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THE ROLE OF TWEAK/Fn14 IN THE PATHOGENESIS OF INFLAMMATION AND SYSTEMIC AUTOIMMUNITY

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1. ABSTRACT

Interactions between members of the TNF ligand superfamily with their cognate TNF receptors play a crucial role in maintaining immune homeostasis in normal individuals, while dysregulation of certain TNF-ligands and receptors contributes to the pathogenesis of autoimmunity. Identification of novel members of the TNF ligand and receptor families will promote our understanding of the pathogenesis of systemic autoimmune diseases, thus facilitating the development of novel therapeutic approaches.

TNF-like weak inducer of apoptosis (TWEAK), a recently identified member of the TNF ligand family, induces PGE2, MMP-1, IL-6, IL-8, RANTES, and IP-10 in fibroblasts and synoviocytes, and upregulates ICAM-1, E-selectin, IL-8, and MCP-1 in endothelial cells**.** The receptor for TWEAK, Fn14, is expressed in various organs including the kidney; it is intriguing that some of these chemokines induced by TWEAK are crucial in the pathogenesis of lupus nephritis. Furthermore, others have described upregulated TWEAK expression on the surface of T cells in human lupus.

In this paper we review the possible roles of TWEAK/TWEAK receptor interactions in the pathogenesis of inflammatory and systemic autoimmune diseases, with particular focus on systemic lupus erythematosus. TWEAK blockade may be helpful therapeutically in restoration of tolerance, but is more likely to modify inflammatory damage in target organs.

2. INTRODUCTION

2.1. TNF ligands and receptors in immunity and autoimmunity

Interactions between TNF-like ligands and their receptors are assuming increasing importance in our understanding of the regulation of normal and pathologic immune responses. In the specific (adaptive) immune response, lymphocyte activation requires not only recognition of antigen by the T and B cell receptors, but also co-stimulatory signals generated by engagement of additional receptor-ligand pairs. The interaction of the TNF family ligand CD154 (CD40 ligand, CD40L) on activated T cells with the TNF-receptor family member CD40 on B cells induces B cell proliferation and formation of germinal centers. Subsequent events taking place in the germinal center that define T cell-dependent antibody responses including isotype switching, somatic mutation, clonal expansion, and terminal differentiation into plasma cells, are also dependent on binding of CD154 to its receptor (1,

2). In lupus, CD40L is overexpressed on T cells, and aberrantly expressed on monocytes and B cells. Furthermore, soluble CD40L is also present in high levels in the serum of lupus patients, where it can induce B cell activation (3, 4). Therapeutically, treatment of young lupus mice (5) or even older mice with already established disease using anti-CD154 treatment decreases the incidence of severe renal disease and prolongs survival (6), while normalizing disease-induced alterations in apoptosis, chemokine expression, and dendritic cells (7). Similarly, Quezada *et al.* recently reported that anti-CD154 was effective in both early and late murine lupus, although the mechanisms of protection was different between these two time points (8). Finally, although human trials needed to be stopped early because of the unexpected development of thrombosis, a short course of an anti-CD40L antibody treatment given to patients with proliferative lupus glomerulonephritis significantly decreased hematuria and anti-dsDNA antibodies levels and increased C3 complement concentrations (9), while markedly decreasing the number of IgG and IgG anti-dsDNA secreting cells (1). Furthermore, Grammer *et al*. recently showed that treatment of human lupus patients with a humanized anti-CD154 antibody disrupted active germinal center responses, decreased the numbers of circulating plasmacytes, and diminished anti-dsDNA serum autoantibody titers (10).

B cell activating factor (BAFF), also know as BlyS, THANK, and TALL-1, is a TNF family member expressed on dendritic cells, monocytes/macrophages, and T cells. Through several TNF-receptor family members (TNFRSF) including BAFF-R, TACI, and BCMA, BAFF acts as a positive regulator of B cell function, while promoting cell survival, activation and differentiation (11). Analogous to the related CD40/CD40L receptor-ligand pair, blockade of the TACI receptor prevents formation of germinal centers, and inhibits T cell dependent B cell responses (12). BAFF-transgenic mice develop high titers of anti-DNA antibodies and kidney immunoglobulin deposition, while treatment of lupus prone NZB x NZW F1 mice with soluble forms of BAFF receptors (TACI-Ig and BAFF-R) improves survival (13, 14). BAFF blockade is currently in clinical trials for the treatment of human SLE, and is believed to be a promising new approach in this disease. Clearly, TNF family ligand-receptor pairs are crucial in immune homeostasis. Moreover, by blocking pathological interactions between these molecules it is possible to restore tolerance in systemic autoimmune disease.

3. TWEAK (TNF-LIKE WEAK INDUCER OF APOPTOSIS), A NOVEL TNF FAMILY MEMBER

The cDNA for TWEAK was discovered fortuitously during cloning of an erythropoietin related gene in peritoneal macrophages (15, 16). This novel gene was assigned to the TNF family of ligands (TNF superfamily [TNFSF]) based upon characteristic sequence motifs, and named TWEAK due to its TNF relatedness and weak apoptotic properties. Although TWEAK is also known as Apo3 ligand (17), several investigators could not confirm its binding to the TNF family receptor Apo3/DR3 (see below), and TWEAK became the name in common usage. Similar to many other members of the TNFSF, TWEAK is a 249-amino acid long type II transmembrane protein with the N-terminus of the molecule inserted inside the cell. Between the transmembrane domain and the 206 amino acid C-terminal extracellular domain is a consensus sequence motif for furin cleavage. TWEAK is synthesized as a membrane bound protein, but similar to TNF itself it is processed early in the synthesis into a soluble form (the C-terminus extracellular receptor binding domain) which circulates (as a trimer) to mediate most of the biologic activities of the molecule. It is important to note that functional TWEAK can be detected in cell-culture medium, even though no TWEAK could be found on the cell surface (18). There is a high degree of homology in the receptor binding domain with other TNF ligand family members, and between mouse and human TWEAK (15, 16). Further aspects of the TWEAK structure can be found in the recent comprehensive review by Wiley and Winkles (16).

TWEAK mRNA is widely expressed, and can be found in the heart, pancreas, intestine, lung, and ovary, and at lower levels in the kidney and liver. Focusing on tissue and cells which are immunologically relevant, spleen, lymph nodes, peripheral blood mononuclear cells (monocytes/macrophages), and peritoneal macrophages, but not bone marrow or thymus, contained high levels of TWEAK message. As will be detailed below, TWEAK expression can also be found in vascular cells, astrocytes, microglia, and certain human tumor cell lines.

About 1 kb downstream from the gene encoding for TWEAK, near the telomeric end of chromosome 17 in humans (and in a syntenic position on mouse chromosome 11) is the gene for APRIL (19). APRIL is a TNFSF member which binds to two receptors also bound by BAFF, TACI and BCMA. Although the exact biologic function of APRIL is unclear, this cytokine promotes tumor growth and is also believed to play an important role in immunomodulation (19). Hahne *et al.* (20) recently described a novel mRNA which they named TWE-PRIL. The TWE-PRIL protein is membrane bound, and consists of the intracellular, transmembrane, and stalk region of TWEAK, fused to the APRIL receptor binding region. TWE-PRIL protein was detected in primary human T lymphocytes and monocytes. This membrane-binding protein binds to the same receptors as APRIL, and is functional, inducing cell proliferation in Jurkat and Ramos cells (19, 20). The question of whether there is synergy between the biological effects of TWEAK and TWE-PRIL, if any, has not yet been explored.

4. Fn14, THE COGNATE RECEPTOR FOR TWEAK

Initially, it was reported that DR3/TRAMP/Apo3, a death-domain containing member of the TNF receptor family, was the receptor for TWEAK (17). However, several tumor cell lines sensitive to TWEAK-induced killing, including HT29, HSC3, and Kym-1 cells, do not express DR3 (21, 22). Moreover, Kaptein *et al.* (23) could not demonstrate binding of TWEAK to DR3 *in vitro*.

Using soluble TWEAK in an expression cloning approach, Wiley *et al.* (24) in 2001 successfully identified a novel receptor for TWEAK in a HUVEC cDNA library, and demonstrated that cross-linking of this receptor induced many of the known effects of TWEAK described in detail below. The TWEAK receptor (TweakR) was found to be identical to Fn14, a previously described gene discovered in fibroblast growth factor stimulated murine NIH 3T3 cells by differential display (25). Fn14 was thought to be involved in fibroblast adhesion and migration. [To prevent confusion, we will use Fn14 as the preferred term for the TWEAK receptor for the remainder of this review]. Although Polek *et al*. (26) recently found that TWEAK ligand induced differentiation into osteoclasts of a murine monocyte/macrophage cell line not displaying Fn14, suggesting the presence of a second TWEAK receptor, the ITEM-4 antibody used for detection of Fn14 in these studies is not optimal for detection of mouse Fn14. The predominance of evidence to date suggests that Fn14 is the receptor for TWEAK, and that Fn14 solely mediates the known biologic effects of TWEAK (22, 27).

The structure of Fn14 includes several interesting features (16, 24, 28) which will be reviewed here only briefly; More details are available in the review referenced above (16). Human Fn14 is 129 amino-acid long type I transmembrane protein containing a single extracellular cysteine rich domain. Although TNF receptor family members usually contain three or four of these domains (which might explain the delay in characterizing Fn14 as a member of this family), two other recently described TNFRSF members, BCMA and BAFF-R, also contain a single extracellular cysteine rich domain. Following proteolytic cleavage of a 27 amino acid N-terminal signal peptide, the mature 102 amino acid Fn14 protein is the shortest known member of the TNFRSF. There is about 90% sequence identity between the mature forms of human and murine Fn14; Moreover, the 29 amino acid cytoplasmic tail of Fn14 which transduces the TWEAK signal via a single TRAF-binding site (see below) is also highly conserved between mouse and human. Although the affinity of TWEAK for Fn14 is much lower than that of TNF for the TNF receptor, it is within the range of affinities known for cognate pairs of TNF-like ligands and receptors (24). It is important to note that TWEAK binds only to Fn14 and not to other known TNFRSF members; similarly, other known TNF family ligands do not bind to Fn14 (24).

Fn14 is widely expressed on a variety of tissues, cells, and cell lines. Fn14 mRNA is present at relatively high levels in brain, heart, aorta, pituitary, adrenal, mammary gland, kidney, lymph node and lung, but is also found in liver, spleen, thymus, bladder and uterus (16). Fn14 is detectable on certain but not all neoplastic tissues, including liver and brain tumors (29, 30), and the following cell lines: pancreatic cancer (31), human colon adenocarcinoma, oral squamous cell, gastric adenocarcinoma, rhabdomyosarcoma (21), and glioma (30). Also worth noting is the upregulation of Fn14 seen in several experimental models during tissue stress, regeneration and repair (24, 32, 33). Finally, of particular interest to systemic autoimmunity and inflammation is the expression of Fn14 on multiple microvascular cell types (reviewed below), fibroblasts, monocytes/macrophages, neuronal cells, astrocytes, and mesangial cells.

5. MECHANISMS OF TWEAK SIGNALING

Some TNF receptor family members contain a defined sequence in the cytoplasmic tail of the molecule which is termed the death domain (DD). Binding of death domain binding proteins such as FADD (Fas associated death domain) to the TNFR DD initiates a program of caspase activation and programmed cell death. The 29 amino-acid long cytoplasmic tail of Fn14 is not, however, of sufficient length for a death domain, but rather contains a binding site for TNF-receptor associated factors (TRAFs). TRAFs are structurally related cytoplasmic adaptor molecules that function to activate downstream signaling (29). Several groups of investigators have identified TRAF 1, 2, 3, and 5 (out of the six structurally similar TRAFs that have been described) as binding to the cytoplasmic domain of Fn14 (24, 29, 34). A Pro-Ile-Glu-Glu sequence present in both human and mouse Fn14 was determined to be the likely site for TRAF binding in transmission of TWEAK signaling. Two threonines flank this putative TRAF binding site, and interestingly are the only potential phosphorylation sites in the entire receptor (16).

Signaling via TNFRSF members that associate with TRAF activates a number of intracellular signaling pathways, including those involving activation of NF-kappaB and the MAP-kinases JNK, ERK, and p38 (16). Several researchers have found that binding of TWEAK to Fn14 activates the NF-kappaB pathway (29, 34, 35). Interestingly, Saitoh *et al.* (35) recently demonstrated that TWEAK can signal via the canonical NF-kappaB pathway involving the IKK complex containing NEMO (similar to TNF), and also by the non-classical, NEMO independent NF-kappaB activation pathway (as do BAFF, CD40 and LTβ). As pointed out by Wiley and Winkles (16), NF-kappaB regulated genes including IL-6, IL-8, RANTES, and ICAM-1 are increased in response to TWEAK, supporting the importance of the NF-kappaB pathway in transducing TWEAK signaling. Donohue *et al.* (36) reported that TWEAK treatment of serum-deprived HUVECs stimulated not only an increase in phosphorylated IkappaB alpha (signifying activation of the NF kappaB pathway) but also increases in phosphorylated ERK 1/2 and JNK 1/2 (but not p38 MAP kinase). Nevertheless, a p38 inhibitor abrogated IL-6 and IL-8 secretion induced by TWEAK in human astroctytes (37), suggesting that multiple intracellular signaling cascades may be involved in the biological effect of this cytokine in various cell types under different conditions.

6. TWEAK-Fn14 INTERACTIONS AND THE INDUCTION OF APOPTOSIS

Several members of the TNF ligand superfamily directly trigger apoptotic death, including TRAIL, Fas, and lymphotoxin. As its name suggests, TWEAK shares this property of induction of apoptosis, albeit with reduced

potency relative to TNF itself. The initial observation of induction of apoptosis was made by Browning *et al.* (15) in the original paper first describing TWEAK. They found that in human gamma-interferon treated HT29 cells (a human adenocarcinoma cell line), cell death was induced by TWEAK within 16-20 hours. Gastric adenocarcinoma KATO-III cells were also weakly sensitive to TWEAK in conjunction with gamma-interferon, while TWEAK alone could induce apoptosis in oral squamous carcinoma HSC3 cells (38). Cytotoxicity against tumor cell lines by gamma-interferon stimulated human monocytes is mediated by TWEAK (38), as is apoptosis of monocytes and macrophages after activation of CD4+ positive T cells (39). It is important to note, however, that TWEAK killing of tumor cell lines is relatively weak, often requiring extended incubation time, relative high ligand concentrations, and co-incubation with sensitizing agents such as gamma-interferon.

What is the mechanism of TWEAK induced cell death? Initially, it was thought that TWEAK induces apoptosis indirectly via endogenously produced TNF-alpha interacting with the TNF receptor, a mechanism of death found in other TNF superfamily members lacking a death domain (40). Indeed, induction of cell death by TWEAK in the Kym-1 rhabdomyosarcoma cell line required the presence of TNF (40). However, TWEAK induction of death in HT29 cells was not found to involve induction of TNF or FasL expression (15), indicating that other, direct mechanisms of death may also be operative. Indeed, Nakayama *et al.* (21) described two additional cell-type specific mechanisms of cell death induced by TWEAK, including caspase-dependent apoptosis and cathepsin B-dependent necrosis. In one example, the cell death of HT29 cells induced by TWEAK is caspase-independent, encompassing features of necrosis and apoptosis (41). It is believed that Fn14 is directly mediating all these various types of death signals (22), although the signaling pathway(s) remain to be defined (41). Finally, it is also possible that TWEAK has non-direct effects that promote killing of tumor cells. It is known that tumors are not effectively infiltrated by leukocytes (particularly monocytes). By inducing some of the pro-inflammatory chemokines which will be described below, TWEAK can promote the migration and retention of inflammatory cells within the tumor environment, thus potentially facilitating a cytotoxic anti-tumor immune response (42, 43).

7. ANGIOGENIC AND PRO-INFLAMMATORY EFFECTS OF TWEAK ON PRIMARY CELLS AND CELL LINES

7.1. Angiogenic effects of TWEAK on endothelial cells

Wiley *et al.* (44) examined the proliferative effect of recombinant TWEAK on a variety of primary human vascular cells, including aortic endothelial cells, umbilical vein endothelial cells (HUVEC), dermal microvasculature endothelial cells, and brain microvasculature endothelial cells. The effect on aortic smooth muscle cells was studied as well. At picomolar concentrations (50 ng/ml), TWEAK induced a two to four fold increase in proliferation of these cell types, as compared to untreated cells. An even more pronounced proliferative effect was observed on HUVEC. Using RNase protection analysis and blocking antibodies to vascular endothelial growth factor (VEGF), Wiley found that the proliferative effect of TWEAK was not mediated via other genes known to be important in angiogenesis. To confirm the proliferative effect of TWEAK on human endothelial cells *in vivo*, the effect of TWEAK on neo-vascularization of rat corneas was evaluated by implantation of a pellet containing the cytokine. Corroborating the *in vitro* data, TWEAK induced formation of new blood vessels that was similar in magnitude to that induced by other known angiogenic stimuli, including basic fibroblast growth factor (bFGF) and VEGF. Similarly, Wiley *et al.* (24) in later work demonstrated that blocking TWEAK signaling with Fn14-Fc in a similar mouse cornea pocket assay inhibited bFGF-induced corneal vessel growth (as reflected in the area of vascularization) and vessel density. Angiogenesis not only involves proliferation; migration of endothelial cells is also a necessary part of this process. To assess the role of TWEAK signaling on endothelial cell migration, the effect of blocking TWEAK signaling in a wound closure assay was also reported in this study (24). It was found that Fn14-Fc treatment significantly inhibits the rate of phorbol myristate acetate (PMA) and epidermal growth factor (EGF) induced closure of circular wounds induced in a primary human microvascular endothelial cell monolayer, indicating that TWEAK also affects endothelial cell migration. Finally, Donohue *et al.* (36) found that TWEAK enhanced bFGF and VEGF stimulated proliferation of HUVECs, and induced endothelial cell migration.

Taken together, these results indicate that TWEAK has a direct effect on angiogenesis, and that at least part of the effect of other growth factors on endothelial cells is mediated by TWEAK-Fn14 interactions. In recent studies, Jakubowski *et al.* reported that depending on the angiogenic context, TWEAK may actually have differential effects on endothelial cells (45). While TWEAK alone did not enhance HUVEC proliferation, TWEAK displayed a synergistic effect with bFGF on endothelial cell proliferation and migration as compared to treatment with bFGF alone. TWEAK however did not affect wound repair or proliferation induced by VEGF. HUVEC proliferation and migration in response to TWEAK was also reported by Harada et al. (27). Another aspect of blood vessel growth, in addition to proliferation and migration, is morphogenetic organization of endothelial cells into capillaries. Jakubowski et al. found that together with bFGF, TWEAK stimulates the formation of lumen containing structures by a variety of human endothelial cells types, as well as the invasion of endothelial cells into a fibrin matrix. Capillary sprout formation induced by bFGF and TWEAK was not inhibited by antibodies to TNF-alpha or IL-8. One way to explain the synergism demonstrated between TWEAK and bFGF lies in the fact that bFGF can upregulate expression of Fn14 in endothelial cells (36). However, in contrast to a synergistic effect in combination with bFGF, TWEAK treatment antagonized the morphogenic response of HUVECs to VEGF. Finally in the study by Jakubowski et al. (45), while TWEAK alone did not affect these different aspects of microvascular growth (proliferation, migration,

morphogenesis), TWEAK treatment promoted HUVEC survival and resistance to apoptosis in culture with growth factor deficient media (45). Thus, TWEAK can surprisingly promote or inhibit angiogenesis, dependent on the prevailing growth factor environment.

7.2. Pro-inflammatory effects of TWEAK on endothelial cells

TWEAK not only has significant effects in modulation of angiogenesis, but also induces pro-inflammatory cytokine secretion in human endothelial cells. Harada et al. (27) found that similar to TNF-alpha and CD40L treatment, TWEAK upregulated cell surface expression of ICAM-1 and E-selectin on HUVEC. This effect was not mediated by induction of endogenous TNF-alpha, but was abrogated by preventing activation of NF-kappa B. Furthermore, secretion of MCP-1 and IL-8 from TWEAK-treated cells was induced to a degree seen with CD40L stimulation.

Thus, TWEAK has a multitude of effects on human endothelial cells. These include influencing proliferation, migration, and capillary formation, promoting cell survival, and increasing secretion of pro-inflammatory cytokines. Whether TWEAK alone had an effect (particular on proliferation and survival) was not always consistent among investigators, perhaps depending on variations in the experimental systems that were used. Nevertheless, based upon the potent effects on endothelial cells, manipulation of the TWEAK pathway might therefore be expected to have profound effects on a variety of neoplastic and inflammatory diseases.

7.3. Pro-inflammatory effects of TWEAK on astrocytes

In the murine brain, astrocyte and microglial cells express TWEAK mRNA (46) while primary human astrocyte cells from different areas of the brain express Fn14 on their cell surface (37). Saas et al. recently reported (37) that while human astrocytes bind to TWEAK, apoptosis is not induced in these cells, either in response to TWEAK exposure alone or after cytokine sensitization. However, treatment with TWEAK led to a dose dependent increase in IL-6 and IL-8 secretion. Production of IL-6 and IL-8 was completely blocked by pretreatment with a p38 inhibitor. Furthermore, while TWEAK did not affect expression of MHC, B7-1, the receptor for TNF, and Fas, ICAM-1 surface expression on astrocytes was significantly enhanced by TWEAK stimulation. Finally, in serum free conditions, astrocyte proliferation was increased twofold in the presence of TWEAK.

Interestingly, it has been recently shown (47) that Fn14 is induced in neurons following axon injury, and in response to treatment with nerve growth factor treatment. Moreover, Fn14 overexpression induced neurite outgrowth and growth cone formation via a Rac1 GTPase-dependent mechanism. These findings would suggest a role of Fn14 also in regenerative axon growth after injury.

7.4. Pro-inflammatory effects of TWEAK on synoviocytes and fibroblasts

To investigate a possible pro-inflammatory role of TWEAK on additional cell types (48), Chicheportiche *et al.* isolated human dermal fibroblasts from normal foreskin and synoviocytes from surgically obtained tissue from patients with rheumatoid arthritis and osteoarthritis. In response to incubation with TWEAK, PGE2, MMP-1, and IL-8 (but not tissue inhibitor of metalloproteinase-1) secretion was increased in both synoviocytes and fibroblasts. A much lesser degree of stimulation was seen for IL-6. Moreover, in synoviocytes and fibroblasts stimulated with TNF or IL-1 beta, addition of TWEAK augmented PGE2, MMP-1, and IL-8 two to four fold. The effects of TWEAK were not impacted by antibodies to TNF, but were completely abrogated by a blocking antibody to TWEAK. In addition, a potent effect on RANTES and IP-10 secretion from fibroblasts and synoviocytes, which was several fold higher than that seen with TNF-alpha stimulation, was observed following TWEAK treatment. Once again, adding TWEAK to TNF or IL-1 beta had a dramatic, synergistic effect. In an RNase protection assay, TWEAK also significantly upregulated RNA message for macrophage inflammatory protein (MIP)-1 alpha in rheumatoid synoviocytes but not dermal fibroblasts. Finally, increased IL-8 secretion in response to TWEAK was also seen in the WI-38 fibroblast cell line (15). The marked effect of TWEAK in inducing secretion of pro-inflammatory cytokines and chemokines from osteoarthritis and rheumatoid arthritis synoviocytes, will no doubt provoke serious investigation into the role of TWEAK/Fn14 interactions in the pathogenesis of chronic inflammatory and non-inflammatory arthritides.

8. THE ROLE OF TWEAK IN AUTOIMMUNE DISEASES

8.1. TWEAK and autoimmune neurological disease

The distribution of TWEAK and TWEAK receptor in the brain, and the demonstrated effects of TWEAK on astrocytes and other cell types, led Boucraut *et al.* (46) to study a possible role of TWEAK/Fn14 interactions in the pathogenesis of experimental allergic encephalomyelitis (EAE), a mouse model of human multiple sclerosis. Forty-eight hours after the onset of EAE induced by myelin oligodendrocyte glycoprotein (MOG) immunization, mice displayed a significant increase in TWEAK mRNA in the spinal cord but not in the encephalon. Boucraut then generated mice transgenic for a soluble TWEAK expression construct under the control of an alpha-anti-trypsin promoter with circulating TWEAK levels of 300-600 ng/ml, and examined the effect of these high circulating TWEAK levels on the induction and severity of MOG-induced EAE. TWEAK transgenic mice were susceptible to MOG-induced TWEAK, with no difference in the incidence of EAE or in disease onset. However, the maximum clinical severity and the mean clinical score were increased in TWEAK-transgenic mice. Moreover, survival was significantly less in the TWEAK transgenic mice.

What may be the role of TWEAK in EAE and other neuroinflammatory disease? Boucraut *et al.* (46) suggest that astrocytes, in response to TWEAK coming from glial cells, infiltrating mononuclear cells, or the astrocytes themselves, are induced to proliferate and induce

cytokines. The cytokines induced by TWEAK can increase the permeability of the blood-brain barrier and facilitate the passage of additional immune cells and mediators into the central nervous system. The blood brain barrier can also be conceivably affected by some of the effects of TWEAK on endothelial cells. Specifically, ICAM-1 on astrocytes and endothelial cells directs cell migration into and through the central nervous system, while IL-6 and IL-8 directly affect barrier permeability (46).

8.2. TWEAK in systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a multisystem, autoimmune disease typically affecting young women. Tissues and organ systems commonly involved in lupus patients include the skin, joints, serosal surfaces, kidney, central nervous system, blood forming elements, heart, and mucous membranes. Involvement of the kidney (lupus nephritis) and central nervous system (neuropsychiatric or central nervous system [CNS] lupus) are of particular importance to clinicians and investigators due to the life threatening nature of disease expression in these organs. While effective treatment is available for many lupus patients, lupus nephritis and CNS lupus are major causes of morbidity and mortality in this disease. Patients with SLE display a wide variety of immunological abnormalities in the specific immune response, including B cells and T cells. Nevertheless, it is clear that a breakdown of tolerance to nuclear antigens in the B cell compartment and the presence of a multitude of autoantibodies are pivotal features in the fascinating and perplexing pathogenesis of this disease.

Richardson *et al.* (49) reported that patients with lupus demonstrate increased apoptosis of autologous but not allogeneic monocytes/macrophages. In investigating the mechanisms underlying this observation, this group reported (50) the presence of a MHC-restricted, autoreactive T cell subset in lupus patients responsible for increased apoptosis. Both CD4+ and CD8+ T cells subsets are necessary for optimal killing. More recently, with the description of the pro-apoptotic effects of TNF ligands, this group studied whether TWEAK and other TNFSF members are involved in monocyte/macrophage apoptosis in lupus (51). Richardson *et al.* (51) evaluated the expression of TWEAK, TRAIL, and FasL in patients with SLE, in comparison to patients with rheumatoid arthritis and normal controls. The percentage of T cells (CD4+ and CD8+), but not B cells, expressing TWEAK in SLE patients was significantly increased. TWEAK expression was pronounced on a subset of activated T cells, and was not due to the effect of medications given to these patients; Similarly, T cells from normal individuals activated with anti-CD3 and anti-CD28 also upregulated expression of apoptotic ligands, including TWEAK. As had been shown previously, co-incubation of TWEAK expressing, lupus T cells with autologous monocytes resulted in cytotoxicity. Lupus monocytes showed the same susceptibility to pro-apoptotic ligand cell death as monocytes from normals, suggesting that increased monocyte cell death is not due to abnormal sensitivity to apoptosis in lupus monocytes/macrophages but rather results from an autoreactive T cell subset characteristic of lupus. Using soluble TWEAK receptor (Fn14-Fc), Richardson was able to demonstrate that TWEAK inhibition could largely prevent monocyte apoptosis induced by T cell upregulation of pro-apoptotic TWEAK. This effect was not due to inhibition of T cell proliferation and activation. Similar inhibition was found with TRAIL or FasL blockade, suggesting redundancy in apoptosis pathways. Furthermore, antibodies to IL-4, IL-10, and interferon did not inhibit T cell induced monocyte cytotoxicity, indicating that the apoptosis was mediated via apoptotic ligand expression on T cells rather than a cytokine effect. In summary, a subset of activated autoreactive T cells promotes cytotoxicity of autologous monocytes in lupus patients, an effect mediated by increased surface expression of TWEAK.

Even less is known about TWEAK and Fn14 expression and interactions in murine lupus (52). In NZB mice, a mouse strain that develops autoimmune hemolytic anemia and is also one of the parental strains of the NZB x NZW F1 lupus prone mouse, TWEAK mRNA was much lower in the liver and kidney at 12 months of age (when active hemolysis was present) as compared to 8 months of age, when the hematocrit was normal. Similarly, kidney TWEAK mRNA levels were decreased at 9 months of age in BXSB mice (a lupus prone mouse strain harboring *Yaa*, the **Y**-linked **a**utoimmunity **a**ccelerator gene) with active glomerulonephritis, when compared to kidney mRNA expression at 5-7 months of age at which time kidney histology is still relatively preserved. Female BXSB mice with normal kidney histology showed no alteration in TWEAK expression at 9 months of age. However, in a different mouse model (NZB.Yaa), down-regulation of TWEAK mRNA expression preceded the appearance of significant disease manifestations, indicating that changes in TWEAK expression can be heavily influenced by background genes. Furthermore, these studies only looked at mRNA levels; whether protein levels of TWEAK are similarly affected was not reported.

8.2.1. The role of TWEAK-inducible chemokines in lupus nephritis

Chemokines, which are chemoattractant cytokines, play a pivotal role in inflammatory processes. Cells challenged with a pathological pro-inflammatory stimulus secrete a variety of different chemokines, which function to regulate and direct leukocyte migration into tissues. These leukocytes are subsequently responsible for much of the tissue injury that can be seen in the affected organ (53). Specifically in human inflammatory renal disease, including a variety of diseases causing acute glomerular and tubulointerstitial inflammation, chemokines are believed to be crucial in disease pathogenesis (reviewed in 54). For example, several chemokines (including MCP-1, RANTES, and IL-8) and their receptors are upregulated in IgA nephropathy, Wegener's granulomatosis, crescentic glomerulonephritis, and acute renal transplant rejection (54). Furthermore, following certain types of injury, many renal diseases do not improve but rather become chronic. Chemokines and chemokine receptors are believed also to

have a central role in regulating the resolution or progression of renal injury (reviewed in 55).

There is ample evidence pointing to chemokine upregulation as a central event in triggering of kidney injury in SLE, or lupus nephritis. MCP-1, RANTES, IP-10, and MIP-1beta expression are all found in kidneys of lupus mice with active nephritis (54, 56). To determine if chemokine upregulation is a primary event in the pathogenesis of nephritis, Schlondorff *et al.* serially analyzed the expression of chemokines, chemokine receptors, and pro-inflammatory cytokines in kidneys of MRL-lpr/lpr lupus prone mice (56). It was found that chemokine upregulation *precedes* infiltration of inflammatory cells and kidney damage, and that this process is limited to areas in which infiltrating cells are subsequently found. Mononuclear cell infiltration occurs together with upregulation of chemokine receptors, with upregulation of pro-inflammatory cytokines and onset of proteinuria only coming later. Conclusive evidence for the centrality of chemokines in lupus pathogenesis can also be found in genetically manipulated lupus mice. Markedly improved renal histopathology and improved survival was noted by Kelly *et al.* (57) in MRL-lpr mice rendered genetically deficient for MCP-1. More recently, it was reported (58) that MRL-lpr mice treated with an MCP-1 antagonist displayed significantly decreased cellular infiltration into the kidney by macrophages and T cells, with improvement in glomerular hypercellularity, crescent formation, and vasculitis. In additional studies by Kelly *et al.* (59), it was discovered that local renal upregulation of RANTES by gene transfer into macrophages transplanted under the kidney capsule of MRL-lpr mice accelerated recruitment of T cells and macrophages and promoted nephritis. It is quite interesting that several chemokines that have been shown to be upregulated by TWEAK treatment of endothelial cells, fibroblasts, and synoviocytes, including MCP-1 and RANTES, are pivotal in the initiation and progression of the histopathological lesions of lupus nephritis. In preliminary studies we have recently demonstrated that mesangial cells from lupus-prone mice display Fn14 on the cell surface, and release MCP-1, RANTES, and IP-10 in response to Fn14 engagement by TWEAK (60), suggesting that resident kidney cells, in response to TWEAK, may secrete chemokines which are crucial in the inflammatory cascade leading to lupus nephritis.

9. IS TWEAK INVOLVED IN THE PATHOGENESIS OF TOLERANCE BREAKDOWN AND TARGET ORGAN DAMAGE IN SLE?

9.1. TWEAK/Fn14 interactions and tolerance to nuclear antigens

A central serologic feature present in lupus is antibodies against nuclear antigens, including autoantibodies to nucleosomes, histones, and dsDNA. Abnormalities in apoptosis are receiving increasing attention in the study of the pathogenesis of SLE, and specifically in understanding the generation of these anti-nucleosomal and anti-DNA autoantibodies. According to this view, an increased amount of apoptotic material and/or a decrease in clearance results in abnormal processing of nuclear antigens (perhaps by non-professional antigen presenting cells), and generation of cryptic epitopes which activate histone and nucleosome specific T and B cells. There is increasing evidence to suggest that an immune response against nucleosomes released during the process of cellular apoptosis may be a pivotal event in the pathogenesis of lupus. Rosen *et al.* (61) made the early observation that ultraviolet light, a known environmental trigger of lupus, induces translocation of nucleoprotein complexes to apoptotic blebs on the cell surface, where these antigens are available for interaction with circulating antibodies. The process of apoptosis itself is associated with the generation of novel antigens to which tolerance has not been established (62). Indeed, hyperimmunization of normal mice with apoptotic cells was sufficient to induce a lupus-like autoantibody response, including antibodies to single stranded DNA and cardiolipin (63). Increased sensitivity of lupus patients to certain forms of environmental inducers of apoptosis can then explain how nuclear antigens in lupus become triggers and/or targets of an immune response. However, an increased apoptotic burden alone may not be sufficient for breakdown of tolerance to nuclear antigens, as clearance of apoptotic cells is obviously a normal physiologic process (64). A defect in apoptotic clearance may also be needed. Indeed, genetic defects interfering with disposal of apoptotic material, including DNAse I, serum amyloid P protein (SAP), mer, and complement deficiencies, have been linked experimentally to lupus (65, 66). In humans, 90% of individuals deficient for C1q component of complement develop SLE. Other genes that have been linked to human lupus, including Fc receptors, mannose-binding protein, and poly (ADP-ribose) polymerase (PARP), are all believed to be important in clearance of apoptotic debris (64).

As discussed above, the most intriguing evidence linking perturbed apoptosis in SLE and TWEAK comes from the work of Richardson *et al.* (51), who demonstrated that upregulated TWEAK on activated lupus T cells can induce apoptosis in autologous macrophages/monocytes. An increased apoptotic burden in and of itself may contribute to nuclear antigen autoimmunization. But it is possible that this specific form of TWEAK-induced apoptosis is a second hit necessary for breakdown of tolerance to nuclear antigens. Accelerated apoptosis of macrophages in lupus, also found by Mevorach *et al.* by itself dramatically decreases the efficiency of clearance of the remaining macrophages (67). Furthermore, under normal circumstances, uptake of apoptotic cells by macrophages is non-inflammatory and tolerogenic. However, if phagocytosis is inefficient, secondary necrosis may occur. Phagocytosis of necrotic cells by dendritic cells may actually cause maturation of dendritic cells, with upregulation of co-stimulatory molecules. Circulating pro-inflammatory cytokines and opsonization by autoantibodies may also contribute to shifting of the balance from the normal, tolerogenic signals induced by clearance of apoptotic cells to a diametrically opposed result. That is, presentation of nucleosomal antigens from apoptotic cells may actually promote dendritic cell activation, increase

MHC and co-stimulatory molecule expression, and prime autoreactive T cells responding to nuclear antigens. Finally, it is interesting that complexes of immunoglobulins from lupus patients and apoptotic monocytes stimulate alpha-interferon production (68), with all the known effects of this cytokine on promoting lupus pathogenesis (69).

9.2. TWEAK/Fn14 interactions and lupus nephritis

TWEAK/Fn14 interactions may not only be influencing lupus pathogenesis centrally in disruption of tolerance, but also can be crucial in local inflammatory cascades in the target organs. Of the multiple types of kidney involvement in human lupus, proliferative lesions including focal and diffuse proliferative glomerulonephritis are associated with the worse prognosis. Glomerular endothelial cells, monocytes, and lymphocytes all contribute to the endocapillary proliferation. Proliferative glomerulonephritis is also the histologic lesion that appears in the classic mouse models of the disease, MRL-lpr/lpr and NZB x NZW F1 mice. Although glomerular disease dominates the clinical picture in lupus nephritis, tubular, vascular and interstitial involvement is commonly seen. Tubulointerstitial findings include tubular basement membrane immune deposits, fibrosis, and infiltration by plasma cells, lymphocytes, and monocytes (70).

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How may TWEAK/Fn14 interactions be contributing to the pathogenesis of the renal lesions seen in lupus? One postulated pathway may be an early upregulation of circulating TWEAK, leading to enhanced secretion of pro-inflammatory chemokines (MCP-1, RANTES) by mesangial, glomerular endocapillary, and possibly other resident renal cells. Chemokine upregulation would direct infiltration of inflammatory cells to the kidney, with the ensuing production of injurious cytokines. Some of the cells infiltrating in the kidney would themselves serve as a source of membrane bound and/or secreted TWEAK, thus creating a positive feedback loop. Both resident cells (endothelial cells, mesangial cells) as well as cells infiltrating the kidney in lupus nephritis (macrophages) are potentially capable of responding to circulating TWEAK, or TWEAK being produced locally. Another important contribution of TWEAK to kidney inflammation may be via its proliferative effects. TWEAK may induce glomerular endocapillary proliferation, a pathologic hallmark of lupus nephritis, as well as changes in interstitial and larger blood vessels. TWEAK-inducible effects, together with other known contributors to the pathogenesis such as anti-DNA antibodies forming immune complexes, would act in concert to induce the histopathologic manifestations of the disease in the kidney. Is indeed TWEAK upregulation an early phenomenon in lupus? Do kidney cells in vivo secrete a pro-inflammatory cocktail of chemokines in lupus similar to that found in other cell types in response to TWEAK? Would blocking the TWEAK pathway result in improvement in lupus nephritis by inhibiting critical, chemokine regulated pathways? The answers to these questions are currently unknown, and will be addressed in future studies.

9.3. TWEAK/Fn14 interactions and neuropsychiatric SLE

Although a neurological presentation is uncommon in lupus patients, eventually up to 75% of patients will develop a neurological or psychiatric syndrome attributable to SLE (71). Not only is CNS disease common, but brain involvement is a significant negative prognostic factor. There are many diverse clinical CNS manifestations in SLE, including headache, seizures, movement disorders, encephalopathy, myelopathy, meningitis, dementia, strokes, and psychiatric disorders. It is unclear whether a single mechanism underlies these very different clinical syndromes. In fact, multiple mechanisms are likely to be involved (71, 72, 73). What is known is that when brain tissue from lupus patients is examined under the microscope, histologic vasculitis with inflammatory infiltration within the walls of affected vessels and fibrinoid necrosis is surprisingly rare. Rather, a picture of small vessel vasculopathy is commonly seen. Pathologic lesions often found in patients with CNS lupus include endothelial cell proliferation and hypertrophy, fibrin thrombi, and extravasation of erythrocytes and fibrin (71, 74, 75). The affected small vessels can be surrounded by clusters of microglia, small infarcts, hemorrhage, white matter necrosis, and perivascular inflammatory infiltrates (72). Cerebral small vessel vasculopathy and the resultant ischemia are believed to be the cause for at least some of the neuropsychiatric manifestions of systemic lupus.

While cytokine abnormalities in sera of lupus patients are commonly found, there is some evidence to support local, intrathecal cytokine production. Trysberg *et al.* (76) reported that cerebrospinal fluid (CSF) levels of IL-6 and IL-8 were increased ten-fold in patients with neuropsychiatric lupus as compared to lupus patients without CNS involvement, while no differences were present in serum levels of these cytokines. Moreover, IL-6 levels in the CSF decreased with remission of neurological symptoms following treatment.

How may TWEAK be related to the pathophysiology of neuropsychiatric lupus? We postulate that the effects of TWEAK on endothelial cells and astrocytes may be instrumental in this process. Stimulation of endothelial cells by this ligand results in endothelial proliferation, possibly a prime contributor to the proliferation and hypertrophy seen in CNS lupus vasculopathy. Furthermore, IL-6 and IL-8 secretion is induced. Moreover, TWEAK can prime the endothelial cells to upregulate adhesion molecule expression, thus promoting leukocyte-endothelial cell adhesion (77). These effects would act in concert to promote the occlusive vasculopathy that is observed. Additional IL-6 and IL-8 is contributed by TWEAK-stimulated astrocytes. Furthermore, as a direct effect of TWEAK stimulation or in response to endogenous or exogenous IL-6 and IL-8, astrocyte proliferation and/or damage is induced. Actively proliferating astrocytes, in turn, may begin to express the target antigen for neuropathic anti-DNA antibodies (78). Supporting the involvement of astrocytes in the pathophysiology of CNS lupus is the recent observation by Trysberg *et al.* (79) that increased levels of glial fibrillary acidic protein, a major structural element of astrocytes, were present in the CSF of patients with CNS involvement.

Furthermore, levels of this protein which is released in response to astrocyte injury, correlated with other clinical and laboratory features of CNS lupus (79).

10. PERSPECTIVE

The interaction of TWEAK, a novel TNFSF ligand, with its recently discovered receptor Fn14 has several pro-inflammatory effects which may be important in the pathogenesis of systemic lupus erythematosus, and perhaps other systemic autoimmune rheumatic diseases. TWEAK induces secretion of multiple chemokines which are pivotal in the early pathogenesis of one of the major manifestations of SLE, lupus nephritis. TWEAK inducible cytokines are conceivably involved also in the pathogenesis of CNS lupus, another life threatening disease manifestation. Such effects of TWEAK in lupus may therefore determine the extent and severity of target organ involvement in disease. An additional biological effect of TWEAK is the induction of apoptosis. Increased apoptosis and/or decreased clearance of apoptotic material are believed to be responsible for the breakdown of tolerance to nuclear antigens in several genetic models of murine lupus. Indeed, a preliminary report found increased TWEAK-mediated killing of autologous macrophages by T cells from lupus patients. Further studies looking at the expression of TWEAK and Fn14 in tissues affected by the autoimmune process in SLE are needed to explore and support a possible role of TWEAK in the pathogenesis of SLE. Blocking the interactions of several TNF ligand superfamily members with their cognate receptors has been shown to be very effective in the treatment of mouse models of SLE, and preliminarily in human lupus patients. It remains to be seen whether novel reagents already in development that block the effects of TWEAK may selectively improve target organ involvement in SLE. Furthermore, it will be interesting to study whether TWEAK blockade, alone or in conjunction with other therapeutic approaches, may contribute to restoration of tolerance to nuclear antigens in SLE.

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