Fas-associated death domain protein and adenosine partnership: fad in RA

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Abstract

Inflammation is the principal hallmark of RA. Different pathways are implicated in the production of pro-inflammatory cytokines, the bona fide mediators of this inflammation. Among them are the TNF pathway and the IL-1 receptor/Toll-like receptor (IL-1R/TLR4) pathway. One of the potential negative regulators of IL-1R/TLR4 signalling is the Fas-associated death domain protein (FADD), which is the pivotal adaptor of the apoptotic signal mediated by death receptors of the TNF family. FADD can sequester myeloid differentiation primary response gene 88 (MyD88), the common adaptor of most TLRs, and hence hinder the activation of nuclear factor-kB (NF-kB), the downstream transcription factor. We recently described a new regulatory mechanism of FADD expression, via the shedding of microvesicles, mediated by adenosine receptors. Interestingly, adenosine is found in high concentrations in the joints of RA patients and has been largely reported as a regulator of inflammation. This review discusses the possible link that could exist between the adenosine-dependent regulation of FADD in the inflammatory context of RA and the potential role of FADD as a therapeutic target in the treatment of RA. We will see that the modulation of FADD expression may be a double-edged sword by increasing apoptosis and at the same time limiting NF-kB activation.

Key words: FADD, rheumatoid arthritis, adenosine receptors, secretion, inflammation.

Introduction

RA is classified as a multifactorial systemic auto-immune disease affecting ~0.5–1% of the world’s population. RA is characterized by substantial infiltration of the synovium by inflammatory cells and local secretion of pro-inflammatory cytokines and growth factors leading to progressive joint and bone destruction with long-term synovial hyperplasia and chronic synovitis [1]. The pro-inflammatory agents released in the joint, among which are IL-1β, TNF-α and IL-6, trigger the activation and proliferation of different cell types, including lymphocytes, neutrophils, osteoclasts, synoviocytes and chondrocytes. These cells participate in the perpetuation of the inflammatory state of the joint and consequently in its destruction. Along with these cytokines, the ligands of the IL-1 receptor/Toll-like receptor (IL-1R/TLR4) pathway (IL-1β), HA, fibronectin) and adenosine, the nucleoside molecule, are in high concentrations in the synovial fluids of RA patients and contribute to inflammation [2–4]. Adenosine is produced by cells following cellular changes, tissue injury or inflammatory conditions. Once produced, adenosine is sensed by autocrine or paracrine pathways via adenosine receptors (ARs). In vitro and in vivo studies have described the potent anti-inflammatory role of adenosine via ARs [3, 5]. Inflammation is therefore a main tag of RA, and most of the drugs commonly used in clinical practice are directed against the principal actors of this inflammatory surge. With the aim of relieving the patient’s distress, various treatment protocols have been set up using either existing drugs (e.g. NSAIDs) and/or newly developed drugs [e.g. anti-TNF-α (infliximab, etanercept, certolizumab, etc.) and anti-R-IL-6 (tocilizumab), anti-B cell (rituximab) or T-cell activation (abatacept)]. However, although many treatments have proved effective in controlling disease activity, none of them can cure the disease.

In spite of the fact that the pathophysiology of RA is not yet fully understood, clear evidence of the role of autoantibodies such as RF have been established. It is now clear that RF participates in the formation of immune
complexes on the site of inflammation but is not specific to RA and is frequently observed in other inflammatory diseases [6, 7].

More recently, a new group of autoantibodies that appeared to be more specific biomarkers of RA than RF were included in the RA criteria from the ACR and the European League Against Rheumatism (EULAR). These antibodies are directed against citrullinated peptides and are known as ACPA or anti-CCP. ACPA can form an immune complex with CCP, inducing immune responses and thereafter increasing inflammation and erosion. The additional presence of RF can worsen the outcome [8]. ACPA levels are correlated with the erosive character of RA [9]. Whether or not autoantibody-producing B cells within the synovium have particular sensitivity or resistance to apoptosis should be the focus of investigations.

One of the important factors of B-cell development is the B-cell activating factor (BAFF), a member of the TNF ligand family that binds to transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), B cell maturation antigen (BCMA) and BAFF-R with different degrees of affinity [10–12]. The binding of BAFF to its receptors leads to the activation of the alternative nuclear factor kB (NF-kB) pathway via recruitment of TNF receptor-associated factor (TRAF) 2, 5 and 6. Contrary to the pro-apoptotic function of Fas-associated death domain protein (FADD), the main role of BAFF is to induce B-cell survival and growth. As we discussed above, B lymphocytes are the source of the RFs and ACPA, and are very efficient antigen-presenting cells. They can therefore activate T cells and they both respond to and produce the chemokines and cytokines that promote leucocyte infiltration into the joints, angiogenesis and synovial hyperplasia [13]. This can explain the high concentrations of BAFF and BAFF-R found in the synovium of RA patients [14]. One theory regarding the persistence of self-reactive B cells in the synovium of RA patients’ cells is that they resist apoptosis, at least in part, due to high levels of secreted BAFF. Besides FADD and adenosine, which are the focus of the present article, several pro-apoptotic and anti-apoptotic molecular regulatory mechanisms have already been reported in RA. The B-cell lymphoma-2 (BCL-2) family of protein is composed of both types of proteins. The anti-apoptotic proteins, namely Bcl-2, Bcl-xL, Bcl-w, have the ability to down-regulate the apoptosis induced by the mitochondrial pathway and are up-regulated in several types of cells in RA, particularly B and T cells [15–19]. The regulation of life and death by these proteins is achieved mainly through their binding to the pro-apoptotic proteins of the same Bcl-2 family released by mitochondria (Bad, Bid, Bax, etc.).

Another class of apoptosis regulators has been described in arthritis. Indeed, it was reported that the expression of different proteins of the inhibitors of apoptosis (IAPs) family (XIAP, cIAP1, cIAP2, etc.) [20], which can bind directly to effector caspases-3, -6, -7 and -9, could be differentially expressed and could play a role in the disease [21, 22].

Furthermore, proteins of the FADD-like interleukin-1 β-converting enzyme (FLICE)/caspase-8 inhibitory proteins (FLIP) family are also well-defined down-regulators of the Fas-mediated apoptotic pathway. While searching for novel apoptosis-regulatory proteins that contain a death effector domain (DED), a new family of viral genes encoding v-FLIPs was identified. Cellular variants were later identified: c-FLIPs. Owing to its structural homology with caspase-8, one of the splicing isoforms of c-FLIPs, namely c-FLIPL, interferes with the activation of caspase-8 at the level of the death-induced signalling complex (DISC) by binding to the latter. Data show that IFN-γ-mediated increased expression of FLIP down-regulates Fas-mediated signalling and apoptotic death in human osteoarthritic chondrocytes [23]. Similarly, high expression of FLIP would account for the resistance of synovial macrophages to programmed cell death [24] as well as fibroblast-like synoviocytes (FLSs) [25, 26]. Thus it appears that numerous pro-apoptotic and anti-apoptotic mechanisms are running together in inflamed synovium and probably affect the numerous types of cells present in the tissue differently.

FADD: from the masterpiece regulation of apoptosis to the potential inflammatory actor in RA

FADD is the key transducer of the apoptotic signal triggered by all death receptors of the TNF family, such as Fas (Fig. 1a), TNF receptor 1 (TNF-R1), death receptor 3 (DR3), TNF-related apoptosis-inducing ligand TRAIL-R1 (DR4) and TRAIL-R2 (DR5) [27–31]. FADD binds directly or indirectly to the intracellular domain of these receptors via its death domain (DD) and transduces the signal to pro-caspase-8 via a homotypic interaction of their respective DEDs [32, 33]. The binding of FADD to the death receptors and to pro-caspase-8 gives rise to the DISC. Consequently, pro-caspase-8 is cleaved into active caspase-8 that will in turn activate effector caspases like caspase-3 (Fig. 1a) to induce apoptosis. Besides its crucial role in apoptosis signalling, FADD is implicated in many other essential biological processes such as embryonic development [34], proliferation [35], cell cycle progression [36], tumour development [37], innate immunity [38, 39] and autophagy [40, 41]. In RA, few apoptotic cells are detected in the joints of RA patients; although Fas is expressed by various cells like synoviocytes, fibroblasts and lymphocytes [42]. Experimental data have shown that macrophages in the joints express both Fas and Fas ligand (FasL). However, though in close proximity with macrophages, a very low apoptosis rate is detected in cells expressing Fas [42]. One explanation for this apparent resistance to Fas-mediated apoptosis is the high expression of FLIP, at least in some of these cells [43]. FLIP is a structural homologue of caspase-8, lacking caspase activity due to mutations in the active site. Together with FLIP, the anti-apoptotic molecule Bcl-2 was shown to be overexpressed in the synovium [44, 45]. Nevertheless, it is hard
Fig. 1 FADD in Fas signalling and NF-κB pathway. (a) The cross-linking of the Fas receptor upon binding to trimer FasL recruits the FADD protein. The latter binds to Fas intracellular domain via their respective DD. FADD in turn recruits pro-caspase-8 via its DED, as pro-caspase-8 is cleaved to generate caspase-8. The latter will recruit activator caspases like caspase-3, which cleaves substrate into the cell. As a result, the cell dies from apoptosis. Fas signalling can be blocked by the action c-FLIP, which resembles pro-caspase-8 but lacks a protease domain. (b) TLRs and IL-1R family members share an intracellular 'Toll-IL-1-R domain'. The triggering of IL-1R or TLR induces the recruitment of the adaptor, MyD88, which in turn recruits IRAK. Subsequently IRAK binds to TRAF-6, which phosphorylates inhibitor of NF-κB (IκB), leading to its degradation by the proteasome. As a result, NF-κB is liberated and induces the transcription of pro-inflammatory genes. FADD can act as an anti-inflammatory molecule by sequestering MyD88 away from IRAK, thus hindering NF-κB activation.
to imagine that these mechanisms are the only ones to operate in the synovium. We could hypothesize that a lack of FADD in the synovium would also result in a lack of apoptosis.

In addition to its role in the apoptotic machinery, FADD proved to be involved in the regulation of inflammation [2, 46]. In a very elegant work, Ma et al. [2] showed that FADD could have an anti-inflammatory role in humans by blocking the lipopolysaccharide (LPS)-induced IL-1R/TLR4 pathway (Fig. 1b). The authors used the in vitro model of mouse macrophages, RAW264.7 cells, transfected with FADD or dominant negative FADD together with an IL-6 luciferase promoter-reporter construct. They showed that expression of FADD or dominant negative FADD inhibited LPS-induced IL-6 expression. Moreover, they demonstrated that a mouse model of collagen-induced arthritis, DBA/1, asserts a new potential role of FADD in the pathophysiology of RA.

FADD secretion: a new regulatory mechanism

Given its role as a leading adaptor in apoptotic signal transduction, the localization of FADD has been restricted to the cytoplasmic compartment. However, with time, a nuclear localization signal of FADD has been described, and concomitantly a role for FADD in the nucleus [47]. This nuclear localization of FADD requires the phosphorylation of Ser 194 (for human FADD). Moreover, this specific site is required for FADD interaction with the nucleocyttoplasmic protein exportin-5 [48]. Studies on the regulation of such a multifaceted protein have constituted an interesting branch of research and brought evidence of regulation via post-translational modifications [49] and differential locations [50]. Recently our laboratory gave a landmark description of a new mechanism of regulation of the FADD protein [33, 51]. We discovered that FADD could be secreted from in vitro cultured mouse organ, following microvesicle shedding. We demonstrated that this process is calcium and adenosine dependent. Moreover, we identified ARs (ADORA2B and ADORA3) as regulators of this mechanism. We showed that FADD secretion is submitted either to positive or negative regulation by these receptors, depending on the organ, and we therefore hypothesized that the balance between expression and/or activation of the different ARs could determine the amount of secreted FADD but also the amount of available FADD inside the cell (Fig. 2a). Consistent with our study, work on red blood cell-shedded vesicles showed that FADD could be found in these vesicles, along with other molecules like Fas, caspase-3 and caspase-8 [52]. The authors reported that the observed secretion was dependent on ATP. Interestingly, it was shown that such microparticles could be found in the SF of RA patients and originated from monocytes, granulocytes and lymphocytes found in the joints [53]. These microparticles were capable of triggering the release of chemokines, cytokines and other inflammatory mediators from FLSs [53]. Besides, considering the IL-1R/TLR-dependent anti-inflammatory role of FADD and the notable concentration of adenosine and IL-1R/TLR ligands in the joints of RA patients, we suggest that there could exist a gripping link between the regulation of FADD via the shedding of microvesicles mediated by adenosine and the establishment and/or progress of the pathology. Indeed, a reduction of intracellular FADD due to adenosine-dependent shedding would result in a defective apoptosis and hyperproliferation of FLSs. The latter would produce a significant amount of pro-inflammatory cytokines and proteases that contribute to cartilage destruction. Moreover, the absence of FADD inside the cells would release MyD88 and therefore allow the maintenance of the inflammatory surge via the IL-1R/TLR4 pathway. Based on our recent demonstration of FADD regulation by adenosine or ADORAs, we sought the presence of FADD and FADD-containing microvesicles in the SFs and sera of RA patients. We observed that FADD was detectable in the fluids of RA patients and was significantly higher than in healthy and OA patients (unpublished results). Furthermore, we have now obtained several results arguing that FADD is, at least in part, secreted along vesicular pathways, and we have been able to purify microvesicles containing FADD (unpublished findings).

Adenosine and ARs: guardians of the body

An extensive discussion of the role of adenosine and ARs in inflammation is beyond the scope of this review and has been presented elsewhere [54–58]. Adenosine is a well-known purine nucleoside. It is released either following the nucleotidase hydrolysis of extracellular adenine...
nucleotides like adenosine triphosphate (ATP) or adenosine monophosphate (AMP) or in response to cell damage. Adenosine can be released from different cells and the extracellular concentration will depend on the level of stress experienced. Once released, the adenosine is rapidly metabolized into AMP and ionosine by adenosine kinase and adenosine deaminase, respectively. The assigned physiological function of adenosine is protection of the body from the deleterious consequences of inflammation and other stresses [59]. Indeed, adenosine mediates tissue repair via the increase of oxygen supply, protection against ischaemic damage and the decrease of inflammation. Adenosine triggers the activation of the four purinergic receptors, known as ADORA1, ADORA2A,
ADORAs and ADORAs. Notably, these receptors have different affinities for adenosine. ARs are expressed by a variety of cells, including platelets, neutrophils, macrophages and lymphocytes, and can mediate pro- or anti-inflammatory signals to these cells depending on the cell type and the conditions [5]. In inflammatory contexts, the expression of receptors can vary positively or negatively according to cell type and pathology. Usually the activation of ADORA signalling is mediated by the stimulation or inhibition of adenylate cyclase. For more than three decades, agonists and antagonists of ADORAs have been designed and commercialized in different pharmaceutical markets to treat various health problems, ranging from renal disorders and cardiac ischaemic disorders to dementia and anxiety disorders [5].

In different models, adenosine and its receptors have proved to be essential in regulating the homeostatic balance, either via direct regulation of cells or via stimulation of regulatory T cells (Tregs). For example, in a rat mast cell line model, adenosine and ADORA3 were shown to be involved in cell degranulation. Upon binding to adenosine, the receptor induces an increase in the intracellular Ca\(^{2+}\) concentration, which consequently leads to enhanced antigen secretion from surrounding cells. This antigen release induces the cross-linking of IgE at the surface of mast cells. As a consequence, mediators of inflammation like histamine, a vasodilator, are liberated following mast cell degranulation [60]. Adenosine can also participate in tempering the immune reaction. In fact, adenosine was shown to be an important mediator of Treg action. It was reported that adenosine could drive Treg production [61]. Also, adenosine binding to ADORAs on Tregs triggers an increase in the intracellular concentration of cyclic AMP (cAMP), which will be transferred to effector T cells via gap junctions, and consequently decrease the inflammatory capacity of effector T cells [54].

ADORAs can thus act either as anti-inflammatory or pro-inflammatory mediators of the immune response, which is basically a process to protect and heal the body after infections or injuries. These examples show the spectrum of action of adenosine.

**Recapitulating the role of ARs in RA**

During RA, the expression of ADORAs varies depending on cell type. Synovial cells express the four types of ADORAs [62–64]. For example, ADORA1 proved to have a pro-inflammatory role in joint disease. The activation of ADORA1 promotes the adhesion of activated neutrophils, important players in the acute phase of inflammation in RA, to the endothelium. Conversely, compelling data identified ADORA2A and ADORA3 as two potent dampers of inflammation in RA [62]. ADORA2A can participate in the attenuation of inflammation by inhibiting T-cell proliferation, activation and cytokine production [65]. ADORA2A overexpression in RA patients was shown to increase the activity of adenylate cyclase, resulting in the high production of cAMP. The latter mediates the decrease of TNF-\(\alpha\) and IL-1\(\beta\), thus decreasing the local inflammatory process in the affected joints [66]. Conversely, ADORA2B is known to have a pro-inflammatory action on cells of the joints. Indeed, the receptor induces the production of cytokines like IL-6 and IL-8 [58]. However, no difference in the density of this receptor is noticed in patients treated with DMARDs compared with healthy individuals [62].

**Therapeutic role of adenosine and ARs in RA**

One of the most important and promising fields in drug research for RA treatment concerns ARs. Interestingly, the concentration of adenosine in the joints of RA patients is 10–100 times higher than in the joints of healthy people; 10–100\(\mu\)M vs <1 \(\mu\)M [67]. One of the keystone drugs used in the treatment of RA, MTX, a conventional DMARD, proved to be highly effective in harnessing the inflammation [68]. Although no single mechanism is sufficient to account for all the anti-inflammatory activities of MTX, there is no doubt that modulation of the release of adenosine from cells is one of the main effector mechanisms [69]. MTX leads to the intracellular accumulation of components of the purine biosynthesis pathway, which in turn induces the release of adenosine in the extracellular space (Fig. 3a). One of the proposed ways to the down-regulation of inflammation could be by the potentiation of the apoptosis of immune cells found in the joint. *In vitro* studies have shown that increased extracellular adenosine concentration increased apoptosis via enhanced caspase-3 activity, up-regulation of p53 expression, down-regulation of Bcl-2 expression and an increase in oxidative stress [70]. A second explanation would be via the down-regulation of the NF-\(\kappa\)B pathway. The presence of MTX proved to increase the affinity of the anti-inflammatory AR ADORA3. Upon adenosine binding, the receptor can induce an increase of adenylate cyclase and thus cAMP. This was shown to dampen the acute inflammation phase of RA [68]. We can also hypothesize that upon treatment with MTX, the increased concentration of adenosine would lead to an increase of Treg activity via cAMP transfer that would participate in the weakening of inflammation.

In light of its anti-inflammatory potential, ADORA3 agonists have been designed to improve the prognosis of RA. Results from mouse and rat models have shown that the use of the ADORA3 agonist CF101 (IB-MECA) reduces inflammation, bone destruction and synovial hyperplasia [71]. A similar study in a rat model of adjuvant-induced arthritis, where the CF101 was used in a combined treatment with MTX, revealed an additive anti-inflammatory effect of the two components [71, 72]. In these studies, the clinical and pathological manifestations of arthritis decreased significantly in the affected animals. The proposed mechanism would be an increase in ADORA3 expression in inflamed tissues and peripheral blood mononuclear cells (PBMCs), induced by MTX. Consequently, the cells were more susceptible to CF101 treatment. In comparison to the mechanism induced by
Fig. 3 FADD as a new anti-inflammatory target in RA. (a) In normal conditions, without MTX, FADD shedding is essentially dependent on adenosine stimulation of activator ARs. With MTX, the expression of ARs can be modulated in favour of an overexpression of inhibitory receptors, which could cause a blockade of FADD secretion. (b) (1) Upon its entry into the joint cell, MTX is polyglutamated. (2) MTX and its polyglutamates can modulate the expression and activation of ARs and increase the concentration of extracellular adenosine. (3) The ARs activated by extracellular adenosine can (4) down-regulate the shedding of microvesicles containing FADD, leading to an increase in intracellular FADD concentration (5). (6) FADD can then bind to MyD88, block the IL-1R/TLR pathway, and thus the activation of NF-κB and the production of pro-inflammatory cytokines, leading to a decrease in inflammation. (8) At the same time, FADD can bind to Fas and caspase-8 and trigger apoptosis, thus decreasing the characteristic synovial hyperplasia in RA.
MTX only, CF101 induced the internalization and degradation of ADORA3, thereby decreasing its overall expression. As a consequence, phosphatidylinositol-3 kinase (PI3K) and downstream actors involved in the activation of NF-κB transcription factor were underexpressed and failed to trigger the NF-κB pathway. We can suggest that depending on the treatment and on the cells implied, regulation of the expression of ADORA3 varies, but might always lead to a decrease in inflammation via a downturn of the NF-κB pathway. Consistently, the phase II trial of CF101 in RA patients gave promising results on the improvement of the signs and symptoms of the disease. At 12 weeks, 55.6, 33.3 and 11.5% of the patients receiving 1.0 mg CF101 achieved ACR criteria 20, 50 and 70% responses, respectively [73]. The expression of pro-inflammatory cytokines like TNF-α and IL-1β was reduced by an increased amount of adenosine, confirming the anti-inflammatory and immune-regulatory properties of adenosine. In an investigative study of the expression and affinity of ARs in MTX-treated RA patients compared with untreated RA patients, the authors reported that the high levels of TNF-α present in the joints of these two groups of patients potentiated an up-regulation of ADORA2A and ADORA3 on lymphocytes and neutrophils via an altered NF-κB pathway [62]. A new agonist of ADORA3, CF502, is currently under investigation.

Results obtained in a mouse model of RA showed that CF502 induced a dose-dependent reduction in FLS proliferation, mediated by a down-regulation of NF-κB signaling. Interestingly, this agonist has been tested in humans and showed particularly high affinity with human ADORA3, therefore holding promise for treating inflammation in humans [74]. The same type of approach has recently been described with ADORA2a agonist in mice [75].

Insights into the therapeutic role of FADD in RA treatment

FADD holds promise as a potent therapeutic target in RA. Evidence from different studies suggests that hyperproliferation of synoviocytes could not account for pannus formation in RA, the main reason being that proliferation is meager in the synovium of RA patients. Instead, it is a dysfunction in the regulation of apoptosis that could explain the apparently uncontrolled proliferation of synoviocytes [76]. Similarly, other studies suggest that the low frequency of apoptosis is due to the overexpression of Bcl-2 in synovial cells [44, 77]. Also, anti-apoptotic molecules like sentrin-1/small ubiquitin-like modifier 1 (SUMO-1) and FLIP have been identified as the principal regulators downstream of Fas signalling in synovial-like fibroblasts [78]. A gene therapy technique was proposed to thwart this dysregulated apoptosis [79]. The authors showed that in vitro transfection of the FADD gene via an adenoviral vector (Ad-FADD) in human cultured RA synoviocytes induced the up-regulation of FADD expression and apoptosis. In their in vivo model, human rheumatoid synovium was implanted in the back of severe combined immunodeficient (SCID) RA mice. Consistent with the in vitro model, the transfer of FADD gene via an adenovirus into the site of engraftment induced the disappearance of most synovial and mononuclear cells of the transplant [79]. With the control Ad-LacZ, no increase in apoptosis was observed in the graft. Consistent with that study, it was shown that in vitro treatment of human RA synoviocytes with monoclonal anti-Fas antibody caused enhanced apoptosis via the recruitment of FADD to the Fas DD, confirming the role of FADD as a prime adaptor of the Fas apoptotic pathway in RA synovium [80]. Additionally, TRAIL has also been described as a pro-apoptotic factor in RA FLS and suggested as a potential drug. It was reported that exposure to TRAIL induced apoptosis in a portion (up to 30%) of RA FLSs within the first 24 h. Conversely, in the cells that survived, TRAIL induced RA FLS proliferation [81] through the mitogen-activated protein kinases extracellular signal-regulated kinase (ERK) and p38, as well as the PI3K/RAC-alpha serine/threonine-protein kinase (Akt) signalling pathway, which is reminiscent of our observation of Fas stimulation in the absence of FADD [82]. This dual functionality of the TNF-R family in stimulating apoptosis and proliferation has important implications for their use in the treatment of RA.

A model of AR-dependent FADD therapy in RA

The new vision of FADD regulation put forward by our laboratory revealed the engagement of different ADORAs in the modulation of FADD expression in and out of cells [33, 51] (Fig. 3a). In light of the role of ARs in RA [58] and the implication of FADD in an IL-1 R/TLR-mediated inflammation, and especially in RA [2] in addition to its well-known role in apoptosis, we suggest a FADD-dependent controlled pathway that could be essential in the pathogenesis of this disease (Fig. 3). We propose a new schema in which the abundant adenosine present in the SFs of patients could activate ADORAs, expressed by the cells participating in the inflammatory process of RA, therefore triggering the secretion of FADD via the shedding of microvesicles (Fig. 3a). Consequently, the apoptotic pathway mediated by death receptors like TNF-α, TRAIL-R or Fas would lack the key actor FADD, essential for the signal transduction leading to the programmed death of the above-mentioned cells. Considering the rapid release of adenosine following cellular stress, hypoxia and cellular damage, all pathophysiological tags of RA, we can infer that FADD would be rapidly released by these cells upon adenosine signalling so as to counter the excessive apoptosis usually induced in stressful situations. In light of these assumptions, a controlled release of FADD, via antagonists or agonists of ARs, could represent a new approach to increase susceptibility to apoptosis, specifically in synoviocytes. Furthermore, the high concentration of IL-1R/TLR ligands and a scarce amount of FADD in the synoviocytes and the surrounding cells like lymphocytes, macrophages or dendritic cells could cause uncontrolled activation of the...
IL-1R/TLR pathway leading to NF-κB activation. This could result ultimately in the extensive production of pro-inflammatory cytokines like IL-6, TNF-α or IL-1β. Upon a controlled activation of ARs in the joints, the shedding of microvesicles containing FADD would also be regulated, and consequently FADD expression in the cells (Fig. 3a). Thus, increasing intracellular FADD concentration in cells involved in the inflammatory process could lead to inhibition of IL-1R/TLR by sequestering MyD88. As a result, there would be a reduction of NF-κB activation, a decrease in the transcription of pro-inflammatory genes and thus a diminution of the inflammatory process (Fig. 3b). It is therefore highly important to investigate the distribution of ARs on joint cells and how they could modulate the secretion of FADD. Consequently, adapted agonists or antagonists could be used to modulate FADD secretion by these cells and therefore participate in the elimination of RA FLS, down-regulation of the NF-κB activation and inflammation.

Conclusion

The FADD protein is the main adaptor of death pathways mediated by receptors of the TNF-R family. In addition, the protein has been implicated in other basic biological processes, among which is an anti-inflammatory role in the IL-1R/TLR pathway. We recently described a new regulatory mechanism of FADD expression via shedding of microvesicles. The modulation of FADD secretion proved to be regulated in part by ADORAs. These receptors are important regulators of inflammatory disorders, among which is RA. Clinical trials using AR agonists and antagonists have proved their efficacy. More importantly, the effect of MTX, the mainstay drug used in RA, was shown to be potentiated by ARs. Considering the unclear understanding of RA pathogenesis, most therapies target the inflammatory pathways involved in disease development. Consistent with this, we propose the FADD molecule as a new actor in RA pathogenesis. This new implication of FADD opens a new field of investigation and paves the way to the development of new therapies for RA. Finally, FADD and adenosine represent two potent biomarkers of RA severity and predictors of therapy response.

Rheumatology key messages

- ADORAs control the level of FADD expression.
- FADD and ADORAs form a new important couple in inflammation.
- The regulation of FADD expression through ADORAs could be a new therapeutic target in RA.

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Clinical Vignette

A case of Behcet’s syndrome presenting as focal myositis

Behcet’s syndrome is a systemic vasculitis leading to protean manifestations. A 30-year-old man presented with acute lower limb myalgia. Examination revealed focal left thigh tenderness. CRP was 213 mg/l. T1-weighted short tau inversion recovery (STIR) MRI demonstrated focal myositis of the left biceps femoris (Fig. 1A). Despite a negative aspirate culture, pyomyositis was diagnosed and symptoms resolved with i.v. antibiotics. Seven months later he developed a further episode of acute lower limb myalgia accompanied by a papulopustular chest rash. MRI demonstrated resolution of the previous myositis with a new focal area of myositis in the right vastus medialis (Fig. 1B) and a ring-like right tibial lesion (Fig. 1C). Cultures were sterile. He subsequently developed acute anterior uveitis and oral ulceration. A diagnosis of Behcet’s syndrome was made. Symptoms resolved with corticosteroids and colchicine. MRI (Fig. 1D) 6 weeks later demonstrated resolution of all abnormalities. He has had no relapses after 14 months of colchicine monotherapy.

Myositis is uncommon in Behcet’s syndrome and is most often a localized lower limb myositis [1].

Corticosteroids are effective for acute myositis. Our case may support the efficacy of colchicine in preventing recurrent myositis [1].

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Fig. 1 Serial T1-weighted STIR MR images demonstrating the appearance and resolution of focal myositis (indicated by arrows). (A) Focal myositis of the left biceps femoris muscle on presentation. (B) New focal myositis of right vastus medialis muscle with resolution of myositis in left biceps femoris muscle on second presentation 7 months later. (C) Ring-like lesion within the proximal right tibial diaphysis with associated bone marrow oedema on second presentation. (D) Resolution of all abnormalities 6 weeks after the second admission.