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Olink® Target 48 Mouse Cytokine

Cytokine changes in the aged tumor microenvironment in a mouse model

Introduction

Aging is known to increase cancer risk, causing genetic mutations in tumor cells. Immune function declines with age, and there are systemic changes in aging like alterations in cytokine levels and inflammation that can affect immune outcomes.

Dr. Debattama Sen's team, with project lead Dr. Alex Chen, at Massachusetts General Hospital — Harvard Medical School, has an ongoing project where they are trying to look at the aged tumor microenvironment and understand how the aging process impacts the immune system's ability to combat cancer. They have a specific focus on CD8⁺ T cell functions and their ability to kill infected cells and cancer cells.

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CD8⁺ T cells are one of the key effector cell populations that help drive anti-tumor immunity and immunotherapy responses. CD8⁺ T cells are very important for immune defence against intracellular pathogens, including viruses and bacteria, and are essential for tumor surveillance.

Mouse models in cancer research

Animal models have been used since the early beginnings of the biomedical sciences, and play an important intermediate role in translating laboratory discoveries into eventual clinical application. This is of particular importance in the pharmaceutical industry, where animal models are necessary during pre-clinical drug development.

Because of the complexity of modeling aging and studying aging in a human population, the project used a very reductionist mouse model to control for variables and isolate the effects of immune aging.

The Cancer-Immunity Cycle

In cancer immunology, there is a concept called the Cancer-Immunity Cycle[1], which essentially lays out some of the key steps that have to occur to mount an effective anti-tumor response.

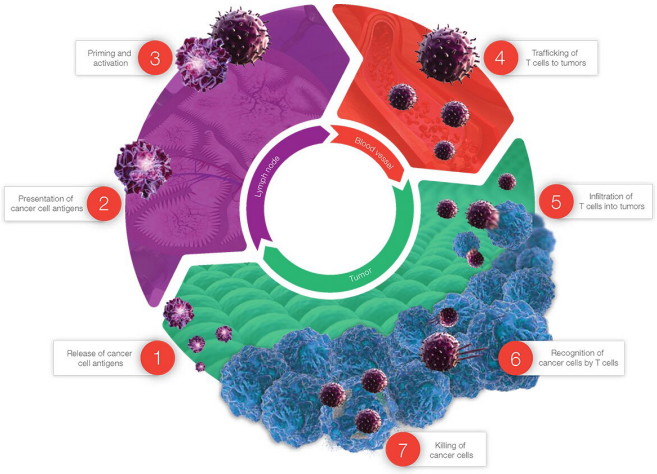

Figure 1 The Cancer-Immunity Cycle, introduced by Chen *et al*[1], and revised by Mellman *et al*[2], Pittet *et al*[3], and Giles *et al*[4].

Previous research by Dr. Debattama Sen's team has shown that a progressive increase in tumor volumes as mice get older was associated with drops in the population of CD8⁺ T cells[5]. The aged tumor microenvironment promotes tumor growth and limits CD8⁺ T cell expansion (steps 6 and 7 in Figure 1).

This group has also shown that that some of the early steps of antigen presentation and priming, and activation, are also impaired in aging[4]. It resulted in reduced communication between dendritic cells and T cells (steps 2 and 3 in Figure 1) is reduced.

In the follow-up experiment described here, they wanted to understand which elements of the tumor microenvironment are driving all of these defects. Might there be global or systemic changes in the milieu of these mice?

Olink analysis and results

With Olink® Target 48 Mouse Cytokine, Dr. Sen's research group tried to match their previous findings, and unlock more information that can only be found through proteomics.

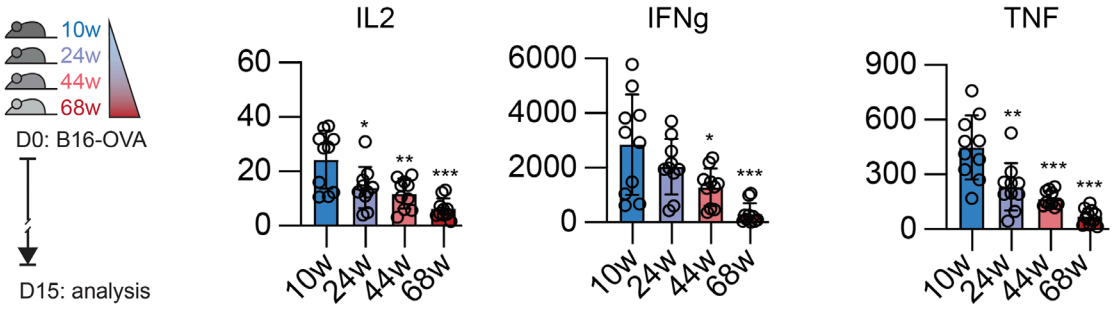

Figure 2 Age-related reduction in expression levels of several cytokines in tumor interstitial fluid.

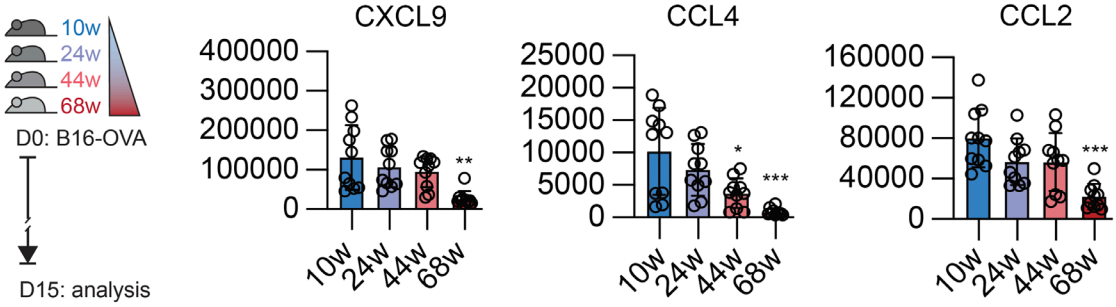

Figure 3 Age-related reduction in expression levels of several chemokines in tumor interstitial fluid.

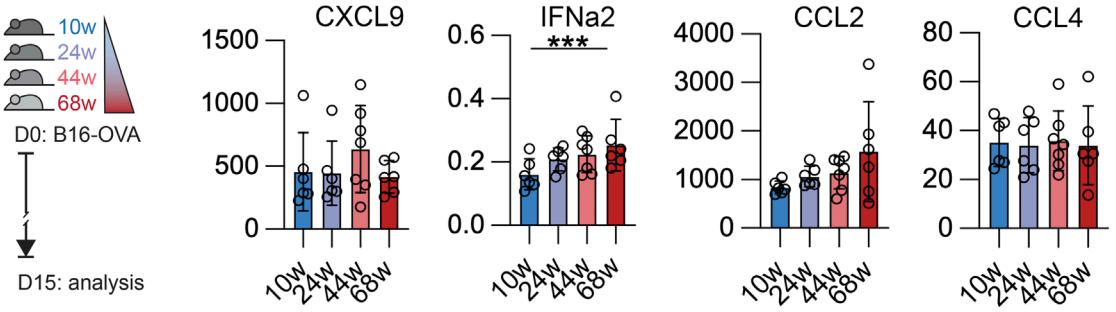

Figure 4 Age-related expression levels in serum of tumor-bearing mice.

Cytokine changes in the aged tumor microenvironment

They used the same cohorts of tumor-bearing mice of various ages used in the previous experiments, and collected tumor interstitial fluid.

The samples were run on Olink Target 48 Mouse Cytokine.

The researchers expected the levels of many different cytokines and chemokines to match previous findings, and as anticipated there were reductions in effector cytokines like IL2, IFNγ and TNF as seen in Figure 2.

They were also able to identify other defects, particularly in chemokines for recruiting immune response cells in the aged tumor microenvironment. CXCL9 is key for recruiting different T cells and other effector cells, whereas CCL4 and CCL2 are important for recruiting myeloid cells (a type of blood cell crucial for immune response). Clear progressive decreases with age can be seen for these chemokines in Figure 3.

To show that this effect related to the aged tumor microenvironment is truly specific to the tumor, they also ran serum samples from the same mice on Olink Target 48 Mouse Cytokine.

Figure 4 shows that CXCL9 does not decrease systemically in the aged mice, with nominal increases actually apparent. Futhermore, CCL2 and CCL4 levels are not lowered systemically either.

Additionally, type I interferons are well-established in aging studies as key drivers of the inflammaging process[6,7]. An increase in interferon alpha 2 was clearly seen in the present study. While further research is necessary to confirm the role of interferon alpha 2, this gave the researchers confidence that the changes noted in both the tumor microenvironment and the serum should correspond with the expected biology.

At least part of the issue in the aged tumor microenvironment is that there is a global reduction, in both the cytokines that help T cells kill tumors and in the chemokines that help recruit different immune cells, particularly myeloid cells, into the tumor.

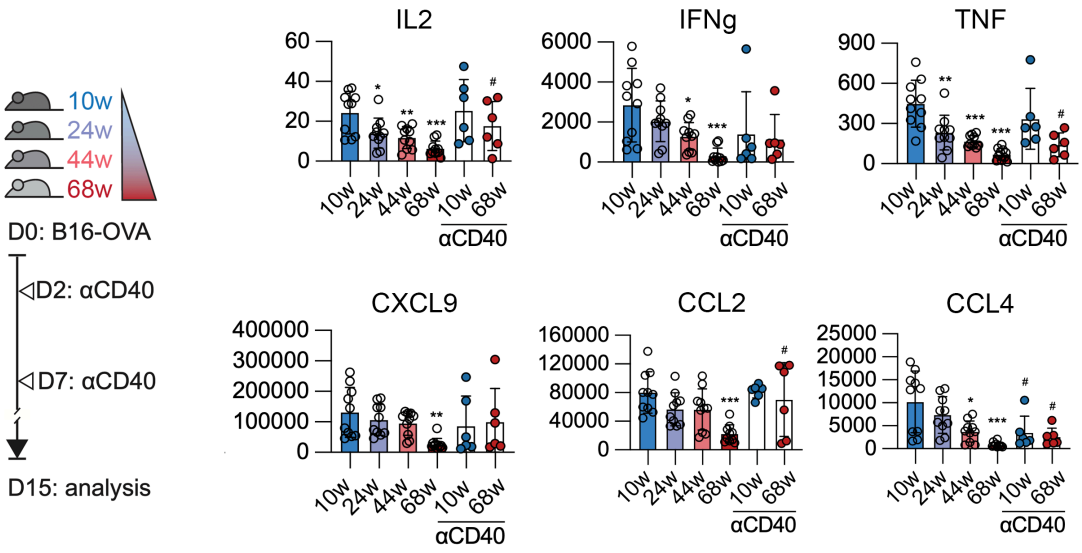

Figure 5 Expression levels in tumor interstitial fluid after CD40 agonism.

CD40 agonism

Dr. Debattama Sen and her group, including project lead Dr. Alex Chen, wanted to try to help and restore normal function and reinvigorate the whole Cancer-Immunity Cycle by targeting the upstream dentritic cells (Steps 2 and 3 in Figure 1) by using a myeloid-targeted therapy called CD40 agonism. They gave either control treatment or CD40 agonist antibody therapy to the mice.

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CD40 agonism refers to the activation of the CD40 receptor, which is found on the surface of antigen-presenting cells like dendritic cells and B cells, leading to a series of immune responses.

When activated, CD40 enhances the ability of antigen-presenting cells to present antigens and and stimulate T cells.

CD40 agonism is a promising strategy in cancer immunotherapy, but it can lead to significant side effects and needs to be researched further.

The young mice controlled tumors better than the old mice, irrespective of treatment. They showed an expansion of conventional type 1 dendritic cells as well as an expansion of $CD8^+$ T cells. The aged mice responded to CD40 agonism, with normalized tumor volumes similar to those seen in young mice. This tells us that therapeutic targeting of the first steps of the Cancer-Immunity Cycle can rescue dendritic cell function and increase priming/expansion of $CD8^+$ T cells.

The data generated in collaboration with Olink, suggests that the chemokine defects characterized in previous experiments are really critical to this process. Figure 5 shows post-therapy changes in the cytokines IL2, IFNγ and TNF on the top and the recruiting chemokines CCXL9, CXL2 and CCL4 at the bottom.

We can see that CD40 agonism almost always rescues the levels in both young and aged mice. In some cases, such as CCL2 and CXCL9, levels are increased to those expected in the tumors of young mice.

Conclusions

The data shows that the aged tumor microenvironment disrupts multiple stages of the Cancer-Immunity Cycle, to ultimately limit $CD8^+$ T cell control. Without any intervention, there are defects in dendritic cell differentiation and activation of $CD8^+$ T cells.

Defects in the recruitment and trafficking of different immune populations to the aged tumor microenvironment occur and this prevents recognition and killing of tumor cells.

Myeloid-targeted therapies like CD40 agonism can help rescue some of these defects. While this therapy has not had much success in translation to the clinic, this data provides proof of concept for a new approach that takes into account the effect of aging on anti-tumor immunity. It also opens the door for other strategies that might reinvigorate different steps of the Cancer-Immunity Cycle to try to rescue these defects.

References

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