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No hiding place for BOB inside myeloma

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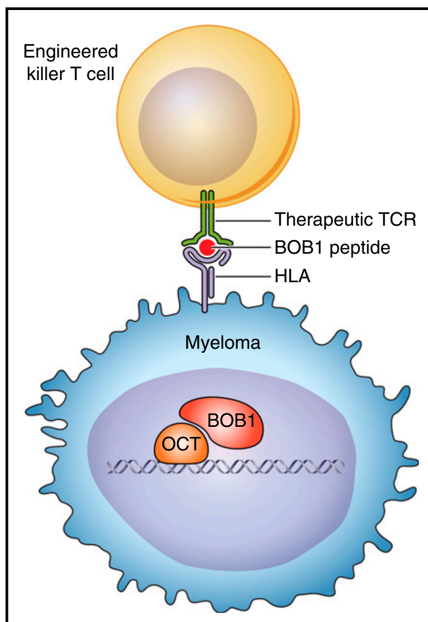
In this issue of *Blood*, Jahn and colleagues have demonstrated that T-cell receptor (TCR) gene transfer can produce cytotoxic T cells that are capable of eliminating myeloma cells in vivo.[1] The therapeutic TCR can equip human T cells with specificity for octamer binding protein-1 (BOB1), a transcription coactivator that is expressed in normal B cells, multiple myeloma, and B-lineage malignancies, thus providing novel options for immunotherapy of B-cell tumors that do not express CD19 or other surface antigens suitable for targeting with chimeric antigen receptor (CAR)–engineered T cells (see figure).

The clinical success of immunotherapy with gene-modified T cells targeting the CD19 molecule has caused excitement among patients, clinicians, and scientists, and has resulted in substantial investment from industry over the past years.[2] CD19 is a cell surface molecule that is expressed in a large proportion of normal and malignant B cells and is efficiently recognized by CARs. A major advantage of CAR-engineered T cells is the direct recognition of the CD19 molecule, which enables treatment of patients independent of their HLA genotype.



Cytotoxic T cells engineered with the BOB1-specific TCR can recognize and kill myeloma cells expressing the BOB1 transcriptional coactivator intracellularly. The TCR is specific for BOB1 peptides presented by HLA class I molecules. Professional illustration by Patrick Lane, ScEYEnce Studios.

A limitation of this form of immunotherapy is that a number of B-cell malignancies, such as multiple myeloma, do not express the CD19 target antigen. Jahn and colleagues have therefore explored whether TCR gene therapy can provide a novel treatment option for all B-cell tumors. Unlike CARs, which require cell surface expression of their target antigen, the HLA-restricted mechanism of antigen recognition enables TCRs to target any intracellular protein. Thus, TCR gene therapy provides access to a large number of intracellular targets that are not accessible for therapy with the CAR technology.

In an elegant piece of research, Jahn et al first screened the expression profiles of normal and malignant B cells to identify *POU2AF1* as a gene with a B-lineage–specific expression profile. The *POU2AF1* gene encodes BOB1 (also known as OCA-B or OBF), which acts in conjunction with the OCT1 and OCT2 transcription factors to activate gene expression.[3] In addition, BOB1 binding to the tyrosine kinase SYK in the cytosol of B cells has been implicated in intracellular B-cell receptor signaling.[4] Next, Jahn et al tested whether BOB1-derived peptides are naturally produced and presented to T cells by screening peptide libraries that were eluted from HLA class I molecules of B lymphoblastoid cell lines. They identified four BOB1 peptides, one naturally presented by HLA-A*0201 and three by HLA-B*07.02. All four peptides were used to generate HLA/peptide tetramer reagents to stain T cells from healthy donors and purify BOB1-specific T cells by single-cell sorting. The use of donors who were negative for HLA-A*0201 and HLA-B*07.02 provided a strategy to circumvent T-cell tolerance to BOB1 peptides presented by these HLA alleles.[5] One HLA-B*07.02–restricted T-cell clone displayed a high level of BOB1 specificity, killing primary tumor cells of patients with chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), mantle cell lymphoma, and multiple myeloma. This clone was used to isolate the genes encoding TCR α and β chains in order to generate retroviral vectors for TCR gene transfer to equip peripheral-blood T cells with BOB1 specificity. As expected, the TCR gene–engineered T cells displayed the same BOB1 specificity as the original T-cell clone, and experiments in a xenogenic mouse model demonstrated that engineered T cells were able to control the growth of multiple myeloma cells in vivo.

The gene expression analyses of primary tumor cells suggest that BOB1 is present in ALL, CLL, and multiple myeloma but absent in myeloid malignancies such as acute myeloid leukemia and chronic myeloid leukemia. This indicates that TCR targeting of BOB1 provides a therapeutic option for treatment of B-lineage malignancies. The gene expression profiles of normal tissues indicate that BOB1 is absent in nonhematopoietic cells, and within the hematopoietic lineage its expression is restricted to the normal B-cell compartment. The side-effect profile of BOB1-specific TCR gene therapy is therefore expected to result in the depletion of the normal B-cell compartment, similar to what has been observed in patients treated with CAR-engineered T cells targeting CD19. The density of the BOB1 and CD19 target antigens in normal and malignant B cells is expected to be vastly different. Whereas BOB1 peptides presented by HLA class I molecules may reach a density of 10 to 100 epitopes per cell, the CD19 expression is in the range of 10 000 to 100 000 per cell. It is possible that the high antigen density results in exaggerated T-cell activation, which may contribute to cytokine storm and the associated toxicity that is observed in patients after treatment with CD19-CAR–engineered T cells. It will be interesting to explore whether the lower antigen density of BOB1 combined with physiologic stimulation via the TCR is associated with less aggressive T-cell activation that is sufficient to achieve tumor protection while reducing the risk of cytokine storm.

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In summary, BOB1 has been validated as an attractive target for TCR gene therapy of multiple myeloma and other B-cell malignancies. The preclinical data provide a solid basis to test this TCR in clinical trials to determine its therapeutic efficacy and the side-effect profile.

Conflict-of-interest disclosure: H.J.S. is advisor and shareholder of Cell Medica and receives research funding from this biotech company.

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DOI 10.1182/blood-2017-01-761510

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IMMUNOBIOLOGY

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A rapid monocyte response team

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In this issue of *Blood*, Hamon et al reveal the existence of a marginated pool of inflammatory monocytes and demonstrate that migration of these cells to inflammatory sites is restricted by CX3C-chemokine receptor 1 (CX3CR1).[1]

It is well recognized that neutrophils can rapidly increase in number in the circulation in response to various inflammatory stimuli. In the setting of infection, this is likely to be an important mechanism for controlling pathogen invasion.[2] However, whether this applies to monocytes is less well understood. Seminal studies have demonstrated that circulating monocytes in mice and men are comprised of 2 main subsets: classical, also known as inflammatory, and nonclassical/patrolling. In addition to bearing different surface markers, these 2 populations are functionally distinct. Nonclassical monocytes migrate intravascularly, attached to the endothelial surface. From this location, they inspect the vascular microenvironment for signs of injury or infection and, if required, coordinate requisite inflammatory responses.[3-5] In contrast, classical monocytes circulate in the bloodstream, from where they can undergo rapid recruitment to sites of inflammation and subsequently promote responses via generation of proinflammatory mediators.[6,7] Monocyte numbers in the blood are controlled in an ongoing fashion by myelopoiesis and subsequent CCR2-dependent release from the bone marrow.[8] However, studies from over 30 years ago indicated that, in response to some stimuli, the number of monocytes in the circulation can almost double within 10 minutes.[9] The time course of this increase is clearly inconsistent with that of a response occurring solely via myelopoiesis. Were these additional monocytes derived from a previously unrecognized intravascular marginated pool?

Hamon et al addressed this issue using state-of-the-art imaging and flow cytometric approaches. Using 2-photon imaging, numerous monocytes were seen to patrol the lumen of the bone marrow vasculature. To characterize these cells more fully, Hamon et al used IV-administered antibodies to label intravascular cells and separate flow cytometric analysis of free cells in the circulation and those remaining attached in the tissue vasculature. Surprisingly, they found a large pool of inflammatory (CCR2$^+$, Ly6C^{high}) monocytes marginated in the bone marrow vasculature, in addition to nonclassical monocytes, identifying the bone marrow as a site of selective monocyte enrichment. Septic peritoneal inflammation resulted in a rapid reduction in the number of intravascular monocytes in the bone marrow. The number in the bloodstream also rapidly decreased in the first hour, but after 4 hours, it was significantly elevated above basal levels. In contrast, monocytes accumulated in the vasculature of the peritoneal lining, ultimately leading to the recruitment of macrophages into the peritoneal cavity (see figure). These results provided evidence of a redistribution of inflammatory monocytes from the bone marrow vasculature to the remote site of inflammation. Unexpectedly, in the absence of CX3CR1, both the monocytosis and the number of macrophages recruited to the peritoneal cavity were markedly elevated above levels in wild-type mice (see figure). Moreover, similar responses could be achieved via antagonism of this receptor using an inhibitory CX3CL1 analog. Interestingly, despite studies indicating that CX3CR1 expression is lower on inflammatory versus patrolling monocytes, inflammatory monocytes bound soluble CX3CL1 as effectively as patrolling monocytes. These findings indicate a previously unrecognized functional role for CX3CR1 on inflammatory monocytes in restricting their release into the bloodstream and migration into inflamed tissue. Furthermore, these findings show that this function can be blocked in real-time with a drug-like reagent.

Prior to this study, nonclassical monocytes were believed to be the major monocyte population patrolling the vascular endothelium. By extending the investigation of intravascular cells beyond those detectable in a straightforward blood sample, Hamon et al reveal that inflammatory monocytes represent a major proportion of the monocytes attached to the endothelium in the bone marrow microvasculature. Moreover, these cells can act as a reservoir of inflammatory monocytes available for rapid release into the circulation in response to local inflammation. However, release of this marginated pool of monocytes is held in check by the CX3CR1/CX3CL1 pathway. In an era where therapeutic uses of inhibiting this pathway are being explored, this study demonstrates that CX3CR1 antagonism can result in increased inflammation-associated monocytosis and increased delivery of macrophages to the target site. As such, in many inflammatory conditions, inhibition of this pathway may have unexpected inflammation-promoting effects associated with increased recruitment of inflammatory monocytes. However, in other settings, such as atherosclerosis, where the vasculature itself is the target of monocyte/macrophage-dependent inflammation, CX3CR1 inhibition reduces

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