The active inner life of naïve T cells

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Published in:
Nature Immunology

DOI:
10.1038/s41590-020-0726-1

Publication date:
2020

Document Version
Peer reviewed version

Title: The active inner life of naïve T cells

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Two sentence summary:

Quantitative systems-level proteomics is cleverly used to reveal a dynamic program of protein turnover in naïve and memory CD4 T cells which, alongside a stockpile of metabolic protein machinery, poises the cells for activation.

Main text:

Inherent to our understanding of adaptive immunity is that naïve T cells with a broad repertoire of antigen receptors patrol the body, remaining in a quiescent, resting state until they encounter their target antigen. Upon recognition of their target antigen they undergo a massive shift in metabolism and biosynthesis, driving an approximate tripling in size over the first day or so, followed by a burst of rapid proliferation and differentiation to produce the effector cells that then clear the invading pathogen. In the face of such massive changes during activation, naïve T cells are frequently overlooked as being inactive, indolent and inert. In the current issue of Nature Immunology, Wolf, Jin and colleagues[1] bust the myth of the "quiescent" naïve T cell; revealing they have rapid protein turnover of key transcription factors, cytokine receptors and adhesion molecules, and an idling reservoir of translational protein machinery and glycolytic enzymes; dynamically maintaining the naïve cells in a state prepared for activation.

This study gives us a powerful new window into understanding the biology of naive T cells. A key strength of this work is the use of 'heavy' labeled amino acid incorporation to delineate newly synthesized proteins, combined with quantitative proteomics and transcriptomics methods to estimate absolute copy numbers per cell of proteins and mRNAs in naïve and

activated T cells over multiple time points. This data is assembled into an easily interrogated website, www.immunomics.ch , and creates a valuable resource to explore the protein turnover kinetics of naïve cells and mRNA to protein translation dynamics as human $CD4^+$ T cells respond to immune activation. This resource sits alongside others that have also used high resolution mass spectrometry to quantitate how human[2] and murine $CD4^+$ and $CD8^+$ T cells reshape their protein signaling, protein expression and intracellular protein complexes as they respond to immune challenge[3, 4, 5]. Proteins are the main mediators of functional processes in cells and the current study highlights once more how quantitative proteomics can provide novel insights that are unobtainable by analysis of mRNA alone.

Wolf et al. cleverly used these quantitative data to gain new insights into T cell biology (Figure 1)[1]. One intriguing observation were the estimated protein synthesis rates across different T cell activation states: naive T cells synthesized ~60,000 proteins min^{-1}; this increased to ~300,000 proteins min^{-1} after 6 h of activation and ~800,000 proteins min^{-1} after 24 h of activation. What were the proteins that rapidly turn over in the naive cells? Interestingly Wolf et al. observed that they include proteins with known roles in maintaining and enforcing T cell quiescence, such as the interleukin 7 receptor alpha chain ($t_{1/2}$ = 3.9 h) , transcription factors Ets1 ($t_{1/2}$ = 53 min) and Foxo1 ($t_{1/2}$ = 5.0 h), cell cycle repressors CDKN1B ($t_{1/2}$ = 13.2 h), and the adhesion molecule CD62L ($t_{1/2}$ = 1.0 h), required for naive T cell homing into secondary lymphoid tissue. The short half-life of proteins that maintain T cell quiescence would ensure that these proteins could be rapidly destroyed once they were transcriptionally silenced in response to immune activation, even if there were no further post-translational regulation to drive their degradation. Conversely, proteins with longer half-lives, such as glycolytic enzymes and ribosomes, were found in this[1] and a previous study[6] to provide the naive T cell with a stockpile of 'idle' machinery that can be rapidly engaged to switch metabolism and increase biosynthesis when T cells activate. The idea that naïve T cells are epigenetically poised for activation is an established concept[7], but the present study has revealed another facet of how T cells are prepared to respond rapidly to immune challenge[1].

Another key question in T cell biology that this quantitative dataset addresses is: how do memory and naive T cells differ? The authors estimate the total translation rate and translation rate per ribosome in naïve, memory and activated T cells and show a higher basal translation rate of human memory $CD4^+$ T cells than naïve T cells is due in part to greater ribosomal activity. This finding gives some new understanding of how memory cells are translationally more capable than naïve T cells, and could more rapidly produce effector cytokines, for which memory cells also have pre-formed mRNA[8], in response to recall challenge. This study by Wolf et al[1] also has a systematic overview of which mRNAs are pre-formed and translationally repressed in the naïve T cells, adding to the small list of specific examples that have previously been identified[6]. In this era of single-cell RNA sequencing to define lymphocyte populations, the current dataset will be especially valuable for understanding which transcripts are likely to be discordant with protein expression.

One caveat to the present study is that the experimental strategy used to obtain estimates of protein turnover relied on the uptake of 'heavy' labeled amino acids from the environment. These experiments are elegant but it is known that naive T cells have very low levels of amino acid transport compared to immune activated cells [3, 9]. Indeed, the increases in protein turnover rates reported in the current study correlate well with what is known about the timing of increases in amino acid transport in response to T cell activation[4, 9]. Estimates of protein turnover based on incorporation of externally provided 'heavy' labeled amino acid could miss the de novo synthesis of proteins fueled by intracellular amino acids made available by recycling processes such as autophagy. In this context, it is well established that that naive T cells are dependent on key autophagy proteins for survival[10], supporting an important role for intracellular recycling of amino acids by autophagy in maintaining protein turnover in naïve cells. However, it should be emphasized that these technical considerations mean that the protein turnover in naive T cells may in fact be higher than predicted herein. The interesting conclusion made by the authors stands: in a quiescent naïve T cell protein degradation and re-synthesis occurs at a rapid rate for a subset of important naïve cell proteins.

In summary, this fascinating study by Wolf et al. sets out a narrative where naïve T cells must actively produce protein to maintain their quiescence and by doing this, they poise

themselves for rapid responses to immune activation. This quantitative exploration of fundamental T cell biology acts as a springboard for posing many interesting questions for the field: How much of this protein turnover is intrinsic to the proteins themselves and to what extent is this controlled by external signals? IL-7 and tonic T cell receptor signaling are known to be critical for naïve T cell survival and homeostasis – how do these contribute to protein turnover in the naïve T cell? Moreover, the current experimental tour du force is limited to $CD4^+$ T cells - do the same principles apply in other T cell subsets? For example, $CD8^+$ T cells, even in the naïve state, have more pre-formed translational machinery [3] – would the protein turnover differ in these cells? Does this contribute to the differences in homeostatic proliferation rates between the $CD4^+$ and $CD8^+$ T cells[11]? What about other T cell subsets, such as regulatory T cells or other immune cell types? Is there a different set of lineage specific proteins that are rapidly degraded and re-synthesized in these cells in order to poise them for activation? The rapid turnover of key proteins that maintain T cell quiescence also raises the question of how much of the amino acid fuel for these proteins comes from external amino acids, linking the maintenance of T cell quiescence to nutrient availability in the diet. Could dietary restriction or a disruption in naïve cell protein translation break quiescence and be a factor in the development of autoimmunity? Importantly, this work brings naïve cells back to the fore as a critical cell we need to understand in order to comprehend adaptive immunity.

Figure 1. Translation dynamics and capacity of naïve and activating T cells

Naïve T cells rapidly lose and replenish expression of a subset of proteins important for T cell quiescence. Naïve T cells have an "idling' pool of ribosomes and low amino acid uptake. As T cells activate total protein translation rate increases, initially by fully engaging the ribosome pool, then by increasing the ribosome number; this transition occurs alongside a rapid increase in amino acid uptake. Once T cells activate proteins important for T cell naivety are destroyed and not replenished.

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T cells:	Naive	6 h activated	24 h activated
synthesis rate:	**60,000** proteins / min (no net change protein content)	**300,000** proteins / min (protein content increased)	**800,000** proteins / min (protein content increased)