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AUTHOR'S VIEW

Neo-antigen specific memory T-cell responses in healthy individuals

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ABSTRACT

The driver mutations in exon 9 of the calreticulin protein have only been identified in patients with myeloid cancers. We recently demonstrated that healthy individuals display strong and frequent T-cell responses towards this mutation. This memory T-cell response is likely evidence of the elimination of mutated cells in healthy individuals.

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Text

The Philadelphia chromosome negative chronic myeloproliferative neoplasms (MPN) are disorders of the hematopoietic stem cells in the bone marrow.[1] Approximately 15% of patients with MPN harbor a driver mutation in exon 9 of the calreticulin (*CALR*) gene, which results in the generation of a novel mutant CALR protein with a 36 amino acid C-terminus.[1,2] We have previously demonstrated that T cells from MPN patients with mutant *CALR* recognize epitopes in the mutant C-terminus,[3] and that T cells isolated and expanded from MPN patients are able to recognize and kill autologous *CALR* mutant cells.[4] The *CALR* mutations have only been identified in patients with myeloid cancers, never in healthy individuals.[2,5] Thus, healthy individuals are not expected to harbor immune responses to epitopes from the mutant CALR C-terminus, as the immune system in these individuals has never been challenged with mutant CALR epitopes.

However, we just recently demonstrated that healthy individuals display strong and frequent T-cell responses to several epitopes derived from the mutant CALR C-terminus.[6] Both *in vitro* stimulated peripheral blood mononuclear cell (PBMC) cultures and PBMC analyzed directly *ex vivo* displayed strong responses to several mutant CALR epitopes. These CALR-mutant-specific T-cell responses identified in *ex vivo* experiments are most interesting as, even in cancer patients, it is extremely rare to detect tumor-associated-antigen-specific T cells without prior stimulation of cells *in-vitro*.[7] Thus, these frequent *ex vivo* responses show that healthy donors harbor a high frequency of circulating T cells specific to mutant CALR epitopes. Additionally, the responses in healthy donors were even stronger and more frequent than the responses in patients with *CALR*-mutant MPN. This finding fits well with the theory of cancer immunoediting, which stipulates that patients with established cancer have a decreased cancer-specific immune response.[8]

The surprising amount of strong T-cell responses to the mutant CALR epitopes spurred us to investigate whether the responding T cells were naïve, or if they could possibly be antigen-experienced memory T cells. This was investigated by enriching memory T cells using either magnetically activated cell sorting (MACS) or fluorescence-activated cell sorting (FACS) and then by analyzing CALR-mutant-specific responses in the memory T cell enriched cultures. Surprisingly, memory T cells were activated upon stimulation with mutant CALR epitopes while naïve T cells isolated by MACS or FACS were not activated upon stimulation with mutant CALR epitopes. Additionally, we showed that $CD4^+$ T-cell clones isolated from $CD4^+$ memory T cells from a healthy donor were able to kill autologous dendritic cells presenting mutant CALR epitopes. We had a great interest investigating the phenotype of the T cells specific to the mutant CALR epitopes, as healthy donors do not harbor CALR-mutations, and thus should not have been challenged with mutant CALR epitopes. Additionally, Pittet and colleagues demonstrated that the immune responses in healthy donors specific to the tumor-associated-antigen MART-1 were indeed from naïve T-cell responses.[9] Nevertheless, we showed that the CALR-mutant-specific T-cell responses in healthy donors were indeed memory T-cell responses. We investigated if a potential sequence homology between the mutant CALR C-terminus and other known epitopes could explain the high frequency of T-cell responses to the mutant C-terminus. However, the mutant CALR C-terminus did not share sequence homology with any other epitopes.

One likely explanation for the frequently occurring CALR mutant-specific immune responses in healthy individuals is that healthy persons occasionally acquire a CALR exon 9 mutation. However, due to the high immunogenic potential of these mutations, the mutant cells are cleared by

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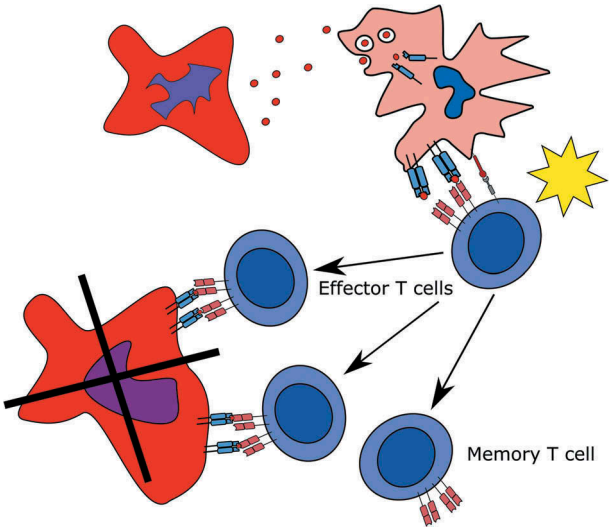

Figure 1. Generation of T cell memory to the CALR-mutations.
(*Top to down*). A CALR-mutant cell dies and sheds its mutant CALR epitopes to an antigen presenting cell which phagocytoses, process and presents mutant CALR epitopes to a naïve T cell. The naïve T cell is primed, starts proliferating and differentiating into either effector T cells, which kill the *CALR*-mutant cells, or to memory T cells.

the immune system and consequently memory T cells specific to the CALR mutations are generated (Figure 1). For a long time, it has been speculated that the immune system is able to spontaneously eliminate neoplastic cells before the establishment of overt malignancy, but such a feat has never been shown in man.[8,10] Additionally, memory T-cell responses to tumor-specific-antigens, such as the CALR-mutations, have never been identified in healthy individuals. We believe our detection of these memory T-cell responses in healthy donors likely provided evidence of the first "E" – elimination – in the theory of cancer immunoediting, and that the immune system in man is able to spontaneously clear neoplastic cells.

Disclosure of Potential Conflicts of Interest

No authors have conflicts of interest to disclose. However, it should be noted that Morten Orebo Holmström, Hans Carl Hasselbalch, and Mads Hald Andersen have filed a patent regarding the *CALR* exon 9 mutation as a target for cancer immune therapy. The patent has been transferred to University Hospital Zealand, Zealand Region and Copenhagen University Hospital at Herlev, Capital Region according to Danish Law concerning inventions made at public research institutions.

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