ARTIST
RUBY ZHENG

CRITICAL REVIEW

Understanding regulatory B cell development with single cell analyses

SAMANTHA VISVA[1] & DR. JOHN-PAUL OLIVERIA[2]

[1]Bachelor of Health Sciences (Honours) Class of 2020, McMaster University
Correspondence: visvas@mcmaster.ca
[2]Assistant Clinical Professor, Division of Respirology, Department of Medicine, McMaster University
Correspondence: oliveria@stanford.edu

ABSTRACT

There is very little research concerning human regulatory B cells and this may in part be due to their inconsistent responses to immunosuppressive cytokines such as IL-10. The purpose of this critical review is to examine our current understanding of regulatory B cell development, such as time points of differentiation, and how *in silico* computer modelling can improve this understanding. Specifically, bioinformatic analysis of the changes in cell surface markers and signalling molecules can help guide our understanding of the timing of cell-fate decisions and regulatory B cell differentiation. Tracking regulatory B cell trajectory with bioinformatics and *in silico* methods may improve our understanding of their role in many neurodegenerative diseases such as multiple sclerosis.

INTRODUCTION

While B cells are most commonly known for their contributions to humoural immunity, a subset of B cells called regulatory B cells (Bregs) are recognized for their role in preventing autoimmunity.[1] While central tolerance eliminates strongly self-reactive lymphocytes, some lymphocytes may bypass these mechanisms, necessitating additional safeguards.[2] Bregs impart some peripheral tolerance functions by releasing immunomodulatory cytokines, such as IL-10, to suppress autoreactive immune responses.[3] IL-10 carries out immune suppression by inhibiting proinflammatory cytokines, expression of co-stimulatory molecules, antigen presentation, and by affecting B cell development and function.[1] Signals stimulating the production of IL-10 are poorly understood, but possible candidates include TLR ligands, dendritic cell-derived IFN-β and CD40L, cell surface markers, and the STAT3 signalling pathway.[1-4] IL-10-independent mechanisms for immune suppression largely rely on the proliferation of regulatory T cells (Tregs) rather than the suppression of pathogenic T cells.[4] For example, some Breg cytokines modulate CD4+ T cell functions when activated and promote the conversion of CD4+ T cells into Foxp3+ Tregs through the release of TGF-β.[4]

Perhaps the heterogeneity of Breg functions can be attributed to differential signals received during Breg development.[1] However, it is unclear if these functions are specific to a Breg subset, whether signals presented during Breg development lead to specific functions, and how functions interact to induce immunosuppression.[1] The powerful immunosuppressive abilities of Bregs suggest therapeutic potential in immune-related diseases such as multiple sclerosis (MS).[2] This review will present our current understanding of Breg development and how *in silico* modelling methods and bioinformatics have the potential to bridge the aforementioned knowledge gaps. *In silico* methods such as CLUSTER algorithms are computer-generated mathematical datasets that attempt to mirror biological systems, whereas bioinformatics uses computer-aided strategies to analyze the empirical data from experimental techniques such as mass cytometry.

BREG DEVELOPMENT

Differential hypotheses about the origin and development of IL-10-producing Bregs exist. Current studies show that any B cell can acquire regulatory properties, supporting the notion that mature B cells may not be in their terminally differentiated state.[4] Proposed models of Breg differentiation include:[4]

1. *Multi-lineage Bregs*: individual progenitors give rise to different Breg subsets.
2. *Single-lineage Bregs*: a single progenitor gives rise to different Breg subsets, based on their expression of a single transcription factor.
3. *Induced Bregs*: with the appropriate microenvironment, any B cell has the potential to differentiate into a Breg cell.

The third model is supported by research showing that treatment with different cytokines, such as IL-10, induces B cell differentiation into different Breg subsets.[4,5] In an inflammatory response, IL-10 is upregulated by activation of BCRs; TLRs including TLR2, TLR4, and TLR9; and costimulatory molecules such as CD40 and CD86/CD80.[5-10] These findings demonstrate that increased levels of IL-10 can be induced by environmental stimuli.

To elaborate, different pathways are involved in BCR, CD40, and TLR stimulation. BCR signalling is required for B cell production of IL-10 by activating proximal kinases and phospholipase C-β2 to initiate store-operated Ca^{2+} entry (SOCE).[5,11] CD40 signalling activates STAT3, NF-βB, and extracellular-signal regulated kinases.[11] In a parallel pathway, TLRs are essential in inducing IL-10 production in naive B cells by altering the chromatin structure at the *IL-10* locus to facilitate transcription.[11] Histone deacetylase 11 and IRF4 are involved in regulating the transcription of the *IL-10* gene to stimulate downstream molecules such as NFAT proteins, which interact with other transcription factors including AP-1, IRF4, GATA-binding protein 3, c-Maf, and the "master transcription factor" in plasma cell differentiation, Blimp1 (Refer to Figure 1).[11] However, these parallel pathways are highly regulated to avoid generalized immunosuppression and non-selective IL-10 secretion.[5]

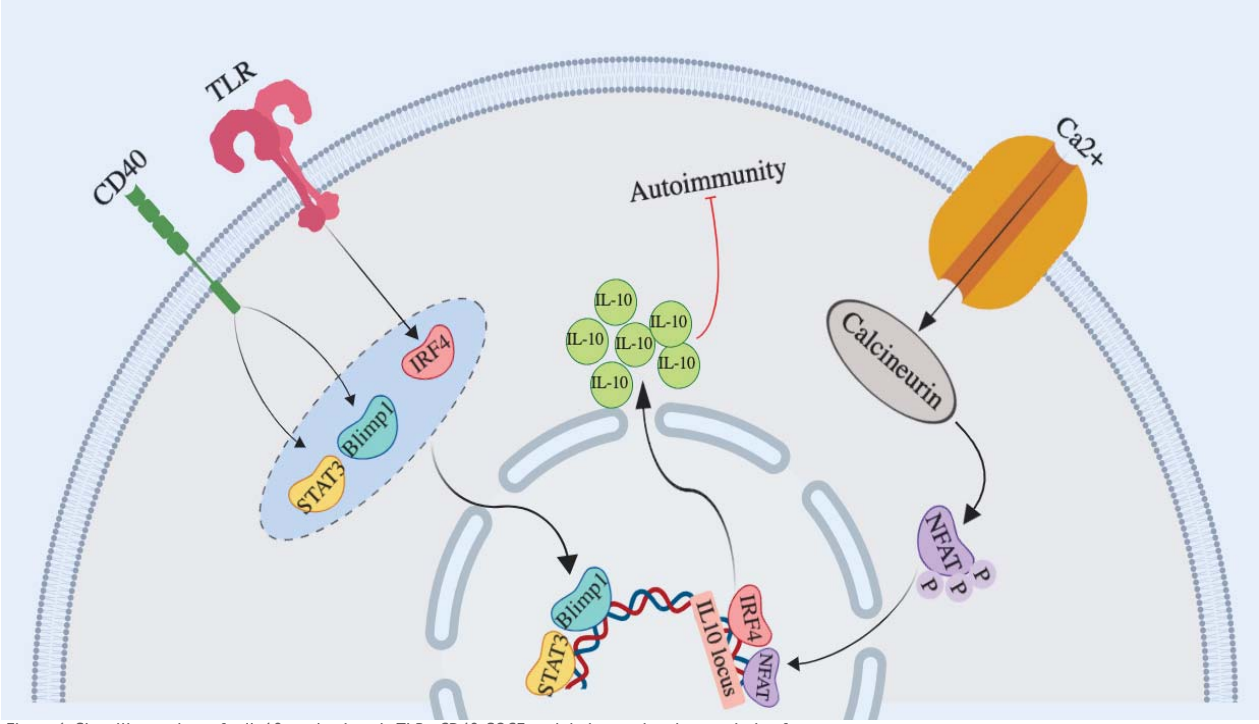

Figure 1: Signalling pathway for IL-10 production via TLRs, CD40, SOCE, and their associated transcription factors.

The third model suggests that although Bregs are phenotypically distinct, they do not express ubiquitous lineage-specific markers. Rather, they are characterized by the production of IL-10 to control powerful T cell-mediated responses. Certain cell types might also possess additional mechanisms of suppression.[3-5] There may be different pathways involved with the differentiation of B cells to Bregs. Bregs share many phenotypic markers with transitional, marginal zone, marginal zone precursor, memory, and B1 B cells. These findings suggest that the environment of developing B cells is critical in their differentiation into Bregs.[12] However, it is still unclear how these Bregs migrate to their respective tissue microenvironments, and how cells from these phenotypic subsets become Bregs, as these cells do not seem to constitute their own lymphocyte lineage.[12]

BIG DATA ANALYSIS

There is a paucity of knowledge concerning Breg development and differentiation.[13] While *in silico* analysis may help to bridge these knowledge gaps, there is little research that explicitly mentions Breg development.[13] Current literature suggests that human B lymphopoiesis mirrors that of murine models as it occurs through certain developmental stages, such as pro-B cells, pre-B cells, and immature B cells before leaving the bone marrow. Throughout development, cells become more restricted to their cell type, and using single-cell technology to analyze cellular features (e.g. phenotypic proteins, transcription factors, regulatory enzymes, cell-state indicators, and regulatory signaling molecules) helps to detect the underlying relationships that drive differentiation.[13] To further illustrate the importance of such features, this review will examine two primary articles that use *in silico* analysis and bioinformatics to understand how cell surface changes directly impact Breg development.

Bendall et al. used single-cell mass cytometry and a Wanderlust clustering algorithm to organize single cells onto a continuum to predict the B cell trajectory from hematopoietic stem cell to naïve B cells using their respective surface markers.[13] A single sample of bone marrow has demonstrated that cells asynchronously transition through these stages, suggesting that hematopoiesis is a continuous process and confirms our current understanding of B cell development from pro- to immature B cells.[13] This data reveals signalling changes corresponding to cell fate decisions, cell-cycle status (i.e. survival, proliferation, and apoptosis), and somatic recombination to paint a bigger picture of B lymphopoiesis.[13] The data also revealed previously unknown stages of pro-B cells characterized by CD34, CD38, CD24, and TdT expression.[13] Furthermore, the study discovered the precise timing of genomic rearrangement in sub-populations of pro-B cells, which is associated with IL-7-induced STAT5 signalling activation.[13] These developmental processes are not a discrete set of steps, but rather a continuous process marked by coordination points and trends.[13] However, without branch points, the model of Bendell et al. suggests that all cells follow the same transition from hematopoietic stem cell to immature B cell. Following the induced hypothesis of Breg development, where cells can be derived from any developmental stage given the right environment, this model is limited.[14]

Probability state modeling (PSM) is an approach that produces an analysis of antigen up- and downregulation

in chronological order from cytometry data.[15] Bagwell et al. used PSM in bone marrow samples to examine B cell stages before and after CD19 upregulation.[15] Prior to CD19 upregulation, there are at least two coordination points, marking three stages: P1, hematopoietic stem cell; P2, common lymphoid progenitor; and P3, pro-B cell.[15] Subsequent to CD19 upregulation, there are three coordination points, forming four stages: B1, pre-B cell; B2, early B cell; B3, immature B cell; and B4, transitional B cell.[15] Transitions between different stages are marked by the upregulation and downregulation of different cell markers. As many of these cell surface markers are also expressed on Bregs, a certain combination of these markers may reflect Breg progression. However, as CD10 and CD19 expression may reflect some stochasticity, there is some variability concerning the labelling of this progression.[15]

Given the lack of research on Breg development, bioinformatics has become a widely sought out solution. *In silico* and gene analysis can provide a greater understanding of the signals that drive Breg differentiation and activation. This analysis would be applicable for research on Bregs and can create healthy developmental trajectories to pinpoint irregularities in disease processes such as MS.[13] For instance, the Th1 plaque formation and demyelination pathogenesis of MS has been oversimplified in the past; now that recent findings have shifted the focus to B cells, computer models may inform improved therapeutic approaches that harness Bregs to ameliorate autoimmune diseases.

CONCLUSION

The maintenance of peripheral tolerance is essential, and Bregs perform regulatory functions to suppress immune responses by secreting IL-10 and TGF-β. No transcription factors have been specifically associated with Breg development; however, the Treg differentiation pathway may provide insight on the unique lineage of Bregs. There has been evidence that Breg subsets may share similar gene expression and cytokine profiles, and this relationship should be further studied. While there is limited research concerning Breg development, bioinformatics and *in silico* methods show immense promise in addressing knowledge gaps and may have even broader implications in understanding autoimmunity.

REVIEWED BY JOSHUA F.E. KOENIG

Joshua F.E. Koenig is currently a PhD Student in the Medical Sciences program at McMaster University. Joshua studies the pathways responsible for generating high-affinity IgE antibodies which mediate allergic reactions to food under the supervision of Dr. Manel Jordana.

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