Biochemistry (Moscow), Vol. 66, No. 5, 2001, pp. 467-475. Translated from Biokhimiya, Vol. 66, No. 5, 2001, pp. 581-591.

REVIEW

Role of Transcription Factor NFAT in the Immune Response

E. M. Kuklina* and S. V. Shirshev

Institute of Ecology and Genetics of Microorganisms, Ural Division of the Russian Academy of Sciences, ul. Goleva 13, Perm, 614081 Russia; fax: (3422) 646-711; E-mail: conf1@ecology.psu.ru

Received July 18, 2000
Revision received November 13, 2000

Abstract—Molecular mechanisms of activation of nuclear factor NFAT in cells of the lympho-myeloid complex are considered. Members of the NFAT family regulate transcription of genes encoding proteins involved in the induction and/or regulation of the immune response. It is possible that altered transcription activity of NFAT under conditions of its deficit or blockade of expression may account for changes in the immune status of an organism.

Key words: NFAT, immune response, apoptosis, lymphocytes, macrophages, mast cells, cytokines

Nuclear factor of activated T-cells (NFAT) plays an important role in immune reactions [1-3]. It is one of the major factors initiating transcription of the interleukin-2 (IL-2) gene required for T-lymphocyte proliferation. Although NFAT is traditionally considered as a key element of antigen-dependent activation of T-lymphocytes [1], some experimental data suggest that it has a negative role in the immune response. Blockade of NFAT expression is accompanied by uncontrolled proliferation of T- and B-lymphocytes leading to hyperplasia of lymphoid organs [2] and augmentation of allergic reactions [2, 3]. In the present review we consider molecular mechanisms underlying these effects of NFAT and responsible for normal adequate immune response.

Abbreviations: AP-1) activating protein-1; BCR) B-cell receptor (antigen receptor of B-lymphocytes); CaM) calmodulin; CaN) calcineurin; CD) cluster differentiation (membrane molecules of lympho-myeloid cells); CD40L) ligand for membrane molecule CD40; CsA) cyclosporin A; CTL) cytotoxic T-lymphocytes; CTLA4) T-lymphocyte membrane molecule; CyPs) intracellular CsA receptors; DAG) diacylglycerol; ERK) extracellular signal regulated protein kinase; FasL) Fas membrane molecule ligand; FcR) Fc receptors; FK506) CaN inhibitor; FKBPs) FK506 binding proteins; GM-CSF) granulocyte-macrophage colony stimulating factor; HIV) human immunodeficiency virus; IFNγ) interferon γ; IL) interleukin; Ins(1,4,5)P_3) inositol-1,4,5-tris-phosphate; JNK) c-Jun-NH_2-terminal protein kinase; MAPK) mitogen activated protein kinase; NFAT) nuclear factor of activated T-cells; PLCγ) phospholipase Cγ; PtdIns(4,5)P_2) phosphatidylinositol-4,5-bisphosphate; TCR) T-cell receptor (antigen receptor of T-lymphocytes); Th) helper T-cell ; TNF-α) tumor necrosis factor α.

* To whom correspondence should be addressed.

FACTORS AND MECHANISMS INVOLVED IN THE IMMUNE RESPONSE

Several types of cells are involved in immune reactions. First, these are the B- and T-lymphocytes that recognize antigen. After antigen binding to their antigen-specific receptors, these cells undergo clonal expansion and differentiation into effector cells. B-Lymphocytes are differentiated into plasmatic cells. Differentiation of "naive" T-lymphocytes into corresponding helper T-cells (Th) or cytotoxic T-lymphocytes (CTL) depends on the type of co-receptor molecule, CD4 or CD8, respectively. Phagocytes and natural killer cells (NK) play an important role in nonspecific resistance. Two types of immune responses, humoral and cell-mediated, are recognized. The former is responsible for the development of defense reactions against bacterial infections; it involves activation of B-lymphocytes producing immunoglobulins (Ig) to antigenic determinants of foreign proteins. The cell-mediated type is formed in response to viruses, tumors, and intracellular parasites. CTL and NK-cells are its main effectors. They kill infected cells via perforin-dependent cytolysis or induction of programmed cell death (apoptosis). The latter involves the production of apoptotic factor, Fas ligand (FasL), which binds to membrane Fas-receptor of target cells [4]. Cytokines producing by the Th sub-population determine preferential development of a certain type of immune response. Th-Cells can be subdivided into two classes, Th1 and Th2, producing different sets of cytokines [5]. Th1-Cytokines such as interleukin-2 (IL-2), IL-12, interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α) stimulate CTL and NK-cells and func-

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tion as the main inducers of the cell-mediated immune response and associated inflammatory reactions [5]. Th2-Cytokines (IL-4, IL-5, IL-10, IL-13) activate B-lymphocytes and therefore determine the humoral immune response [5]. Th1 and Th2 differentiate from naive $CD4^+$ T-lymphocytes. This differentiation includes the stage of a less committed precursor, Th0, and subsequent differentiation depends on the nature of the antigen, its concentration, presentation, and the presence of cytokines and colony stimulating factors [6]. The contribution of phagocytes to the immune response is determined not only by pathogen disintegration and presentation of its antigen determinants, but also by the production of inflammatory transmitters [7].

In addition to Th, other cells also produce cytokines (such as para- and autocrine regulators) involved in the immune response. CTLs produce IFN-γ; NK-cells and macrophages produce IFN-γ and TNF-α. B-Lymphocytes and mast cells produce IL-10/TNF-α and IL-4/IL-13, respectively.

MECHANISMS OF NFAT ACTIVATION IN CELLS OF THE LYMPHO-MYELOID COMPLEX

Five members of NFAT family are now recognized: NFAT1 (NFATp, NFATc2), NFAT2 (NFATc, NFATc1), NFAT3 (NFATc4), NFAT4 (NFATx, NFATc3) [8-11], and NFAT5 [12]. At least three of them (NFAT1, NFAT2, and NFAT4) are expressed by immune system cells: thymocytes [9, 13], T- and B-lymphocytes [8, 11, 14, 15], and NK-cells [16] and by descendants developed from myeloid cells (macrophages and mast cells) [15, 17]. Thymocytes preferentially express NFAT4 [9], whereas mature lymphocytes and NK-cells synthesize NFAT1 and NFAT2 [8, 11, 16]. NFAT1-4 have an N-terminal regulatory domain with an adjacent region of moderate homology (300 amino acid residues) including a series of conservative Ser- and Pro-rich sites and highly conservative DNA-binding sequence similar to that of Rel-family transcription factors [9, 10, 18, 19].

In resting cells, phosphorylated NFAT-proteins are located in cytoplasm. Their activation requires intracellular Ca^{2+} mobilization. Activation of these proteins is induced by stimulation of various membrane proteins such as antigen receptors of T- and B-cells (TCR and BCR, respectively), Fc-receptors for IgG and IgE (FcRγ and FcRε, respectively), and CD40 (a co-stimulator molecule of B-lymphocytes) [14-16, 20]. Binding of corresponding ligands to these receptors usually initiates an intracellular signal common for all leukocytes (Fig. 1). This results in activation of membrane phospholipase Cγ (PLCγ) and hydrolysis of phosphatidylinositol-4,5-bisphophate ($PtdIns(4,5)P_2$) with formation of two main second messengers, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$). The former (DAG) activates protein kinase C (PKC) whereas the latter stimulates $Ins(1,4,5)P_3$-dependent Ca^{2+}-channels; release of Ca^{2+} from the intracellular store, endoplasmic reticulum, and entry of extracellular Ca^{2+} increases cytoplasmic Ca^{2+} concentrations [21-23]. This is accompanied by activation of two Ca^{2+}-binding proteins, calmodulin (CaM) and calcineurin (CaN). The latter is a Ca^{2+}/CaM-dependent serine/threonine phosphatase; this enzyme directly regulates the activity of NFAT protein. CaN binds to the regulatory subunit of NFAT and dephosphorylates these proteins. This promotes their translocation to the nucleus, binding to DNA, and activation of transcription of various genes [24-26].

CaN is a heterodimer that consists of catalytic and regulatory Ca^{2+}-binding subunits [27]. Besides the catalytic domain, the catalytic subunit also contains a CaM binding site [28, 29] and a C-terminal autoinhibitory domain. Removal of this domain results in permanent activation of CaN [29, 30]. The autoinhibitory domain forms a loop blocking the active site of the catalytic domain; in resting cells, this prevents interaction of the catalytic domain with NFAT [31].

Binding with CaN and dephosphorylation of NFAT depends on two factors. The first is cytoplasmic Ca^{2+}. Both processes (binding and dephosphorylation) are initiated by the calcium ionophore ionomycin; its removal from the cultivation medium or addition of the Ca^{2+} chelator EGTA abolishes these processes [24]. The second factor required for CaN-dependent activation of NFAT is CaM; the latter is ineffective without Ca^{2+} [32]. Analysis of mechanisms of NFAT-protein interaction with CaN revealed the involvement of at least two functionally distinct sites of the catalytic domain of CaN. One site overlaps with the active site and is accessible for NFAT binding only in the case of CaN activation by CaM [32], which results in removal of the autoinhibitory domain and opening of the CaN active site [31].

The phosphatase activity of CaN is sensitive to specific inhibition by cyclosporin A (CsA) and FK506 [33, 34]. These compounds in a complex with intracellular immunophilin receptors (cyclophilin (CyPs) for CsA and FKBPs for FK506) bind to the catalytic subunit of this enzyme and inhibit its activity [31, 33]. Since CsA inhibits activation of NFAT and NFAT-dependent transcription of cytokine genes in activated T- and B-lymphocytes, NK, and mast cells [15, 16, 35, 36], it is widely used in clinical practice as an effective immunosuppressant.

CaN-dependent dephosphorylation of NFAT can be reversed; even in the presence of ionomycin, CaN inhibition by CsA initiates re-phosphorylation of NFAT, its return to the cytoplasm, and a decrease of its DNA-binding activity to the basal level [24]. A similar effect was observed after removal of ionophore or addition of EGTA. Stimulation of plasma membrane receptors also causes short-term phosphorylation of NFAT. Later stages

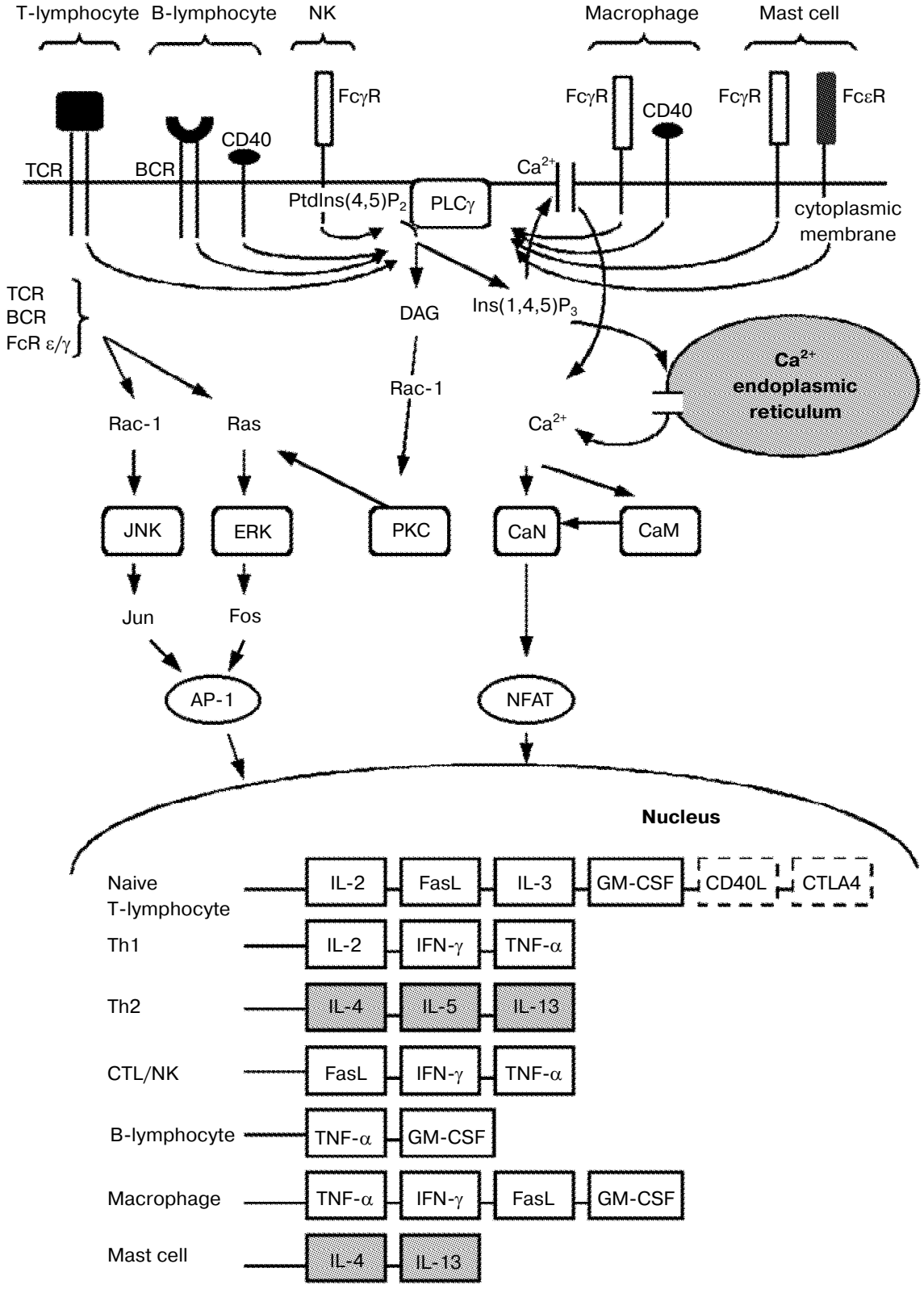

Fig. 1. Molecular mechanisms of NFAT activation in cells of lympho-myeloid complex. In cytokine genes opened and closed rectangles symbolize NFAT-dependent activation and inhibition, respectively. The broken contour shows only the presence of an NFAT-binding site in the promoter.

of activation are characterized by re-phosphorylation of the major proportion of NFAT, which correlates with the decrease in Ca^{2+} due to triggering of negative feedback mechanism(s) [24]. Two factors responsible for NFAT re-phosphorylation have been recognized, c-Jun-NH_2-terminal protein kinase (JNK) [37] and protein kinase A [38].

Activated NFAT is translocated into the nucleus where it binds to promoter and/or enhancer sequences of various genes and regulates their transcription [9, 24-26] (Fig. 1). Binding of NFAT to DNA usually requires cooperative interaction with another transcriptional factor, activating protein-1 (AP-1). The latter is formed during homo- or heterodimerization of Jun and Fos proteins. It is induced in the process of initiation of the intracellular signal involving activation of Ras and Rac-1 proteins, both PKC- and tyrosine kinase-associated membrane receptors. This signal transduction stimulates mitogen activated protein kinases (MAPK), extracellular signal regulated protein kinase (ERK), and JNK [39]. Addition of antibodies against Jun and Fos proteins [40] or blockade of the MAPK signal [41] partially or completely abolish NFAT-dependent gene transcription in cell culture. Study of the interaction of NFAT and AP-1 with DNA revealed that an NFAT-binding DNA sequence flanks the AP-1 binding site [42, 43]. The presence of NFAT results in a 10-fold increase in AP-1 affinity for its binding site [43], and the presence of AP-1 causes a 10-fold increase in dissociation time of NFAT–DNA complex [44]. Thus, NFAT and AP-1 mutually stabilize their binding to DNA.

The considered properties are the characteristic features of the first four members of the NFAT family of transcription factors. NFAT5 was identified only recently, and its characterization is in the very beginning. However, it is already known that NFAT5 significantly differs from other "classic" NFAT proteins; in contrast to them, it is permanently located in the nucleus irrespectively to CaN activation, and during binding to DNA it does not form a complex with AP-1 [12].

NFAT-DEPENDENT TRANSCRIPTION

Binding sites for NFAT proteins have been identified in promoter and enhancer regions of many inducible genes encoding cytokines and various membrane receptors including IL-2, IL-3, IL-4, IL-5, IL-13, INF-γ, TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF), membrane co-stimulating molecules CD40L and CTLA-4, and apoptotic factor FasL [1, 18, 45-48].

NFAT was originally identified as one of the main factors initiating IL-2 gene transcription [1]. Blockade of NFAT expression in T-lymphocytes is accompanied by reduction of IL-2 promoter activity and repression of this cytokine synthesis [1]. Five NFAT binding sites were identified in the promoter of the IL-2 gene; four of them are composite ones (they bind NFAT in the complex with AP-1) [49]. All five sites are required for complete activation of the promoter in response to TCR stimulation of lymphocytes [49]. Thus, NFAT is an important component of the signal pathway regulating expression of the IL-2 gene. All NFAT proteins of lymphocytes—NFAT1, NFAT2, and NFAT4—exhibit stimulating activity, the promoter of the IL-2 gene possessing low selectivity with respect to NFAT-type initiating transcription [10]. This explains why blockade of expression of one NFAT factor (e.g., NFAT1) produces a weak effect (if any) on the expression of IL-2 provided that NFAT2 and/or NFAT4 are available [50].

Data on the role of NFAT in the regulation of IL-4 gene transcription are not unequivocal. It was shown that at least two NFAT proteins, NFAT1 and NFAT2, interact with purine-rich promoter sequences (P0-P5), and this interaction significantly increases transcription of the IL-4 gene [45, 50, 51]. Moreover, there is a direct correlation between the amount of NFAT1 in the nucleus and expression of the IL-4 gene in lymphocytes [19]. Blockade of IL-4 gene transcription by CsA and FK506 also support the important role of NFAT-dependent activation [8].

However, some data also illustrate a negative role of NFAT proteins in the regulation of IL-4 gene transcription. For example, *in vivo* and *in vitro* T-lymphocytes of transgenic mice lacking NFAT1 ($NFAT1^{-/-}$) exhibit significant increase of IL-4 production in response to ovalbumin stimulation compared to cells of normal (non-transgenic) mice [52]. T-Lymphocytes of double-negative animals ($NFAT1^{-/-}/NFAT4^{-/-}$) respond to TCR-dependent stimulation by more pronounced expression of IL-4: production of this cytokine in the culture of double-negative T-cells is 75 and 600 times higher than in normal cells at the primary and secondary immune response, respectively [2].

The most probable explanation for this evident discrepancy consists in the phenomenon of late phase inhibition of gene transcription by NFAT1 [53]. In normal mice, stimulation of T-cells is accompanied by a transient increase in IL-4 mRNA (followed by rapid decline of its content), whereas in T-lymphocytes of $NFAT1^{-/-}$ mice the amount of IL-4 transcripts remained at a high level. These results were obtained both *in vitro* and *in vivo* using various ways of cell stimulation [53]. Since the half-life of these transcripts was similar in normal and $NFAT1^{-/-}$ cells, the high level of IL-4 transcripts in $NFAT1^{-/-}$ cells is obviously maintained due to high rate of gene transcription rather than mRNA stabilization [53]. Thus, NFAT proteins are involved not only in initiation but also in termination of IL-4 gene transcription and, therefore, they finally play a negative role in this process. The molecular mechanisms responsible for NFAT-dependent inhibition of IL-4 gene transcription remain unknown. Apparently, NFAT1 regulates activation of genes encod-

ing proteins that directly or indirectly block transcription of IL-4 in later stages of the immune response.

Two other Th-2 cytokines, IL-5 and IL-13, are regulated similarly to IL-4. Cells of NFAT1$^{-/-}$ and NFAT1$^{-/-}$/NFAT4$^{-/-}$ mice exhibit significant increase in the production of these cytokines in both *in vitro* and *in vivo* experiments [2, 52]. Moreover, the time-course of expression of these (IL-5 and IL-13) genes in NFAT1$^{-/-}$ mice is similar to that of IL-4. Stimulation-induced levels of IL-5 and IL-13 transcripts reached a plateau and remained at this level at later stages of activation (however, they were twice less than that of IL-4) [53]. Since these effects were not abolished by antibodies against IL-4 [53], it is possible that the IL-5 and IL-13 genes are directly controlled by NFAT1. Obviously, transcription of the whole cluster of IL-4/IL-5/IL-13 genes is coordinately regulated by this transcription factor.

Expression of TNF-α, the most important Th1-cytokine, depends on NFAT: it is blocked by CaN inhibitors (CsA and FK506) [47] and is significantly reduced in NFAT1$^{-/-}$/NFAT4$^{-/-}$ mice [2]. Four binding sites with various affinities for NFAT1 are recognized in the TNF-α promoter [47].

Expression of another Th1-cytokine, IFN-γ, is also controlled by NFAT: NFAT1$^{-/-}$/NFAT4$^{-/-}$ mice are characterized by significant reduction of IFN-γ transcripts and production of this cytokine [2]. A similar effect was observed during the CsA effect [46]. Two NFAT binding sites (one of which is the composite) have been identified in the promoter of the IFN-γ gene [46, 54]. Both sites are required for maximal induction of the IFN-γ promoter. In contrast to IL-2, IL-4, or TNF-α, which respond to the increased expression of NFAT1 and other NFAT-proteins by corresponding increase of transcription of their genes, IFN-γ lacks the effect [46]. It is possible that basal levels of these transcriptional factors are sufficient for full activation of the IFN-γ promoter. This may reflect differences in promoter structures and a different role of NFAT in their induction. For example, from three to five NFAT-binding sites have been identified on the promoters of the IL-2, IL-4, and TNF-α genes, whereas the promoter of IFN-γ has only two sequences effectively interacting with this transcriptional factor.

NFAT proteins are effective activators of transcription of hemopoietic growth factors IL-3 and GM-CSF [55]. In contrast to the cytokines above considered, the composite binding sites for NFAT1/AP-1 and NFAT2/AP-1 are located not in the promoter of the IL-3 and GM-CSF genes, but in the enhancer element common for both genes; this enhancer, which is sensitive to CsA, is required for correct regulation of promoter activity [56, 57].

The expression of various cytokine genes depends on the duration of the presence of NFAT in the nucleus [58]. Activation of GM-CSF and IL-13 gene transcription requires short-term translocation of NFAT to the nucleus, whereas transcription of the IL-2, IL-3, IL-4, and IFN-γ genes is activated after the prolonged presence of NFAT in the nucleus [58].

NFAT binding sites have also been recognized in promoters of genes encoding T-lymphocyte membrane molecules—CD40L and CTLA-4 [59, 60]. The mechanisms of NFAT-dependent regulation of these genes remain unknown.

The role of NFAT in control of FasL transcription in response to TCR-dependent stimulation is better understood. Its expression is blocked by CsA [61], and it is significantly lower in NFAT1$^{-/-}$ mice [62]. Moreover, NFAT activation was shown to be ultimately important for FasL induction [48]. Two NFAT binding sites have been identified in the promoter of the FasL gene, and both are important for optimal expression of FasL in activated T-cells [63].

NFAT binding sites have also been identified in the promoter of human immunodeficiency virus type 1 (HIV1) [64] and in enhancer regions of HIV1 and HIV2 [65]. The promoter activity of HIV1 in CD4^{+} T-lymphocytes is sensitive to independent inhibition by CsA and FK506 and expression of dominant-negative NFAT1 protein [64]. The latter interacts with viral Tat factor and thereby inhibits Tat-mediated activation of viral genes, whereas Tat potentiates NFAT1-dependent transcription of T-lymphocyte cytokine genes [66].

ROLE OF NFAT IN THE IMMUNE RESPONSE

Lack or impairment of NFAT protein expression *in vivo* is accompanied by marked alteration of the immune reaction of the body [2, 3, 52, 62]. A shift in the activity of immune competent cells is one of the most pronounced impairments [2, 3, 62]. Non-selective inhibition of activation of NFAT-proteins by CsA significantly suppressed T-lymphocyte proliferation [67], whereas selective knockout of genes encoding each of the NFAT proteins causes various functional effects. For example, knockout of the NFAT2 gene attenuated the proliferative response of T-lymphocytes [68, 69], whereas blockade of expression of NFAT1 and/or NFAT4 is accompanied by augmentation of both spontaneous and antigen-stimulated T- and B-cell proliferation and by an increased proportion of activated cells in both populations [2, 3, 62]. Clonal lymphocyte expansion is the earliest event in the cell response to antigen stimulation. It is regulated by two opposite mechanisms: growth factor-induced proliferation and FasL/Fas-dependent apoptosis [70, 71]. For example, IL-2 is the main growth factor for T-lymphocytes. It is secreted by "naive" T-cells during TCR-dependent activation and initiates their proliferation, acting as a paracrine or autocrine regulator. Under normal conditions, FasL expression in such a cell is blocked; however, during inadequate activation (very low antigen signal or

absence of colony stimulating signals), T-lymphocytes secrete this factor, which acts as a paracrine regulator inducing apoptosis. Thus, preferential activation of FasL or IL-2 expression in response to antigen stimulation determines the subsequent fate of "naive" T-lymphocytes (corresponding death or proliferation with subsequent differentiation into CTL and Th). Since all three NFAT proteins expressed by lymphocytes activate transcription of the IL-2 gene, the increase of T-lymphocyte proliferation in NFAT1$^{-/-}$ and NFAT4$^{-/-}$ animals may obviously be attributed to blockade of FasL transcription. In fact, NFAT1$^{-/-}$ mice are characterized by a lower level FasL mRNA than normal mice; in double-negative animals (NFAT1$^{-/-}$/NFAT4$^{-/-}$) expression of this factor is totally blocked [2]. This explains the increased proportion of activated cells in these animals in response to stimulation [2, 72]; under normal conditions, inadequately stimulated cells are subjected to apoptosis, but in the case of blockade of FasL expression, they replenish the population of activated T-lymphocytes. Blockade of CTLA4 gene transcription also makes some contribution to augmentation of T-cell proliferation in NFAT1$^{-/-}$ and NFAT4$^{-/-}$ mice [73]. (T-Lymphocytes express this membrane molecule at later stages of activation, and CTLA4 inhibits their proliferation). In the case of NFAT2 blockade or non-selective inhibition of NFAT protein activation, the inhibition of proliferative response of T-lymphocytes suggests that NFAT2 is not involved (or does not play) a special role in FasL gene transcription. However, it is possible that NFAT2 is the most effective activator of IL-2 gene transcription. Thus, NFAT2 obviously plays a positive role in control of T-cell proliferation, whereas NFAT1 and NFAT4 have the opposite effect. The positive effect of NFAT2 is mediated by IL-2 gene transcription, whereas the negative effect of NFAT1 and NFAT4 may be associated with initiation of FasL expression. These data are consistent with recently demonstrated differential expression of different NFAT proteins in T-lymphocytes: cell activation is accompanied by rapid augmentation of NFAT2 transcripts and reduced expression of NFAT1 and NFAT4 [2].

The Th2 cytokines IL-4, IL-5, and IL-13 are the main growth factors of B-lymphocytes. Increased proliferation of these cells in NFAT1$^{-/-}$ and NFAT4$^{-/-}$ animals may be associated with increased production of these cytokines or with abolishment of FasL/Fas-dependent apoptosis.

Inhibition of cell-mediated reactions represents another change of immune reactivity under conditions of NFAT blockade. This is observed on inhibition of NFAT-dependent transcription induced by various treatments. CsA is an effective inhibitor of transplantation immunity [67], and directed NFAT blockade is accompanied by reduced resistance to intracellular parasites [53]. This effect is realized via several mechanisms. 1) Blockade of NFAT expression causes inhibition of gene transcription of Th1-cytokines, IL-2, IFN-γ, and TNF-α, inducing cell-mediated reactions [5] and also gene transcription of IL-3 and GM-CSF, polypotent stimulators of hemopoiesis and leukocyte activation [74]. 2) Blockade of NFAT expression is accompanied by blockade of apoptotic factor FasL expression. Blockade of FasL expression at the stage of "naive" lymphocyte activation increases proliferative response. For CTL and NK cells, this effect means abolishment of one of the two most important mechanisms responsible for elimination of "affected" cells [4]. TNF-α can also act as an apoptotic factor, and decrease of its expression in NK cells can also contribute to inhibition of NK-dependent apoptosis. The occurrence of both mechanisms in the body results in decreased resistance of NFAT-deficient animals to viruses, tumors, and parasite infections. The ability of NFAT to inhibit Tat-induced activation of HIV1 genes plays an important role in the antiviral defense of the body [65]. However, Tat-dependent augmentation of NFAT1 transcriptional activity may be a reason for a temporary increase in the production of IL-2 and other Th1-cytokines detected in HIV1-infected patients [66]. Thus, NFAT prevents replication of viral RNA and stimulates immune reactions responsible for elimination of virus-infected cells. This may be a universal mechanism use for protection against other viral infections.

In contrast to cell-mediated immunity, the humoral immune response is increased under conditions of NFAT expression blockade; this results in significant increase in IgG1 and IgE in blood serum, eosinophil accumulation, and increased allergic reactions [2, 52]. Since these effects are blocked by antibodies against IL-4 and IL-5, they are related to increased production of Th2-cytokines registered, for example, in NFAT1$^{-/-}$ and NFAT4$^{-/-}$ animals [2, 52]. This phenomenon may be attributed to the ability of NFAT to inhibit the late phase of transcription of Th2-cytokine genes IL-4, IL-5, and IL-13 [53]. Permanent activation of these genes in the absence of NFAT leads to increased proliferation of B-lymphocytes and IL-4/IL-13-dependent switch of Ig classes to IgG1 and IgE. The latter binds to mast cells FcεR in the presence of antigen, and this induces release of vasoactive transmitters, chemotactic factors, and cytokines such as IL-4 and IL-13. Their synthesis in mast cells also depends on NFAT. These effects result in the development of immediate hypersensitivity type reactions [75]. IL-5 stimulates differentiation and proliferation of eosinophils, and degranulation of these cells also contributes to the development of allergic reactions [76].

During immune response, proteins of the NFAT family activate transcription of most cytokine and other inducible genes and thus they control proliferation, differentiation, and effector functions and also programmed death of immune competent cells. Figure 2 schematically summarizes results of NFAT activation in lympho-myeloid cells and the role of NFAT in immune response.

NFAT proteins are involved in simultaneous stimulation of expression of growth (IL-2) and apoptotic (FasL)

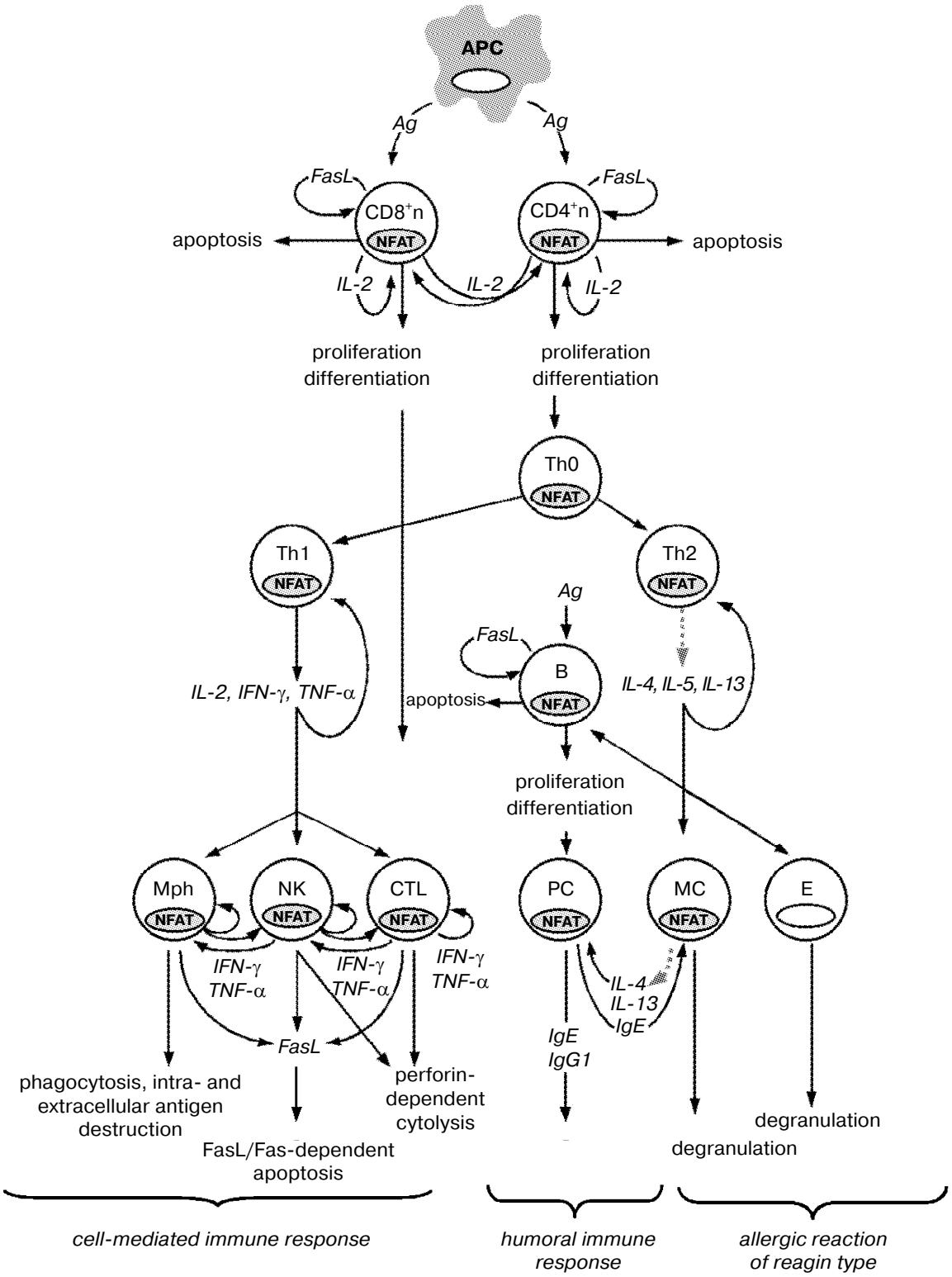

Fig. 2. Role of NFAT in immune response. Solid and dotted lines show stimulation and inhibition, respectively; the presence of NFAT in the nucleus means its activation in the cell. The following abbreviations have been used: Ag) antigen; $CD4^+$n and /$CD8^+$n) "naive" T-lymphocytes (which have not contacted antigen); NK) natural killers; Th) helper T-lymphocytes; APC) antigen presenting cells; B) B-lymphocyte; Mph) macrophage; PC) plasmatic cells; MC) mast cells; CTL) cytotoxic T-lymphocyte; E) eosinophil.

factors in "naive" $CD4^+/CD8^+$ T-lymphocytes responding to antigen-dependent activation. This induces clonal expansion of lymphocytes but restrains it within certain limits and prevents uncontrolled proliferation.

Proteins of the NFAT family are effective activators of transcription of Th1-cytokine genes (IL-2, IFN-γ, TNF-α) in Th1 and other cells involved in the immune response: CTL, NK-cells, macrophages. Th1-cytokine-mediated preferential differentiation and activation of Th1 also contributes to NFAT-dependent increase in production of IL-2, IFN-γ, and TNF-α. Th1-cytokines induce activation of CTL and NK and, consequently, destruction of "affected" cells by perforin-dependent cytolysis or FasL/Fas mediated apoptosis. Besides cytokine gene induction, direct expression of NFAT in CTL and NK-cells initiates transcription of the FasL gene. This represents an autonomic mechanism of NFAT-dependent augmentation of cell-mediated immune reactions. Direct or Th1-cytokine mediated macrophage activation initiates the development of inflammatory reactions resulting in pathogen destruction. Thus, NFAT-induced increase in cell-mediated immunity occurs via activation of transcription of genes encoding both regulatory (Th1-cytokines) and effector (FasL) molecules (Fig. 2).

NFAT proteins exert negative control of Th2-cytokine IL-4, IL-5, and IL-13 gene transcription. This causes inhibition of proliferation and differentiation of B-lymphocytes and the production (by B-lymphocytes) of IgE and IgG1 accompanied by the inhibition of humoral immunity. The NFAT-dependent decrease in Th2-cytokine production inhibits activation of mast cells and eosinophils involved in the development of reagin-type allergic reactions (Fig. 2).

NFAT protein-dependent control of expression of the most important regulator (cytokines), effector (FasL), and membrane (CTLA4) molecules of lympho-myeloid cells determines the central role of this protein family of transcriptional factors in the immune response. In contrast to a generally accepted notion on the positive role of NFAT in immunity, NFAT-dependent transcription results in activation of cell-mediated reactions and also in inhibition of humoral immunity. Opposite regulation of Th1- and Th2-cytokine gene expression is the basis for the opposite effects of NFAT.

It should be emphasized that proteins of the NFAT family exert diverse functional effects and the final result of NFAT-dependent transcription is determined by the selective expression of these factors in lympho-myeloid cells and their balanced activation.

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