Immunoregulatory potential of T2-MZP B cells

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B cells with the capacity to inhibit inflammatory immune responses have now been described in a number of experimental models of autoimmunity, infection and cancer. Just as the identification of regulatory T cells offers a possible target for the control of aberrant immune responses, a well-defined regulatory B-cell subset may have the potential to be manipulated *in vivo* for the treatment of conditions such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and inflammatory bowel disease. In this review, we discuss the latest findings showing that immature transitional-2 (T2-MZP) B cells can produce IL-10 and are able to prevent or ameliorate collagen-induced arthritis. These results ascribe T2-MZP B cells with a previously unrecognized regulatory capacity.

In 1996, Charles Janeway demonstrated that, unlike wild-type controls, B-cell-deficient (μMT) mice, following immunization with the NH-terminal myelin basic protein encephalitogenic peptide Ac1–11, failed to recover from experimental autoimmune encephalomyelitis (EAE) [1]. This finding suggested that in a normal B-cell-sufficient mouse, B cells, or a particular subset of B cells, could play an active role in controlling aberrant immune responses. Janeway *et al*. further hypothesized that these B cells, by virtue of their ability to present antigens, might be necessary for maintaining Th2 responses in diseases that are predominantly mediated by the production of Th1 cytokines. It was not until the early 2000s that more direct immunoregulatory mechanisms for this protection, including B-cell production of IL-10 and the induction of $CD4^+CD25^+$ regulatory T cells (Tregs), were revealed [2,3].

In the past 10 years, B cells with a regulatory activity have been identified in a number of other immunological disorders. In models of autoimmunity, B cells have been shown to be protective against disease progression in both Th1 ($G\alpha i2^{-/-}$) and Th2 ($TCR\alpha^{-/-}$) models of intestinal inflammation, and in the collagen-induced arthritis (CIA) model of rheumatoid arthritis (RA). They can also prevent non-obese diabetic mice from developing diabetes [4–10]. In studies of parasitic infections, B cells have been implicated in the inhibition of $CD4^+$ T-cell-mediated granuloma formation following *Schistosoma mansoni* worm plus egg infection, and in the resistance of mice to systemic anaphylaxis following *S. mansoni* worm infection [11,12]. Studies of B cells in cancer have shown enhanced antitumor immunity and natural killer T-cell and $CD8^+$ T-cell activity (both *in vivo* and *in vitro*) following B-cell depletion [13,14]. Finally, IL-10-producing host B cells are thought to be important in the protection of transplant recipients from graft versus host disease [15]. Recent reviews by Mauri [16] and Mizoguchi cover these grounds extensively [17].

Until recently there was no general consensus describing a unifying phenotype, mechanism of protection or physiological location that would suggest that immunoregulation observed in the above models could be ascribed to a single B-cell subset. However, we have now described a subset of immature transitional B cells, transitional-2 marginal-zone precursors (T2-MZPs), that possesses regulatory capacity in the CIA model of RA [18]. This subset of B cells reconciles several of the features described in the different experimental models, and may be the likely candidate for regulatory B cells (Bregs).

Adoptive transfer of T2-MZP Bregs prevents or ameliorates arthritis

The term 'transitional B cell' was coined by Carsetti and colleagues to describe immature B cells that are phenotypically and functionally in transition between the early immature B cells in the bone marrow and mature B cells in the periphery [19]. Transitional B cells have been further subdivided into subsets that reflect discrete maturation stages in the periphery [20–24]. The exact number and phenotype of these subsets is still controversial, but there is a growing consensus dividing transitional B cells into transitional-1 (T1) B cells (whose precursors are the earliest IgM-positive immature B cells emigrating from the bone marrow), T2 B cells (derived from T1 B cells), and a further intermediate subset between T2 B cells and mature MZ B cells,

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T2-MZP B cells [21]. In this model, proposed originally by Allman *et al*., mature follicular (FO) B cells derive mainly from T2 B cells, while MZ B cells arise mainly from T2-MZP B cells [25,26]. In the mouse, these B-cell subsets can be distinguished by their expression of some, or all, of the markers IgM, IgD, CD1d, CD21, CD23, CD24 and CD93, with T2-MZP identified as $CD19^{+}IgM^{hi}IgD^{hi}CD1d^{hi}CD21^{hi}CD23^{hi}CD93^{+}$ B cells [25,27–29].

We originally reported that adoptive transfer of anti-CD40-treated splenic B cells to syngeneic DBA mice could protect recipient mice from developing arthritis in the CIA model of RA [8]. This protection appeared to be mediated by the release of the immunoregulatory cytokine IL-10 by the transferred B cells. A follow-up study of these 'IL-10-producing Bregs' revealed that they were phenotypically indistinguishable from the previously identified T2-MZP B cells [18]. Indeed, most $IL\text{-}10^{+}$ B cells isolated from naive, as well as from immunized, mice expressed identical levels of IgM, IgD, CD1d, CD21, CD23, CD24 and CD93 to those expressed by 'conventional' T2-MZP B cells [18]. Moreover, these IL-10-producing Bregs, isolated from naive mice, or mice in the remission phase of arthritis, expressed Hes1 and Deltex1, the immediate downstream targets of the Notch-2 signaling activity that is required for T2-MZP and MZ development and survival, supporting their classification as T2-MZP B cells [30,31].

Adoptive transfer of T2-MZP B cells isolated from the spleens of DBA/1 mice in the remission phase of arthritis significantly reduced the incidence and the severity of disease in syngeneic recipient mice. By contrast, mice that received FO or MZ B cells or PBS developed a severe arthritis. T2-MZP B cells also had a therapeutic effect if transferred at the time of disease onset. From an immunological perspective, the protection observed following transfer of T2-MZP B cells was accompanied by the inhibition of $CD4^{+}$ T-cell proliferation and dampening of inflammatory Th1 responses [18].

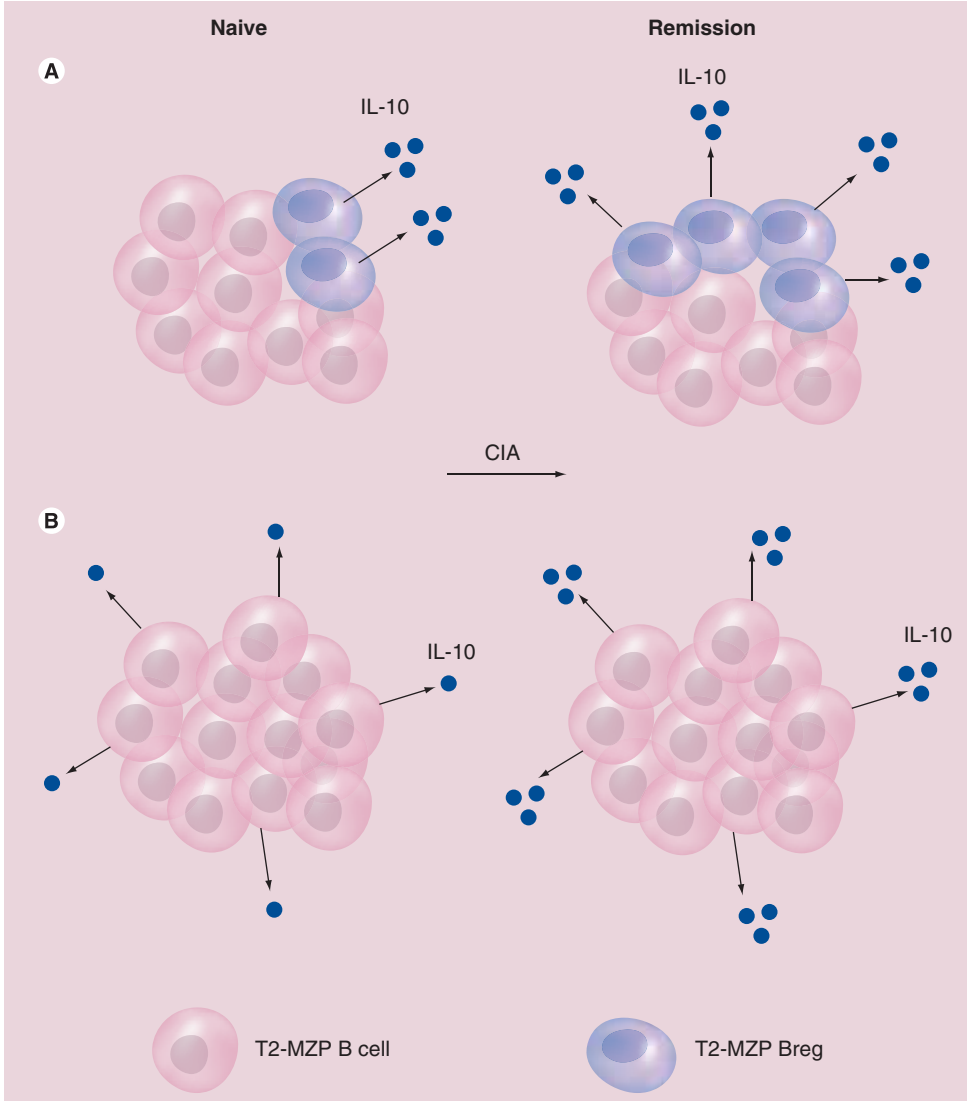
Protection from arthritis was also seen when the T2-MZP B cells were isolated from naive mice. To achieve the same degree of protection as observed following transfer of 'remission' T2-MZP B cells, twice as many naive T2-MZP B cells had to be transferred. Interestingly, mice in remission from CIA have increased numbers of IL-10-producing T2-MZP B cells compared with mice before or during acute arthritis [18]. It is not yet clear whether each T2-MZP B cell has the potential to be regulatory in addition to its role as an MZ B-cell precursor, or whether there is a functionally distinct, but phenotypically identical (in terms of the above markers), Breg subset [18]. Therefore, we could argue that undergoing arthritis, and the resolution of inflammation that follows, might either enrich the T2-MZP pool for T2-MZP Bregs, or that antigenic stimulation is required to enhance the suppressive activity of all T2-MZP B cells (i.e., by increasing the production of IL-10 per cell) (Figure 1).

Another important issue concerns the fate of the transferred T2-MZP Bregs during the course of arthritis. While it is relatively well established that T2-MZP B cells transferred to immune-deficient mice differentiate into mature MZ B cells, there are virtually no data relating to the fate of transferred T2-MZP B cells and, more importantly, those with regulatory function, in mice developing autoimmune disease [26]. It has previously been shown that MZ B cells produce high levels of IL-10; therefore, it would be feasible to hypothesize that transferred T2-MZP B cells protect only after differentiating into mature MZ B cells [9]. However, in the CIA model, transfer of MZ B cells not only failed to protect mice from developing arthritis, but appeared to exacerbate the disease.

Could Bregs from other models also be T2-MZP B cells?

In the autoimmune models described above, Bregs were isolated from the spleen (EAE, $G\alpha i2^{-/-}$ and $TCR\alpha^{-/-}$ colitis) and mesenteric lymph nodes ($TCR\alpha^{-/-}$ and $G\alpha i2^{-/-}$ colitis) [2,4,5]. However, T2-MZP B cells have so far been described exclusively in the spleen [20]. Therefore, those Bregs isolated from the mesenteric lymph nodes are unlikely to be conventional T2-MZP B cells. Nevertheless, there are similarities between T2-MZP B cells and Bregs in other autoimmune models. For example, like T2-MZP Bregs, Bregs in the $TCR\alpha^{-/-}$ colitis model express high levels of CD1d [5], while Bregs isolated in CIA and EAE share a requirement for CD40 [2,8]. Moreover, the spontaneous disease in the $G\alpha i2^{-/-}$ colitis model is associated with a decrease in the absolute numbers of T2-MZP B cells in this strain, suggesting a potential regulatory role for T2-MZP in this model, although this has not yet been formally proven [4].

Figure 1. Alternative views of the expansion of transitional-2 marginal-zone precursor immunoregulatory potential following collagen-induced arthritis resolution.



The resolution of the inflammatory process associated with CIA may either **(A)** induce the expansion of a population of specialized T2-MZP Bregs within the T2-MZP pool, or **(B)** stimulate the general T2-MZP pool to make more IL-10.
Bregs: Regulatory B cells; CIA: Collagen-induced arthritis; T2-MZP: Transitional-2 marginal zone precursor.

How do T2-MZP B cells protect mice from autoimmune disease?

IL-10 is a key regulator of inflammation and has been shown to inhibit both Th1- and Th2-type immune responses [32,33]. As mentioned above, T2-MZP B cells were identified as possible regulatory cells as a result of their ability to produce ILH10, and indeed their ability to protect recipient mice against development of arthritis is dependent on their production of IL-10. Transfer of T2-MZP B cells isolated from the spleens of IL-10-deficient mice is unable to protect recipient mice from arthritis. *In vitro* the inhibition of IFNγ production, by naive $CD4^+$ T cells, observed following coculture with remission T2-MZP B cells, is abrogated in the presence of anti-IL-10 receptor/anti-IL-10 monoclonal antibody [18]. IL-10 production is a common feature of Bregs in the CIA, EAE and $TCR\alpha^{-/-}$ chronic colitis experimental models and is a defining feature of Bregs in mice [2,5,8]. TGF-β may also have an important role, as LPS-treated B cells appear to protect

non-obese diabetic mice from diabetes via the production of TGF-β [10]. It remains to be established whether T2-MZP Bregs also work via the expression/production of TGF-β.

In addition to a requirement for IL-10, Tregs have been found to be important in mediating the protective effect of Bregs in different models of autoimmunity [3,4]. In the Gαi2$^{-/-}$ model of intestinal inflammation, B cells can only control disease if CD4$^+$CD8α$^+$ Tregs are present, and it has recently been shown that transfer of B cells to μMT mice mediates recovery from EAE by promoting FoxP3 expression in the CNS [3,4]. However, T2-MZP B cells appear to control autoimmunity independently of endogenous Tregs, as transfer of T2-MZP B cells protects recipient mice from arthritis even after CD25$^+$ cells have been depleted by administration of anti-CD25 monoclonal antibody [18].

Can we use T2-MZP Bregs in the therapy of autoimmune diseases?

It is not as yet clear why endogenous Bregs do not prevent mice from developing disease. This inability could be due to a lack of activation, insufficient numbers or a defect that renders them unable to control the initial onset of disease. In experimental arthritis, resolution of disease is accompanied by an increase in the size of the T2-MZP B-cell pool, suggesting that endogenous Bregs may contribute, at a later stage, to the suppression of inflammation in the CIA model. This finding holds hope that manipulation of Bregs *in vivo*, as well as *in vitro*, to activate them or increase their numbers, is a potential new therapeutic strategy for the treatment of autoimmune inflammation.

Conclusion

- Bregs have been described in several models of autoimmunity and inflammation;
- Transfer of T2-MZP B cells is able to prevent the development of arthritis in the DBA/1 CIA model of RA, and can ameliorate established arthritis;
- IL-10 production is necessary for the immunoregulatory function of T2-MZP B cells;
- The action of T2-MZP B cells does not require the presence of CD4$^+$CD25$^+$ regulatory T cells.

Future perspective

While a number of questions remain to be answered regarding T2-MZP Bregs in the mouse, such as their antigen specificity, where they exert their function and whether they can be specifically expanded *in vivo*, the major goal of Breg research is now to find an equivalent human population. The presence of T2 B cells has recently been tentatively hypothesized in human peripheral blood, giving an ideal starting point for research [34]. It is possible that the induction of Bregs or the identification of pathways leading to their expansion will become an alternative option to treating autoimmune conditions with B-cell depletion.

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Executive summary

- B cells have now been described that have an anti-inflammatory, regulatory function similar to that ascribed to regulatory T cells (Tregs).
- These regulatory B cells (Bregs) have been identified in mouse models of the autoimmune diseases multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease and diabetes.
- In these autoimmune mouse models, depletion of these Bregs exacerbates disease whilst adoptive transfer of these cells confers protection from disease.
- It has been proposed that Bregs in the collagen-induced arthritis model of rheumatoid arthritis have a phenotype identical to that of the immature B-cell subset transitional-2 marginal zone precursor (T2-MZP) B cells, although it is possible that these Bregs could be a developmentally distinct subset.
- Bregs inhibit inflammation by downregulating CD4$^+$ T-cell expression of inflammatory cytokines and via induction of Tregs.
- The regulatory capacity of T2-MZP Bregs is mediated by IL-10.
- Expansion or potentiation of the suppressive capacity of T2-MZP Bregs, *in vivo* or *ex vivo,* may provide an alternative method for the treatment of autoimmune diseases.

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