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Inhibitory immune checkpoints cause exhaustion of viral immunity in coronary artery disease

In patients with coronary artery disease, stabilizing post-translational modifications to the mRNA of the immune-checkpoint inhibitor CD155 result in an immunosuppressive macrophage phenotype and impair activation of T cells in response to viral infection.

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The question

Coronary artery disease (CAD) is a metabolic condition but also an inflammatory condition, with immune cells, such as macrophages, contributing to all stages of vascular damage[1,2]. When, where and how the immune system fails remain unknown. Driven by metabolic signals, macrophages in CAD are reprogrammed and overexpress PD-L1 (programmed death ligand 1), an immune-checkpoint inhibitor, which curbs T cell responses to varicella zoster virus[3,4]. Pre-existent CAD resulted in a predisposition to severe and fatal infection with the coronavirus SARS-CoV-2 during the recent pandemic[5], indicative of dysfunctional adaptive immunity. T cells recognize antigens on antigen-presenting cells, endowing the host with lasting immunity to pathogens and malignancies. However, the intensity and duration of T cell responses after antigen exposure depend on the co-recognition of stimulatory or inhibitory ligands, which bolster or dampen the induction of T cell immunity. Ligands that transmit inhibitory signals (immune-checkpoint inhibitors) have emerged as important therapeutic targets in the stimulation of anti-cancer immunity.

The discovery

We probed the competence of anti-viral immunity in patients with CAD and age-matched healthy control participants. We quantified production of interferon-γ (IFNγ) and responses of CD4$^+$ helper T cells expressing the early activation antigen CD69 and CD40L (which promote T cell proliferation and cytokine production) against two viral antigens (SARS-CoV-2 spike protein and Epstein–Barr virus glycoprotein 350). We phenotyped antigen-presenting macrophages, antigen-recognizing T cells and tissue macrophages by multi-parameter flow cytometry. Macrophages from patients with CAD were only half as efficient as those from control participants in inducing IFNγ production and mobilizing antigen-reactive CD4$^+$ T cells (Fig. 1a,b). Through the use of blocking antibodies or knockdown via small interfering RNA, we found that CAD macrophages had high expression of the T cell inhibitory (immunosuppressive) ligand CD155 (Fig. 1c). Antibodies to CD155 or knockdown of *CD155* mRNA (also known as *PVR*, poliovirus receptor) restored T cell–mediated anti-viral immunity. High expression of CD155 resulted from increased N^6-methyladenosine (m^{6}A) post-translational modifications on *CD155* mRNA transcripts, which increased the mRNA stability (assessed by decay assays). Targeted transcriptomics indicated that CAD macrophages had high expression of *METTL3*, which encodes the catalytic subunit of N^6-adenosine-methyltransferase,

a key component of the METTL3–METTL14 complex that adds m^{6}A onto RNA (Fig. 1d). We predicted potential m^{6}A sites by searching for DRACH consensus motifs in methylated RNA–immunoprecipitation sequencing data. By relying on m^{6}A-dependent suppression of retrotranscription, we mapped positions 1635A and 3103A in *CD155* mRNA as being functionally relevant for the binding of CD155 to TIGIT on T cells and validated these results by site-specific mutation. TIGIT is an immune-checkpoint molecule, and by binding to CD155, it prevents T cell activation (Fig. 1e,f). We screened plasma from people with CAD for inducers of the immunosuppressive macrophage phenotype, and low-density lipoprotein (LDL) cholesterol–rich plasma and oxidized LDL stood out as potent inducers of this phenotype of high expression of METTL3 and CD155, which is already present on undifferentiated monocytes in patients with CAD and on most tissue macrophages in atherosclerotic arteries.

The implications

The data define a state of immunodeficiency in patients with CAD, elicited by antigen-presenting macrophages that are sensing metabolic changes (such as increased levels of LDL cholesterol). Given the functional importance of CD155, the patients' immunoincompetence probably extends beyond viral antigens to include all microbial pathogens and cancer cells. In our study, this immunocompromised state was 'rescued' by blockade of the immune-checkpoint inhibitor, similar to immunostimulatory therapy in cancer.

RNA modifications, specifically m^{6}A methylation, are involved in the regulation of immunity-relevant genes and antigen presentation, but additional macrophage functions could be regulated by the exposure of macrophage to lipids.

The current study did not establish a timeline for when this immunological defect begins. The inductive role of oxidized LDL cholesterol could place the defect in the pre-disease period, but longitudinal studies of appropriate patient cohorts are needed.

Future studies will address the question of how LDL cholesterol interferes with the expression of *METTL3* and *CD155*, and which functions, beyond antigen presentation, depend on m^{6}A methylation of RNA. Elucidation of the effector functions of macrophages with high expression of CD155 in atherosclerotic tissue lesions could yield important insights to resolve the causal relationship between atherosclerosis and immunodeficiency.

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EXPERT OPINION

" This paper is very original and has a major impact on how we should interpret immunity in a cardiovascular disease setting." **Esther Lutgens, Ludwig–Maximilians University, Munich, Germany.**

FIGURE

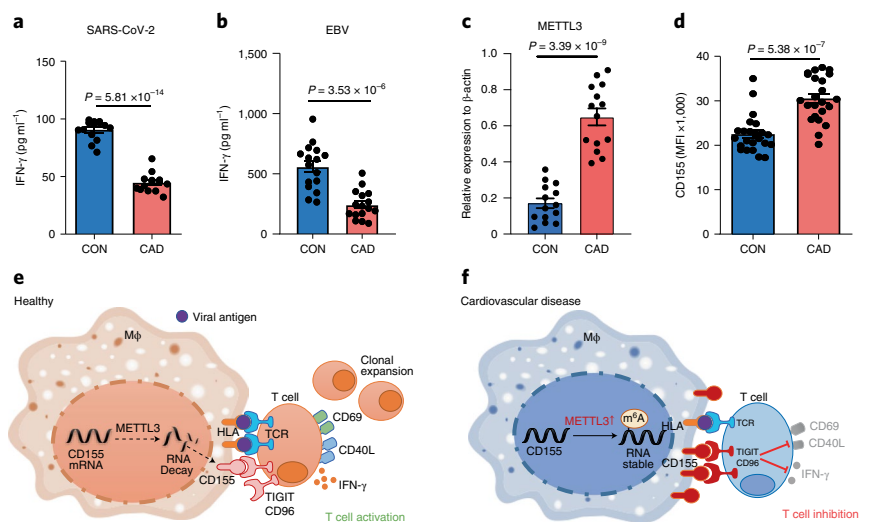**Fig. 1 | Immunosuppressive macrophages in CAD. a,b,** IFNγ concentrations obtained after activation of macrophages from patients with CAD and control participants (CON) in response to viral antigens from SARS-CoV-2 (**a**) or Epstein–Barr virus (EBV) (**b**). **$P < 0.01$; ***$P < 0.001$. **c,** *METTL3* mRNA expression in macrophages (presented relative to control *ACTB* (β-actin) mRNA). ***$P < 0.001$. **d,** CD155 expression in macrophages, presented as mean fluorescence intensity (MFI). ***$P < 0.001$. **e,f,** By binding to TIGIT and CD96 (also known as T cell surface protein tactile), CD155 on macrophages (MΦ) stops protective T cell immunity by preventing clonal expansion of viral antigen–activated T cells, which results in immunodeficiency in patients with CAD. HLA, human leukocyte antigen; TCR, T cell receptor. © 2022, Zhao, T. V. et al.

BEHIND THE PAPER

CAD is currently considered a lipid-storage disorder; the emerging paradigm recognizes atherosclerosis as an immune-mediated disease, inextricable from anti-pathogen and anti-cancer immunity. For immunologists, CAD is a disease full of questions and surprises. Macrophages are usually associated with debris removal, but many cells in the atheroma are T cells, indicative of antigen-recognition events. Which antigens are recognized? Is the maladaptive wound healing reaction dependent on recognition of self antigens? Are T cells 'good', 'bad' or both? Surprisingly, immune cells explicitly sense intracellular and extracellular nutrients and metabolites, which indicates that metabolism, antigen recognition, host defense and tissue healing are connected. Cancer is now understood as a disease of immune system failure. Here, atherosclerosis joins the club, emerging as a disease process in an immunocompromised host. Immune exhaustion, shared in patients with cancer, infection and atherosclerosis, emerges as a new frontier for immunologists. **C.M.W.**

REFERENCES

1. Libby, P. The changing landscape of atherosclerosis. *Nature* **592**, 524–533 (2021).
A Review that presents current concepts in the pathobiology of atherosclerosis.

2. Fernandez, D. M. et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat. Med.* **25**, 1576–1588 (2019).
An Article that describes the profile of immune cells in the atherosclerotic lesion.

3. Watanabe, R. et al. Pyruvate controls the checkpoint inhibitor PD-L1 and suppresses T cell immunity. *J. Clin. Invest.* **127**, 2725–2738 (2017).
An Article that reports excessive expression of the checkpoint ligand PD-L1 on CAD macrophages.

4. Shirai, T. et al. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *J. Exp. Med.* **213**, 337–354 (2016).
An Article that describes the bias of CAD macrophages toward pro-inflammatory function.

5. O'Gallagher, K. et al. Pre-existing cardiovascular disease rather than cardiovascular risk factors drives mortality in COVID-19. *BMC Cardiovasc. Disord.* **21**, 327 (2021).
A cohort study that examines the association between established cardiovascular disease and mortality in COVID-19.

FROM THE EDITOR

" The study by Zhao et al. expands the understanding of why patients with chronic diseases show compromised immune responses. In particular, the authors show that in patients with CAD, the macrophages are primed, possibly by exposure to LDL cholesterol, and are overly immunosuppressive, thereby blunting the response of T cells to viral antigens." **Elvira Forte, Associate Editor,** *Nature Cardiovascular Research.*