

Role of the Cyclosporin-sensitive Transcription Factor NFAT1 in the Allergic Response

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Proteins belonging to the NFAT (nuclear factor of activated T cells) family of transcription factors are expressed in most immune cell types, and play a central role in the transcription of cytokine genes, such as IL-2, IL-4, IL-5, IL-13, IFN- γ , TNF- α , and GM-CSF. The activity of NFAT proteins is regulated by the calcium/calmodulin-dependent phosphatase calcineurin, a target for inhibition by CsA and FK506. Recently, two different groups have described that mice lacking the NFAT1 transcription factor show an enhanced immune response, with tendency towards the development of a late Th2-like response. This review evaluates the possible role of NFAT proteins in the Th2 immune response and in the eosinophil-mediated allergic response.

Key words: nuclear factor of activated T cells - interleukin - cyclosporin

The cytokine profiles of T cells differentiating down the Th1 and Th2 pathways have been described, Th1 cells preferentially produce IL-2 and IFN- γ , whereas Th2 cells produce IL-4, IL-5, IL-10 and IL-13 (Paul & Seder 1994, Carter & Dutton 1996). In many pathological situations, the balance between Th1 and Th2 immune response determines the outcome of different immunologically-mediated clinical syndromes including infectious, autoimmune, and allergic diseases (Carter & Dutton 1996).

Allergic disease is a broad range of disorders including rhinitis, conjunctivitis, systemic anaphylaxis, and asthma (Casolaro et al. 1996, Drazen et al. 1996). Atopic allergy is characterized by increased synthesis of IgE antibodies through the actions of IL-4 and IL-13 in B cell Ig isotype class switching, directed at groups of antigens that activate the CD4-dependent Th2-like immune response (Romagnani 1995). The IgE produced binds to Fc ϵ receptors present on the surface of mast cells and basophils, priming them for activation by antigen, and triggers the release of vasoactive mediators, chemotactic factors and cytokines (Romagnani 1995, Drazen et al. 1996). In addition, eosinophils are also involved in the pathogenesis of allergic reactions, as these cells accumulate at the sites of allergic inflammation and significantly contribute to the tissue damage (Desreumaux & Capron 1996).

In asthma, some data suggest that the severity of the disease is related to the degree of inflammation (Peters 1990, Broide et al. 1991, Pare & Bai 1995), and that the magnitude of the asthmatic response is related to the number of eosinophils present in the lung (Bradley et al. 1991). Moreover, suppression of eosinophil accumulation at the site of inflammation impairs the development of asthma disease (Wagner et al. 1990, Foster et al. 1995). These data suggest that eosinophils could be a central mediator of the pathogenesis of allergic disease. We can hypothesize three sequential and interacting events for how eosinophils mediate inflammation at the site of allergic response, described as follows: first, eosinophil differentiation and maturation in the bone marrow; second, rolling, adhesion, and migration in the inflamed vascular endothelium; and third, activation and degranulation in the target organ (Fig. 1).

Eosinophil tissue infiltration is coordinated by an interacting network of cytokines, chemokines, adhesion molecules, and inflammatory mediators. In fact, GM-CSF, IL-3 and IL-5 have been described as factors that induce differentiation, maturation and proliferation of bone marrow eosinophils (Sanderson et al. 1985, Lopez et al. 1986, Clutterbuck & Sanderson 1988, Takatsu et al. 1988, Warren & Morre 1988, Yamaguchi et al. 1988). However, blood eosinophilia is not related to eosinophil tissue accumulation (Dent et al. 1990, Desreumaux et al. 1996), suggesting that overproduction of eosinophils is not enough for tissue infiltration by these cells, and chemoattractant production at the site of inflammation is essential for eosinophil recruitment.

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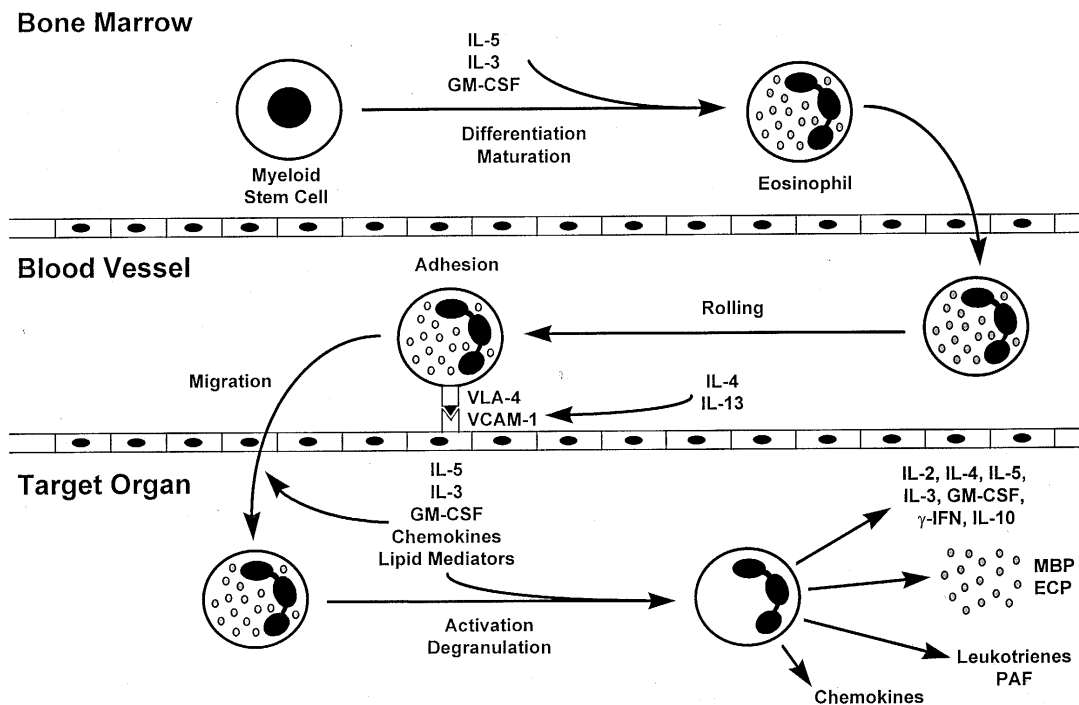


Fig. 1: a simplified view of eosinophil differentiation and maturation, in the bone marrow; rolling, adhesion and migration, in the blood vessel; and activation and degranulation, in the target organ. Cytokines, growth factors, adhesion molecules, chemokines, and lipid mediators that play a role in each step are indicated.

Rolling and adhesion of eosinophils on vascular endothelium is the first step for eosinophil infiltration in the target organ, and may depend on several adhesion molecules (Desreumaux & Capron 1996). However, eosinophils are the only granulocytes that express VLA-4, and may selectively bind to endothelial cells via VCAM-1 (Weller et al. 1991, Pretolani et al. 1994, Nakajima et al. 1994, Wardlaw et al. 1994). Moreover, it has been described that IL-4 and IL-13 upregulate VCAM-1 on human endothelial cells (Bochner et al. 1995), suggesting that interaction between VLA-4/VCAM-1 play a central role in eosinophil migration during the allergic response.

Eosinophil migration into inflamed tissue involves several chemoattractant mediators, including cytokines, chemokines and lipid mediators, and occurs after adhesion to the vascular endothelium (Desreumaux & Capron 1996). GM-CSF, IL-3 and IL-5, are the key cytokines influencing eosinophil migration and activation (Broide et al. 1992, Weller 1993, Sullivan & Broide 1996). In fact, several reports demonstrated that IL-5 plays the central role in eosinophil-mediated allergic responses, since this cytokine is a selective chemoattractant for eosinophils (Sehmi et al. 1992), and has the ability to

prime and activate these cells (Coeffier et al. 1991, Sehmi et al. 1992, Warringa et al. 1992). In addition, IL-5 deficient mice do not show eosinophilia (Kopf et al. 1996), and fail to develop airway hyperresponsiveness and eosinophil infiltration in an experimental model of asthma (Foster et al. 1995). Other important chemoattractants and activators of eosinophils are the C-C subfamily of chemokines (Desreumaux & Capron 1996). The eosinophil active chemokines include RANTES, MCP-2, MCP-3, MCP-4, MCP-5, MIP-1 α , and eotaxin (Jia et al. 1996, Kita & Gleich 1996, Sarafi et al. 1997). Eotaxin, first described in guinea pigs and subsequently in mice and humans, is a potent and specific eosinophil chemoattractant (Jose et al. 1994, Gonzalo et al. 1996, Ponath et al. 1996), and disruption of the eotaxin gene partially reduces tissue eosinophil infiltration in a model of allergic response (Rothenberg et al. 1997).

Once eosinophils infiltrate the inflamed tissue, they degranulate and secrete several proinflammatory mediators and cytokines (Weller 1993). Activated eosinophils release their granule proteins, including the major basic proteins, eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (Desreumaux & Capron

1996). They also secrete lipid mediators, chemokines, and cytokines, which amplify the response and generate a feedback loop that perpetuates the allergic inflammatory response (Drazen et al. 1996). Together, these inflammatory mediators and cytokines generate tissue damage that could be related with the clinical symptoms of the different allergic diseases.

NFAT TRANSCRIPTION FACTORS IN IMMUNE RESPONSE

Many of the cytokines that regulate eosinophil function are under the control of proteins belonging to the NFAT (nuclear factor of activated T cells) family of transcription factors. These proteins play a key role in the regulation of cytokine gene transcription during the immune response (Crabtree & Clipstone 1994, Rao 1994, Jain et al. 1995b). The NFAT family encodes four distinct classes of proteins: NFAT1 (formerly NFATp), NFAT2 (NFATc), NFAT3 and NFAT4 (NFATx) (Rao et al. 1997). NFAT1, the first identified member of the family, was cloned from murine (Ar-5) and human (Jurkat) T cell cDNA libraries (McCaffrey et al. 1993, Luo et al. 1996). A distinct protein, NFATc (NFAT2), later was also cloned from a Jurkat T cell cDNA library (Northrop et al. 1994). cDNA clones encoding three other NFAT proteins: NFAT3, NFAT4 and NFATx (isoform of NFAT4), were isolated from Jurkat T cell, human peripheral blood (PBL) and human thymus cDNA libraries (Ho et al. 1995, Masuda et al. 1995).

Despite their name, NFAT proteins are expressed not only in T cells, but also in other classes of immune and non-immune cells. At the protein level, NFAT1 and NFAT2 are expressed in peripheral T cells and T cell lines, and NFAT1 is also expressed in B cells, mast cells, NK cells, monocytes and macrophages (Ho et al. 1994, Aramburu et al. 1995, Ruff & Leach 1995, Wang et al. 1995, Weiss et al. 1996). Moreover, NFAT1 is expressed in a neuronal cell line and in the nervous system (Ho et al. 1994), and an endothelial cell line (Cockerill et al. 1995a, Wang et al. 1995). NFAT1 and NFAT2 mRNAs are expressed in peripheral lymphoid tissue (spleen and PBL), and NFAT2 mRNA is upregulated in activated T cells and NK cells (Northrop et al. 1994, Aramburu et al. 1995, Hoey et al. 1995, Masuda et al. 1995, Park et al. 1996). NFAT4 mRNA is expressed at high levels in the thymus (Hoey et al. 1995, Ho et al. 1995, Masuda et al. 1995), and NFAT3 is expressed at low levels in lymphoid tissues (Hoey et al. 1995).

Several isoforms have been described for NFAT1, NFAT2 and NFAT4. Sequence homology represented in all the isoforms suggests two different domains, comprising the DNA-binding do-

main (DBD) and the NFAT homology region (NHR) (Jain et al. 1995a, Luo et al. 1996). The DBD, which is located between amino acid residues 400 and 700, is highly conserved within the NFAT family, and shows moderate sequence similarity to the DNA-binding domains of Rel-family proteins (Nolan 1994, Jain et al. 1995a, Chytil & Verdine 1996). This domain contains the highly conserved RAHYETEG sequence in which residues contact DNA (Jain et al. 1995a, Chytil & Verdine 1996). The NHR is located in the N-terminal region, comprising 300 amino acids, and shows a strong conservation of several sequence motifs characteristic of the NFAT family (Ho et al. 1995, Hoey et al. 1995, Masuda et al. 1995, Luo et al. 1996) (Fig. 2).

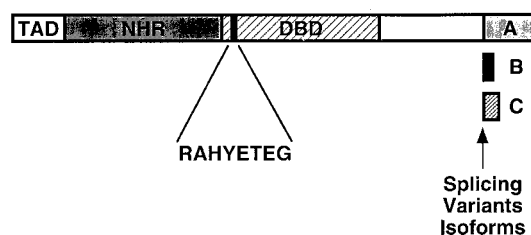


Fig. 2: schematic diagram of the primary structure of the NFAT1 protein, as deduced from analysis of cDNA clones. The region of highest homology within NFAT proteins is the DNA-binding domain (DBD), which shows similarity to the Rel homology region of Rel-family transcription factors, and encodes the amino acids that contact DNA. Other regions such as transactivation domain (TAD), NFAT homology region (NHR), and splicing variants isoforms are indicated.

NFAT transcription factors are cytosolic proteins constitutively expressed in resting cells (Rao et al. 1997). NFAT proteins are activated by stimulation of receptors coupled to calcium mobilization, such as the antigen receptors on T and B cells (Yaseen et al. 1993, Choi et al. 1994, Crabtree & Clipstone 1994, Rao, 1994, Venkataraman et al. 1994, Jain et al. 1995b, Serfling et al. 1995, Loh et al. 1996b), Fcε receptors on mast cells and basophils (Hutchinson & McCloskey 1995, Prieschl et al. 1995a, Weiss et al. 1996), the Fcγ receptors on macrophages and NK cells (Aramburu et al. 1995), and receptors coupled to heterotrimeric G proteins (Desai et al. 1990, Wu et al. 1995, Boss et al. 1996). Three different steps of activation have been defined for NFAT proteins: dephosphorylation, nuclear translocation, and DNA binding. In resting cells, NFAT proteins are phosphorylated and cytoplasmic, and show a low affinity for DNA (Shaw et al. 1995). Stimuli that trigger calcium mobilization result in rapid dephosphorylation of

NFAT proteins and their translocation to the nucleus, and dephosphorylated proteins show increased affinity for DNA (Shaw et al. 1995, Loh et al. 1996a,b). Receptor stimulation and calcium mobilization result in activation of the calmodulin-dependent phosphatase calcineurin (Weiss & Littman 1994). Each step of NFAT activation is blocked by the calcineurin inhibitors CsA or FK506, suggesting that calcineurin is a major upstream regulator of NFAT proteins, and that dephosphorylation is the initial step of NFAT activation (Fig. 3).

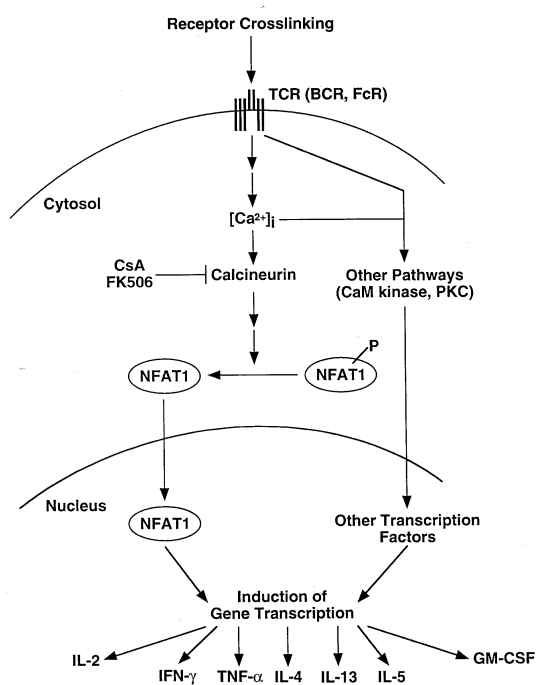


Fig. 3: signal transduction mechanisms leading to transcription of cytokine genes in activated T cells (and other cells of the immune system) upon stimulation through surface receptors capable of mobilizing calcium. Abbreviations: TCR, T-cell receptor; BCR, B-cell receptor; FcR, Fcγ and Fcε receptors; CsA, cyclosporin A; PKC, protein kinase C; CaM kinase, calmodulin-dependent kinase; P, phosphorylation.

Stimulated cells inducibly transcribe a large number of genes, such as genes encoding transcription factors, signalling proteins, cytokines, cell surface receptors, and other effector proteins (Leonard et al. 1987, Crabtree 1989, Cockerill et al. 1995b, Kelly & Siebenlist 1995). NFAT was first identified in T cells as a rapidly-inducible nuclear factor binding to the distal antigen receptor response element of the human IL-2 promoter (Shaw et al. 1988). Over the next few years, studies from several laborato-

ries indicated that the promoter/enhancer regions of different activation genes possessed binding sites for NFAT family proteins, including the cytokines IL-2 (Shaw et al. 1988, Mattila et al. 1990, Randak et al. 1990, Brabletz et al. 1991, Ullman et al. 1991, Jain et al. 1993a, Rooney et al. 1995a), IL-4 (Casalero et al. 1995, Rooney et al. 1995b), IL-5 (Prieschl et al. 1995a,b), IL-13 (Dolganov et al. 1996), IFN-γ (Brown et al. 1991, Campbell et al. 1996), TNF-α (McCaffrey et al. 1994, Tsai et al. 1996), and GM-CSF (Cockerill et al. 1995a,b), as well as the cell surface receptors CD40L (Schubert et al. 1995, Tsytsykova et al. 1996), and CTLA-4 (Perkins et al. 1996). Based on comparison of these sequences, the NFAT binding site is presented as a 9 bp element, possessing the consensus sequence (A/T)GGAAA(A/N)(A/T/C)N (Rao et al. 1997). In addition, NFAT proteins show a characteristic ability to cooperate with AP-1 proteins in DNA-binding and transactivation (Rao 1994). The interaction between NFAT proteins and AP-1 involves binding of these transcription factors to adjacent sites on DNA, and results in stabilisation of the NFAT-DNA interaction (Jain et al. 1993a,b, Chen et al. 1995). Note that cytokines that are inhibited by CsA or FK506 have present in their promoter regions binding sites for NFAT proteins, suggesting that NFAT transcription factors are major targets of these immunosuppressive drugs (Rao et al. 1997).

Cytokine expression by different cells of the immune system plays a central role in the immune response, and each cell type produces a characteristic pattern of cytokines (Paul & Seder 1994). The immune response is coordinated by an interacting network of transcription factors that dictate expression of different cytokines (Crabtree 1989, Paul & Seder 1994). To study the unique functions of the NFAT1 transcription factor in the *in vivo* immune response, two different groups generated mutant mice carrying a disrupted NFAT1 gene (Hodge et al. 1996, Xanthoudakis et al. 1996). Although all reports indicate that the NFAT1 transcription factor is a positive regulator of cytokine expression, surprisingly, certain primary and secondary immune responses in mice lacking NFAT1 gene were enhanced, such as increased intrapleural accumulation of eosinophils and increased serum IgE levels in an *in vivo* model of allergic inflammation (Xanthoudakis et al. 1996), increased serum IgE levels in response to immunization with TNP-ovalbumin (Hodge et al. 1996), and more efficient differentiation towards a Th2 phenotype in spleen cells stimulated *in vitro* with IL-4 and anti-CD3 (Hodge et al. 1996). In the next section we discuss the possible role of the NFAT1 transcription factor in the Th2 immune response and eosinophil-mediated allergic inflammation.

ROLE OF NFAT1 TRANSCRIPTION FACTOR IN THE ALLERGIC RESPONSE

The response of the immune system to antigen is coordinated by an interacting network of transcription factors that dictate expression of different effector proteins that regulate the immune response (Crabtree 1989, Paul & Seder 1994). However, it is not known how the same stimuli can be responsible for encoding the specificity of cellular response. Recently, it has been described that different calcium signalling patterns can activate different transcription factors, demonstrating that the same second messenger can drive specificity in signalling to the nucleus (Dolmetsch et al. 1997). Nevertheless, the molecular basis for the tissue-specific expression of Th1/Th2-like cytokines has remained elusive. Over the next few years, several groups have been described important advances in signaling and gene transcription in the immune system using *in vivo* gene disruption.

In order to address the specialized functions of NFAT1 transcription factor in the *in vivo* immune response, mutant mice carrying a disrupted NFAT1 gene have been described (Hodge et al. 1996, Xanthoudakis et al. 1996). In both cases the targeted exon was in the DNA-binding domain encoding the Rel-homology region (see above), and the disruption resulted either in the expression of a truncated protein without DNA-binding activity (Hodge et al. 1996), or in no protein expression (null phenotype) (Xanthoudakis et al. 1996). Except for a moderate degree of splenomegaly, NFAT1-deficient mice developed normally, did not exhibit any obvious behavioral deficiencies, and were immunocompetent.

In the primary immune response, NFAT1-deficient mice showed no impairment in IL-2, IL-4, IFN- γ and TNF- α production by *in vitro* stimulation of spleen cells with anti-CD3 antibody or Con A (Xanthoudakis et al. 1996). However, in an *in vivo* model of primary response NFAT1-deficient mice showed an early impairment of several cytokines, such as IL-4, IL-13, TNF- α and GM-CSF, and cell surface receptors, including CD40L and FasL (Hodge et al. 1996). These results suggest that the NFAT1 protein played an important role in the primary *in vivo* immune response that could not have been predicted from the *in vitro* experiments.

Surprisingly, certain primary and secondary immune responses were markedly enhanced. In fact, CD4 T cells hyperproliferated in an *in vitro* response to anti-CD3 antibody, and an *in vitro* model of T helper (Th) differentiation, NFAT1-deficient mice showed an increased level of IL-4 production at later timepoints (Hodge et al. 1996).

In addition, these mice presented high serum IgE levels in response to immunization with ovalbumin (Hodge et al. 1996, Xanthoudakis et al. 1996). Moreover, NFAT1-deficient mice consistently showed a marked increase in the secondary immune response using two different experimental models. First, cells from draining lymph nodes of mice that had been sensitized with ovalbumin hyperproliferated after a secondary *in vitro* stimulation with the same antigen (Xanthoudakis et al. 1996). Second, an allergic/inflammatory response to antigen was assessed *in vivo*. Mice that had been previously sensitized to ovalbumin were restimulated by intrapleural injection of antigen, and the accumulation of eosinophils in the pleural cavity was assessed. NFAT1-deficient mice showed a marked increase in the number of eosinophils in the pleural cavity and a corresponding increase in the level of serum IgE (Xanthoudakis et al. 1996).

The immune phenotype of NFAT1-deficient mice illustrates three important points. First, these mice are immunocompetent rather than immunodeficient and do not show any gross impairment in the production of NFAT-dependent cytokines, indicating that the lack of NFAT1 is compensated for by the presence of other NFAT proteins. Second, the increased secondary immune responses and increased cell proliferation observed in NFAT1-deficient mice suggests that NFAT1 may actually have an overall negative effect on immune responsiveness in normal mice. This behaviour is not unprecedented: for example, in signal transduction pathways, kinases that are activated early during a response often activate feedback processes that contribute to the late downregulation of the same response. Finally, the unusual hyper-eosinophilia of NFAT1-deficient mice in a model of allergy, and their tendency towards the late production of Th2-type cytokines, suggests that NFAT1 critically influences Th differentiation during the normal immune response. NFAT1 could act to promote the transcription of genes encoding immunosuppressive cytokines, cytokines that skew T cell differentiation towards the Th1 pathway, or cytokines that suppress differentiation towards the Th2 pathway. Alternatively, NFAT1 could inhibit the production of cytokines having the opposite effect. These possibilities are not mutually exclusive. Given the importance of Th1-Th2 cytokine production in asthma, allergy, and other clinical situations, it is of considerable interest to understand the mechanisms by which NFAT1 exerts its profound effects on T cell differentiation and function.

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