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POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

TNFα-Driven Interplay between Hematopoietic Stem Cells and Antigen-Specific Cytotoxic T Cells

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Clonal hematopoiesis (CH) is governed by aberrant hematopoietic stem cell (HSC) clones with leukemia-associated somatic mutations that arise spontaneously during organismal aging or after exposure to genotoxic insults. Cells with a somatic mutation can be eliminated through presentation of neoantigens on MHC class I (MHC-I) to antigen-specific cytotoxic T cells. Indeed, many of the CH-related mutations, such as *JAK2V617F*, are reported or predicted to elicit antigen-specific cytotoxic T cell response. On the other hand, accumulating evidence suggests that inflammatory environment facilitates clonal expansion of abnormal HSCs. However, much remains to be elucidated on whether HSCs expressing such non-self peptides can be eliminated by antigen-specific cytotoxic T cells and how inflammation affects the T cell-mediated HSC regulation.
Our RNA-seq analyses revealed that genes involved in MHC-I-dependent antigen presentations, such as *Nlrc5*, *H2-kb* and *B2m*, are most abundantly expressed in HSCs (Lin $^{-}$/c-Kit $^{+}$/Sca-1 $^{+}$/Flk2 $^{-}$/CD48 $^{-}$/CD150 $^{+}$) among murine bone marrow immature hematopoietic cells. Using transgenic mice that ubiquitously express a model antigen ovalbumin (OVA) and a monoclonal antibody that specifically recognizes MHC-I-OVA complex, we found that while MHC-I protein is abundantly expressed on the surface of hematopoietic cells except granulocytes, HSCs and multipotent progenitor cells (MPPs) possess greater capacity to present intracellular antigens through MHC-I compared to myeloid progenitor and mature cells. Remarkably, in vitro co-culture experiments revealed that whereas OVA-expressing (OVA $^{+}$) HSCs, MPPs and myeloid progenitors could directly activate OVA-specific OT-I CD8 $^{+}$ T cells through MHC-I-dependent antigen presentation, HSCs are the most susceptible population to the killing effect by cytotoxic T cells. Indeed, co-infused OT-I CD8 $^{+}$ T cells totally abolished the long-term repopulation capacity of OVA $^{+}$ HSCs in a serial transplantation setting, and adoptive transfer of OT-I CD8 $^{+}$ T cells to mixed chimeric mice that harbor both wild-type and OVA $^{+}$ hematopoietic cells completely and specifically eliminated hematopoiesis governed by OVA $^{+}$ HSCs.
TNFα is not only a prototypical inflammatory cytokine but also one of the major cytokines that are secreted by CD8 $^{+}$ cytotoxic T cells. Notably, genes directly involved in T cell regulation including *Cxcl9*, *Cd274*, and *Pdcd1lg2* were induced in HSCs, but not myeloid progenitor cells, upon exposure to TNFα (Yamashita and Passegué, Cell Stem Cell 2019). Indeed, upon co-culturing OT-I CD8 $^{+}$ T cells produced TNFα as well as IFNγ and Granzyme B and OVA $^{+}$ HSCs but not myeloid progenitor cells produced CXCL9 largely in a TNFα receptor-dependent manner. Interestingly, such HSC response to T cell-derived TNFα was critical for naïve CD8 $^{+}$ T cells to rapidly differentiate to effector cytotoxic T cells, as TNFα receptor-deficient (*Tnfr1* $^{-/-}$*Tnfr2* $^{-/-}$) OVA $^{+}$ HSCs stimulated proliferation and cytokine production of OT-I CD8 $^{+}$ T cells but failed to stimulate Granzyme B production, whereas IFNγ receptor deficiency (*Ifngr* $^{-/-}$) did not have such effect. As a result, *Tnfr1* $^{-/-}$*Tnfr2* $^{-/-}$ OVA $^{+}$ HSCs, but not *Ifngr* $^{-/-}$ OVA $^{+}$ HSCs, could escape from the killing by OT-I CD8 $^{+}$T cells. In sharp contrast, prior exposure to environmental TNFα, but not to IL-1β or IL-6, before interaction with OT-I CD8 $^{+}$ T cells rendered OVA $^{+}$ HSCs resistant to the killing by OT-I CD8 $^{+}$ T cells largely through upregulation of PD-L1 and PD-L2. Of note, upon 24-month aging or acquisition of *Jak2V617F* mutation, OVA $^{+}$ HSCs still maintained robust MHC-I-dependent antigen presentation activity and high susceptibility to the killing by OT-I CD8 $^{+}$ T cells, though prior exposure to TNFα allowed for their immune escape. Consequently, adoptive transfer of OT-I CD8 $^{+}$ T cells completely and specifically eliminated *Jak2V617F*-mutant OVA $^{+}$ HSCs and reversed myeloproliferative neoplasms in the mixed chimeric mice.

Taken together, our results reveal the robustness of HSC quality control via MHC-I-dependent antigen presentation to antigen-specific cytotoxic T cells. They also highlight a critical but complex role of TNFα in active interplay between HSCs and antigen-specific cytotoxic T cells as well as immune evasion by HSCs during inflammation. Specifically targeting abnormal HSCs by antigen-specific cytotoxic T cells may pave the way to eradicate mutant HSC clones and prevent CH-associated disease.

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