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Extra domain A-positive fibronectin-positive feedback loops and their association with cutaneous inflammatory disease

John P. McFadden, MD[a,*], David A. Basketter, DSc[a], Rebecca J. Dearman, PhD[b], Ian R. Kimber, PhD[b]

[a]*Department of Cutaneous Allergy, St John's Institute of Dermatology, St Thomas' Hospital, SE1 7EH London, UK*
[b]*Faculty of Life Sciences, University of Manchester, Simon Building, M13 9PL Manchester, UK*

Abstract Cutaneous inflammation can show Th1 or Th2 predominance, but the precise mechanisms by which such selectivity is determined are unknown. A recent study has demonstrated that Th1 cells, but not Th2 cells, produce an endogenous ligand for Toll-like receptor (TLR) 4, namely extradomain A+ fibronectin containing extra type III domain A (FnEDA+). As TLR4 stimulation leads to production of proinflammatory cytokines that recruit (via altered endothelial adhesion molecule expression and chemokine production) more Th1/Th17 cells, a positive feedback mechanism for Th1/Th17 inflammation exists. We propose that FnEDA+ positive feedback loops are a potential driver of Th1/Th17 inflammation. Conversely, the inflammatory EDA+ fibronectin loop is negatively regulated in atopic dermatitis, Th2 cytokines actively suppress TLR4 expression of Th1 cytokines, and recruited Th2 cells do not produce FnEDA+. In psoriasis, there are multiple FnEDA+ loops, comprising inflammatory, keratinocyte, and autoimmune loops. In allergic contact dermatitis, a single inflammatory loop operates. In atopic dermatitis, the FnEDA+ loop is actively suppressed by Th2 cytokines, and recruited Th2 cells do not "feedback" FnEDA+. We review endogenous ligands for TLR in relation to inflammatory disease, FnEDA+ function, and the potential role for FnEDA+ in psoriasis, allergic contact dermatitis, and atopic dermatitis.

Introduction

It is now apparent that within the skin, there are important interfaces between tissue trauma or damage, the innate immune system, and adaptive immunity. Signs of damage, danger, or threats, manifested as pathogen-associated molecular patterns, are recognized by cellular components of the adaptive immune system by way of pathogen recognition receptors.[1] Such pathogen recognition receptors include the Toll-like receptors (TLR), the nucleotide oligomerization domain-like receptors, and the C-type lectins. TLRs are found at the plasma membrane (TLR1, 2, 4, 5, 6, and 10) or the endosomal membrane (TLR3, 7, 8, and 9).[2] One important ligand for TLR4 is an embryonic form of cellular fibronectin (Fn) generated by alternative splicing that bears an extra type III domain A (EDA). FnEDA+ has a number of properties relevant for skin biology. Thus, FnEDA+ activates cutaneous dendritic cells (DC), stimulating their maturation and the production of cytokines.[3] Moreover, FnEDA+ stimulates the entry of keratinocytes into the cell cycle.[4]

* Corresponding author.
E-mail address: john.mcfadden@kcl.ac.uk (J.P. McFadden).

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Here, we hypothesize that the behavior of FnEDA+ and the pattern of its interaction with surrounding cells and tissues may profoundly affect the pathophysiology and persistence of inflammatory skin diseases. In support of this, we describe evidence for the existence of discrete FnEDA+ feedback loops that directly or indirectly affect the behaviors of skin cells of several types and thereby influence disease process:

- In psoriatic skin, there is an elevated expression of FnEDA+ that drives keratinocyte proliferation.[5] Proliferating keratinocytes in turn stimulate the production of additional FnEDA+ production, that is then able to feedback, in autocrine fashion, stimulating further keratinocyte turnover—*the keratinocyte loop.* Furthermore, via activation of TLR4, FnEDA+ will provoke the maturation of skin DC (epidermal Langerhans cells and dermal DC) and stimulate the production of an array of cytokines that will, among other effects, cause the infiltration into the skin of Th1 and Th17 cells.
- Recent evidence has emerged that Th1 cells are able to produce FnEDA+, thereby creating the opportunity for another positive feedback loop—*the inflammatory loop.*
- The third loop is based on the observation that proliferating keratinocytes produce keratins, including keratins 6, 16, and 17. The argument is that these keratins can be recognized by T lymphocytes as autoantigens,[6] and that keratin-driven activation of responsive Th1 cells will in turn result in more production of FnEDA+ with the creation of a third loop—*the autoimmune loop.*

We postulate that the inflammatory FnEDA+ feedback loop is a central driver for Th1/Th17 inflammation and is operative in allergic contact dermatitis (ACD). In contrast, in atopic dermatitis, cytokines, such as interleukin (IL)-4, IL-13, and vasoactive intestinal peptide, downregulate TLR4, and recruited Th2 cells do not produce EDA+Fn. Therefore, the inflammatory FnEDA+ loop appears to be actively suppressed in atopic dermatitis, allowing for Th2 dominance. Thus, the inflammatory FnEDA+ loop is central to Th1/Th17-driven cutaneous inflammatory disease, whereas multiple FnEDA+ loops feature in psoriasis and there is active downregulation of the inflammatory loop in atopic dermatitis.

Endogenous ligands of TLRs

TLRs are one type of receptor (Table 1) involved in the rapid innate immune response against invading microorganisms by their recognition of conserved motifs of microbial origin, known as pathogen-associated molecular patterns.[7] In the skin they are present on many resident cells, including DC, keratinocytes, and mast cells. TLR signal-transduction leads to the release of proinflammatory cytokines such as IL-1β and tumor necrosis factor (TNF)-α.[8] TLRs are members of the IL-1 receptor (IL-1R) family and are characterized by the leucine-rich repeat domain in their extracellular region. Ten have been identified to date (TLR 1-10) and, in addition, TLR1, TLR6, and TLR9 form heterodimers with TLR2.[2,9]

Table 1 Endogenous ligands for toll-like receptors[a]

Ligand	Target TLR for ligand				
EDA+ fibronectin		TLR4			
Tenascin		TLR4			
Fibrinogen		TLR4			
Surfactant protein-A		TLR4			
Heparan sulphate		TLR4			
Soluble hyaluronan		TLR4			
β-Defensin 2		TLR4			
Heat shock protein 60	TLR2	TLR4			
Heat shock protein 70	TLR2	TLR4			
Gp96	TLR2	TLR4			
High-mobility group protein B1	TLR2	TLR4			
Messenger RNA					TLR3
Single-stranded RNA				TLR7	TLR8
DNA, DNA-containing immunocomplex					TLR9

[a] Most endogenous ligands of toll-like receptors (TLR) stimulate TLRT4 so with initial mechanical or chemical insult (skin trauma producing psoriasis, cutaneous hapten application) one may expect an initial TLR4 stimulation and resulting Th1/Th17-associated inflammation. The extracellular matrix proteins listed in particular appear to exclusively stimulate TLR4, whereas stress protein ligands stimulate TLR4 and TLR2, and intracellular proteins stimulate a variety of TLRs.

Bacterial lipopolysaccharide, a major cell-wall component of gram-negative bacterial cell wall, was first described as a microbial ligand for TLR, in this case TLR4.[10] Since then, various microbial components have been described as being able to stimulate different TLRs, including double-stranded RNA, flagellin, and peptidoglycan.[7] Thus TLRs are able to recognize conserved molecular signatures of potentially pathogenic bacteria, viruses, and fungi. TLRs are located on different resident and migrating cells within the skin, including keratinocytes, macrophages, mast cells, and certain T cells.

Certain endogenous molecules are able to activate the innate immune system through triggering of these TLRs. Heat shock protein (HSP) 60 was found to be an endogenous ligand for TLR4, with proinflammatory effects such as the production of IL-1, TNF-α, IL-6, IL-12, the release of nitric oxide and chemokines from DCs and macrophages, and maturation of DCs. Since then, HSP 60 has also been found to stimulate TLR2.[7,11] TLR4 stimulation also leads to release of IL-23 and the promotion of Th17 cell activation.[12] To date, endogenous ligands for TLR 2, 4 (extracellular), and 3, 7, 8, and 9 (intracellular) have been found. Of particular interest here is FnEDA+ as an endogenous TLR ligand.

Extra domain A+ fibronectin

Fn is a multifunctional glycoprotein found in plasma and in the extracellular matrix of tissue and is expressed by various cells.[13] In addition to cell adhesion and migration, the role of Fn in cell-extracellular tissue interaction is vital in embryogenesis, hemostasis, wound healing, and maintenance of tissue integrity.[13] Classical interaction between Fn and cells is through integrins, which are adhesion sensing and signalling molecules that promote stable interactions between cells and their environment. The arginine-glycine-aspartic acid (RGD) amino acid sequence within Fn represents an important integrin binding site; for example, it binds to $\alpha5\beta1$, which is important not only in angiogenesis[14] but also in multiple other functions, including keratinocyte proliferation, wound healing, and internalization and carriage of microbes in pharyngeal cells.[14] RGD also binds to several other ligands, including $\alpha9\beta1$, $\alpha3\beta1$, and $\alpha4\beta1$ (vascular endothelial growth factor 4).[13]

A Fn monomer consists of three different types of homologous repeating domains: type I (12 units), type II (2 units), and type III (15 units). Splicing Fn with EDA causes conformational changes with 180° rotation, which enhances exposure of different ligand binding sites by "stretching" the Fn molecule through incorporation on one (or both) of the alternatively spliced type III domains EDA and B.[13] Incorporation of EDA into Fn leads to enhanced exposure of the RGD site, thereby increasing interactions with integrins.[13]

As well as having prothrombotic actions through increased binding to integrins on platelets, FnEDA+ also has immune-stimulatory properties that mimic the effect of lipopolysaccharide and include the induction of genes encoding proinflammatory cytokines and matrix metalloproteinases through stimulation of TLR4 on macrophages, DCs, and keratinocytes.[3] FnEDA+, but not other Fn domains, activates TLR4, with subsequent nuclear factor-κB activation and matrix metalloproteinase expression.[3] FnEDA+ can stimulate, via TLR4, TNF-α, IL-1β, Il-6, IL-12, Il-18, Il-23, and interferon-α.[3,12,15,16] Release of such cytokines will tend to promote Th1- and Th17-dominated inflammation rather than Th2 cell activation.

FnEDA+ has other immunostimulatory properties. It can also facilitate antigen processing and presentation of peptide to cytotoxic T cells by DCs, increasing the magnitude of the lymphocyte response.[17]

FnEDA+ can stimulate different cascades of cell stimulation. Some of the cells targeted by these cascades for promotion, such as Th1 cells or, in psoriatic individuals, proliferating keratinocytes, can release FnEDA+ themselves.[4,18] The possibility therefore exists for positive feedback loops of FnEDA+ in inflammatory cutaneous diseases. We will compare the possible roles of FnEDA+ loops between psoriasis and ACD and how the inflammatory loop appears to be actively suppressed in atopic dermatitis.

Psoriasis: A cutaneous inflammatory disease characterized by multiple FnEDA+ feedback loops?

This has been addressed,[5] but will briefly be summarized here. There are at least two, and possibly three FnEDA+ feedback loops operative in psoriasis (Figure 1).

The keratinocyte FnEDA+ loop

FnEDA+ stimulates the keratinocyte growth cycle, which appears to be mediated through the RGD motif interacting through $\alpha5\beta1$ keratinocyte surface integrin.[19] FnEDA+ is more potent than FnEDA– in inducing the G1-S phase of cell cycling, with induced expression of the proliferative cyclin-D1 and tyrosine phosphorylation of $p130^{cas}$.[20,21] FnEDA+ increases cell cycle entry in keratinocytes from uninvolved psoriatic skin, but not from normal skin.[19,22] Hyperproliferating keratinocytes are themselves an important source of FnEDA+. Studies with cultured keratinocytes show that total Fn and FnEDA+ and the EDA+/EDA– ratio increases with proliferative state and peaks in highly proliferating cells.[4] Therefore, a simple autocrine feedback loop for FnEDA+ could be operative, uniquely, in psoriasis.

The inflammatory FnEDA+ loop

This involves the proinflammatory effects of Fn. Skin trauma is one of the best-known stimuli for psoriasis plaque formation (the Koebner phenomenon). After wound injury, TGF-β upregulates FnEDA+ and $\alpha5\beta1$ integrin expression.[23] TLR4 (present in the skin on keratinocytes and DC/macrophages) stimulation by FnEDA+ can lead to production of IL-1β, TNF-α, IL-6, IL-12, and IL-23.[3,12,16,17] These cytokines will in turn lead to endothelial expression of intercellular adhesion molecule-1, E-selectin, and vascular cellular adhesion molecule-1, with augmentation of T-cell recruitment into the lesional skin.[24] Such cytokine production should lead to promotion of Th1 and Th17 cell activation; and psoriasis is indeed characterized by such a cytokine and T-cell profile.[25] Experimental stimulation of TLR4 leads to production of chemokine (C-C motif) ligand (CCL) 20 and recruitment of CCL20-responsive chemokine receptor (CCR) 6+ Th1/Th17 cells.[26] Psoriasis is also characterized by tissue expression of CCL20 and infiltration by CCR6+ T cells.[25] In psoriasis, high levels of C-X-C motif chemokine 10 (CXCL10), which attracts Th1 cells, are present[27] as is likewise observed for a TLR4-stimulated model of skin inflammation[28] and in ACD.[29] Th1 cells recruited into skin will produce significant amounts of FnEDA+,[18] thus completing another positive feedback loop that can be operative. Although it has been shown that Th1 cells produce FnEDA+ and that Th2 cells specifically do not, it remains to be established whether Th17 cells produce

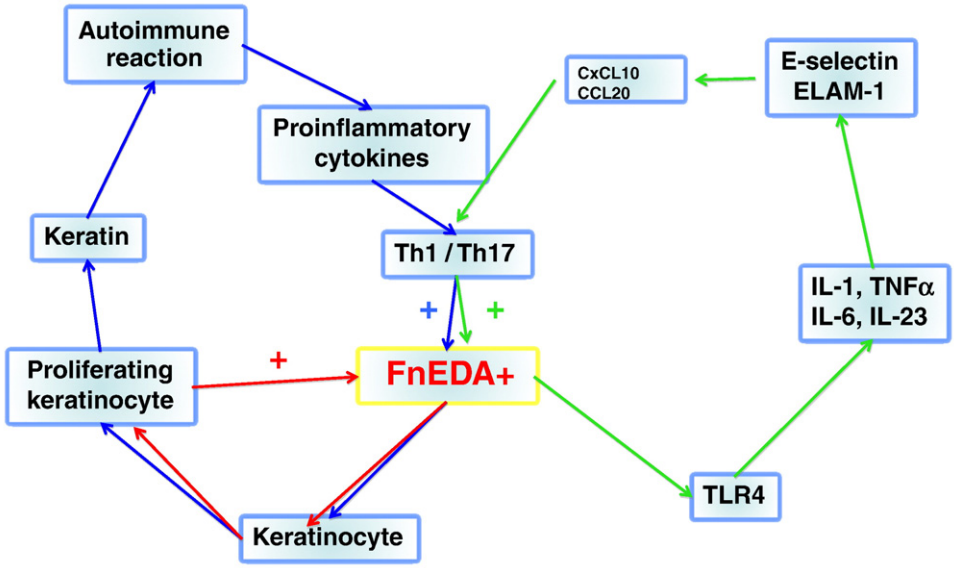

Fig. 1 Multiple FnEDA+ loops in psoriasis. FnEDA+ stimulates TLR4, leading to the expression of IL-1β, TNF-α, IL-6, IL-12, IL-18, and IL-23. These cytokines, in turn, upregulate E-selectin and endothelial cell leukocyte adhesion molecule-1 (ELAM-1) adhesion molecule and chemokine expression such as CXCL10, which facilitate the recruitment of Th1/Th17 cells. As Th1 cells produce FnEDA+, a positive feedback loop is operational (inflammatory loop). Uniquely in psoriasis, FnEDA+ promotes keratinocyte hyperproliferation. The proliferating keratinocytes produce high quantities of FnEDA+, thereby completing another loop (keratinocyte loop). Proliferating keratinocytes will also produce hyperproliferative keratins, which have been claimed to act as autoantigens in psoriasis; a resulting autoimmune reaction will recruit more Th1 cells, producing a further FnEDA+ putative autoimmune loop.

FnEDA+ and as a consequence contribute to these positive feedback loops.

The autoimmune FnEDA+ loop

FnEDA+ will cause keratinocytes from psoriatic individuals (but not nonpsoriatic) to proliferate.[4,19] Hyperproliferating keratinocytes produce proliferative keratins such as keratins 6, 16, and 17.[30] The observation of close homology between proliferative keratin and streptococcal M protein[31] led to the proposal that T cells that recognize epitopes in both proteins are involved in an autoimmune reaction in psoriasis. The frequency of peripheral blood T cells that reacted to both M protein and proliferative keratin was much higher in psoriasis patients than in controls.[5] Such an autoimmune reaction will lead to proinflammatory cytokine release, which in turn will lead to recruitment of Th1 and Th17 cells. Th1 cells will produce FnEDA+, completing the positive feedback loop. It remains to be established whether T cells within psoriasis lesions react to keratin, so the autoimmune loop remains as a hypothetic possibility, although the blood T cells, which have been shown to cross-react with streptococcal M protein and proliferative keratin, were skin-homing.[5]

Multiple genes/candidate genes variants have been associated with psoriasis.[32] Many of these genes can be accommodated onto a model of psoriasis based on multiple FnEDA+ loops (Figure 2).

Thus multiple FnEDA+ loops (Figure 1) may be operative in psoriasis and override regulatory attempts to switch off cutaneous inflammation, which may explain why psoriatic inflammatory plaques have the potential to persist indefinitely.

ACD: A Th1 driven cutaneous inflammatory disorder characterized by an inflammatory FnEDA+ feedback loop?

Although keratinocyte hyperproliferation is not a feature of ACD, and therefore keratinocyte or keratin-related autoimmune FnEDA+ feedback loops would not be operative, there is evidence for involvement of an inflammatory FnEDA+ feedback loop (Figure 3). In one study, the earliest histologic change noted after hapten application was the expression of Fn (although not specifically stated if this was FnEDA+ as one would expect after mechanical injury).[43] This "chemical insult" of hapten application mimics mechanical insult/injury, where Fn/FnEDA+ expression is one of the earliest changes found (detected within 1 hour after injury).[44] After cutaneous hapten application, expression of Fn precedes monocyte chemoattractant protein-1, which is produced after TLR4 stimulation.[43] Loss of TLR4 and IL-12 function completely blocked contact sensitization.[43] TLR4 activation could partially bypass the need for IL-12 through IL-23 activation. IL-23

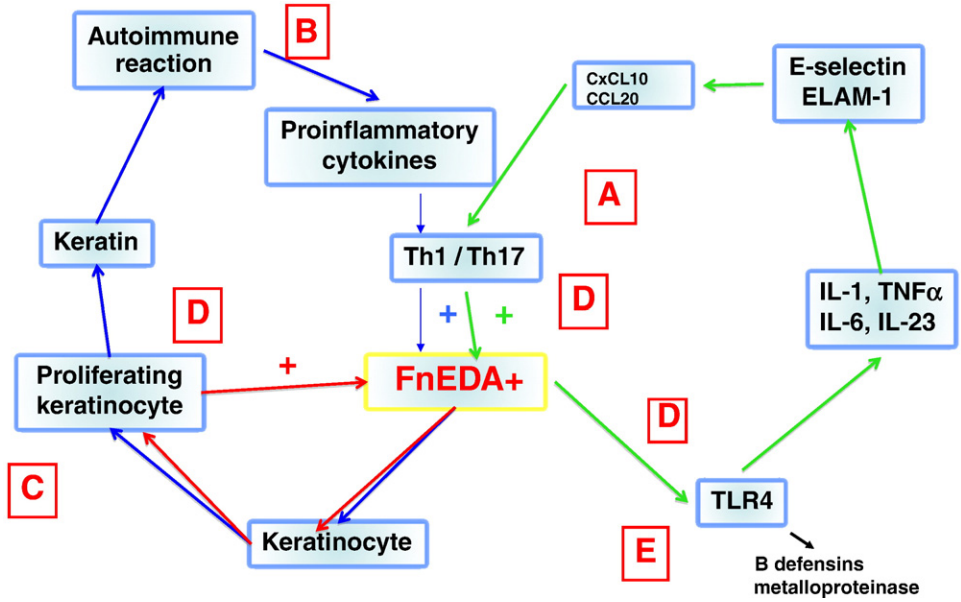

Fig. 2 Genetic variants/candidate genes associated with psoriasis mapped onto a model of psoriasis based on multiple FnEDA+ loops. There appears to be 5 major groupings: (A) Associated with Th1/Th17 promotion differentiation and activation, namely *IL-12B* (encodes the p40 unit for IL-12/Il-23), IL-23R (IL-23 receptor gene), IL-6, TNF, IL-15 (cytokine that affects T cell activation), PTPN22 (intracellular protein tyrosine phosphatase involved in T cell receptor signalling), and STAT4 (associated with Th1 activation). IL-4/IL-13 (Th2 cytokines) could also be accommodated here through a defective function. (B) Associated with autoimmune reactions. It has been postulated that *HLACw6* may facilitate antigen presentation of proliferative keratin. (C) Associated with keratinocyte proliferation/differentiation; namely, late cornified envelope genes (linked to keratinocyte proliferation) and corneodesmosin (involved in physiologic desquamation). Other than *HLACw6* other HLA B/C genes have been associated with psoriasis/psoriatic arthritis such as *HLA B13 B40 B57* and *Cw7*. These HLA genes have also independently been linked to reduced natural killer cytotoxicity. Reduced cytotoxicity by natural killer cells will help prevent apoptosis of proliferating keratinocyte, whilst the natural killer cell production of γ-interferon will promote Th1 differentiation. Natural killer immune-globulin-like receptors help regulate the natural killer response and therefore the degree of keratinocyte cytotoxicity. (D) Associated with nuclear factor (NF)-κB signalling pathways. NFκB is a transcription factor that regulates the expression of a number of proinflammatory genes. The NFκB pathway is associated with TLR4 signalling, control of keratinocyte proliferation, and T-cell activity. NF-κB signalling proteins, namely TNFAIP3, TN1P1, and TNFAIP8L3 have genetic variants that associate with psoriasis. ZNF313, a ubiquitin ligase, has also been associated with psoriasis (ubiquitin ligation is involved in NF-κB pathways). (E) Associated or interacting with TLR4. These include genetic variants of *DEFB* (encodes human β defensin genes, a ligand for TLR4) *ADAM 33* (a disintegrin and metalloprotease) gene associated with both psoriasis and elevated Fn and lipoprotein (apolipoprtein A, which affects lipid metabolism but which may also affect T cell activation). Fn and lipoprotein are both ligands for TLR4. (Not allocated is CDKAL1). Sources: Duffin et al.,[32] with additional material from Chang et al,[33] Coto-Segura et al,[34] Dubey et al,[35] Danik et al,[36] Elder et al,[37] Gudjonsson et al,[38] Sarkar et al,[39] Settin et al,[40] Zervou et al,[41] and Zhang et al.[42]

is a member of the IL-12 family, participates in DC activation and in the enhancement of delayed-type hypersensitivity response,[45] and is inducible by TLR4.[46]

Expression of vascular cellular adhesion molecule-1, endothelial cell leukocyte adhesion molecule-1, and intercellular adhesion molecule-1 adhesion molecule is increased on cutaneous endothelial cells after hapten exposure compared with simple irritant exposure.[47] One would expect increased expression of these molecules secondary to TNF and IL-1β[24] release, both of which are expressed after TLR4 stimulation (Figure 4).

To complete the FnEDA+ feedback loop, it has been observed that infiltrating T cells in ACD show a substantial amount of Fn expression—but relatively negligible amounts of other extracellular matrix proteins—and the infiltrates contained more Fn than biopsy specimens from lesions induced by an irritant.[47] Because Th1 cells are known to be involved in ACD, then one would expect this FN expression to be of EDA+ type.[18] FnEDA+ and TLR4 may be therefore be involved in sensitization phase of ACD, and inflammatory FnEDA+ loops appear to be operative in ACD elicitation.

The expression of endogenous ligands such as FnEDA+ may be one means by which haptens deliver their "danger signal." Indeed, expression of other endogenous ligands for TLRs, such as HSP 70,[48] hyaluronan,[49] and β defensin[10] has been reported after cutaneous hapten application. It is of interest that because only a single EDA+ loop may be operative in ACD, the Th1 dominance is not as marked as in psoriasis. Although the inflammatory cellular infiltrate in ACD is remarkably similar to psoriasis,[50] cytokine profiles of p-phenylenediamine reactive lymphocytes showed Th1 and some Th2 cytokine profiles.[51] Th1 attracting CXCL10 has been found in ACD as well as in experimental inflammation from TLR4 signalling.[28,29]

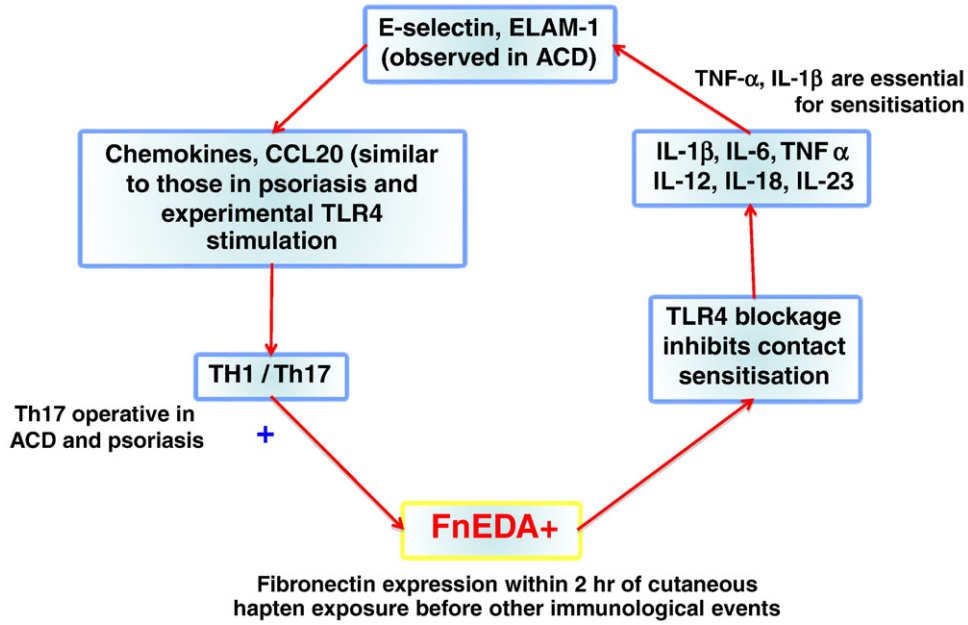

Fig. 3 Inflammatory FnEDA+ loop operative in allergic contact dermatitis. TLR4 stimulation is required for experimental sensitization. IL-1β and TNF-α expression, seen after TLR4 stimulation, is required for dendritic cell migration during sensitization and endothelial adhesion molecule expression is required for elicitation/inflammation. There is a similar cytokine and chemokine profile between experimental inflammation from TLR4 stimulation and inflammation of allergic contact dermatitis. Recruited Th1 cells express FnEDA+ leading to a positive feedback loop.

It is feasible that the inflammatory EDA+ loop could be operative in some systemic Th1/Th17 inflammatory conditions. For example, FnEDA+ expression, TLR4 signalling, and recruitment of Th1 cells are observed in conditions such as rheumatoid arthritis and in inflammation associated with atherosclerosis.[52-56]

Atopic dermatitis: Active suppression of the inflammatory FnEDA+ feedback loop?

Th2-associated cytokines operative in atopic dermatitis appear to actively suppress TLR4 function and the

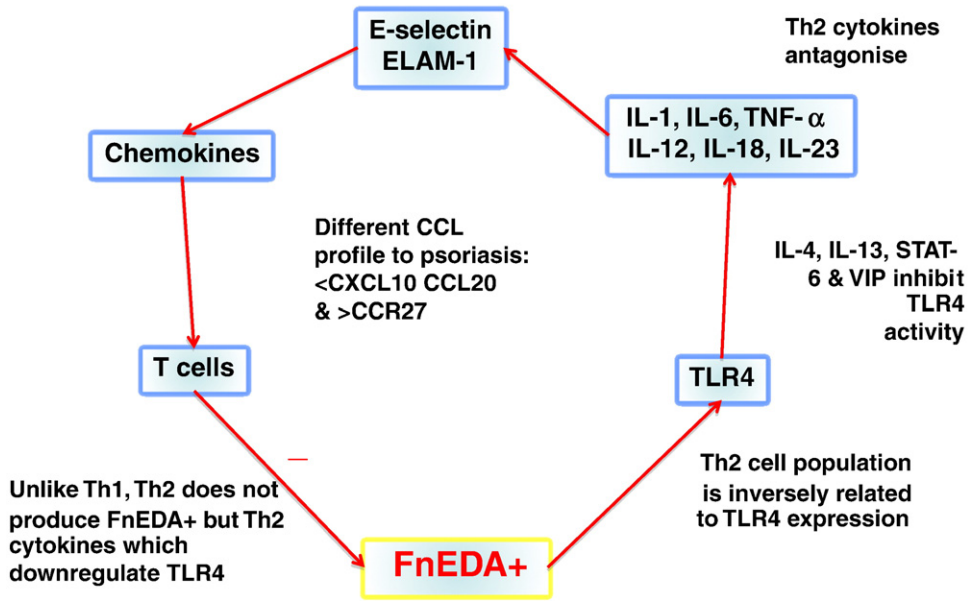

Fig. 4 The inflammatory FnEDA+ loop is actively suppressed in atopic dermatitis. IL-4, IL-13, vasoactive intestinal peptide (VIP), and STAT-6 activity inhibit TLR4 function and expression. They also antagonize the Th1 cytokines produced from TLR4 stimulation. Chemokine expression is geared towards recruiting Th2 rather than Th1 cells. Recruited Th2 cells, unlike Th1 cells, do not produce FnEDA+.

inflammatory FnEDA+ loop (Figure 3). IL-4 and IL-13 significantly decrease TLR4 messenger RNA expression in *in vitro* models.[57] The IL-4 downregulation of TLR4 expression appears to involve signal transducer and activator of transcription (STAT)-6, which is active in atopic disease.[58] Similarly, IL-13 downregulation of TLR4 is also STAT-6–dependent.[59] A dominant Th2 cytokine phenotype is associated with decreased TLR4 monocyte expression.[60] Th2 cytokines would therefore appear to interfere with the Th1/Th17 cytokine expression usually observed after TLR4 stimulation.

Whereas TLR4 signalling chemoattracts DC migration through production of CCL20,[61] IL-4 induces recruitment of myeloid DCs independent of TLR4 signalling.[62] There may thus be mechanisms by which different antigen-representing cells can be recruited in Th1/Th17- or Th2-dominant disease.

There are also characteristics in chemokine expression and their leukocyte receptors in atopic dermatitis that could be related to TLR4 suppression. TLR4 stimulation leads to CCL20 chemokine production and attraction of CCR6 T cells.[61] In atopic dermatitis, there is increased expression of CCL23[63] but reduced levels of CCL20,[64] which is the chemoattractant for CCR6+ Th1/Th17 cells present in lesional psoriasis to a significant degree.[25] Accordingly γ-interferon–producing CCR6 T cells are decreased in atopic dermatitis compared with psoriasis.[64] In contrast to observed changes in experimental TLR4 stimulation, ACD, and psoriasis, there is no evidence for upregulation of the Th1-attracting chemokine CXCL10 in atopic dermatitis.[65]

Vasoactive intestinal peptide is another cytokine secreted by Th2 cells, but not Th1 cells,[66] that may actively downregulate the inflammatory FnEDA+ loop. Vasoactive intestinal peptide protects Th2 cells (but not Th1 cells) by downregulating granzyme B expression and preventing activation-induced cell death.[67] Vasoactive intestinal peptide reverses the expression of TLR4-stimulated pathways, impairing production of IL-6 and RANTES (regulated upon activation normal T cell expressed and secreted)/CCL5 after lipopolysaccharide stimulation,[68] and in another study, it inhibited TLR4-stimulated macrophage secretion of IL-1, IL-6, IL-12, and TNF.[69] Vasoactive intestinal peptide interferes directly with IL-12 and STAT-4 signalling pathways[70] and enhances IL-4 and IL-10 production by peripheral blood lymphocytes.[71] In contrast Th1 cell-derived FnEDA+ promotes IL-6 secretion and downregulates IL-10 production.[18] CXCL10 (IP-10) acts as a chemokine on CXCR3 expressed on activated Th1 cells, and CCL22 (myeloid DC) acts on CCR4 and CCR8 expressed on activated Th2 cells. Vasoactive intestinal peptide upregulates CCL22 and downregulates CXCL10 *in vivo* and *in vitro*,[69] thereby encouraging Th2 and discouraging Th1 cell recruitment. Finally, although FnEDA+ has proangiogenic properties, vasoactive intestinal peptide inhibits angiogenesis.[72]

The abundant evidence from the literature therefore suggests that the inflammatory FnEDA+ loop that promotes Th1/Th17 dominance is actively suppressed in atopic dermatitis, leading the way for Th2 predominance. Th2 cells recruited into atopic dermatitis skin do not produce FnEDA+, excluding the possibility of an inflammatory feedback loop.

Conclusions

FnEDA+ is an endogenous ligand for TLR4, stimulation of which leads to proinflammatory cytokine release that in turn promotes the recruitment of Th1/Th17 cells through expression of endothelial adhesion molecules and chemokines. Because Th1 cells themselves produce significant amounts of FnEDA+ there exists the potential for an inflammatory FnEDA+ positive feedback loop in Th1/Th17-driven inflammatory disease. An inflammatory loop of this kind appears to be operative in ACD. Multiple feedback loops have been proposed for psoriasis, comprising not only inflammatory but also keratinocyte and possibly autoimmune loops. In contrast in atopic dermatitis, the inflammatory FnEDA+ loop is actively suppressed. The inflammatory FnEDA+ positive feedback loop therefore appears to be operative in Th1/Th17 cutaneous inflammatory diseases, and could be a main driver for Th1/Th17 inflammation. The downregulation of this in atopic dermatitis would then encourage preferential Th2 inflammatory responses to predominate.

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