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Single-cell sequencing on granulomas opens new therapeutic approaches for sarcoidosis

Granulomas are an accumulation of immune cells in the tissue, often the result of an overactive immune response. Granulomas contribute to several inflammatory systemic diseases such as sarcoidosis, berylliosis, and rheumatoid arthritis. For the first time, scientists at CeMM, the Medical University of Vienna and the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases have thoroughly characterized granulomas in the skin in immense detail. The results provide numerous insights into the composition, structure and signaling pathways of granulomas, providing clues for new therapeutic approaches. The study was published in the journal *Immunity*.

(Vienna, 6 February 2023) Granulomatous diseases like sarcoidosis pose significant challenges to physicians and researchers. With an unknown origin, sarcoidosis manifests as an inflammatory disorder with an overactive immune response, resulting in the formation of granulomas: clusters of cells that take on a nodular form. These granulomas are most frequently located in the lungs, followed by the skin, and can affect any other organ as well. Out of 100,000 individuals worldwide, between 1 and 35.5 people are diagnosed with sarcoidosis depending on the geographical region. For half of those affected, a spontaneous regression occurs within two years, while around 20% experience a chronic progression.

“Sarcoidosis of the skin can appear in a variety of colors and forms. For example, granulomas in the skin may appear at itchy clusters around old tattoos or scars, after filler procedures, or as a purple discoloration on the tip of the nose and cheeks, a condition also known as lupus perino,” says Anna Redl, PhD student and co-author of the study under project leader Georg Stary, Associate Professor at the Medical University of Vienna and CeMM Adjunct Principal Investigator. Stary adds, “The issue becomes particularly problematic when granulomas spread throughout the body, forming in organs, and causing problems which can lead to organ failure.”

Together with the research group led by CeMM Principal Investigator Christoph Bock, who is also a Professor at the Medical University of Vienna and co-study leader, the research team was able to create a detailed picture of the composition, structure, and components of granulomas from tissue samples for the first time using single-cell sequencing and spatial analysis methods.

The granuloma structure: macrophages, T cells, fibroblasts

According to Christoph Bock, granulomas are composed of various components of our immune system, but they have become disordered. “The cells communicate with each other, but they no longer understand each other – the signaling pathways do not function,” Bock says. “This is how granulomas

are formed. More and more cells gather, activate each other, and lead to inflammation, for which there is no logical biological explanation."

For the study, first authors Thomas Krausgruber and Daniele Barreca, scientists in Bock's research group, together with Anna Redl, analyzed tissue samples from 12 patients with sarcoidosis and compared the granulomas to seemingly healthy skin. By using single-cell sequencing, the researchers were able to characterize immense detail of the granulomas. As Krausgruber explains, "We see that granulomas are primarily comprised of macrophages that serve as scavenger cells in the body, mainly to defend against unwanted viruses, bacteria, and toxins. In addition, we observed T cells, particularly the Th1 and Th17 subtypes, which are associated with inflammation processes. Around the macrophages and t cells, we see fibroblasts, which play an important role in remodeling connective tissue and give the granuloma its shape."

Georg Stary adds, "We believe that the fibroblasts remodel collagen around it to allow the granuloma to grow, but the fibroblasts are also trapped within the structure at the same time." Generally, the study shows that the granulomas on the skin bear a striking resemblance to those found in other regions of the body such as the lungs. Characterizing granulomas in the skin can provide insights into the treatment of sarcoidosis in other organs or granuloma-associated diseases.

Potential target for new therapeutic approaches: tissue enzymes

The protein MMP12, which plays an important role in tissue remodeling, was found to be strongly expressed in macrophages. Thomas Krausgruber explains: "This enzyme seems to play a significant role in the formation of granulomas. Initial in-vivo experiments in mouse models have already shown that inhibiting MMP12 reduces swelling. This is seen as an important starting point for the development of new therapies, but other results of our analysis also show potential for clinical testing."

Currently, patients with sarcoidosis are treated with cortisone preparations such as systemic glucocorticoids, which are administered over a long period of time and in high doses. The relapse rate for such treatments is also high. New targeted therapies that are based on the findings of this study, can greatly improve the possibilities and treatment for patients with granuloma-associated diseases.

Pictures attached
Picture 1: Immunofluorescence staining of a skin biopsy with cutaneous sarcoidosis. Accumulations of macrophages (stained in red) and scattered T cells (stained in white) are seen. Macrophages and T cells are surrounded by fibroblasts (stained in green), which hold the structure together. Each dark blue dot represents a vascular nucleus and thus a cell. © Anna Redl
Picture 2: The study authors Georg Stary, Anna Redl, Thomas Krausgruber, Christoph Bock, © Laura Alvarez, CeMM

The study "Single-cell and spatial transcriptomics identify aberrant developmental programs driving granuloma formation" was published on 6 February 2023 in the scientific journal *Immunity*, DOI: 10.1016/j.immuni.2023.01.014

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Funding: The study was funded with support from the Vienna Science, Research and Technology Fund (WWTF), grant number WWTF LS18-058.

Georg Stary is a dermatovenerologist with direct patient contact and researches organ-specific immunological processes. After his clinical residency, he was a postdoctoral fellow at Harvard Medical School (2010 – 2014). He the became Assistant Professor in Dermatology (2015) at the Medical University of Vienna and is now Associate Professor at the Department of Dermatology, Medical University of Vienna (since 2016). He was appointed Adjunct Principal Investigator at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences (2018) and Co-Director of the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (2019). Stary's research focuses on various aspects of host-pathogen interactions, the contribution of tissue-resident leukocytes to physiological and pathological immune responses, and rare skin diseases. He has received many national and international research awards, including the Oscar Gans Prize for Experimental Dermatology of the German Society for Dermatology and the Ferdinand von Hebra Prize of the Austrian Society for Dermatology and Venereology.

Christoph Bock has led a research group at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences (ÖAW) since 2012 and has been Professor of Medical Informatics at the Medical University of Vienna since 2021. Prior to his time in Vienna, Christoph Bock was a postdoctoral fellow at the Broad Institute of MIT and Harvard and Harvard University (2008-2011) and a PhD student at the Max Planck Institute for Computer Science (2004-2008). He is also scientific coordinator of the Biomedical Sequencing Facility of CeMM and MedUni Vienna, "Key Researcher" at the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases (LBI-RUD), Fellow of the European Lab for Learning and Intelligent Systems (ELLIS) and elected member of the Young Academy of the ÖAW. He has received major research awards, including the Otto Hahn Medal of the Max Planck Society (2009), an ERC Starting Grant (2016-2021), an ERC Consolidator Grant (2021-2026), the Overton Prize of the International Society for Computational Biology (2017), and the Erwin Schrödinger Prize of the Austrian Academy of Sciences (2022). He has been on Clarivate Analytics' Highly Cited Researchers list (ISI Web of Science) every year since 2019.

The **CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences** is an international, independent and interdisciplinary research institution for molecular medicine under the scientific direction of Giulio Superti-Furga. CeMM is oriented towards medical needs and integrates basic research and clinical expertise to develop innovative diagnostic and therapeutic approaches for precision medicine. Research focuses on cancer, inflammation, metabolic and immune disorders, and rare diseases. The Institute's research building is located on the campus of the Medical University and the Vienna General Hospital.
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Medical University of Vienna (MedUni Vienna) is one of the most traditional medical education and research facilities in Europe. With almost 8,000 students, it is currently the largest medical training centre in the German-speaking countries. With 6,000 employees, 30 departments and two clinical institutes, 13 medical theory centres and numerous highly specialised laboratories, it is one of Europe's leading research establishments in

the biomedical sector. MedUni Vienna also has a medical history museum, the Josephinum.
www.meduniwien.ac.at

The **Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases** (LBI-RUD) was founded by the Ludwig Boltzmann Society in April 2016 together with the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, the Medical University of Vienna and St. Anna Children's Cancer Research. The three partner institutions, together with CeRUD - Vienna Center for Rare and Undiagnosed Diseases, represent the main collaborating partners of LBI-RUD. The goal of LBI-RUD is to establish a coordinated research program, involving the expertise of its partner organizations, that incorporates and considers not only the scientific but also the social, ethical and economic aspects of rare diseases.
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