·Review·

Potential role of nuclear receptor ligand all–trans retinoic acids in the treatment of fungal keratitis

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Abstract

• Fungal keratitis (FK) is a worldwide visual impairment disease. This infectious fungus initiates the primary innate immune response and, later the adaptive immune response. The inflammatory process is related to a variety of immune cells, including macrophages, helper T cells, neutrophils, dendritic cells, and Treg cells, and is associated with proinflammatory, chemotactic and regulatory cytokines. All –trans retinoic acids (ATRA) have diverse immunomodulatory actions in a number of inflammatory and autoimmune conditions. These retinoids regulate the transcriptional levels of target genes through the activation of nuclear receptors. Retinoic acid receptor α (RAR α), retinoic acid receptor γ (RAR γ), and retinoid X receptor α (RXR α) are expressed in the cornea and immune cells. This paper summarizes new findings regarding ATRA in immune and inflammatory diseases and analyzes the perspective application of ATRA in FK.

• **KEYWORDS:** nuclear receptor; all-trans retinoic acid; fungal keratitis; cornea

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INTRODUCTION

Fungal keratitis (FK) is well known as an immunoinflammatory disease. Antifungal therapy is not effective for some fungal strains, which can cause fungal endophthalmitis and blindness [1-4]. The recognition of infectious agents by the Toll-like receptor (TLR) system initiates the primary innate immune response and, later, the adaptive immune response [5,6]. A new category of drugs is needed to treat fungi infections that are resistant to currently available treatments[7,8]. All-trans retinoic acids (ATRA) is an active metabolite of vitamin A with anti-inflammatory and immunoregulatory effects [9,10]. Transcriptional control of ATRA may provide new targets for pharmacological intervention in chronic diseases [11]. These retinoids regulate the transcriptional levels of target genes through the retinoic acid receptor α (RAR α), β, and γ as well as retinoid X receptor α (RXR α), β, and γ and RAR α, RAR γ, and RXR α, which are expressed in the cornea, conjunctiva, and all of their constitutive cells [12]. Retinoic acids (RA) acts as a mediator directly controlling CD4 (+) T cell differentiation and immunity [13]. As an antiinflammatory and immunoregulatory agent, the potential role of ATRA in FK therapy needs to be investigated.

METHOD OF LITERATURE SEARCH

Papers and abstracts of relevant studies for this review were obtained from the MEDLINE database. The following search words (inclusive MESH headings) were used: FK and innate immunity, ATRA and innate immunity, FK and transcription factors, ATRA and transcription factors, FK and Th1 cytokines, ATRA and Th1 cytokines, FK and Th2 cytokines, ATRA and Th2 cytokines, FK and chemokines, ATRA and chemokines, FK and cellular immunity, ATRA and cellular immunity, FK and matrix metalloproteinases (MMPs), ATRA and MMPs, FK and humoral immunity, and ATRA and humoral immunity. The search covered publications from 1970 to 2014, and articles published in English and German were included. Additional sources included publications cited in relevant articles. Criteria for inclusion or exclusion of articles were originality, importance for ophthalmoscopic evaluation of FK and ATRA characteristics. Articles were appraised critically, and pertinent information was included in this review and cited accordingly.

ALL –TRANS RETINOIC ACID AND INNATE IMMUNITY

Innate immunity mechanism of FK: for the mononuclear phagocyte system, Dectin-1 and TLR play key roles in innate immune responses [14-16]. Dectin-1 has been shown to mediate cytokine production, neutrophil recruitment, and fungal survival [17]. TLR_2 siRNA treatment attenuated FK by suppressing corneal inflammation and preventing fungal invasion[18-20]. Innate immunity has been shown to be essential

to the recognition of fungi and to host defense[21-23] (Figure 1). ATRA has a potential role in FK innate immunity: ATRA induces the activation of macrophages, which induces the inflammatory response [24-26]. ATRA can be converted into immunogenic APCs with the help of activated natural killer T (NKT) cells to induce incremental immune responses [27]. ATRA can also induce U937 cell differentiation [28]. ATRA supplementation enhances the immune system by increasing T lymphocyte number. Activation of NOS II through RAR α contributes to the host inflammatory/immune response [29,30]. Lack of innate immunity which can be initiated by ATRA increases the chance of FK occurrence. ATRA represents a new target for treating inflammation in humans[31].

ALL-TRANS RETINOIC ACID AND TRANSCRIPTION FACTORS

TLR triggers the activation of transcription factors that induce the expression of inflammatory cytokines and chemokines resulting in the recruitment of neutrophilic granulocyte (PMN) to the cornea and the generation of corneal ulcers [32]. Interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) in infected corneas are activated by the mitogen-activated protein kinases (MAPKs) and jun-terminal kinase (JNK) which are regulated by NF-kappa B [33]. The activation of $TLR_{2,4}$ by Aspergillus fumigatus through NF-kappa B may contribute to keratomycosis [34] (Figure 1). ATRA downregulates the expression of receptor activator of NF-κ B ligand in CD4(+) T cells and in rat liver injury [35-37]. ATRA suppressesd chemokine expression in THP-1 cells and various cells in part *via* the c-Raf-MKK1/2-ERK/MAPK pathway [38-40]. ATRA can also perform its biological effect by activating Smad signaling and PI3K/Akt [41-44]. These data indicate that ATRA can modulate transcription factors to generate its antiinflammatory effects. We will provide a summary of ATRA's involvement in the gene expression of cytokines and chemokines gene through its impact on transcription factors.

ALL -TRANS RETINOIC ACID AND CYTOKINES AND CELLULAR IMMUNITY

Mechanism of cellular immunity to FK and cytokines involved in FK: interleukin-1β (IL-1β) and chemotactic cytokines, such as MIP-2, recruit PMN in response to bacterial infection. Interferon-γ (IFN-γ) production in dominant T-helper (Th) 1 responder strains contributes to corneal destruction and perforation, while IFN-γ is associated with bacterial killing and less corneal destruction in dominant Th2 responder strains [45]. The proinflammatory cytokines IL-1β and tumor necrosis factor-α (TNF-α) are elevated in the early stage of aspergillus and fusarium corneal infections. Immune damage is likely orchestrated by the recognition of antigen-presenting cells (APC) by CD4 (+) T-cells, which then activate naïve CD4 (+) Th0 cells, initiating a cascade of immune reactions that are determined by the cytokines they produce. Autoantigen recognition and the above effector mechanisms are opposed by regulatory T-cells[46]. In the early stage of infection, the Th1 cytokines have an immunity protection effect. Immunity initiation is essential in the early stage of infection, and immune intervention should be underlied in the late stage for the severe host response induced by cytokines in the late stage of infection leads to corneal destruction and perforation[16,47](Figure 1).

Potential role of ATRA in FK cellular immunity: receptors for ATRA have been shown to promote regulatory T cell (Treg) differentiation and to suppress Th17 development. Increased Treg activity reduces inflammation [48-54]. The survival of antigen-specific T cells can be differently modulated by ATRA. These results have a significant implication for T cell adoptive immunotherapy in different settings [55] (Figure 2). Th1/Th2 cytokines play a key role in immune responses by controlling macrophage activation [56]. Stromal keratitis is a chronic immunopathological lesion of the eye orchestrated by Th1 cells and, to a lesser extent, Th17 cells. The reduction of Th1 type proinflammatory cytokines, chemokines, PMN recruitment and antiinflammatory factors is a useful approach for the treatment of corneal infection[57-58]. IL-4, 6, and 10 cytokines may be protective during Gram-positive corneal infection [59]. ATRA promotes the synthesis of type 2 cytokines, including IL-4, IL-5, IL-10 and IL-13, while decreasing IFN-γ and TNF-α expression in activated human T cells. RAR-α is involved in the regulation of genes and proteins involved in human T cell activation and type 2 cytokine production [60-63]. Th1 cells respond to corneal destruction, while Th2 cells respond less to corneal destruction. ATRA decreases the severe destruction of inflamed tissue by modulating the ratio of Th1/Th2 cytokines. Inhibition of Th1 cytokines and activation of Th2 cytokines is beneficial for the control of fungi keretitis[52,60,64-67]. ATRA is a potential therapy for inflammatory conditions [68] (Figure 2).

ALL-TRANS RETINOIC ACID AND CHEMOKINES

FK inflammation at the early stage of infection requires the activation of cytokines and chemokines, which attract PMN infiltration to clear the pathogen [69-70]. Some chemokines are critical regulators of PMN recruitment [71-72]. Although chemokines attract PMN at the early stage of infection, which is essential to clear the pathogen during FK, the treatment of FK must suppress excessive corneal inflammation and prevent fungal invasion in the late stage[19]. ATRA leads to massive changes in gene expression involved in immune function and increases dendritic cell (DC) markers, co-stimulatory molecules (CD80, CD83 and CD86), adhesion molecules (CD40) and chemokine receptors (CCR6). ATRA stimulates T cell proliferation and presents antigens to T cells [73]. ATRA and its receptors augment a diverse Th1-, Th2-, Treg- and inflammation-associated

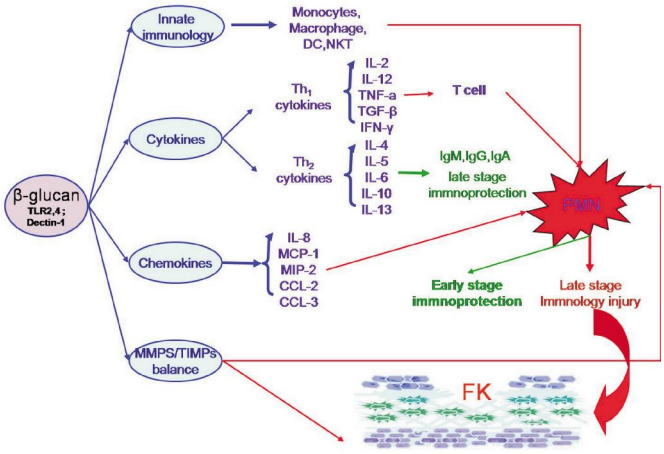

Figure 1 TLR and Dectin−1 recognize β−glucan of the fungi cell wall initiating primary innate and, later, adaptive immune responses. Proinflammatory cytokines were elevated in infected corneas. The cytokines trigger Th cell generation and recruitment to the cornea. The Th1 cytokines act as immunity protection in the early stage and immunological damage in the late stage. Th2 cytokines act as immunity protection in the late stage. Cytokines, chemokines, MMPs and innate immunity related cells attract PMN infiltration; thus, in the late stage, PMN leads to FK.

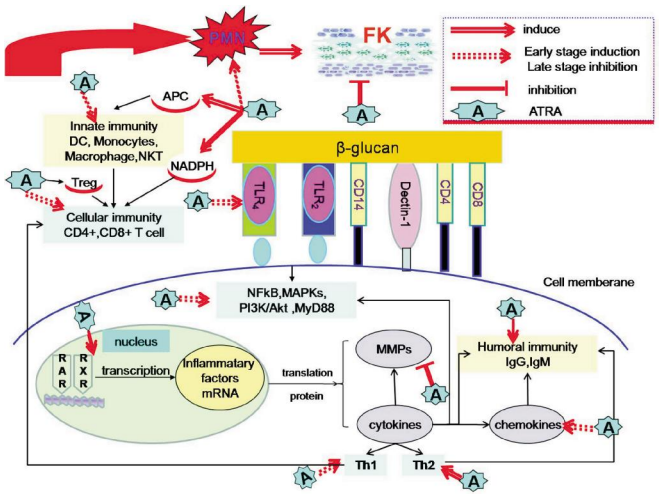

Figure 2 During FK, ATRA initiates the innate and adaptive immune systems by modulating transcription factors in many pathological processes to perform its antiinflammatory and immunoregulatory effects, including T−cell activation, cytokine regulation, chemotaxis, inflammatory response, immune response, signal transduction and MMPs production. ATRA may be a potential therapy for FK.

response that may be related to increased chemokine [vascular endothelial growth factor, macrophage colony-stimulating factor (M-CSF) and MCP-1] production by macrophages [63,74]. The proposed anti-inflammatory action of the ATRA-dependent induction of intercellular adhesion moleculeICAM-1 CC-chemokines and IL-8 have positive roles in the therapeutic response to retinoids [75,76]. ATRA regulates multiple chemokines, M-CSF, MCP-1, ICAM, CXCL10, CCL2, CCL3, CCL22, CCL24, CXCR4, CXCL16/SR-PSOX, and eotaxin, in different cell lines and different pathological processes [63,77-82]. ATRA has the potential to act as a new therapeutic and steroid-sparing drug [83]. These data suggest that ATRA might also be effective for the treatment of inflammatory processes[84]; thus, it has potential for the treatment of FK (Figure 2).

ALL −TRANS RETINOIC ACID AND MATRIX METALLOPROTEINASES

MMPs play important roles in the degradation of cornealextracellular matrix (ECMs).The hyphal growth pattern and invasive depth are dependent on the different degradations of ECMs and are different in various FK infections [85]. The increased immunoreactivity of MMPs-2,8,9, and 13 in FK is related to neutrophil chemo-attraction [86-89]. Targeting MMPs may be a novel therapeutic strategy for FK [90]. ATRA modulates MMP and TIMP expression, shifting different cell lines from a matrix-degrading phenotype to a matrix-preserving phenotype [91-96]. Thepro-MT_1-MMP and pro-MT_2-MMP are greatly attenuated by ATRA, inhibiting MMP activity [97-98]. ATRA can modulate protease/antiprotease balance in a manner that may have impacts on disease pathogenesis [79,99]. ATRA treatment is promising for the inhibition of MMPs and FK (Figure 2).

ALL −TRANS RETINOIC ACID AND HUMORAL IMMUNITY

ATRA augments immunoglobulin synthesis of cord blood mononuclear cells by enhancing the synthesis of certain cytokines. Nearly all species of fungi contain the conserved carbohydrates β-glucan and chitin within their cell walls, and these may be targets of innate and adaptive immunity which prevents extensive tissue damage. Natural antibodies bind fungal organisms and enhance host defenses against Pneumocystis in the early stages of infection. Immunoglobulin M (IgM) antibodies influence the recognition of fungal antigen by DC. IgM antibodies are required for Th2 and Th17 cell differentiation and guide B cell isotype class-switch recombination during host defense. IgM isotype shapes the earliest steps in the recognition and clearance of fungi [100]. The humoral response elicited by Candida keratitis plays an important role in host protection against secondary Candida keratitis infections [101]. Immunologic memory is induced by C. albicans keratitis, and previous contact with Candida preparation enhanced the resistance of the host to subsequent corneal challenge with the same fungus. Active immunization might be an effective strategy for preventing FK in populations at high risk. Reactions to pathogens usually tend to affect immunity and limit tissue damage [102]. Vaccination using DC may help patients with chronic hepatitis B to clear the infection [103]. After induction of an immune response with a DNA vaccine, ATRA pushes that immune response in the Th2 direction and acts as a candidate adjuvant and immunomodulatory

molecule [30,104,105]. ATRA appears to be useful to increase memory T cell responses and protect mucosal sites from viral infection, and it may facilitate the development of more effective vaccines against mucosally transmitted pathogens, such as HIV [106]. ATRA prevents both human monocytes and mice bone marrow-derived monocytes/macrophage cells from differentiating into osteoclasts. These data suggest that ATRA is an effective treatment modality for RA patients[35]. In summary, ATRA can induce beneficial humoral immunity and prevent immune tissue injury and damage, which is essential for the treatment of FK (Figure 2).

Although we conducted a Pubmed search, we did not find any direct link between FK and ATRA. The results of the search show that deficiency in Vitamin A increases the severity of experimental corneal herpes simplex virus infections and P.aeruginosa infections [107,108]. Vitamin A deficiency plays a role in FK with corneal xerosis[109]. This is useful evidence for future studies.

CONCLUSION

FK is an immunopathological lesion of the eye and a common cause of blindness in humans. ATRA was shown to promote the resolution of several inflammatory and immune diseases by regulating target genes through the activation of nuclear receptors. The recognition of infectious agents by the TLR system initiates primary innate immune responses and, later, adaptive immune responses. Activation of transcription factors induces the expression of inflammatory cytokines and chemokines, which recruit PMN to the cornea and result in a corneal ulcer. ATRA initiates the innate and adaptive immune systems by modulating the transcription factors involved in many pathological processes to generate its antiinflammatory and immunoregulatory effects. ATRA can also prevent immune injury in the late stage of inflammatory disease. ATRA inhibits the expressions of MMPs, which play a key role in the FK process. In this review, we summarized the processes of T-cell activation, cytokine production, chemotaxis, inflammatory response, immune response, signal transduction and MMP production in FK and extrapolated the potential approach of ATRA treatment to control lesion severity in FK.

We will generate an animal FK model using ATRA nanoparticle to investigate our proposed FK treatment strategy.

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