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Overrepresentation of Th1- and Th17-like Follicular Helper T Cells in Coronary Artery Disease

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Abstract T cells and B cells play substantial roles in the process of coronary artery disease (CAD). Here, we examined the role of circulating follicular helper T (Tfh) cells in CAD. Compared to non-CAD controls, CAD patients had increased levels of circulating Tfh. Also, circulating Tfh in CAD patients exhibited increased frequencies of Th1- and Th17-like phenotypes and aberrant cytokine expressions. Coculture experiments with B cells showed that Tfh from CAD patients were more potent at inducing antibody production from B cells, enhancing plasmablast differentiation and suppressing B10 cell differentiation. Importantly, we found that the skewing of circulating Tfh toward the Th1/Th17-like cells was directly correlated with B cell inflammation and low density lipoprotein level in CAD patients. Together, our data demonstrated a skewing of blood Tfh composition in CAD patients, which resulted in significant changes in B cell inflammation.

Keywords Follicular helper T cells · Coronary artery disease · B cell · Inflammation

Infiltrating of $CD4^+$ helper T cells in atherosclerotic arteries is frequently observed and is thought to play a key role in modulating local immune responses in atherosclerotic lesion, with functions including exacerbating proinflammatory responses and promoting plaque formation [1]. Recently, the human blood equivalent of tonsillar follicular helper T (Tfh) cells has been identified [2]. These cells are named as circulating Tfh and are characterized by $CD4^+CXCR5^+ICOS^+$ expression. Circulating Tfh can be distinguished into Th1-, Th2-, and Th17-like subsets and are capable of inducing naive B cell differentiation toward plasmablasts and supporting antibody production through IL-21 and IL-10. Altered Tfh subset composition has been discovered in autoimmune disorders, including juvenile dermatomyositis and systemic autoimmune erythematosus [2, 3]. This, and the observation that depletion of B cells by anti-CD20 antibody could reduce atherosclerotic lesion [4, 5], inspired us to examine the circulating Tfh in CAD patients.

Peripheral blood mononuclear cells (PBMCs) were collected from 22 CAD patients and 22 age-, gender-, and BMI-matched non-CAD controls by venipuncture and Ficoll density centrifugation. CAD patients presented significantly higher total cholesterol, LDL cholesterol, and triglycerides levels, as well as lower LDL cholesterol levels than non-CAD subjects. Patients with other factors that may independently affect the inflammatory status, such as smoking, obesity, diabetes, and hypertension were excluded. Circulating Tfh cells in PBMCs were identified as $CD4^+CXCR5^+ICOS^+$ T cells, and we observed that compared to non-CAD controls, CAD patients had

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significantly upregulated percentage of circulating Tfh cells in total $CD4^+$ T cells and increased absolute number of circulating Tfh cells per liter of blood (Fig. 1a, $P<0.001$ and $P<0.001$). Based on CXCR3 and CCR6 expression, circulating Tfh cells can be distinguished into Th1-like ($CXCR3^+CCR6^-$), Th2-like ($CXCR3^-CCR6^-$), and Th17-like ($CXCR3^-CCR6^+$) subsets, with the Th17-like subset most efficient at inducing B cell antibody secretion, followed by the Th2-like subset [2]. We observed that the Th1- and Th17-like Tfh cells were significantly upregulated in CAD patients ($P=0.033$ and $P<0.001$) while the Th2-like Tfh cells were significantly downregulated, compared to non-CAD controls (Fig. 1b, $P<0.001$).

We then examined cytokine expression in purified Tfh cell subsets. The IL-10 expression is significantly downregulated in Th2-like ($CXCR3^-CCR6^-$) and Th17-like ($CXCR3^-CCR6^+$) Tfh cells in CAD patients ($P=0.005$ and $P<0.001$). IFN-gamma was primarily expressed by Th1-like ($CXCR3^+CCR6^-$) Tfh cells and was upregulated in CAD patients ($P=0.013$). Among IL-4, IL-5, and IL-13, which were mostly found in Th2-like Tfh cells, the expressions of IL-4 and IL-5 were significantly reduced in CAD patients ($P<0.001$ and $P=0.036$). Function analysis revealed that circulating Tfh cells from CAD patients induced significantly higher productions of IgM, IgG, and IgA than those from non-CAD controls. Furthermore, we investigated B cell differentiation after coculture and found that blood Tfh cells from CAD patients induced higher frequencies of plasmablasts ($P<0.001$) and lower frequencies of B10 cells ($P<0.001$). Subsequently, we observed that the frequencies of plasmablasts and B10 cells were associated with the overrepresentation of Th1- and Th17-like Tfh cells and the underrepresentation of Th2-like Tfh cells. These data, again, highlighted the functional differences between different blood Tfh subsets. Plasmablasts are capable of IL-10 secretion and can exert regulatory function in a mouse experimental autoimmune myelitis (EAE) model. Interestingly, in our coculture, we observed an increase of plasmablasts and concurrent decrease of IL-10-producing B cells in CAD patients ($P<0.001$ and $P=0.008$).

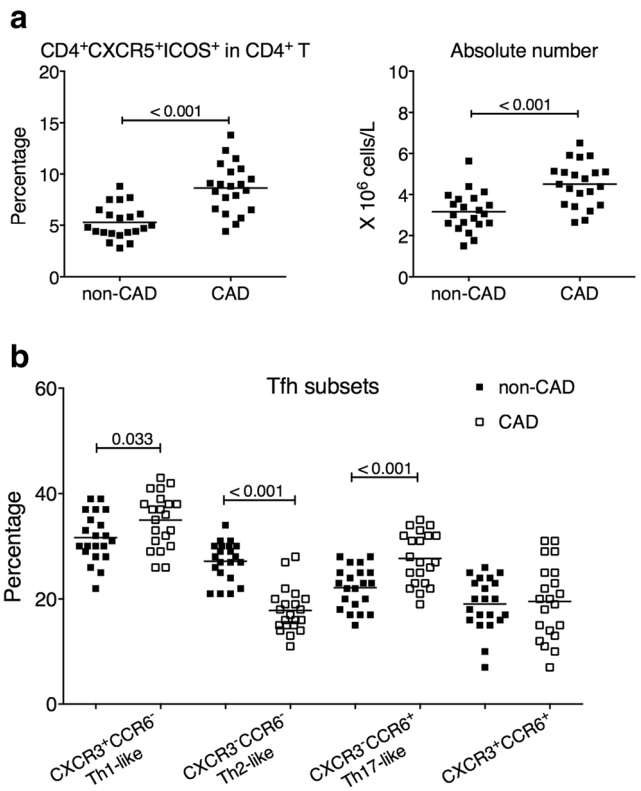

Fig. 1 CD4+CXCR5+ICOS+ Tfh frequency and subset composition in blood. **a** The frequency and absolute numbers of blood Tfh cells, identified by CD4+CXCR5+ICOS+ expression, in non-CAD controls and CAD patients. **b** The composition of Th1-, Th2-, and Th17-like blood Tfh cell subsets based on CXCR3 and CCR6 expression. Student's *t* test

To investigate the mechanism of Tfh subset changes in CAD patients, we examined physiological characteristics of the study subjects. The skewing of T cells toward proinflammatory Th1 and Th17 subtypes was previously observed in metabolic syndromes such as diabetes and obesity, two risk factors for atherosclerosis and CAD. We found that the upregulation of Th1- and Th17-like Tfh cells were directly correlated with LDL level in both CAD patients and controls ($P=0.002$, $r=0.64$, and $P=0.018$, $r=0.51$, respectively), which suggested that the Th1/Th17 skewing was in line with the immune profile alterations in patients with metabolic and inflammatory disorders.

In conclusion, our results demonstrated an overrepresentation of circulating Tfh cells in CAD patients, together with a skewing of subset composition toward Th1- and Th17-like Tfh cells and away from Th2-like Tfh cells. The positive correlation between Tfh skewing and LDL levels suggested a potential cause of Tfh skewing. How this Tfh skewing is involved in the exacerbated T cell inflammation in CAD will require further examinations.

Compliance with Ethical Standards

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