A fate determination fork-in-the-road for germinal center Tfh and T memory cells

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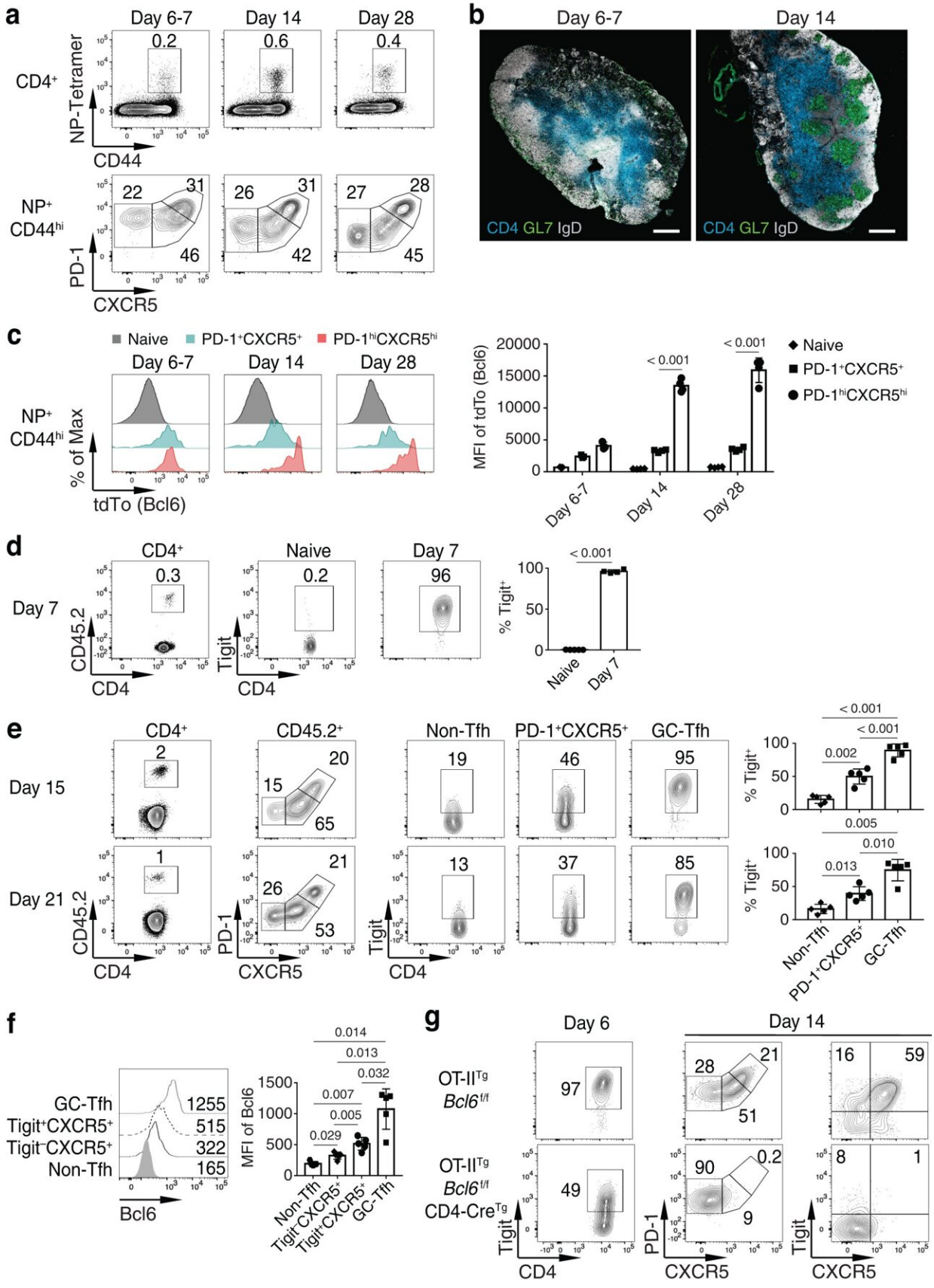
a
Day 6-7
Day 14
Day 28
CD4+
NP-Tetramer
CD44
0.2
0.6
0.4
NP+ CD44hi
PD-1
CXCR5
22
31
46
26
31
42
27
28
45
b
Day 6-7
Day 14
CD4 GL7 IgD
CD4 GL7 IgD
c
Naive
PD-1+CXCR5+
PD-1hiCXCR5hi
Day 6-7
Day 14
Day 28
NP+ CD44hi
% of Max
tdTo (Bcl6)
MFI of tdTo (Bcl6)
20000
15000
10000
5000
0
< 0.001
< 0.001
Naive
PD-1+CXCR5+
PD-1hiCXCR5hi
Day 6-7
Day 14
Day 28
d
CD4+
Naive
Day 7
Day 7
CD45.2
CD4
Tigit
CD4
0.3
0.2
96
% Tigit+
100
50
0
< 0.001
Naive
Day 7
e
CD4+
CD45.2+
Non-Tfh
PD-1+CXCR5+
GC-Tfh
Day 15
Day 21
CD45.2
CD4
PD-1
CXCR5
Tigit
CD4
2
20
15
65
19
46
95
1
21
26
53
13
37
85
% Tigit+
< 0.001
< 0.001
0.002
0.005
0.010
0.013
Non-Tfh
PD-1+CXCR5+
GC-Tfh
f
GC-Tfh
Tigit+CXCR5+
Tigit−CXCR5+
Non-Tfh
1255
515
322
165
Bcl6
MFI of Bcl6
1500
1000
500
0
0.014
0.013
0.032
0.007
0.005
0.029
Non-Tfh
Tigit−CXCR5+
Tigit+CXCR5+
GC-Tfh
g
Day 6
Day 14
OT-IITg Bcl6f/f
OT-IITg Bcl6f/f CD4-CreTg
Tigit
CD4
PD-1
CXCR5
Tigit
CXCR5
97
49
28
21
51
90
0.2
9
16
59
8
1

Sustained Tigit expression in CXCR5+CD4+ T cells is associated with GC-Tfh cell differentiation. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-39299-3

Follicular helper T cells, or Tfh cells, have a crucial role in immune defense. Without Tfh cells, B cells cannot form germinal center (GC) responses during which high-affinity antibodies are generated.

When naïve CD4-positive $CD4^+$ T cells receive news of an infection elsewhere in the body, they become activated with additional cell-surface markers, and they differentiate in two directions, becoming either PD-1^+CXCR5^- or PD-1^+CXCR5^+ T cells. PD-1 and CXCR5 are two different cell-surface markers, and the plus and minus signs tell whether they are present or absent.

The PD-1^+CXCR5^+CD4^+ T cells have the capacity to differentiate, move into B-cell follicles and mature into GC-Tfh cells. These GC-Tfh cells are essential for the formation of germinal centers, the place where B cells generate high-affinity antibodies.

But there was a mystery about these PD-1^+CXCR5^+CD4^+ T cells. While some PD-1^+CXCR5^+CD4^+ cells enter the follicle, others do not. Now Hui Hu, Ph.D., and colleagues at the University of Alabama at Birmingham have identified a cell-surface marker that indicates which T cells will go into the follicles and which ones will stay out. A sustained appearance of the cell-surface protein, Tigit, identifies the Tigit-positive PD-1^+CXCR5^+CD4^+ T cells, or pre-Tfh, that will become GC-Tfh cells, they report in a study published in *Nature Communications*.

"Which of the PD-1^+CXCR5^+CD4^+ T cells will differentiate into

PD-1^{hi}CXCR5^{hi} GC-Tfh cells while the others have a different fate is a long-standing question in the Tfh field," said Hu, an associate professor in the UAB Department of Microbiology.

Importantly, the Tigit-negative PD-1^{+}CXCR5^{+}CD4^{+} T cells are found to be the precursors to long-lived memory CXCR5^{+}CD4^{+} T cells that will quickly awaken the immune response to a later infection by the same pathogen.

Hu and co-first authors Fangming Zhu, and Ryan J. McMonigle, M.D., Ph.D., UAB Department of Microbiology, also looked into this formation of memory T cells. It has been observed that the surface marker CCR7 is re-expressed in CXCR5^{+}CD4^{+} T memory cells rather than CXCR5^{-}CD4^{+} T memory cells, leading to the recognition by some of CCR7^{+}CXCR5^{+}CD4^{+} T cells as central memory CD4^{+} T cells.

"In our study, using both influenza virus infection and protein immunization models, we have discovered conditions under which CCR7 and CD62L can be biased to CXCR5^{+} T cells or equally expressed between CXCR5^{-}CD4^{+} T memory cells and CXCR5^{+}CD4^{+} T memory cells," Hu said. Hu and colleagues found that the strength of the T cell response—as influenced by the competition within the local environment and the location in the draining versus non-draining secondary lymphoid organs—and the type of challenge, viral infection or antigen immunization, play critical roles in determining the central memory phenotype of CD4^{+} T cells.

"Given the historical context and the current controversy surrounding CXCR5^{+} 'central memory' CD4^{+} T cells, and also the differences between CXCR5^{-}CD4^{+} T cells and CXCR5^{+}CD4^{+} T cells, our findings will prompt the field to reassess how we describe the different memory CD4^{+} T cells," Hu said. "To what extent that GC-Tfh cells give rise to memory CXCR5^{+}CD4^{+} T cells is also an open question," Hu added.

Numerous previous studies and many current studies still do not separate PD-1^{+}CXCR5^{+}Bcl6^{+} pre-Tfh cells from PD-1^{hi}CXCR5^{hi}Bcl6^{hi} germinal center-Tfh cells, Hu says. The UAB researchers also showed that pre-Tfh cells undergo extended differentiation in transition to GC-Tfh cells, as seen in substantial changes in differential gene expression—where genes are either activated or turned off. They also found differences in chromatin accessibility during differentiation—where the packaging of DNA in the chromosome is loosened to allow gene expression.

"Importantly, we found that c-Maf, a transcription factor that has been reported to be involved in Tfh cell differentiation, exerts a critical function specifically in the transition step from pre-Tfh cells to GC-Tfh cells," Hu said. "More excitingly, we have identified Plekho1, a c-Maf downstream factor, as a novel regulator that plays stage-specific roles in GC-Tfh cell differentiation."

"Our study has clarified several important but controversial points in Tfh cell differentiation and provided compelling evidence to elucidate the intricacy of GC-Tfh cell differentiation and the varied CD4^{+} memory T cells," Hu said.

These findings have opened many new avenues to study the fundamental mechanisms in Tfh cell differentiation and CD4^{+} T cell memory, Hu says. How a cell takes on one fate over another and how that fate is maintained is one of the most fundamental questions in biology. Understanding the various stages, cell subsets and the underlying regulatory mechanisms of Tfh cell differentiation can facilitate the design of new strategies to treat infectious diseases and autoimmune disorders and can aid vaccine development for new pandemic threats.

More information: Fangming Zhu et al, Spatiotemporal resolution of germinal center Tfh cell differentiation and divergence from central memory CD4+ T cell fate, *Nature Communications* (2023). DOI:

10.1038/s41467-023-39299-3