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Mini-Review

FAN (factor associated with neutral sphingomyelinase activation), a moonlighting protein in TNF-R1 signaling

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ABSTRACT

TNF-α is a pleiotropic cytokine involved in the regulation of various biological effects, including cell survival and proliferation, cell differentiation, and cell death. Moreover, TNF-α triggers proinflammatory responses, essentially through its ability to promote the expression of various proinflammatory genes. Most of the biological effects initiated by TNF-α rely on its ability to bind to and activate TNF-R1. As a consequence, molecular complexes are being formed, resulting from the recruitment of multiple adaptor proteins to the intracellular TNF-R1 DD. The adaptor protein FAN constitutively binds to a proximal membrane domain of TNF-R1 called NSD. Herein, the role of FAN in TNF-α-induced cell signaling and biological responses is discussed. *J. Leukoc. Biol.* 88: 897–903; 2010.

TNF-α TRIGGERS MULTIPLE SIGNALING PATHWAYS AND BIOLOGICAL EFFECTS

TNF-α is a single-pass type II membrane protein of 233 aa in length, which is secreted after proteolytic cleavage, generating a soluble form being a noncovalently bound homotrimer of 17 kDa subunits. TNF-α was first described as a cytotoxic molecule able to induce necrosis of some tumors in mouse models [1]. In vitro, TNF-α has been reported to trigger apoptosis or necrosis in various cell types including cancer cells [2, 3]. However, TNF-α-induced toxicity was not restricted to cancer cells, limiting anti-cancer therapy based on systemic TNF-α injection. In addition, TNF-α is now well-established as a pleiotropic cytokine, essentially involved in inflammation, modulating acute and chronic inflammatory as well as innate and adaptive immune responses [4–7]. As a matter of fact, TNF-α or TNF-R1-deficient mice, as well as patients treated with TNF-neutralizing agents, display higher susceptibility to infection, indicating that TNF-α is essential for the clearance of pathogens, particularly intracellular microorganisms [7–11]. TNF-α- or TNF-R1-deficient mice display an alteration in specific immune responses, including a humoral response to thymo-dependent antigens [12–14]. TNF-α is also involved in acute inflammatory responses such as peritonitis and fulminant hepatitis in mice [15–17]. Moreover, studies based on the analysis of TNF-α knockout mice or transgenic mice overexpressing TNF-α revealed the important function of TNF-α in experimental autoimmune encephalomyelitis [18]. In humans, TNF-α is involved in various autoimmune diseases such as rheumatoid arthritis, psoriasis, multiple sclerosis, and Crohn's diseases, and anti-TNF-α therapies are used successfully to limit inflammatory disorders [4–7].

TNF-α binds specifically to TNF-R1 (CD120a) and TNF-R2 (CD120b) with high and low affinity, respectively. Most of the signaling pathways and subsequent biological effects triggered by TNF-α depend on its ability to stimulate TNF-R1 [4]. For instance, TNF-R1 mediates activation of kinases of the MAPK family, transcription factors such as NF-κB, and caspase cascade, modulating cell survival, proliferation, and apoptosis, as well as inflammatory responses [19–21]. Distinct intracellular domains of the TNF-R1 have been described. The DD, which

Abbreviations: BEACH=Beige and Chediak-Higashi, caspase=cysteinyl-aspartate-specific proteinase, CB1=cannabinoid receptor 1, CHS=Chediak-Higashi syndrome, cPLA2=cytosolic phospholipase A2, DD=death domain, FADD=Fas-associated with death domain protein, FAN=factor associated with neutral sphingomyelinase activation, Gbp2=guanylate-binding protein 2, IFI16=IFN-γ-inducible protein 16, IPA=Ingenuity Pathways Analysis, Lyst=lysosomal trafficking regulator, MEF=mouse embryonic fibroblast, NSD=neutral sphingomyelinase domain, NSMAF=neutral sphingomyelinase activation factor, PH=plekstrin homology, PIP2=phosphatidylinositol (4,5) bis phosphate, RACK1=receptor for activated C-kinase 1, RIP1=receptor-interacting protein 1, SMPD1–5=sphingomyelin phosphodiesterase 1–5, TAPBP=transporter associated with antigen processing-binding protein, TRADD=TNF-R1-associated death domain protein, TRAF2=TNF-R-associated factor 2, WD=tryptophan-aspartic acid

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contains an 80-aa sequence located at the C-terminal part of the TNF-R1, interacts with TRADD, an adaptor protein enabling the activation of proinflammatory and apoptotic pathways through the sequential formation of two molecular complexes upon TNF-α treatment [22, 23]. Complex 1 is formed initially by the association of TNF-R1, TRADD, TRAF2, and the serine/threonine kinase RIP1. Formation of this complex leads notably to the activation of transcription factors such as NF-κB, which is involved in the expression of genes encoding survival and inflammatory proteins [20, 22]. Complex 2, which is formed afterward upon TNF-R1 internalization, couples TRADD to FADD, leading to the recruitment and activation of initiator procaspase 8 [22]. Complex 2 is therefore responsible for caspase cascade activation via mitochondria-dependent and -independent signaling pathways, leading to apoptosis [22] (**Fig. 1**).

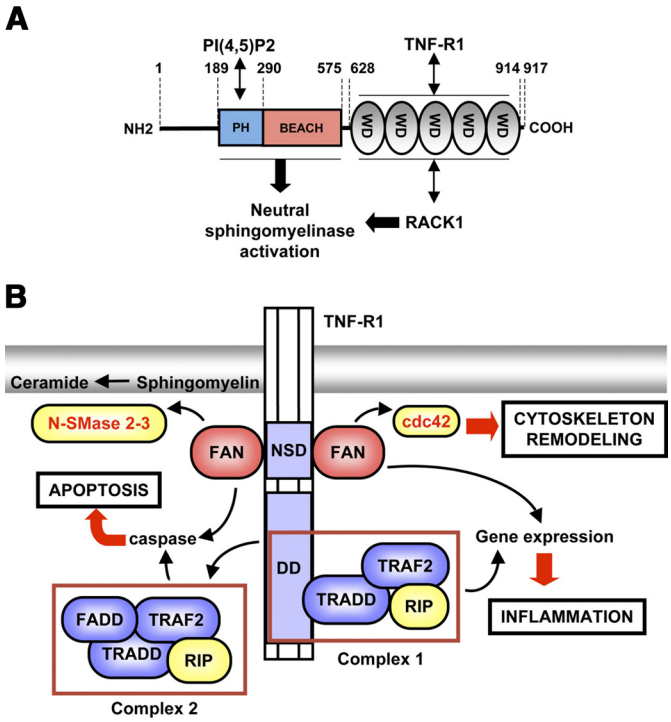

Figure 1. Structure of the FAN protein and its role in TNF-R1 signaling pathways. (A) The FAN carboxy-terminal portion possesses five WD repeats, which constitutively interact with TNF-R1 and the adaptor protein RACK1. The FAN amino-terminal portion possesses a PH domain that binds specifically to PIP2, allowing plasma membrane localization of FAN. The PH domain interacts physically with the BEACH domain and are both functionally required, together with RACK1, for TNF-induced neutral sphingomyelinase activation. The numbering of amino acids in the human FAN protein is indicated. (B) FAN constitutively binds to the TNF-R1 NSD and stimulates neutral sphingomyelinases 2 and 3 (N-SMase 2-3), which trigger activation of the "sphingomyelin-ceramide pathway." FAN is required for cdc42 activation and cytoskeleton remodeling. In addition to complex 1 proteins, FAN modulates proinflammatory gene expression. FAN is also involved, together with complex 2 proteins, in caspase cascade activation and apoptosis.

THE NSD AND ITS ASSOCIATED FAN ADAPTOR PROTEIN

The NSD has been identified first as required for TNF-α-induced neutral sphingomyelinase activation [24]. This TNF-R1 domain is a short intracellular sequence, closer to the plasma membrane between aa 309 and 319 [24]. Deletion of NSD on the TNF-R1 sequence abolished the ability of TNF-α to stimulate neutral sphingomyelinase but not acidic sphingomyelinase, the activation of which is dependent on the DD [24]. Using the NSD as a bait in a yeast-interaction trap system, FAN was identified by screening a cDNA library from Jurkat cells [25]. Interaction between FAN and NSD has also been demonstrated using a peptide-scanning library, indicating direct binding between TNF-R1 and FAN [25]. Coimmunoprecipitation experiments showed constitutive interaction between TNF-R1 and flag-tagged or endogenous FAN [25, 26].

FAN STRUCTURE, SUBCELLULAR LOCALIZATION, AND PROTEIN EFFECTORS

The *NSMAF* gene encoding FAN is located on chromosomes 4 and 8 in the mouse and human genomes, respectively [25, 27, 28]. Little is known about the regulation of *NSMAF* gene expression. FAN mRNA is found in a wide variety of human tissues, indicating that similarly to TNF-R1, FAN is expressed ubiquitously [25]. Under microgravity conditions, *NSMAF* expression is up-regulated in fibroblasts [29].

Human FAN protein is 917 aa in length with a predicted molecular mass of 104 kDa belonging to the WD repeat protein family, which is composed mostly of regulatory proteins involved in signal transduction [25, 30]. The C-terminal part of FAN, which is composed of five WD repeat sequences, constitutively interacts with the NSD domain of TNF-R1 [25], as well as with another WD repeat protein, RACK1 [31]. The N terminus of FAN possesses BEACH and PH domains [32]. The overexpressed GFP-tagged FAN protein is located mainly at the plasma membrane, and FAN PH domain interaction with PIP2 is essential for such a localization [33]. Moreover, the FAN BEACH domain interacts physically and functionally with the FAN PH domain, and both domains are required for TNF-induced neutral sphingomyelinase activation [32] (Fig. 1A).

FAN's BEACH domain is homologous to that of the Lyst/CHS protein [32], which is involved in vesicular trafficking modulation. The Lyst/CHS protein is inactivated in patients affected with CHS, who display hypopigmentation and immunological and neuronal disorders [34, 35]. Cells derived from CHS patients exhibit giant intracellular vesicles as a consequence of vesicular transport alteration between the endolysosomal compartment [34, 35]. Of note, FAN-deficient fibroblasts displayed bigger lysosomes as compared with their wild-type counterparts [36], suggesting that FAN may somehow modulate vesicular traffic to and/or from the endolysosomal compartment. The role of FAN in vesicular trafficking modulation remains to be established.

TNF-α modulates actin cytoskeleton remodeling and cell motility essentially through TNF-R1 engagement [37]. NSD

and FAN have been reported to modulate filamentous actin polymerization in response to TNF-α [33, 37]. As a matter of fact, the TNF-induced filamentous actin increase and filopodia formation were impaired in FAN-deficient MEFs [33]. Modulation of actin is dependent on the activation of members of the GTPase family, including cdc42, Rac, and Rho [38]. Whereas TNF-α-induced activation of Rac and Rho was not altered by FAN deficiency, cdc42 was not activated in TNF-α-treated FAN-deficient MEFs, indicating the critical role of FAN in cdc42 activation in response to TNF-α [33] (Fig. 1B). FAN-dependent cdc42 activation and filamentous actin reorganization may be involved in TNF-α-triggered cell migration. Of note, we have documented recently the role of FAN in TNF-α-induced neutrophil migration in mouse peritonitis models [17]. Whether FAN regulates neutrophil migration through its ability to mediate TNF-α-triggered cdc42 activation and subsequent actin cytoskeleton remodeling remains to be established.

FAN IS REQUIRED FOR TNF-α-INDUCED NEUTRAL SPHINGOMYELINASE ACTIVATION

TNF-α triggers the activation of the sphingomyelin-ceramide pathway through stimulation of neutral and acidic sphingomyelinases [39]. This pathway is initiated by the hydrolysis of sphingomyelin to ceramide, which is considered as a putative bioactive sphingolipid in cell signaling [40]. Whereas overexpression of wild-type FAN enhanced TNF-α-induced neutral sphingomyelinase activation in mammalian cells, expression of the C-terminal part of FAN, harboring the WD repeat domains and acting as a dominant-negative form of FAN, abrogated neutral sphingomyelinase activation in response to TNF-α [25], and TNF-induced neutral sphingomyelinase activation was also impaired in cells derived from FAN-deficient mice [27]. Finally, TNF-α-induced sphingomyelin breakdown and ceramide generation were impaired in SV40-transformed fibroblasts stably expressing the dominant-negative form of FAN, further illustrating FAN's role in sphingomyelin-ceramide pathway activation [41].

Deleterious mutations of the *SMPD1* gene, which encodes acidic sphingomyelinase, are responsible for Niemann-Pick disease, a sphingolipid storage disorder characterized by sphingomyelin accumulation within lysosomes [42]. Four genes, namely *SMPD2*, *SMPD3*, *SMPD4*, and *SMPD5*, have been identified so far as encoding neutral sphingomyelinases 1, 2, 3, and 4, respectively [43–46]. Of note, only neutral sphingomyelinases 2, 3, and 4 but not neutral sphingomyelinase 1 display in vivo sphingomyelin-cleaving activity [46, 47]. TNF-α has been reported to activate acidic and neutral sphingomyelinases 2 and 3 [39, 45, 48]. Whereas TNF-α induces activation of acidic sphingomyelinase in a FADD-dependent manner [49], it likely activates neutral sphingomyelinases 2 and 3 under the control of FAN [45, 50]. Indeed, overexpression of a dominant-negative form of FAN alters TNF-α-induced neutral sphingomyelinase 3 activation in MCF-7 (human breast cancer cell line) cells [45]. It has been shown recently that FAN may participate in the formation of a molecular complex, which includes RACK1, embryonic ectodermal development, and neutral sphingomyelinase 2 [50]. Thus, this molecular complex, which is formed in a NSD-dependent manner, physically and functionally couples TNF-R1 to neutral sphingomyelinase 2, leading to an increased neutral sphingomyelinase activity [50] (Fig. 1).

FAN IN APOPTOSIS SIGNALING

TNF-α triggers cytotoxicity in numerous cell types, including cancer cells. TNF-α-induced apoptosis is dependent on TNF-R1 stimulation. The intracellular C-terminal TNF-R1 DD has long been known to signal the cytotoxic effects of TNF [51]. More recently, the NSD has been shown to participate in TNF-induced apoptosis [52]. Although previous observations demonstrated that the NSD by itself is unable to mediate the cytotoxic response [24], deletion of this short domain reduced TNF-α-induced apoptosis significantly [51, 52]. This latter observation demonstrates that the NSD provides a concurrent signal with those emanating from the DD for a full apoptosis signaling activation. Accordingly, we reported for the first time that FAN is involved in the cytotoxic response to TNF-α in human and mouse fibroblasts [41]. Indeed, stable expression in human fibroblasts of a dominant-negative form of FAN attenuated caspase cascade activation and cytochrome c release from mitochondria and markedly inhibited TNF-α-triggered apoptosis. Fibroblasts from FAN-knockout mice significantly resisted TNF-α-induced caspase activation and apoptosis. Finally, genetic correction of FAN expression using the retroviral vector restored TNF-α-induced apoptosis in FAN-deficient fibroblasts [41]. The role of FAN in TNF-α-induced apoptosis has been documented further by Gores' group in a rat hepatoma cell line [53]. Overexpression of a dominant-negative form of FAN impaired initiator and effector caspase activation and an increase in lysosomal permeability and cathepsin B release from the lysosome [53]. Collectively, the last two studies highlight the previously unrecognized role of FAN in cell death signaling induction by TNF-α, including caspase cascade activation associated with mitochondrial and lysosomal membrane permeabilization (Fig. 1B). The implication of FAN in cell death is not restricted to TNF-R1 signaling. Indeed, CD40 ligand-triggered apoptosis was also impaired in human fibroblasts, overexpressing a dominant-negative form of FAN, as well as in FAN-deficient fibroblasts [26]. In addition, whereas overexpression of FAN significantly enhanced hypoxia/reoxygenation-triggered apoptosis in rat cardiomyocytes, expression of a dominant-negative form of FAN impaired cell death [54].

The molecular mechanisms by which FAN mediates apoptosis are not yet fully established. As FAN is required for neutral sphingomyelinase activation, one can speculate that the ability of FAN to enhance cell death signaling depends on its role in neutral sphingomyelinase activation, leading to sphingomyelin breakdown and concomitant generation of ceramide, a putative bioactive molecule in cell death [40, 55, 56]. As a matter of fact, ceramide has been reported to increase permeability of mitochondrial and lysosomal membranes (see ref. [40] for review). This hypothesis is reinforced by the finding that overexpression of a dominant-negative form of FAN abrogates

TNF-α-induced ceramide generation from sphingomyelin hydrolysis [41]. Moreover, pharmacological inhibition of TNF-R1 internalization impairs apoptosis signaling, significantly but not totally, without affecting neutral sphingomyelinase activation in response to TNF-α [57]. More recently, neutral sphingomyelinase activation has been reported to trigger caspase-dependent apoptosis initiated by TNF-R1 lacking the cytoplasmic TNF-R1 internalization domain, which is required for TNF-α-induced TNF-R1 internalization and FADD-dependent caspase activation [23, 52]. It is important to note that MEFs expressing a NSD-deleted human TNF-R1 mutant strongly resisted TNF-induced apoptosis in comparison with cells expressing wild-type human TNF-R1 [52]. Thus, NSD and FAN trigger an apoptotic signaling pathway, which does not require TNF-R1 internalization and likely amplifies the FADD-dependent signaling (Fig. 1B). Another challenging hypothesis is to consider that FAN somewhat facilitates the formation of complex 2 [22], enhancing caspase-8 activation upstream of mitochondrial and lysosomal molecular events. This tenet is supported by the marked inhibition of caspase-8 and cytochrome c release in human fibroblasts overexpressing a dominant-negative form of FAN [41]. The role of FAN in TNF-α-induced formation of protein complex 2 remains to be evaluated.

FAN INVOLVEMENT IN INFLAMMATION AND IMMUNE RESPONSES

TNF-α induces inflammatory responses essentially through its ability to generate proinflammatory eicosanoids as a consequence of the activation of cPLA2 and to stimulate proinflammatory gene expression [4, 21]. Krönke and coworkers [58] have reported previously that TNF-induced activation of cPLA2 occurs independently of FAN. By analyzing the responses of MEFs derived from TNF-R1 and TNF-R2 double-knockout mice, which express wild-type or NSD-deleted human TNF-R1 [52], we have shown recently that the expression of some proinflammatory cytokines is mediated partly by TNF-R1 NSD [17]. Whereas cells expressing wild-type TNF-R1 produced MCP-1 and CXCL-2 in response to TNF-α, cells expressing the NSD-truncated receptor secreted only MCP-1. Thus, deletion of the NSD selectively perturbs TNF-α-induced gene expression. Moreover, TNF-α-induced proinflammatory gene expression was selectively altered in FAN-deficient MEFs, as evaluated by microarray analysis, real-time RT-PCR, and ELISA [17] (Fig. 1B). Essentially, we showed that FAN is required for full expression of genes encoding CXCL2 and IL-6, which are upregulated upon activation of NF-κB and AP1. Although one report indicates that FAN is necessary for cRaf-1 kinase activation in response to TNF [59], TNF-α-triggered activation of NF-κB, ERK, and JNK seems to be unaltered in FAN-deficient MEFs as compared with their wild-type counterparts [17, 27, 41, 58]. Thus, the molecular mechanism coupling FAN to selective gene expression is currently unclear.

FAN-deficient mice have been generated by homologous recombination in C57BL/6J mice by partially replacing exon 2 of *Nsmaf* [27]. Inactivation of *Nsmaf* did not cause gross and reproduction abnormalities. In sharp contrast to TNF-R1-deficient mice, FAN-deficient mice did not exhibit alterations of lymphoid organ development. In addition, FAN-deficient mice displayed normal leukocyte composition within blood and lymphoid organs. In vitro cytotoxicity activity of T lymphocytes and NKs was unaffected by FAN deficiency [27] (**Table 1**).

As the TNF-R1 signaling pathway is essential in the host defense against various pathogens [10, 21], we investigated the role of FAN in the clearance of bacterial and parasite infections. We challenged mice with i.p. injection of *Listeria monocytogenes*, *Streptococcus pneumoniae*, or *Toxoplasma gondii*. In contrast to TNF-α-deficient mice, FAN-deficient mice did not display a higher susceptibility to infection, indicating that FAN does not have a crucial function in an innate immune response against microorganisms [17] (Table 1). However, we have documented that the humoral response toward a thymo-dependent antigen was altered significantly in FAN-deficient mice as compared with their wild-type littermates, indicating that FAN plays a critical role in the establishment of a specific immune response [17] (Table 1). As FAN interacts physically with CD40 [26] and TNF-R1 [25], both of which have important functions in humoral responses, the decreased antibody

TABLE 1. Phenotype of FAN-Deficient Mice

Function	Phenotype of FAN-deficient mice	References
Development, growth, reproduction	No alterations	[16, 27]
Immune system and immune responses	Normal development of lymphoid organs	[27]
	Normal peripheral blood, thymus, spleen, lymph node leukocyte composition	[17, 27]
	No alteration of NK and CTL activities	[27]
	No increased susceptibility to infection	[17]
	Reduced thymo-dependent humoral response	[17]
Acute inflammatory responses	Partial resistance to fulminant hepatitis	[16]
	Reduced neutrophil recruitment in peritonitis models	[17]
	Reduced *in vivo* cytokine production	[16, 17]
Cutaneous barrier	Increased transepidermal water loss	[27]
	Decreased cutaneous barrier repair	[27]
	Decreased epidermal cell proliferation	[27]
CNS	Resistance to TNF-induced sickness behavior	[60]

response in FAN-deficient mice might be a consequence of the alteration of CD40 and/or TNF-R1 signaling. The weak humoral response was associated with a reduced spleen leukocyte number in FAN-deficient animals [17]. As a matter of fact, spleen macrophages, neutrophils, and dendritic and lymphoid cells were reduced in FAN-deficient mice as compared with their wild-type counterparts [17]. Thus, FAN is likely involved in the recruitment of a diverse leukocyte population within secondary lymphoid organs, contributing to the establishment of the specific immune response.

The molecular mechanisms by which FAN modulates immune responses remain to be clarified. An IPA network of genes exhibiting a FAN-dependent optimal expression in response to TNF indicates that FAN may play a role in the specific immune response [17]. Indeed, focusing on genes encoding proteins involved in the modulation of immune and inflammatory responses, the main functions associated with the subnetwork are antigen presentation and cell-mediated and humoral immune responses (**Fig. 2**). For instance, some genes encoding MHC proteins and proteins involved in the regulation of antigen presentation, such as TAPBP and the CD74 antigen, are optimally up-regulated in a FAN-dependent manner in response to TNF. Whether FAN is involved in antigen presentation and the establishment of other types of immune responses requires additional investigations. Interestingly, IL-6 seems to be connected directly or indirectly to most of the genes presented in this subnetwork (Fig. 2). For instance, not only TNF but also TLR agonists induce IL-6 expression in a NF-κB-dependent manner [61, 62]. Under some experimental conditions, IL-6 triggers the expression of mRNA, encoding IFI16 [63] as well as Gbp2 and MHC proteins [64]. IL-6 plays a major role in immune responses, including T cell-dependent antibody response [65]. Thus, some biological functions of FAN may depend on its ability to favor IL-6 production.

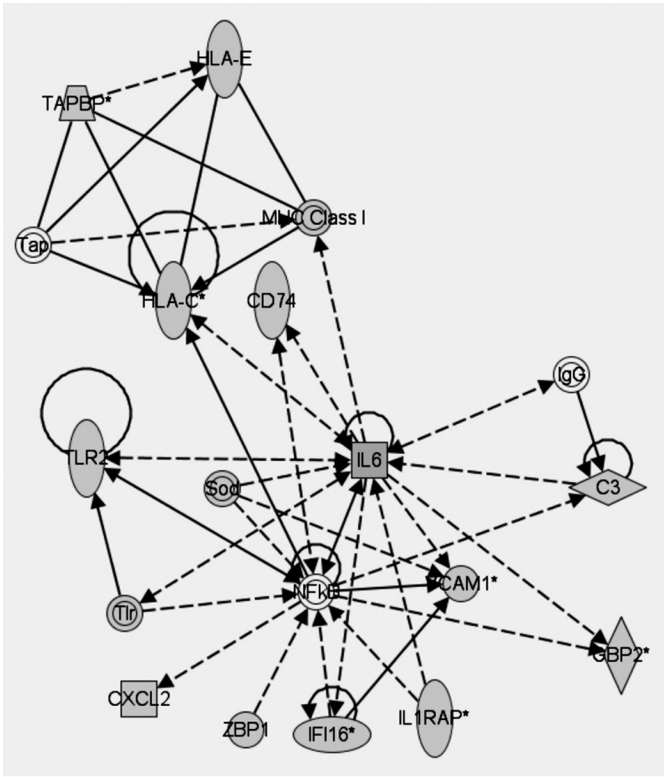

Figure 2. Network analysis for genes exhibiting a FAN-dependent optimal expression in response to TNF-α. The subnetwork of genes, which are up-regulated in a FAN-dependent manner and involved in immune response, is depicted. Direct interactions are represented by solid lines and indirect ones by dashed lines. Molecules in red are the focus genes induced in wild-type MEFs and to a lesser extent, in FAN-deficient MEFs. Molecules in white have been added by IPA based on their high connectivity with focus genes. Note that MHC genes are referred to as HLA by IPA. Tap, Transporter associated with antigen processing; Sod, superoxide dismutase; VCAM1, vascular cell adhesion molecule 1; ZBP1, Z-DNA binding protein 1; ILIRAP, IL-1R accessory protein.

Various other phenotypic alterations have been reported in FAN-deficient mice, highlighting the important contribution of FAN to diverse pathophysiological conditions (Table 1). The role of FAN in acute inflammatory responses has been evaluated in fulminant hepatitis triggered by LPS or TNF-α in D-galactosamine-sensitized mice [16]. FAN-deficient mice were significantly resistant to both fulminant hepatitis models [16]. Noteworthy was the finding that the resistance of FAN-deficient animals was associated with a reduction of systemic IL-6 production [16], indicating for the first time the putative role of FAN in cytokine gene expression in vivo. This tenet has been supported recently by findings about peritonitis models triggered by TNF-α or thioglycollate [17]. Indeed, cytokine secretion into the peritoneal fluid was selectively reduced in FAN-deficient mice as compared with wild-type animals in both peritonitis models. As a matter of fact, whereas MCP-1 secretion was not affected by FAN deficiency, IL-6 and CXCL-2 were produced less in the peritoneal cavity of FAN-deficient mice challenged with TNF-α or thioglycollate [17]. Consequently, recruitment of neutrophils, but not macrophages, into the peritoneal cavity was reduced significantly in FAN-deficient mice. Collectively, our recent data argue for a selective role of FAN in gene expression, leukocyte recruitment, and the establishment of acute inflammatory responses [17] (Table 1).

The role of FAN is unlikely to be restricted to the modulation of inflammatory and immune responses. Indeed, FAN-deficient mice showed an increase in transepidermal water loss and a delayed kinetics of recovery after cutaneous barrier disruption, which was associated with a reduced keratinocyte proliferation [27] (Table 1). In another study, FAN has been reported to be involved in TNF-induced expression of peroxisome proliferator-activated receptor β/δ, a nuclear receptor involved in keratinocyte differentiation [66]. These observations indicate the important physiological role of FAN in epidermal barrier homeostasis and repair. Moreover, an intracerebroventricular injection of TNF-α did not trigger sickness behavior in FAN-deficient mice, highlighting the essential role of FAN in the TNF-α-mediated central action [60] (Table 1).

CONCLUDING REMARKS

FAN has been described initially as an adaptor protein that constitutively binds to the NSD of TNF-R1 and is required for

TNF-α-triggered neutral sphingomyelinase activation [25]. Recent findings indicate that FAN is necessary for the activation of neutral sphingomyelinases 2 and 3 [45, 50]. A growing body of evidence in the literature demonstrates that NSD and FAN modulate TNF-induced caspase cascade activation and apoptosis [41, 52, 53], actin cytoskeleton remodeling [33, 37], and gene expression [17]. Moreover, FAN-deficient mice exhibit various phenotypic alterations including reduced cutaneous barrier repair [27], inflammatory and immune responses [16, 17], and sickness behavior [60]. Thus, FAN likely modulates several biological and physiological responses mediated by TNF-R1 and can be considered as a moonlighting protein involved in apoptosis and inflammatory signaling. Moreover, FAN's biological functions are not restricted to TNF-R1, as it also physically and functionally interacts with CD40 [26] and the G protein-coupled CB1 cannabinoid receptor [67]. Indeed, CD40 and CB1 contain intracellular sequences that share some homology with the NSD of TNF-R1, interact with FAN in coimmunoprecipitation experiments, and trigger sphingomyelin breakdown in a FAN-dependent manner [26, 67].

Whether FAN's role depends on its ability to favor the generation of biologically active sphingolipid metabolites via neutral sphingomyelinase activation remains an open issue. Deciphering the FAN-dependent molecular pathways is essential to better clarify the role of this protein in cell signaling and to determine putative, new pharmacological targets for the treatment of TNF-dependent, proinflammatory diseases. Anti-TNF-α therapy in autoimmune diseases is associated with enhanced susceptibility to infection. As FAN-deficient mice did not exhibit higher susceptibility to infection while exhibiting partial resistance to inflammatory responses triggered by TNF-α [16, 17], FAN and/or downstream effectors may constitute potential therapeutic target(s) to attenuate the deleterious inflammatory effects and preserve the TNF-α-dependent, anti-infectious response.

AUTHORSHIP

A.M. and B.S. wrote the manuscript; P.G.P.M. conceived of Figure 2 and edited the manuscript; and T.L. and H.B. improved the manuscript.

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