Published in final edited form as:

Cell Calcium. 2017 May ; 63: 66–69. doi:10.1016/j.ceca.2017.01.014.

Calcium–NFAT transcriptional signalling in T cell activation and T cell exhaustion

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Abstract

A cornerstone of the adaptive immune response is the T cell receptor (TcR)–calcium–calcineurin signalling pathway leading to T cell activation. The ‘nuclear factor of activated T cells’ proteins NFAT1, NFAT2, and NFAT4 are transcription factors that promote expression of a panel of genes required for activation. It has become apparent that these same NFAT transcription factors underlie an alternative transcriptional program in T cells that serves to limit the immune response. This duality in NFAT transcriptional functions raises the possibility that NFAT transcriptional complexes could be targeted therapeutically to alter the relative strength of the effector and alternative transcriptional programs, thereby modulating immune responses.

T lymphocytes recognize antigens through a TcR, and ‘decide’ on a response by integrating the resulting intracellular signals with other signals impinging on stimulatory and inhibitory cell surface receptors. Key steps in T cell activation include expression of cell surface receptors and receptor ligands to communicate with other cells, induction of cytokines to orchestrate the functions of other immune cells, and— in CD8⁺ T cells— expression of cytolytic effector proteins. The central role of NFAT-family transcription factors in expression of these activation markers has long been recognized [Figure 1A]. To appreciate that NFAT transcriptional complexes could be therapeutic targets, it is helpful to review the original experiments that implicated NFAT in T cell activation, and the newer evidence linking NFAT to a second opposing transcriptional program [Figure 1B].

NFAT in T cell activation

The earliest tangible definitions of ‘NF-AT’ were as a nuclear factor that could bind to the NFAT-1 (alternatively termed IL2-IIA) binding site in the IL2 promoter [1,2] or to the CLEO element in the GM-CSF promoter [3]. This factor was soon parsed experimentally into an NFAT subunit and an AP1 family dimer [4–6]. The NFAT-family members NFAT1 and NFAT2 were purified based on their affinity for the ARRE2 site of the IL2 promoter and

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subsequently cloned [7,8]. In all there are four calcium– calcineurin-activated NFAT proteins in mammalian cells, NFAT1–4 [7–9], with NFAT1, NFAT2, and NFAT4 relevant in T cells.

Subsequent studies cemented the association of NFAT with T cell activation. The known block of the appearance of a nuclear NF-AT complex by cyclosporin A (CsA) [10–12] was explained by the direct demonstration that CsA inhibits the dephosphorylation and therefore the activation of NFAT [13,14]. The recognition of NF-AT target sites in the promoter/enhancer regions of an increasing number of T cell activation genes, and evidence for an essential role of NFAT in the induction of numerous effector cytokines and T cell activation markers, also reinforced the association. However, as detailed below, the NFAT-dependent activation program is only one facet of the story.

These insights into NFAT signalling shed additional light on the requirement for costimulation in the T cell activation program— that is, the fact that the T cell activation program is not turned on effectively by stimulation through the TcR alone, or by artificial provision of the cytoplasmic calcium signal that is a primary intermediary of TcR stimulation. NFAT-family proteins are activated by the calcium signal downstream of TcR stimulation, but strong induction of other transcription factors, including AP1, depends on parallel activation of a second intracellular signalling pathway. The NF-AT complex binds to composite sites in DNA, consisting of closely spaced subsites recognized individually by its components NFAT and AP1, as illustrated in the crystal structure of NF-AT bound to a site from the IL2 promoter [15]. Composite NF-AT sites in the gene regulatory regions for cytokines and cell surface markers induced upon T cell activation characteristically exhibit relatively weak binding of NFAT in the absence of AP1 [16]. This weak binding, paired with the low cellular levels of NFAT, explains the impaired upregulation of the NFAT-dependent genes of the T cell activation program when NFAT binding is not stabilized by AP1.

T cell unresponsiveness

Paradoxically, stimulation through the TcR alone, without costimulation, is not merely a poor activating stimulus, but can in fact induce a state of sustained unresponsiveness or reduced responsiveness to a later physiological stimulus. A thoughtful review of the literature in this area up to 2002 [17] sets the historical context for the line of experimentation that tied NFAT to T cell unresponsiveness. The field has continued to evolve, and its current competing definitions of ‘tolerance’, ‘anergy’ in CD4⁺ helper T cells, ‘exhaustion’ in CD8⁺ cytolytic T cells, and of differing degrees of unresponsiveness, will not be reviewed here. Rather, the focus will be on the common theme of reduced responsiveness to TcR stimulation, and on the NFAT-driven genetic program(s) underlying this reduced responsiveness.

NFAT was first convincingly linked to T cell unresponsiveness in a study of CD4⁺ T cell ‘anergy’ [18]. That study confirmed earlier evidence that a TcR signal or calcium signal unaccompanied by a second costimulatory signal leads to sustained unresponsiveness [19] and that CsA prevents the switch to an anergic state [20]. Two important new findings were that the switch is much less pronounced or absent in NFAT1^{-/-} T cells, and that mere prior expression in T cells of NFAT1 engineered to be constitutively active can reduce the

response to physiological stimulation [18]. The latter finding is not an artifact of NFAT1 overexpression, because the constitutively active NFAT1 is in fact present at lower levels than the endogenous NFAT1 in wildtype T cells, probably because the active form of NFAT is more susceptible to protein degradation.

A second NFAT genetic program

These findings pointed to the existence of a second calcium–calcineurin–NFAT-dependent transcriptional program in T cells. A set of ‘anergy-associated’ genes was defined as mRNAs preferentially induced in CD4⁺ T cells by a calcium signal alone, under the conditions that induce anergy [18]. The set includes genes encoding tyrosine phosphatases, several proteins capable of negatively regulating cellular signalling, E3 ligases and other components of cellular proteolytic pathways, and negative transcriptional modulators [18,21]. Consistent with their identification as part of an NFAT-dependent program, many of the anergy-associated genes show significantly lower expression, after stimulation, in NFAT1^{-/-} CD4⁺ T cells than in wildtype cells [18]. Importantly, the anergy program does not depend on NFAT-AP1 transcriptional complexes, since NFAT1(RIT)— an engineered version of NFAT1 that cannot interact effectively with AP1 [22]— elicits the same unresponsiveness as wildtype NFAT1 and upregulates at least a subset of the identified anergy-associated genes [18]. Although the programmed unresponsiveness of CD8⁺ cytolytic T cells is not expected to mirror identically that of CD4⁺ T cells, the general finding that NFAT1(RIT) elicits reduced responsiveness is also true of CD8⁺ T cells [23].

The engineered NFAT1(RIT) has afforded one way to compile a list of NFAT-dependent genes that are upregulated in T cells in the absence of the T cell activation program. In both CD4⁺ and CD8⁺ T cells, many of these genes encode negative regulatory proteins that impinge on early steps in TcR/costimulatory signalling, or negative transcriptional modulators [23]. Inhibitory cell surface receptors that have become clinical targets in ‘checkpoint blockade’ therapy [24,25] are also selectively induced by NFAT(RIT) [23]. Chromatin immunoprecipitation–DNA sequencing finds a strong enrichment of NFAT(RIT) binding in gene promoter regions [23], indicating that NFAT binds directly to the regulatory regions of anergy/exhaustion genes in many cases, as was established previously for effector genes.

The genes upregulated in T cells expressing constitutively active NFAT1(RIT) overlap considerably with the genes previously found to be upregulated *in vivo* in anergic CD4⁺ T cells or exhausted CD8⁺ T cells [23,26–28]. There is not a one-to-one correspondence, nor is an exact correspondence expected. In part, the differences reflect the fact that expression of constitutively active NFAT1(RIT) by itself does not mimic NFAT activation together with cooperating transcription factors during physiological stimulation. In part, they reflect the real differences in CD4⁺ and CD8⁺ T cells, and the existence of diverse states of reduced responsiveness [29]. Beneath the diversity, though, is the commonality that NFAT-family proteins have an important role in promoting unresponsiveness in both CD4⁺ and CD8⁺ T cells, and participate directly in upregulating a set of genes associated with unresponsiveness.

Practical implications

RNA sequencing shows that both the activation and the unresponsiveness gene programs are invoked simultaneously during T cell activation [18,23]. Since NFAT by itself binds weakly even to a consensus NFAT site, and since NFAT concentrations in the nucleus are likely to be limiting, the extent to which individual genes are upregulated will depend on the availability of specific NFAT partner proteins and the stability of the corresponding NFAT-partner complexes. This logic suggests that it would be possible to rebalance T cell responses by targeting specific NFAT transcriptional complexes [Figure 2].

As a concrete example, the NFAT-AP1 contact surface that is a physical basis for the T cell activation program is conserved across the NFAT family [15], and the data obtained with NFAT(RIT) show that this interface is not needed for the NFAT-dependent unresponsiveness program [18,23]. Therefore a compound that inhibits formation of NFAT-AP1 transcriptional complexes would be expected to curtail an unwanted immune response, and a compound that stabilizes the NFAT-AP1 interaction would favor an enhanced response. The converse logic could be applied to NFAT partners underlying the opposing transcriptional program, once they have been identified. Targeting NFAT transcriptional complexes, if successful, could be a valuable new approach to modulating immune responses.

Acknowledgments

The author's work on calcium signalling and on T cell transcriptional programs is funded by US National Institutes of Health grants AI109842, AI084167, GM110397, and AI040127.

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Highlights

NFAT-AP1 complexes drive a transcriptional program of T cell activation

Other NFAT complexes favor upregulation of genes that oppose activation

Specific NFAT complexes could be therapeutic targets in immune disease

Figure 1.

A, Parallel signalling pathways from the T cell receptor and a costimulatory receptor (for instance, CD28) converge in the T cell nucleus, where NFAT and AP1 are critical players in expression of a panel of activation genes. B, Signalling from the T cell receptor alone, without costimulation, acts through NFAT to upregulate a panel of genes opposing T cell activation.

Figure 2.

Cartoons depicting NFAT complexes bound to gene regulatory sites. The complex with AP1 upregulates genes associated with T cell activation. Complexes with an unidentified partner protein (or partner proteins) upregulate genes associated with T cell unresponsiveness. The NFAT-AP1 protein-protein interface involved in T cell activation is not needed for the opposing transcriptional program, suggesting that the two classes of NFAT complexes could be targeted separately to modulate immune responses.