RESEARCH HIGHLIGHT OPEN

B-cell immune checkpoint TIM-1: a potential target for tumour immunotherapy

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Recently, Lloyd Bod et al. published a study in *Nature* that identified the B-cell immune checkpoint T cell immunoglobulin and mucin domain 1 (TIM-1) and mechanistically elucidated that targeting TIM-1 enhances the responses to type I interferon (IFN-I), promotes B cells antigen presentation and activation, subsequently enhances anti-tumour responses of $CD4^+$ and $CD8^+$ T cells and inhibits tumour growth. This study provides important implications for anti-tumour therapy.[1]

Tumour immunotherapy, such as treatment with immune checkpoint blockade (ICB), has revolutionised the approach to dealing with tumours and has significantly improved long-term survival rates.[2] Currently, immune checkpoint inhibitors (ICIs) have made significant advancements in anti-tumour clinical applications, representing a breakthrough in the field of tumour therapy.[3] Some of the well-established ICIs include cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1). Additionally, there are several ICIs currently undergoing preclinical or clinical studies, such as cluster of differentiation 47 (CD47), T cell immunoglobulin domain and mucin domain 3 (TIM-3), and lymphocyte-activation gene 3 (LAG-3).[4] However, not all patients respond to immunotherapy, which highlights the need to identify specific mechanisms and biomarkers that can enhance its effectiveness and application.

While boosting T cells and NK cells function has primarily been the focus of tumour immunotherapy, the function of B cells, a crucial component of the immune system, in anti-tumour immunity remains a subject of debate.[1] TIM-1, expressed on a subset of peripheral B cells, binds to phosphatidylserine exposed on apoptotic cells to promote tissue tolerance.[1] However, its potential role in cancer has not been thoroughly investigated.

To investigate how B cell subsets regulate anti-tumour immune responses, B cells were globally depleted by anti-CD20 monoclonal antibodies, leading to an increase in B16F10 melanoma tumour growth. Moreover, it was observed that the expression profiles of tumour-infiltrating B cells were specific through RNA-sequencing (RNA-seq). These findings suggest that the presence of total B cells possesses tumour-suppressive properties and showed a unique phenotype upon infiltration in melanoma tumour.

Furthermore, to investigate total B cell heterogeneity, $CD45^+$ cells in tumour microenvironment (TME), draining lymph nodes (dLN), and non-draining lymph nodes (ndLN) were analysed during growth of B16F10 melanoma tumour. Then, the population of B cell that expanded in tumour, dLN and ndLN or over time were searched based on transcriptional states or B cell receptor (BCR) clones. The results revealed that the frequency of a specific cluster of B cells in dLN cells increased with tumour growth, which suggests that B16F10 melanoma tumour growth induces a unique subset of B cells. Furthermore, by combining COMET, flow cytometry analysis and bulk RNA-seq, the authors found an overexpression of the genes associated with the activation and proliferation of B cells in TIM-1^+ B cell subset from dLN. Additionally, B cells bearing TIM-1 from mice with B16F10 melanoma tumour exhibited higher expression levels of synergistic inhibitory and immunoregulatory factors that are commonly found on T cells. Then, to investigate how these factors impact the anti-tumour immunity, selected removal of TIM-1 was conducted. The results demonstrate that the loss of *Havcr1*, which encodes TIM-1, significantly inhibits tumour growth in various tumour models, highlighting the importance of TIM-1 expressing B cells in suppressing tumour growth.

Next, to validate how targeting TIM-1 regulates tumour growth, a high-affinity anti-TIM-1 antibody (3B3) was administered to B16F10 mice. It was observed that 3B3 did not exhibit therapeutic effect in μMT mice or *hCD20.TamCre* × *Havcr1*$^{fl/fl}$ (*Havcr1*^{BKO}) mice, which indicates that the presence of B cells is a prerequisite for the therapeutic effect of anti-TIM-1 antibody. Moreover, treating the spontaneous melanoma model, *Tyr-cre*^{ERT2}*Bra*$^{fCA/WT}$*Pten*$^{lox/lox}$ mice, with the anti-TIM-1 antibody resulted in a significant reduction of tumour growth. Further, combining PD-1 blockade with anti-TIM-1 antibody resulted in a more effective inhibition of tumour growth. Notably, treatment with the anti-TIM-1 antibody alone or with PD-1 blockade increased the fraction of activated $CD4^+$ and $CD8^+$ T cells. These findings indicated that targeting TIM-1 therapeutically reduces tumour growth and requires B cells expressing TIM-1.

Besides, to investigate the influence of TIM-1 expressing B cells on regulating tumour growth, the flow cytometry analysis to $CD45^+$ cells was performed in dLN, ndLN and TME after the inoculation of B16F10 cells in *Havcr1*^{BKO} mice. Additionally, the percentage of activated $CD4^+$and $CD8^+$ T cells within tumour-infiltrating leucocytes (TILs) from *Havcr1*^{BKO} mice were higher than those of control mice. Moreover, $CD45^+$ cells from dLN, ndLN and TME were profiled using single-cell RNA- and TCR-seq (scRNA/TCR-seq). It was confirmed that the clonal expansion and tumour infiltration of $CD8^+$ T cells were enhanced in *Havcr1*^{BKO} tumours. Interestingly, clonally amplified $CD8^+$ T cells from *Havcr1*^{BKO} tumours have effector/cytotoxic phenotype-associated genes expression. Meanwhile, staining with H-$2K^b$-$OVA_{257–264}$ dextramer and Ki-67 expression of TILs from mice with B16-OVA demonstrated a higher proliferation of OVA-specific $CD8^+$ T cells in *Havcr1*^{BKO} tumour than that from control group.

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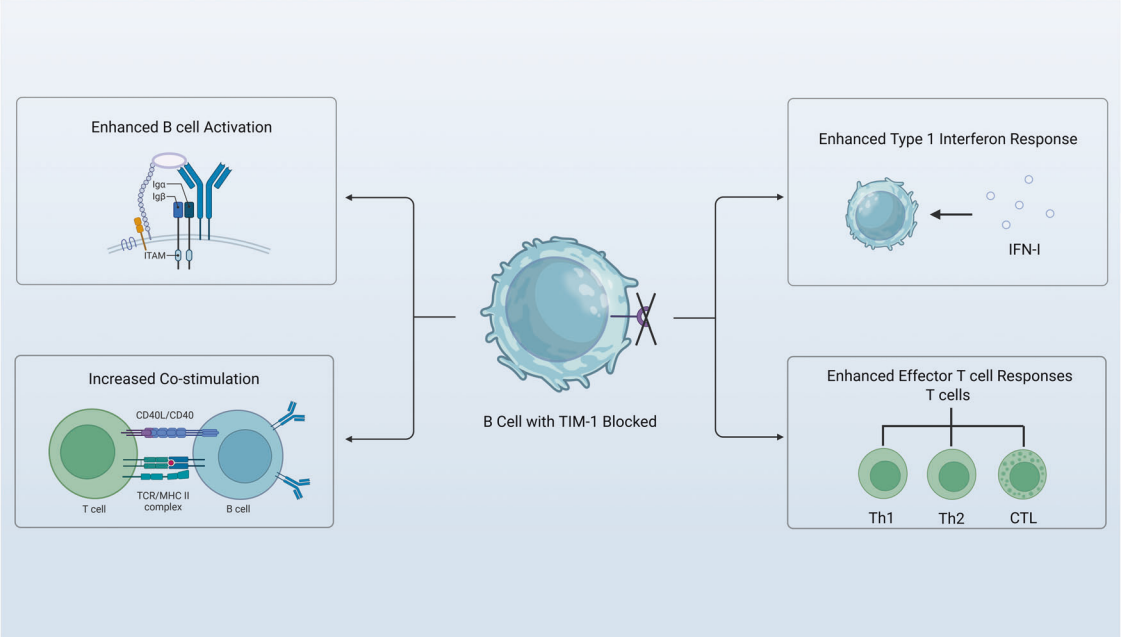

Fig. 1 TIM-1 blockade in B cells enhances the sensing of IFN by B cells. This leads to an augmented IFN-I response, increased B cell activation, enhanced antigen presentation, and improved co-stimulation. Consequently, there is an increase in inflammatory cytokine production and responsiveness, resulting in enhanced effector T cell responses. Created with BioRender.com

Additionally, to investigate the regulatory function of B cell loss in the anti-tumour responses mediated by T cells, *Havcr1* deletion was performed in B cells. The scRNA-seq analysis of *Havcr1*^{BKO} B cells from tumours and dLNs indicated a remarkable improvement for BCR sensing, activation, and immune co-stimulation gene signatures of B cells, along with increased expression of characteristics associated with IFN response. The results suggest that the deletion of *Havcr1* had minimal impact on humoral immunity. Furthermore, to investigate how the genetic deletion of *Havcr1* affected antigen presentation of B cells to CD4$^+$ T cells, the proliferating frequency of T cells was determined. As the results show, a higher level of T cell proliferation was found to be induced by *Havcr1*^{BKO} B cells in MHC II presentation-dependent manner.

Moreover, the impact of *Havcr1*^{BKO} B cells on T cells was assessed by analysing the expression levels of IFNγ, ICOS, and FOXP3. The analysis revealed that a larger percentage of IFNγ$^+$ cells and a substantial increased expression of ICOS were induced by *Havcr1*^{BKO} B cells promote the expression of IFNγ and ICOS in CD4$^+$ T cells, while inhibit the expression of FOXP3. In addition, naive CD4$^+$ T cells from CD45.1 OT-II donors were adoptively transferred into congenic CD45.2 *Havcr1*^{BKO}, in which tumour-derived CD45.1$^+$CD4$^+$ T cells showed increased IFNγ expression and reduced FOXP3 expression. These findings indicate that TIM-1 could suppress antigen presentation by B cells. Notably, ex vivo *Havcr1*^{BKO} B cells were found to exhibit an IFN-I gene signature, which is associated with increased responsiveness to IFN-β and enhanced IFNα/β receptor (IFNAR) expression. Additionally, blocking IFNAR1 reversed the tumour growth control in *Havcr1*^{BKO} mice. These two findings indicate that the expression of TIM-1 is regulated by both IFN-I and IFN-II, and loss of TIM-1 enhances the sensing of IFN-I and IFN-II by B cells.

In summary, this study suggests that TIM-1 functions as an immune checkpoint to B cell activation. Furthermore, deficiency of TIM-1 leads to an upregulation of B cells IFN-I response, resulting in enhanced activation, antigen presentation of B cells and immune co-stimulation (Fig. 1). Activation of the adaptive immune system by blocking TIM-1 can potentially improve the effectiveness of cancer immunotherapy and provides novel strategies for immune check point blockade.

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AUTHOR CONTRIBUTIONS

D.T. and X.Z. offered main direction and significant guidance of this manuscript. X.T. drafted the manuscript and made the figure for the manuscript. All authors have read and approved the article.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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