Background Chronic myeloproliferative neoplasms (MPN) are a group of diseases that are characterized by the hyperproliferation of mutated hematopoietic stem cells (HSCs) in the bone marrow. Clinically, they can progress to myelofibrosis and acute myeloid leukemia. Megakaryocytic hyperplasia is known to drive fibrotic progression through the secretion of fibrosis-promoting factors such as transforming growth factor beta (TGFβ). Previous work from our group has demonstrated the existence of proinflammatory TGFβ-specific CD4+ and CD8+ T cells that are able to recognize and react towards cancer cells and autologous myeloid cells in a TGFβ-dependent manner. In this study, we investigated the presence of TGFβ-specific T cells in patients with MPN and examined their ability to target key cell types involved in the disease pathogenesis, including megakaryocyte progenitors.

Methods T-cell responses towards a TGFβ-derived peptide epitope were assessed in peripheral blood mononuclear cells (PBMCs) from 102 patients with MPN and 34 healthy donors (HDs) using interferon gamma (IFN-γ) ELISPOT and intracellular cytokine staining. TGFβ-specific CD4+ T-cell cultures were generated from several patients with MPN by enrichment of reactive T cells following in vitro stimulation and subsequent rapid expansion. Autologous megakaryocyte progenitors were generated through in vitro differentiation of HSCs and expression of megakaryocytic surface markers was demonstrated using flow cytometry. The functional recognition of autologous megakaryocyte progenitors by TGFβ-specific CD4+ T cells was assessed through co-culturing and subsequent intracellular cytokine staining. The production of TGFβ in megakaryocyte progenitors was quantified using ELISA and western blotting.

Results We describe the presence of TGFβ-specific CD4+ T cells in patients with MPN. We demonstrate stronger TGFβ-specific T-cell responses in patients with essential thrombocytethemia (ET) as compared to both HDs and patients with polycythemia vera (PV) or primary myelofibrosis (PMF). We show that TGFβ-specific T cells initiate a proinflammatory cytokine response upon recognition of autologous megakaryocyte progenitors. We validate that these megakaryocyte progenitors produce significant amounts of TGFβ and express human leukocyte antigen (HLA)-class II on their surface. We are currently investigating the presence of TGFβ-specific T cells in the bone marrow of patients with MPN using T-cell receptor (TCR) sequencing.

Conclusions Our findings highlight the ability of TGFβ-specific T cells to directly target megakaryocyte progenitors, which is a key cell type contributing to the manifestation and progression of disease in patients with MPN. As such, these results warrant further investigation into TGFβ-based immunomodulatory vaccination in patients with MPN.

Ethics Approval Patient protocols were approved by the scientific ethics committee for the Capital Region of Denmark. Subjects gave written informed consent to participate in the study before taking part.

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