD19^{+}CD21^{hi}CD23^{hi}CD24^{hi}IgM^{hi}IgD^{hi}CD1d^{hi}$ phenotype. Among the different B-cell subsets residing in the spleen of mice with arthritis, this specific Breg cell type is responsible for IL-10 production after collagen stimulation. T2-MZP Bregs were discovered to have decisive suppressing properties both in vitro and in vivo, and the mechanism of suppression includes inhibition of pathogenic Th1 responses via producing IL-10 [65]. IL-10-producing T2-MZP B cells are shown to exert immunomodulatory properties in various immune-mediated pathologies, including autoimmune diseases, cancer, and allergy [21, 65, 66]. Recently, Oleinika et al. reported a novel role of $CD1d^{+}$ T2-MZP Bregs in the induction of immunosuppressive invariant Natural Killer T (iNKT)-cells that downregulate excessive Th1/Th17 responses partially via secreting IFN-γ and limit inflammation in experimental arthritis [17]. Recently, T2-MZP Breg cells have been linked as the precursors of B10 Bregs, but the interrelation between these two Breg subsets needs to be further established [21].

### 5.2.2 Human Breg Subsets

Similar to mouse Bregs, human Breg cells also play an important role in the maintenance of tissue homeostasis. Mauri et al. in an extensive study demonstrated that $CD19^{+}CD24^{hi}CD38^{hi}$ B cells with a phenotype very similar to immature B cells produce the highest fraction of IL-10 in healthy human peripheral blood upon CD40 stimulation [20]. Separately, Tedder et al. also categorized human Breg cells as $CD24^{hi}CD27^{+}$, a phenotype related to memory B cells [22]. Furthermore, Bosma et al. reported that due to altered CD1d recycling in B cells, defect in B-cell-mediated iNKT expansion was observed in SLE patients [67]. Human Bregs exert immunomodulatory properties through their actions on various immune cell types such as inhibiting cytokine production in monocytes [22]; inducing immunosuppressive NKT cells [67], restraining IFN-α production from pDCs [68]; and regulating $CD4^{+}$ T cell proliferation [69], inhibition of Th1 and Th17 differentiation, and conversion

of $CD4^+$ T-cells into $CD4^+CD25^+$ cells along with enhancing FOXP3 and PD-1 expression on Tregs [20, 70, 71]. In humans, research on Bregs is mainly restricted due to lack of access to the human spleen, the primary site of the Bregs population. Thus, the majority of identified human Bregs are from peripheral blood where Bregs ranging from immature B cells to differentiated plasmablasts are found. Other phenotypes of human Bregs comprise $CD19^+CD25^+CD71^+CD73^-$ B regulatory 1 (Br1) cells [72], $CD19^+CD27^{int}CD38^+$ plasmablasts [73]. Furthermore, human Bregs (i.e., equivalent to B10 of mice) with the $CD19^+CD24^{hi}CD27^+$ phenotype along with $Tim1^+$ Bregs are preferentially found in the transitional B cells [22, 74]. Thus, it is important to describe different defined subsets of human Bregs.

#### 5.2.2.1 $CD19^+CD24^{hi}CD38^{hi}$ Bregs

Human B cells with regulatory function have been described in $CD19^+CD24^{hi}CD38^{hi}$ immature subset of peripheral blood B cells. After CD40 stimulation, this subpopulation isolated from peripheral blood of healthy individuals is known to inhibit the differentiation of Th1 cells via IL-10 production and CD80 and CD86 engagement [20]. However, $CD24^{hi}CD38^{hi}$ cells isolated from SLE patients lacked regulatory capacity [20]. Recently, in patients with SLE, an expanded population of $CD19^+CD24^{hi}CD38^{hi}$ Bregs was observed with deficient IL-10R expression, which is correlated with compromised Breg function despite showing enhanced IL-10 expression [75]. Thus, targeting the 'Bregs/IL-10/IL-10R' axis may prove to be a novel therapeutic approach in the treatment of SLE. In addition to inhibiting Th1 and Th17 differentiation, these cells also convert $CD4^+CD25^-$ into Tregs [70]. Both numerical and functional impairment has been observed in a number of autoimmune diseases such as SLE [20, 75] and RA [70]. Recent studies showing reduced capacity of $CD19^+CD24^{hi}CD38^{hi}$ Bregs to secrete IL-10 in GVHD patients as compared to transplant tolerant and healthy controls indicated their important role in preventing graft rejection by promoting tolerance. Moreover, Cherukuri et al. in 2014 found low IL-10/TNF-α ratio by $CD19^+CD24^{hi}CD38^{hi}$ transitional B cells in renal patients with graft rejection when compared with healthy controls, further highlighting their role in establishing transplant tolerance [76] TIM-1 is also a marker for $IL\text{-}10^+$ Bregs and around 50% of $IL\text{-}10^+$ B cells were $TIM\text{-}1^+$. On evaluating TIM-1 expression on human B cell subsets, this transitional subset was enriched in $TIM\text{-}1^+$ subset [74]. In the same study, authors found a decreased number as well as impaired function of $TIM\text{-}1^+$ in patients with systemic sclerosis [74]. In 2015, Kristensen et al. stated that in humans, 40% of $IL\text{-}10^+$ B10 cells expressed TIM-1 [77]. Supporting this study, Liu et al. found that compared to HIV-infected patients, healthy controls have more than 75% of peripheral B10 cells expressing TIM-1. These studies highlight the role of TIM-1 as a marker of Bregs and will open new avenues for the isolation of Bregs that could be utilized for achieving immune homeostasis.

#### 5.2.2.2 $CD19^+CD24^{hi}CD27^{hi}$ Bregs

The IL-10-producing B cells, named B10 in humans, are predominantly $CD19^+CD24^{hi}CD27^+$ memory subset of B cells, known to be a major source of IL-10 after stimulation with LPS or CpG along with CD40 ligation B cells. B10 cells

also express CD48, and CD148 [22]. IL-21 has the potential to further induce IL-10 production from CpG- or LPS-treated $CD19^+CD27^+$ memory B10 cells [78]. Among other subsets, B10 cells are also present in the tonsils, spleen, and newborn cord blood [76]. Interestingly, an increase in the number of B10 cells was observed in a number of autoimmune diseases [22, 79, 80]. In patients with RA, B10 cells are highly capable of expressing receptor activator of nuclear factor-κB ligand (RANKL) compared to those in the healthy controls, suggesting a possible mechanism by which B10 cells are involved in RA pathogenesis [81]. At the molecular level, Zheng et al. in 2017 reported that microRNA-155 (miR-155) positively regulates IL-10 expression in B10 cells, which is impaired in patients with Crohn's disease (CD), leading to miR-155-induced expression of TNF-α by monocytes. These findings further suggest a novel miRNA-mediated approach in developing Breg-based strategies to control the progression of autoimmune diseases.

#### 5.2.2.3 Br1 Bregs

This subset of human Bregs with the $CD19^+CD25^+CD71^+CD73^-$ phenotype was identified by Van de Veen et al. in 2013. These IL-10-producing Br1 Bregs share homology with the Tr1 subtype of T cells. Due to the low CD73 expression on their surface, the immunosuppressive function of Br1 cells was considered to be independent of adenosine and could thus be IL-10 dependent. In support of this, further studies substantiated the role of IL-10 in imparting immunosuppressive functions to Br1 cells. This $IL\text{-}10^+$ subset of Bregs is reported to induce tolerance toward allergens by repressing the proliferation of allergen-specific $CD4^+$ T cells as well as by producing allergen-specific anti-inflammatory IgG4 antibodies [72], thus contributing to peripheral tolerance. This subset of Bregs can induce tolerance against bee venom allergen and PLA2 (phospholipase A2) in an IL-10-dependent manner and also showed tolerance toward various food allergens like casein (cow milk protein). Van de Veen et al. used flow cytometry and whole-genome sequencing to further show that human Br1 cells express the inhibitory ligand PD-L1 (programmed death ligand-1), which binds PD-1 on T cells to inhibit T cell activation and promote the maintenance of Tregs cells.

#### 5.2.2.4 Plasmablasts

This subset of Bregs is known to be derived from both naïve and immature B cells in humans with the $CD19^+CD27^{int}CD38^+$phenotype, which secretes IL-10 [73]. In the presence of IL-2, IL-6, CpG, and IFN-α, immature B cells undergo differentiation, leading to expansion of plasmablasts with increased expression of IRF4, Blimp1, and XBP1 [73]. In normal tissues, CD30 expression is limited to a few T and B cells, whereas in B cell lymphoma, CD30 expression is upregulated on B cells. Recently, in a mouse model of B cell lymphoma, higher CD30 expression on B cells was found to promote the differentiation of plasma B cells to plasmablasts via NF-κB activation and enhanced phosphorylation of STAT3, STAT6, and nuclear factor IRF4 [82]. Interestingly, exacerbation of inflammatory symptoms in MS patients upon treatment with Atacicept, which deplete antibody-secreting cells,

further suggests the regulatory function of plasmablasts [83]. Patients with immunoglobulin G4 (IgG4)–related disorder (IgG4-RD), primary Sjögren's syndrome [84, 85], and SLE [86] have increased plasmablast number, indicating their expansion could be the result of inflammatory conditions. In 2019, Arbore et al. further reported that microRNA-155 (miR-155) plays an important role in the survival and proliferation of plasmablast B cells [87].

#### 5.2.2.5 Granzyme B (GrB$^+$) Bregs

Granzyme B–expressing Bregs are known to display the characteristic phenotype of CD19$^+$CD38$^+$CD1d$^+$IgM$^+$CD147$^+$ [88]. Expression of Granzyme B on Bregs (GrB$^+$ Bregs) mediates their inhibitory effect on T cells by suppressing their proliferation and inducing apoptosis. In various inflammatory conditions such as SLE [89] and in acute viral infections [90], the percentage of GrB$^+$ Bregs is relatively high. Peripheral B cells stimulated in the presence of IL-21 are reported to produce and secrete GrB. These cells mediate their suppressive function by repressing T cell proliferation, partly via downregulation of the TCR zeta chain, thereby promoting T cell apoptosis [88]. In the case of RA, the proportion of GrB$^+$ Bregs is significantly reduced due to the lowered expression of IL-21R, which in turn impairs the negative regulation of Th1/Th17 by GrB$^+$ Bregs [91], suggesting that impaired GrB$^+$ Bregs are associated with RA pathogenesis.

#### 5.2.2.6 iBregs (Induced Bregs)

B cells like other immunosuppressive cells differentiate into induced Breg (iBreg) cells when subjected to certain stimuli and express indoleamine 2,3-dioxygenase (IDO) and TGFβ. T cells expressing cytotoxic T lymphocyte–associated protein 4 (CTLA-4) enhance the induction of iBregs, which then convert T cells into TGF-β- and IL-10-producing Tregs, thereby modulating various immune responses [92].

#### 5.2.2.7 IgA$^+$ Bregs

This subset of Bregs has been identified recently by Fehres et al. in 2019. They described that overexpression of APRIL (A Proliferation-Inducing Ligand) instead of BAFF induces activation of IL-10$^+$ human Bregs that further repress inflammatory immune reactions. These APRIL-induced IgA$^+$ Bregs suppress the effector function of T cells and macrophages and induce Tregs via IL-10 and PD-L1 expression [93]. These findings collectively suggest the importance of the novel APRIL-induced Breg subset with IgA$^+$ phenotype, both in the immunopathology and homeostasis of immunological reactions. In colorectal cancer patients, a higher proportion of IgA$^+$ Bregs was observed at the tumor site due to lowered expression of microRNA15A (miRNA15A) and microRNA16–1 (miRNA16–1). These microRNAs exhibit the ability to regulate proliferation, drug resistance, and apoptosis. These studies thus concluded that microRNAs and IgA$^+$ Bregs are negatively correlated and that a lower level of microRNAs along with higher proportion of IgA$^+$ Bregs reduces the survival rates in cancer patients [94].

## 5.3 Bregs in Health and Diseases

The discovery of various defined subsets of Bregs has now compelled researchers to revisit the understanding of B cell biology in the context of various immune-mediated diseases. Vaccines have been ideally responsible for eradicating several diseases via the specific activation of B cells. Similarly, cancer immunotherapies demonstrate their course of action via production of different B cells. Moreover, B cell deficiencies lead to various devastating impacts on health and immunity. It is now well established that B lymphocytes produce antibodies and are associated with various immunomodulatory properties. Bregs are now extensively studied for their novel immune-regulatory roles, as mice deprived of B cells are reported to demonstrate higher incidences of immune-related disorders. Bregs are known to produce various cytokines and immunomodulatory factors responsible for proper functioning of the host immune system [95]. A cohort study indicated that targeted depletion of B cell populations serves as a treatment in autoantibody-mediated autoimmune disorders such as SLE [96]. Thus, Bregs undoubtedly play an important role in host pathology, thereby opening Pandora's Box in harnessing the potential of Bregs in mediating health. In the following sections, we focus on the role of Bregs in selected diseases/pathologies.

### 5.3.1 Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disorder occurring due to T and B cell hyperactivation, leading to demyelination and axonal damage in the central nervous system (CNS). Apart from the role of B cells as pathogenic cells, they also modulate immune responses in MS. IL-10-producing Bregs were first observed in MS patients infected with helminthes; these Bregs were found to suppress the proliferation and IFN-γ production in T cells in vitro [97]. The role of Bregs in MS was further substantiated by diminished levels of IL-10 production in MS patients. In relapsing-remitting MS patients, a significantly reduced number of IL-10-producing naïve Bregs were observed compared to that in the controls [98]. Further, treatment of MS patients with IFN-β, fingolimod, or alemtuzumab is reported to increase the number and function of Bregs [99, 100]. In EAE, one of the most widely studied animal model of MS, the importance of Bregs in alleviating EAE progression was recently illustrated [52, 58, 101, 102]. The suppressive functioning of Bregs involves binding to the BCR co-receptor CD19, which plays an inhibitory role in the development of EAE by modulating the Th1/Th2 cytokine balance [103]. Fillatreau et al. found that B-cell-depleted mice have a persistent type I immune response in EAE and that their recovery was dependent on myelin oligodendrocyte glycoprotein (MOG)-specific IL-10-producing B cells [3]. Further studies indicate that Bregs with the $CD1d^{hi}CD5^{+}$ phenotype are effective in inhibiting EAE progression. $CD1d^{hi}CD5^{+}$ Bregs possess highly decisive immunomodulatory properties in controlling the pathogenesis of the initial and late phase of EAE [52, 58]. Further, depletion of $CD20^{+}$ B cell enhances the pathogenesis of EAE. This was evident from a

simultaneous increase in the expression of various inflammatory cytokines in the CNS and an increased number of autoreactive $CD4^+$ T cells due to absence of the IL-10-producing $CD1^{hi}CD5^+$ Bregs subset [52, 58].

### 5.3.2 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a highly deteriorating inflammatory condition of the intestine, usually represented by Crohn's disease (CD) and ulcerative colitis (UC) [104, 105]. Recently, an alarming rise in the prevalence and incidence of IBD has been observed globally [105]. Numerous studies have reported the functions of Bregs in regulating intestinal inflammation. Mizoguchi et al. [106] credited B cells and autoantibody production as important factors in protecting T cell receptor (TCR) α chain-deficient ($TCR\alpha^{-/-}$) mice, which are highly susceptible to develop chronic colitis. They showed that $CD1^+$ B cells producing higher levels of IL-10 upon induction in the gut-associated lymphoid tissues in $TCR\alpha^{-/-}$ mice reduced the intestinal inflammation and disease incidence [4]. IL-10-producing Bregs have now been linked with downregulating the inflammatory cascade associated with IL-1 and signal transducer and activator of transcription 3 (STAT3) without tweaking T cell responses. Wei et al. demonstrated that adoptive transfer of B cells from mesenteric lymph nodes could repress IBD by enhancing the Tregs population [107, 108]. A numerical (number/percentage of Bregs) defect in IL-10-producing Bregs has also been described in patients with both CD and UC [109].

### 5.3.3 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is designated as a systemic multigene autoimmune disorder characterized by higher production of autoantibodies with simultaneous deposition of immune complexes, resulting in tissue inflammation and damage to the skin, kidneys, and joints. This phenomenon results in proteinuria and large-scale renal tubule inflammation (glomerulonephritis), which eventually affects the immune system [110, 111]. Both B- and T-cell abnormalities have been found to be responsible for the occurrence of SLE in mammals [112]. SLE-affected individuals usually show a reduced number as well as decreased functional activity of circulating Bregs. This defect usually arises as immature B cells ($CD19^+CD24^{hi}CD38^{hi}$) fail to differentiate into Bregs [20, 68, 113]. Various mouse models have been identified to study the role of regulatory B cells in spontaneous lupus. Recently two well-defined models, New Zealand Black (NZB) × New Zealand White (NZW) F1 hybrid (NZB/W) mice and MRL/lpr mice, have been used to investigate the inhibitory role of Bregs in regulating the severity of SLE [59, 112]. Depletion of Bregs in infant mice resulted in higher severity of SLE, whereas deletion of Bregs from adult mice did not affect SLE progression. Thus, Bregs have been found as predominantly effective during the initiation phase of SLE rather than during disease progression [59, 112]. Additionally, the higher therapeutic interventions of Bregs have come

into play due to their role in enhancing the number of Tregs after the transfer of splenic $CD1d^{hi}$ $CD5^{+}$ B cells from wild-type NZB/W F1 mice to $CD19^{-/-}$ NZB/W F1 [95]. Blair et al. further observed that anti-CD40-induced T2 Breg cells significantly improved the survival rate in MPL/lpr mice via higher expression of IL-10. Collectively, these findings indicate that T2-MZP B cells as well as B10 cells effectively help in protecting mice from severe SLE [21].

### 5.3.4 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease with a worldwide prevalence of 0.3–1%. It is responsible for increased societal dependency with simultaneous reduction of mobility and working ability [114]. RA is characterized by autoimmune inflammatory responses at synovial membranes and joint capsules, resulting in significant morbidity and mortality due to synovial proliferation, cartilaginous injury, and bone erosion [115]. B cells produce various factors including autoantibodies like anti-citrullinated protein antibodies (ACPAs) and rheumatoid factor (RF) that are responsible for severe disease activity in RA [116]. Moreover, reduced numbers of Bregs such as IL-10-producing Bregs, $CD19^{+}TIM\text{-}1^{+}IL\text{-}10^{+}$ Bregs, $CD19^{+}CD5^{+}CD1d^{hi}$ B cells, and $CD19^{+}CD5^{+}CD1d^{+}IL\text{-}10^{+}$ Bregs were observed in RA patients compared to those in healthy controls upon stimulation with CpG or LPS along with phorbol myristate acetate and ionomycin [117, 118]. Further, the function of Bregs was found to be impaired in RA. One study demonstrated that $CD24^{hi}CD38^{hi}$ Breg cells from healthy individuals inhibited Th1 and Th17 differentiation and favored the conversion of $CD4^{+}CD25^{-}$ T cells to Tregs via IL-10 expression. In contrast, $CD19^{+}CD24^{hi}CD38^{hi}$ cells from RA patients were unable to reduce Th17 development and induce Tregs differentiation [70]. In 2017, Banko et al. showed that $CD19^{+}CD27^{+}IL\text{-}10^{+}$ Bregs are significantly reduced in RA patients compared to those in the controls and that the existing Bregs showed a reduced ability to suppress IFN-γ production by T helper cells. Breg-deficient mice demonstrate higher incidences of autoimmune arthritic conditions due to enhanced induction of Th1 and Th17 cells along with simultaneous suppression of Treg cells [113]. Bregs have thus been found instrumental in suppressing inflammation via restoring or modulating the Th1/Th2 balance in various T-cell-mediated autoimmune diseases such as EAE and RA [113].

### 5.3.5 Type 1 Diabetes

Type 1 diabetes (T1D) is an autoimmune disease caused by the obliteration of insulin-producing pancreatic β cells mediated by $CD4^{+}$ and $CD8^{+}$ T cells [119]. Onset of T1D usually occurs around 13–15 weeks of age in non-obese diabetic (NOD) mice, a model of human T1D. The prevalence of T1D in NOD mice is higher in females with about 80% females and 20% males affected by this disease

by 30 weeks [120]. B cells are particularly found to be responsible for the development of pathogenesis of T1D. B cell penetration into the pancreatic islets of NOD mice results in selective propagation of T cells within lymphoid structures, leading to an increased number of autoreactive B cells [121]. Treatment of 5-week-old NOD female mice with anti-CD20 mAbs was found to deplete 95% of B cells, thereby arresting insulitis; however, at 15 weeks, the same treatment was inefficient to hinder the progression of T1D [8, 122]. Grey et al. found that the increased population of $CD4^+CD25^+Foxp3^+$ Treg cells due to B cell depletion reduced the occurrence of diabetes [123]. Smith and Tedder further postulated that B-cell-depleted NOD mice remained free from diabetes even after reconstitution with B cells [124]. Among various types of B cells, IL-10-expressing B cells have been primarily found to be responsible for decreasing the pathogenicity of insulitis and reducing T1D incidence. Simultaneously, various Th1 immune-related responses were curbed, leading to the diversion of $CD4^+$ T cells toward the Th2 phenotype upon introduction of activated B cells in pre-diabetic NOD mice [125]. Tian and colleagues further established that LPS-activated B cells mediate apoptosis of diabetogenic Th1 cells in NOD mice via expression of FasL and secretion of TGF-β [24]. These findings provide new insights into treating human T1DM via targeting the T cell-B cell interaction. Reduced numbers of IL-10-producing Bregs have been reported in patients with T1D [126]. There is substantial evidence that Bregs are either insufficient in number and/or functionally compromised in autoimmune diseases. Thus, further studies are needed to understand their mechanisms of action in these diseases.

### 5.3.6 Infectious Diseases

The role of B cells in infectious diseases has been studied extensively. In contrast, the role of Bregs in intracellular infections is unclear. Studies on Bregs in infections will uncover the valuable targets/potent markers in developing therapeutic interventions to treat various infectious diseases. Recent studies have shown that successful treatment of *Mycobacterium tuberculosis* infection induces Bregs with the ability to express FasL and IL-5RA in TB patients. Thus, these molecules could be potentially utilized as indicators of monitoring treatment responses during infections [127, 128]. Various studies have demonstrated the suppressive role of Bregs in chronic hepatitis B virus infection. Das et al. [129] first demonstrated that Bregs are responsible for regulating antigen-specific $CD8^+$ T cells in hepatitis B virus infection. They also found that inhibition of IL-10 may reestablish HBV-specific $CD8^+$ T cells in vitro. Various studies have reported that in HIV infection, Bregs impaired T cells via expression of IL-10 and programmed death (PD)-L1, contributing to immune dysfunction [130]. In 2014, Jiao et al. found that the frequency of Bregs in HIV patients was negatively correlated with the $CD4^+$ T cell count but was positively correlated with the viral load. Supporting this, it is also observed that following anti-retroviral treatment, the frequency of Bregs was decreased along with a concomitant step-wise increase in the $CD4^+$ T cell count.

### 5.3.7 Allergy and Asthma

Bregs also exert protection against allergic airway inflammation [131]. Through antigen- specific/non-specific immunomodulatory mechanisms, it is apparent that Bregs demonstrate allergen tolerance and contribute to suppress allergic diseases. Allergic inflammation is reported to be suppressed by IL-10-producing Bregs and involves a delicate balance between IL-10 induced parasite responses and detrimental IL-4-mediated allergic responses [132]. Br1 and Br3 cells increase in response to casein in milk-tolerant individuals [133] but not in milk-allergic individuals. Thus, both Br1 and Br3 cell types are critical for immune tolerance in non-IgE-mediated food allergies related to atopic dermatitis. Patients with allergic asthma and allergic rhinitis have a decreased number of IL-10-producing $CD24^{hi}CD27^{+}$ Bregs [134]. In a similar manner, beekeepers also develop tolerance against bee venom allergen, i.e., Phospholipase Az (PLAz)–specific to BR1 cells producing IgG4 antibodies by suppressing T cell responses in an IL-10-dependent manner [71, 135]. In allergic asthma, treatment with oral corticosteroids (OCS) significantly affects the frequency of Bregs as well as their ability to express IL-10 in a Breg subset–specific manner [136].

### 5.3.8 Osteoporosis

Osteoporosis represents one of the most common bone loss conditions, leading to higher fragility and bone fractures often related to advanced age and post-menopausal conditions [137, 138]. Osteoporosis is often a neglected disease with more than 200 million affected individuals worldwide, thus also referred as a "silent killer" [139, 140]. In the bone marrow, B cells are a major source of the osteoclastogenesis inhibitor osteoprotegerin (OPG), in the presence of activated T cells signaled by CD40L-CD40 interaction on B cells. Moreover, a CD40L-CD40-deficient mice showed reduced bone mass compared to the control mice. B cells also express RANKL along with OPG, which in the long run affects bone physiology. Furthermore, mice with B cell deficiency show suppressed OPG production and high prevalence of osteoporosis [141]. Bregs suppress various proinflammatory cytokines such as IL-1 and TNF-α, which are osteoclastogenic in nature, therefore leading to enhanced bone loss. The ratio of Th1/Th2 is an important parameter defining bone strength [142], including the rate of bone resorption and the resulting bone loss. Moreover, several subtypes of Bregs have now been reported with the suppression of Th1-, Th2-, or Th17-mediated autoimmune responses with a subsequent increase in $Foxp3^{+}$ Treg cells along with conversion of effector T cells into Tr1 cells ($CD4^{+}$ $Foxp3^{+}IL\text{-}10^{+}$ Treg 1 cells). Bregs have also been observed to suppress the expression of Th17 cells [59, 109], which are responsible for enhanced osteoclastogenesis and bone loss [142]. Recent observations (unpublished) from our lab clearly demonstrate the role of $CD19^{hi}CD1d^{hi}CD5^{hi}IL\text{-}10^{hi}$ Bregs in modulating bone health. Thus, further research is needed to establish the precise role of Bregs in regulating bone health.

## 5.4 Therapeutic Potential of Bregs: From Bench to Bedside

The present global scenario arising from various studies using experimental models and human disorders validate the vital role of Bregs in several diseases. Together, these studies indicate that Bregs have the potential to modulate a number of immune pathologies. Tedder et al. demonstrated that Bregs are involved in autoimmune responses and also provide protection to host tissues during the immunopathogenesis of infectious diseases [143]. More importantly, understanding the basic principle underlying the induction of Bregs will help in tweaking cellular tolerance and amend the influence of disease. As a small number of Bregs are inefficient in inhibiting inflammation, mechanisms that can enhance both the number and effector functions of Bregs can result in enhanced immune-suppressive functions. In the context of immunological conditions such as autoimmunity and transplantation, long-term usage of immunosuppressive drugs increases the likelihood of life-threatening infections. In certain conditions such as during graft transplantation, autoimmune diseases, and so on, expansion of the immunosuppressive Bregs population is needed. Thus, strategies that can be exploited by therapeutically targeting Bregs can open new avenues in treating various immune-mediated diseases such as the following: (a) ex vivo expansion of Bregs: stimulation of B cells in patient-derived PBMCs, leading to expansion of Bregs, followed by adoptive transfer of Bregs sorted by FACS may suppress the inflammation and re-induce tolerance. (b) in vivo modulation of Bregs for expansion: stimuli that can shift the differentiation of B cells toward immunosuppressive regulatory B cells. Some evidence suggest that pro-inflammatory cytokines such as B cell–activating factor (BAFF), IL-1β, IL-6, IL-21, IFN-α, and IFN- γ [23, 68] are the key cytokines that expand the Bregs population upon exposure. Interestingly, in arthritic mice, the gut microbiota has the potential to induce the expression of IL-1β and IL-6, which further promote Bregs differentiation and production of IL-10 cytokine [23]. (c) Depletion of Bregs: B cell depletion therapies (viz. rituximab), usage of targeted B cell therapies, that can target a specific subtype of B cells is more advantageous than total B cell depletion. Thus, further in-depth studies are required to develop Breg-dependent immunotherapies and to enhance their applications in treating various immune disorders and pathologies.

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