CORRESPONDENCE

CD5 and B lymphocyte responses: multifaceted effects through multitudes of pathways and channels

Taher E. Taher¹, Jonas Bystrom², Olivier Mignen³, Jacques-Olivier Pers⁴, Yves Renaudineau^{4,5} and Rizgar A. Mageed²*Cellular & Molecular Immunology* (2020) 17:1201–1203; <https://doi.org/10.1038/s41423-020-0490-z>

CD5⁺ B lymphocytes, also known as B1 cells, play key roles in the immune system. This subset of B cells is a focus of renewed interest because it is an innate-like B cell population that is responsible for producing natural antibodies, rapid humoral immunity, and immune regulation and has a tendency to undergo leukemic transformation.^{1,2} It has been unclear, however, whether CD5 is a mere marker of B1 cells or whether it effectively modulates B cell responses and, if so, how. CD5 is a member of the scavenger receptor cysteine-rich (SRCR) superfamily and has a cytoplasmic tail with no enzymatic activity but with a motif that serves as docking sites for phosphorylated Src homology 2 (SH2) domain-containing kinases. In a recent study published in *Cellular and Molecular Immunology*, we reported that CD5 directly modulates B cell biology.³ These studies revealed that CD5 promotes the activation of Erk1/2 and multiple downstream signaling pathways. Activation of these pathways induces IL-10 production and modulates survival and metabolism. These effects were dependent on extracellular calcium (Ca²⁺) influx facilitated by upregulation of the transient receptor potential channel 1 (TRPC1) protein. Interestingly, TRPC1 is also typically upregulated in CD5⁺ B cell chronic lymphocytic leukemia (B-CLL). CD5-induced IL-10 production was independent of B cell receptor (BCR) engagement, as confirmed by targeted CD5 and TRPC1 suppression with siRNAs. Our findings facilitate a better understanding of the mechanisms of CD5⁺ B cell roles in health and diseases.

CD5 is characteristically expressed in T lymphocytes and is expressed at lower levels in B1 cells, a subset of B cells. B1 cells constitute a large subset in the human fetal spleen and umbilical cord blood but decline to ~10% in adult blood. CD5 is also abundantly expressed in B cells in most patients with B cell malignancies, including B-CLL, mantle cell lymphoma, and marginal zone B cell lymphoma.² Compelling and, at the time, paradoxical evidence also indicates that CD5⁺ B cells play important roles in providing rapid first line immunity (innate immunity) and a level of immune regulation.^{1,2} However, it has been difficult to differentiate between CD5 as a phenotypic marker and as an effective modulator of B cell biology. In a series of experiments, we confirmed that CD5 directly modulates B lymphocyte responses.³ Using cell transfection, we observed that CD5-mediated induction of mitogen-activated protein kinase (MAPK) Erk1/2 activation is key to inducing IL-10 production. This effect was Ca²⁺-dependent and involved the nonselective Ca²⁺ channel TRPC1. Using a comprehensive approach to analyze

kinase activation with kinome arrays and traditional Western blotting, we discovered that these effects of CD5 were distinct from its suppressive action on BCR-mediated signaling. Studies have also revealed that CD5 induces the activation of a range of important kinases, including Syk, Btk, and PLC γ 2, which are upstream effectors of canonical Erk1/2 activation cascades. CD5 also increased the phosphorylation of SHP1, as previously observed in B-CLL cells.⁴ Immunoprecipitation experiments confirmed that CD5 was associated with SHP1 and c-Cbl but not with the BCR complex (CD79a) when the BCR was not engaged. However, when the BCR was engaged, CD5 coprecipitated with CD79a.

CD5 induced at least three biological pathways that are key for cell survival, metabolism and cytokine, and autoantibody production (Fig. 1). These pathways are the MAPK (Ras/Erk) pathway, the Ca²⁺-calmodulin-calcineurin-NFAT pathway and the PI3-K/Akt/mTOR pathway. Activation of PI3-K/Akt/mTOR is related to the association between CD5 and the p85 unit of PI3-K. Single-cell video microscopy revealed that CD5 induced an increase in the basal level of intracellular Ca²⁺ that was dependent on TRPC1. Studies of signaling pathways revealed that changes in intracellular signaling prompted by CD5 were similar to those of anergic B cells and B-CLL cells.⁵

To verify the role of CD5 in modulating B cell responses, patients with B-CLL were segregated into two groups based on CD5 expression and high and low phosphorylation of Erk1/2 (phospho-Erk1/2^{high} and phospho-Erk1/2^{low}). Only CD5⁺ phospho-Erk1/2^{high} B-CLL cells expressed high levels of TRPC1 and produced IL-10. Furthermore, inhibiting CD5 or TRPC1 with siRNA suppressed IL-10 production in B-CLL cells. The data indicate that in phospho-Erk1/2^{high} B-CLL cells, CD5 promotes IL-10 production through a BCR-independent Ca²⁺-dependent pathway involving TRPC1. Furthermore, overexpression of TRPC1 and Ca²⁺ was associated with NFAT2 activation, which is a signaling cascade that leads to cytokine/chemokine production. Based on previous studies and observations made in our study, it appears possible that upregulation of TRPC1 in CD5⁺ B cells is involved in promoting B-CLL cell survival. However, the full molecular effects of CD5 on B cell transformation and biology remain to be further explored. This is in part due to the capacity of CD5 to prime, activate or inhibit multiple kinases and phosphatases and their associated biological pathways and gene expression.

After CD5 cells were first discovered, these cells were implicated in the pathogenesis of autoimmune rheumatic diseases based on

¹Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK; ²Experimental Medicine and Rheumatology, William Harvey Research Institute, Queen Mary University of London, London, UK; ³Canalopathy and Calcium Signaling, INSERM UMR1078, Brest, France; ⁴INSERM ERI29/EA2216, Réseau Epigénétique and Réseau Canaux Ioniques du Cancéropôle Grand Ouest, Brest, France and ⁵Laboratory of Immunology and Immunotherapy, CHU Morvan, Brest, France
Correspondence: Rizgar A. Mageed (r.a.mageed@qmul.ac.uk)

Received: 31 May 2020 Accepted: 8 June 2020

Published online: 1 July 2020

Fig. 1 Key signaling pathways modulated by CD5 expression in B cells. A schematic depicting key kinases and signaling pathways whose activities are modulated by CD5 expression in B cells. Only major targets that are influenced by CD5 expression are shown and are based on kinome array and Western blot analyses from our recent study⁴ and data from the literature. In addition to promoting IL-10 production and downregulating BCR-mediated signaling, CD5 induces activation of at least three signaling pathways that are key for cell survival, metabolism and cytokine, and autoantibody production, the MAPK (Ras/Erk) pathway, the Ca^{2+} -calmodulin-calcineurin-NFAT pathway and the PI3K/Akt/mTOR pathway

their frequency and autoreactive repertoire.² This proposition was subsequently questioned by the discovery that CD5 negatively regulates BCR-mediated signaling.⁶ Indeed, CD5 expression is associated by many with immunological energy and regulation of a subset of regulatory B cells (Bregs).² Thus, intracellular signaling in CD5⁺ cells has been shown to be similar to that of anergic B cells and exerts regulatory effects following BCR or CD40 engagement. In our recent study, we observed that IL-10 production by CD5 was independent of BCR or CD40 engagement. These findings suggest that CD5 can prime B cells to become Bregs. Indeed, the ability to prime B cells to become IL-10-producing B cells can be critical to their proposed role in immune regulation.

The findings that CD5 enhances the activation of the PI3-K/mTOR, S6K, and calmodulin-calcineurin-NFAT pathways have implications for understanding the metabolism, survival and IL-10 production of CD5⁺ B cells. In addition, to promote survival and proliferation, activation of PI3-K can suppress Ig class switching by activating Akt, which represses Forkhead Box (Foxo) transcription factors. This outcome is relevant to the observation that CD5⁺ B cells express IgM and do not generally switch their Ig isotype. Activation of the PI3-K/mTOR and S6K pathways indicates that CD5 can also modulate B cell metabolism. Thus, PI3-K and its downstream effector Akt activate mTOR, which regulates aerobic glycolysis.⁶ This activation enhances glucose uptake by upregulating the expression of glucose transporters and activating key glycolytic enzymes, such as hexokinase. mTORC1 can also promote glycolysis by upregulating hypoxia-inducible factor 1, which converts pyruvate to lactate.⁷ Glycolysis can further be enhanced by mTORC2-mediated activation of Akt. Unlike PI3-K pathway-mediated modulation of metabolic processes, increased Ca^{2+} ions regulate B cell metabolism differently. The increase in cytoplasmic Ca^{2+} activates stromal interaction protein 1 on the endoplasmic reticulum (ER), eliciting the release of the ER store to further increase extracellular Ca^{2+} influx.⁷ The consequent increase in intracellular Ca^{2+} enhances the expression of adenosine monophosphate activated protein kinase, which stimulates the glycolytic pathway.⁸ The increase in cytoplasmic Ca^{2+} promotes mitochondrial uptake of Ca^{2+} , which, in turn,

leads to activation of the tricarboxylic acid cycle to generate more ATP through oxidative phosphorylation.^{7,8}

The relationship between CD5 expression, Erk1/2 activation, and B cell transformation has often been highlighted. Analysis of kinases whose activities are upregulated or downregulated following CD5 expression indicate that the Erk1/2 pathway is activated through the Ras pathway. In CD5-negative cells, the Ras-Erk pathway is activated following BCR engagement or the binding of cytokines to their receptors. Activation of the Ras-Erk pathway by CD5 was independent of BCR engagement/cytokine binding and occurred through activation of Fyn, Src, MAPKs, and PKC. Activation of these kinases was also associated with the upregulation of TRPC1, which was also observed in CD5⁺ B-CLL cells. Overexpression of TRPC1 was also previously described in the transformed CD5⁺ chicken DT-40 cell line and was linked with the increase in intracellular Ca^{2+} and activation of NFAT2, which led to cytokine/chemokine production in B-CLL cells. In addition, activation of Akt by PI3-K could overlap with other mechanisms that underpin B-CLL pathogenesis. Thus, in the Eμ-Tcl1 transgenic mouse model of CLL, the oncogene Tc11 activates Akt, which appears to be involved in the leukemic transformation of B cells at 2 months of age.⁹

Finally, can the induction of IL-10 production by CD5 expression promote leukemic B cell transformation? The relationship between IL-10 production and B cell transformation is complex. Our studies showed that CD5 expression promotes IL-10 production independent of BCR/CD40 engagement and Btk activation. Other studies have highlighted the need to engage the BCR/CD40 and activate Btk. IL-10 has paradoxical effects on B cell responses and, arguably, transformation. Thus, IL-10 is suggested to be a negative regulator of BCR signaling in normal B1 cells, leading to reduced expansion of self-reactive B-1 cells.¹⁰ This IL-10-mediated regulatory effect is lost in malignant CD5⁺ B cells, since neutralizing IL-10 or blocking IL-10R in Eμ-Tcl1 cells did not affect the survival of these cells.⁹ IL-10, however, could play a role in promoting an immunosuppressive niche that allows B-CLL cells to proliferate.

The findings reported in our study may help in developing novel therapeutic strategies, particularly for CLL patients. Such strategies could involve targeting TRPC1 or CD5. Alternatively,

modulating kinases/phosphatases induced by CD5 expression could provide another strategy for treating patients with B-CLL and autoimmune diseases.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

- Hayakawa, K. et al. B cells generated by B-1 development can progress to chronic lymphocytic leukemia. *Ann. N.Y. Acad. Sci.* **1362**, 250–255 (2015).
- Wong, J. B. et al. B-1a cells acquire their unique characteristics by bypassing the pre-BCR selection stage. *Nat. Commun.* **10**, 4768 (2019).
- Garaud, S. et al. CD5 expression promotes IL-10 production through activation of the MAPK/Erk pathway and upregulation of TRPC1 channels in B lymphocytes. *Cell Mol. Immunol.* **15**, 158–170 (2018).
- Tibaldi, E. et al. Lyn-mediated SHP-1 recruitment to CD5 contributes to resistance to apoptosis of B-cell chronic lymphocytic leukemia cells. *Leukemia* **25**, 1768–1781 (2011).
- Muzio, M. et al. Constitutive activation of distinct BCR-signaling pathways in a subset of CLL patients: a molecular signature of anergy. *Blood* **112**, 188–195 (2008).
- Gary-Gouy, H., Harriague, J., Dalloul, A., Donnadieu, E. & Bismuth, G. CD5-negative regulation of B cell receptor signaling pathways originates from tyrosine residue Y429 outside an immunoreceptor tyrosine-based inhibitory motif. *J. Immunol.* **168**, 232–239 (2002).
- Feske, S., Skolnik, E. Y. & Prakriya, M. Ion channels and transporters in lymphocyte function and immunity. *Nat. Rev. Immunol.* **12**, 532–547 (2012).
- Ma, E. H., Poffenberger, M. C., Wong, A. H. & Jones, R. G. The role of AMPK in T cell metabolism and function. *Curr. Opin. Immunol.* **46**, 45–52 (2017).
- Pekarsky, Y., Drusco, A., Kumchala, P., Croce, C. M. & Zanesi, N. The long journey of TCL1 transgenic mice: lessons learned in the last 15 years. *Gene Expr.* **16**, 129–135 (2015).
- Alhakeem, S. S. et al. Role of B cell receptor signaling in IL-10 production by normal and malignant B-1 cells. *Ann. N.Y. Acad. Sci.* **1362**, 239–249 (2015).