

An overview of possible pathogenesis mechanisms of *Alternaria alternata* in chronic rhinosinusitis and nasal polyposis

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ABSTRACT

Chronic Rhinosinusitis (CRS) is a multifactorial disease, and different etiologies like metabolism and immunity disorders, bacterial superantigens, biofilms, and fungal allergens are known to develop this disease, especially the CRS with nasal polyps. *Alternaria alternata* (Alternaria) is one of the most prevalent airborne fungal species in the nasal discharge, which might have vigorous immunologic activities in nasal epithelial cells and play an essential role in the pathogenesis of CRS. Moreover, the interaction between this fungus and the innate and adaptive immune systems leads to the development of chronic inflammation. This inflammation may consequently instigate the CRS and nasal polyposis. The attenuation of surfactant protein synthesis or intracellular reserves and mucus hypersecretion could prevent the clearance of Alternaria from sinuses and may be correlated with colonization and re-infection of airborne fungi. Furthermore, higher expression of cathelicidin, thymic stromal lymphopoietin, toll-like receptors, and T helper 2-dominant immune responses can result in an IgE-mediated pathway activation and eosinophils degranulation. Moreover, higher local Alternaria-specific IgE was shown to be correlated with eosinophilic cationic proteins and might relate to nasal polyps. However, the role of genetic and environmental factors affecting CRS and nasal polyposis is not well studied. Likewise, further animal and clinical studies are required to better understand the role of *Alternaria* in CRS disease. The current article reviews the recent findings around the Alternaria-induced CRS and nasal polyposis.

1. Introduction

Chronic Rhinosinusitis (CRS) is the chronic inflammation of the nasal cavity and paranasal sinuses with non-infectious inflammatory immune responses characterized by rhinorrhea and obstruction, facial pain and pressure, and loss of sense of smell which can last beyond 12 weeks [1, 2]. In general, the prevalence rate of CRS was reported to be around 4.5–12%, while this rate varied between 0.5 and 4.3% among patients with nasal polyposis. In western countries, CRS is more prevalent among males, the elderly, and asthmatics. CRS is phenotypically divided into two types; the CRS with and without nasal polyps (CRS_{wNP} and CRS_{sNP}, respectively) [3–5].

Nasal polyps, known as inflammatory cell infiltrations, can damage epithelial cells, and to date, increased local inflammatory mediators, extracellular matrix accumulation, and mucosal epithelial damages

have been reported to be the most common factors in their development [6,7]. Furthermore, following the release of cytotoxic granule proteins, eosinophils can play an important role in the pathogenesis of CRS with polyps [8]. Thus, according to the dominant inflammatory cell types, CRS can be further classified as either eosinophilic or non-eosinophilic CRS [9]. An indirect symptom of the eosinophilic CRS is the generation of type-2 cytokines and eosinophilic inflammatory infiltration. Eosinophilic CRS, due to its strong association with asthma as well as the high probability of its recurrence despite medical and surgical treatments, significantly affects the quality of life [5,10,11].

Osteitis, bacterial superantigens, and mucosal defense disorders have been reported as etiologic factors of CRS disease [12,13]. Additionally, fungal allergens are correlated with dysregulation of the immune response, epithelial barrier dysfunction, exacerbation of local inflammatory reactions, and CRS [14]. Noteworthy, the non-invasive types of

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mycotic CRS such as Fungal Rhinosinusitis (FRS), fungus ball, and Allergic Fungal Rhinosinusitis (AFRS) are more prevalent than invasive types [15]. Recent studies have reported that *Alternaria alternata* (Alternaria) is a widespread fungal species in the airway discharge, and the pathogenic hypersensitivity and inflammatory reactions correspond to the development of CRS, bronchial asthma, and allergic rhinitis [13, 16,17]. Airborne fungi induce an inflammatory reaction by secretion of proteins and enzymes in the human body, and a protease of these fungi triggers desquamation of respiratory epithelial cells and synthesis of an inflammatory mediator [13]. However, owing to the multiple and inconsistent hypotheses on CRS, our knowledge about this disease is still evolving and the role of Alternaria in the pathogenesis of CRS and nasal polyposis remains unclear. Therefore, the present review paper is an attempt to investigate the possible underlying mechanisms of Alternaria in triggering CRS and nasal polyposis.

2. Epidemiology

It was estimated that 5–15% of CRS cases were related to fungal allergens. However, there are reports that show fungi were the primary cause of CRS, while Alternaria was among the highly colonized agents, with 44.3%, in samples recovered from CRS patients [18–20]. The agents responsible for AFRS are diverse among different studies. For instance, in an investigation on 168 positive cases, 87% and 13% of agents belonged to dematiaceous fungi and *Aspergillus* spp., respectively [21]. Notably, among dematiaceous fungi, Alternaria, *Bipolaris* spp., *Drechslera* spp., and *Curvularia lunata* are respectively shown to have the highest frequencies [18,22–24].

2.1. Geographical distribution

One of the possible factors in the development of CRS disease is geographical location. Typically, AFRS is frequently reported in India, the Middle East, Africa, and some parts of the USA [18,25–28]. However, it is also reported in other parts of the world. In this regard, a recent study in Serbia found Alternaria fungus in five (8%) out of 60 samples from Allergic Bronchopulmonary Aspergillosis (ABPA) patients [29]. In this study, rhinosinusitis and the existence of fungi in sinuses were confirmed by Computer Tomography (CT) scan. Notably, ABPA-positive AFRS patients were affected with a more severe type of CRS; often with nasal polyps [29]. In another study on FRS in India, results demonstrated that five out of 84 (5.95%) samples were verified to contain Alternaria spp. [30]. Interestingly, out of 53 patients with CRS in Taiwan, Alternaria genome particles were detected by Polymerase Chain Reaction (PCR) in 79.3% and 73.6% of inferior turbinate and ethmoid sinus specimens that were collected during surgery, respectively [31]. In line with this, a study in Germany reported that the Alternaria genome was present in all 27 surgical specimens of CRS patients, while it was not detected in healthy control groups [32]. Furthermore, results of a study in Iran showed that according to the data acquired from the medical records of 127 patients with sinonasal polyposis, 12 patients (9.45%) were proven to have allergic fungal sinusitis. Among these 12 cases, eight were positive for *Aspergillus* spp. and three were positive for Alternaria spp. [33]. This study was in agreement with the findings of another research which demonstrated that geographical variability appeared to be influential in the incidence of allergic fungal sinusitis and fungal species related to the disease, as *Aspergillus* spp. have been mostly reported in the Middle East [27,34]. On the other hand, according to a report in Egypt, a Middle Eastern country, *Aspergillus* spp. was found in 42 out of 68 patients (61.7%) with AFRS, while Alternaria was isolated from only one sample [35]. In a study performed in another Middle Eastern country, Iran, fungi were detected in 12 out of 162 (7.4%) participants with chronic sinusitis, of which nine cases were Alternaria spp. [36]. The authors suggested that Alternaria was the most causative agent and was more prevalent in patients from 20 to 40 years old [36].

Altogether, Alternaria isolates were observed in patients with

different subtypes of sinusitis, however, a discrepancy was detected in the results from cases all over the world. It appears that further studies are required to better find the link between geographical location and the causative agent of fungal sinusitis.

2.2. Socioeconomic status

Socioeconomic status is a controversial factor as some studies have considered the significant role of low socioeconomic status in the prevalence of fungal sinusitis, while some others could not find any correlation between them [37,38]. However, most AFRS patients with intraorbital and intracranial extension were shown to reside in rural areas where the healthcare system was in low standards [39]. AFRS patients were also reported to reside in the USA counties with lower median income [40]. Nevertheless, there is no specific report available about the impact of socioeconomic status on the occurrence of Alternaria-induced CRS, and more studies would be required to determine the actual role of socioeconomic status in Alternaria CRS infections.

2.3. Seasonal variation

Fungi usually grow in hot and humid environments during summer and fall seasons and thus the seasonal variation could be a determining factor in fungal-borne diseases. It was demonstrated in a study that among 108 patients diagnosed with CRSwNP, 60 cases (63%) were culture positive and fungus culture rates were higher in the summer and fall. Cladosporium was the main species isolated in the fall, while Alternaria was observed more in summer. The authors hypothesized that Alternaria might prefer a hot and humid environment [41]. Another research investigated nasal mucus in 233 cases and demonstrated that the seasonal fluctuation had a remarkable role in the recurrent prevalence of *Cladosporium* spp., *Alternaria* spp., and *Aureobasidium pullulans*. The maximum prevalence rate was observed in the late summer and early autumn, while a minimum rate was in the late winter and early spring [42].

3. Pathogenesis

In recent years, various studies have proposed underlying pathogenesis mechanisms of Alternaria in CRS and nasal polyposis disease. This section reviews the most critical interactions of this microorganism with the innate and adaptive immune systems that may lead to the development of different subtypes of CRS and nasal polyposis.

3.1. Innate immune system

3.1.1. Innate immune system mediators

The innate immune system is one of the most critical barriers against fungal pathogens because, in addition to eliminating this microorganism, it plays a significant role in preventing infection by activating the adaptive immune system. Consequently, any disorder in the innate immunity caused by genetic diseases and various fungal pathogens can prevent the elimination of microorganisms and leads to chronic infections [43]. Respiratory epithelial cells secrete Surfactant Protein (SP)-D, which is an essential innate immune modulator and has a critical role in the immune system by enhancing chemotaxis, cytokines production, and phagocytosis. A previous study has reported that SP-D is expressed in the human nasal mucosa and may affect allergic inflammation upon the downregulation of allergic hypersensitivity and inflammation [44,45]. In this regard, SP-D was shown to remain undetected in patients with allergic fungal sinusitis, indicating possible attenuation of SP-D synthesis or intracellular reserves [46]. Furthermore, the authors reported a decreased SP-D level in patients with CRS and Non-Allergic Non-Fungal Eosinophilic Sinusitis (NANFES), compared to control groups. They suggested that decreased SP-D level

during the progression of CRS could lead to an inability to clear microorganisms and thus increasing the chances of re-colonization and re-infection. Notably, *Alternaria* allergens increased the expression of SP-D in patients with CRS. Therefore, SP-D is a natural defense peptide in the human nasal mucosa, which may have an essential role in fungal allergen-induced inflammation. Besides, depletion of this protein reserve during chronic infection can prevent the clearance of *Alternaria* from sinuses [46].

Mucus hypersecretion is known as one of the symptoms of inflammation in the upper and lower airways. Nasal polyp and mucosal hypertrophy can increase surface area and lead to the greater generation of inflammatory cytokines and mucus. In this regard, local inflammation was shown to induce the upregulation of mucin genes and the production of mucin, which may have adverse effects on mucosal protection and promote predisposition to disease development [47,48]. It should be noted that upregulation of *MUC4*, *MUC5AC*, *MUC5B*, and *MUC8* genes was observed in chronic sinusitis or nasal polyp tissue [49]. In this regard, a recent study reported that *Alternaria* could increase the expression of *MUC4*, *MUC5AC*, and *MUC8* in nasal polyp epithelial cells, while *Aspergillus fumigatus* (*Aspergillus*) could only upregulate *MUC5AC* mRNA levels [50]. Additionally, the authors reported synergistic effects between airborne fungal exposure and Rhinoviruses on the upregulation of mucin gene in nasal polyp epithelial cells. They suggested that strong induction of mucus production might be associated with *Alternaria* and aggravation of respiratory symptoms [50].

In response to fungi, the production of cathelicidin antimicrobial peptides was increased in nasal epithelial cells. These peptides play an essential role in the innate mucosal defense against airborne pathogens. Previous research reported that LL-37, the human cathelicidin hCAP18, had a higher expression level in nasal polyps, compared to the normal inferior turbinates [51,52]. Accordingly, it was shown that *Alternaria* extracts upregulated LL-37 in the nasal tissue of CRS patients [53]. LL-37 was reported to induce the production of chemokine and cytokine in leucocytes and local cells, and it had chemotactic effects on a large number of immune cells such as CD4 T lymphocytes, monocytes/macrophages, and neutrophils. Thus, in cooperation with other immune mediators and through multiple pathways, LL-37 could trigger innate immune responses [54]. Abnormal immune responses to fungi may be an essential factor in the pathophysiology of CRS, and *Alternaria* allergens may trigger mucosal innate immune responses in CRS patients [53]. As noted above, fungal allergens can lead to chronic inflammations in the nasal cavity by stimulating innate immune mediators. Genetic background can also play a crucial role in forming these interactions because an abnormal decrease or increase in any of the innate immune mediators can yield hypersensitivity and inflammation in response to *Alternaria* allergens. Further investigations seem necessary to elucidate these interactions.

3.1.2. Pattern recognition receptors (PRRs)

Toll-Like Receptors (TLRs) regulate the innate and adaptive immune responses by recognizing conserved molecular structures of pathogens. The interface between the external environment and the respiratory tract's mucosal surface is composed of epithelial cells. These cells express *TLR* genes which are known as the primary innate recognition system of microbial invaders [55]. *TLR2* and *TLR4* genes were expressed in nasal epithelial cells while they showed an increased levels in CRS patients [56]. These observation has raised interests in investigating the interaction between fungal pathogens and TLRs in sinusitis. In this regard, a study reported that *Alternaria* was a potent stimulant to increase *TLR2*, *TLR3*, and *TLR4* mRNA expression in nasal epithelial cells. Notably, cysteine protease inhibitors only hindered the expression of *TLR3*. It appears that various components of this fungus such as proteases and cell wall components are associated with increased expression of *TLR* genes [57]. Another study detected that expression of *TLR3* mRNA and its protein, which were induced by dsRNA, were hindered by *Alternaria*. Indeed, this microorganism suppresses dsRNA-induced

chemokine *CXCL10* and *CXCL11* (IP-10 and I-TAC, respectively) and interferon (IFN- β) production. Since this phenomenon remained active under heat treatment, which inactivates *Alternaria* protease, the authors suggested that fungal chitin and glycan might play pivotal roles in this gene suppression. Given that *TLR3*, directly or indirectly, senses viral double-stranded RNA and triggers the immune system against viral infection, the inhibition of *TLR3* by *Alternaria* can exacerbate symptoms of CRS patients by increasing viral infections. Nevertheless, more investigations are needed to verify the interaction between *Alternaria* and viral infection in sinuses [58].

The correlation between *Alternaria* and Thymic Stromal Lymphopoietin (TSLP) was reported in multiple investigations. TSLP is synthesized in different cells such as epithelial, fibroblast, and dendritic cells (DCs). TSLP triggers myeloid DCs and leads to the differentiation of naive T cells toward the helper T cells (Th) 2, which play a vital role in the development of allergic inflammation. This role is through the synthesis of cytokines such as Tumor Necrosis Factor (TNF)- α and Interleukin (IL)-4, IL-5, and IL-13 [59,60]. Nasal fibroblast produces TSLP, and its expression is increased in nasal polyps and allergic rhinitis [61]. The results of a recently published paper showed that *Alternaria* could enhance TSLP production in nasal fibroblasts, especially Nasal Polyp Fibroblasts (NPF) [16]. In this study, TSLP showed a higher expression level in NPF than normal cells, which may indicate a higher immunological activity against fungal pathogens. The authors reported that the expression of *TLR2* and *TLR5* was increased in NPF upon *Alternaria* stimulation. Thus, accurate identification of *Alternaria*-TLR interaction, which prompts increased production of TSLP, can help control CRS. Since TSLP can trigger airway mucosal DCs, which consequently facilitate the development of Th2 immune response in sinonasal mucosa, it may be involved in the initiation and amplification of CRSwNP [16].

Calprotectin is secreted from immune and epithelial cells and plays a distinct role in inflammation. The Receptor for Advanced Glycation end products (RAGE) and *TLR4* are specific receptors for this protein [62]. The results of a recent study indicated that calprotectin triggered the *Alternaria*-induced production of TSLP and IL-25 in airway epithelial cells [63]. Indeed, after exposure to airborne allergens, calprotectin was secreted from airway epithelial cells, which then triggered TSLP and IL-25 production via *TLR4* and RAGE, in airway epithelial cells. Compared to control non-eosinophilic CRS patients, allergen-induced calprotectin biosynthesis was significantly stimulated in primary nasal epithelial cells of patients with eosinophilic CRS [63]. Therefore, TSLP and IL-25 could be involved in the development of eosinophilic CRS, while calprotectin was shown to enhance and modulate these inflammatory responses in airway epithelial cells [63].

Another study also reported that *Alternaria* induced the expression of *TLR2* and *TLR5* in NPF, whereas these two *TLR* genes were connected with the production of IL-6 and IL-8 [64]. Consequently, as mentioned earlier, *Alternaria* has different interactions with TLRs. In this regard, the correlation between *Alternaria* and *TLR2* and *TLR5* has received the highest praise from researchers. These TLRs have an influential role in nasal fibroblast in the production of immune mediators such as IL-6 and IL-8. However, *TLR5* is usually not associated with the immune mechanism triggered by fungi, and more studies are required on this subject. Altogether, *Alternaria* could activate an immune pathway in nasal mucosa by increasing the expressions of TLRs and it may induce chemical mediator production in NPF and inferior turbinate fibroblasts. Notably, fungal pathogens cannot penetrate healthy nasal mucosa, but injuries resulted from fungal protease and mechanical hurts help with the penetration of antigens into the aggravate local. Afterward, fungal antigens interact with nasal fibroblasts and trigger local inflammations in the nasal cavity. Overall, *Alternaria*, by maintaining or increasing local inflammations in the nasal cavity, may play a role in the initiation and development of CRS and nasal polyposis [64–66].

3.2. Adaptive immunity

In addition to the disorders of innate immune systems that may increase chronic inflammation and lead to CRS, recent studies have reported an increase in the amount/number of T lymphocytes, IL-5, IL-13, and INF- γ in sinus mucosa of patients with CRS and nasal polyps [67, 68].

3.2.1. Type-2 inflammation

A recent investigation has reported an IgE-mediated pathway in the pathogenesis of CRS and nasal polyposis. In this regard, a local humoral immune response to *Alternaria* in nasal polyps was reported, which shows a higher prevalence of *Alternaria*-specific IgE in CRSwNP patients, compared to the CRSsNP patients [69]. Interestingly, in 60% of CRSwNP patients, *Alternaria*-specific IgE was not detected in the serum, supporting the development of local inflammation. Besides, there was an association between eosinophil cationic protein and the amount of IgE in nasal polyps. This result reveals that eosinophilic degranulation in nasal polyps depends on IgE and may play a role in polyp formation. It seems that the eosinophils covered with IgE could bind to *Alternaria* proteins and lead to eosinophils degranulation, the release of cationic proteins, and localized eosinophilic inflammation [69]. Two years later, another study also reported no variation in the serum *Alternaria*-specific IgE, IgA, and IgG levels in patients with and without nasal polyps. On the other hand, *Alternaria*-specific IgE levels were remarkably higher in polyp tissue than the non-polyp tissue, and there was a positive correlation between these antibodies and eosinophilic cationic protein [70].

Other groups have reported that increased local IgE response to common aeroallergens such as *Alternaria* allergens might play a role in nasal polyp formation in patients with CRS who lacked evidence of systemic atopy. A high IgE level was detected in polyps compared to the turbinate of CRSwNP patients, and the authors suggested that detection and inhibition of these tissue-specific IgEs could attenuate nasal polyp formation in CRS patients [71]. A positive correlation between antigen-specific IgEs and eosinophil cationic protein in CRSwNP patients was also identified [72]. In this manner, *Alternaria*-specific IgEs had a higher prevalence rate in the sinus mucosa, but not in serum, followed by the mite-, *Aspergillus*-, and *Staphylococcal Enterotoxin B* (SEB)-specific IgEs [72]. Thus, in addition to local mite- and *staphylococcal enterotoxins*-specific IgEs in the pathogenesis of CRSwNP, fungal allergens, especially *Alternaria*, prompted local inflammation [72–74]. Finally, dispersed nasal polyp cells are reported to produce IL-5 and IL-13 when exposed to *Alternaria* [75]. These cytokines cause eosinophilic inflammation by mediating eosinophil differentiation, recruitment, and survival. Although fungal antigens could produce IL-5 and IL-13 in nasal polyp cells, the ability of these antigens to induce eosinophilia-associated cellular responses in nasal polyps was lower than SEB [75]. Therefore, IgE-mediated hypersensitivity to *Alternaria* antigen may act as a significant pathogenic mechanism that drives localized eosinophilic inflammation in CRSwNP patients, which may be relevant to the pathogenesis of CRS and nasal polyposis.

3.2.2. T cells

T-helper lymphocytes, central cells of the adaptive immune response, when activated by different antigens, are converted into significant effector subtype cells including Th1 or Th2. These cells release a particular cytokine and stimulate various immune responses. In this context, Th1 produces macrophage-activating factors, INF- γ and TNF- α , and targets intracellular pathogens. On the other hand, upon secretion of IL-4, IL-5, IL-10, and IL-13, Th2 adopts humoral immune and allergic inflammatory responses [76]. Luong et al. reported that fungal antigens, especially *Alternaria*, could trigger the production of Th2 cytokines including IL-4 and IL-5 in Peripheral Blood Mononuclear Cells (PBMCs) in patients with AFRS, but not in healthy control individuals. On the contrary, a considerable elevation in the IL-10 expression level was detected in healthy control PBMCs upon exposure to fungal antigens

[76]. IL-4 induces human respiratory epithelial cells to synthesize eosinophil chemoattractant cytokines, while IL-5 causes eosinophil induction. The production of these cytokines in response to fungal antigens can be considered as the characteristic eosinophilic mucin within the paranasal sinuses [77,78]. On the other hand, IL-10 suppresses inflammatory cytokines and regulates Th2 responses in allergic asthma [79]. Thus, in healthy individuals, higher production of IL-10 in interaction with fungal antigens may act as a protective response.

Another investigation reported that PBMC of CRS patients was activated vigorously by fungal pathogens, especially by *Alternaria* [80]. These cells produced INF, IL-5, and IL-13 that were consistent with those expressed in CRS patients' airways. Furthermore, CRS patients showed higher circulating IgG in response to *Alternaria* than normal cases. The results of this study showed that both Th1 and Th2 cytokines, regardless of atopy status, were expressed in CRS patients when exposed to *Alternaria* [80]. Similarly, a recent study performed by Shin et al. also reported that *Alternaria* could induce the production of both Th1 and Th2 in the nasal epithelial cell. This study showed that *Alternaria* triggered the production of IL-33 and TSLP in nasal epithelial cells, and enhanced the production of these mediators through NF- κ B, Activator Protein 1 (AP-1), and Mitogen-Activated Protein Kinase (MAPK) pathways [81]. This inconsistency between different studies may be due to employing various research methods and existence of multiple pathophysiological pathways in different CRS subtypes [82]. For instance, Shin et al. used normal nasal epithelial cells, which could not represent the CRS or nasal polyp epithelial cells [83], while the immune responses would have been different if they had used pathologic mucosal epithelial cells.

Finally, *Alternaria* antigens were shown to lead to the proliferation of peripheral blood CD4⁺ and CD8⁺ T-cell subsets in healthy cases, Allergic Rhinitis with Fungal Allergy (ARFA) cases, and most CRSwNP patients, whereas CD8⁺ T-cell response was surprisingly absent in AFRS and eosinophilic mucus CRS patients. Moreover, in most individuals, CD4⁺ and CD8⁺ T-cell were involved in stimulating anti-fungal-specific responses, while in patients with AFRS and eosinophilic mucus CRS, the response was mainly caused by CD4⁺ cells [84]. These results suggested that fungal-specific memory CD4⁺ and CD8⁺ cells were present in healthy cases, and both T-cell subsets were essential in the immune response against fungal pathogens. Therefore, CD8⁺ cells, absent in anti-fungal immunity, may cause dysfunctional immune activity and enhance fungal accumulation in AFRS and eosinophilic mucus CRS sinuses [84,85]. Different aspects of reported interactions of *Alternaria* with the immune system are illustrated in Fig. 1. These studies have been performed in *ex vivo* conditions, and more animal studies are needed to determine the exact pathogenesis mechanism of *Alternaria* in CRS and nasal polyposis.

4. Animal studies

Because of the difficulty of CRS induction in animal models, *in vivo* analysis of *Alternaria*-induced CRS remains unfulfilled. However, in this section, we review the available reports on the possible mechanisms of *Alternaria* in the induction of CRS in animal models. A recent study has used mice models to establish the immunological effects of *Alternaria* on sinonasal mucosa inflammation and the immunopathologic characteristics of the *Alternaria*-induced CRS [1]. The authors instilled *Alternaria* intranasally for 8–16 weeks and then evaluated different immune responses. The results showed that *Alternaria* caused significant inflammation in sinonasal mucosa regardless of the allergic predisposition status. This fungus triggered a Th2 immune response with an allergic predisposition, while Th1 and Th2 immune responses were triggered without allergic predisposition. Notably, higher eosinophilic inflammatory responses were detected in allergic CRS [1]. Additionally, the authors reported that *Alternaria* could trigger mucus production, tissue inflammatory cell infiltration and also increase the serum IgE level regardless of allergic status. Therefore, Th2-dominant immune responses and eosinophils were caught in an allergic predisposition state,

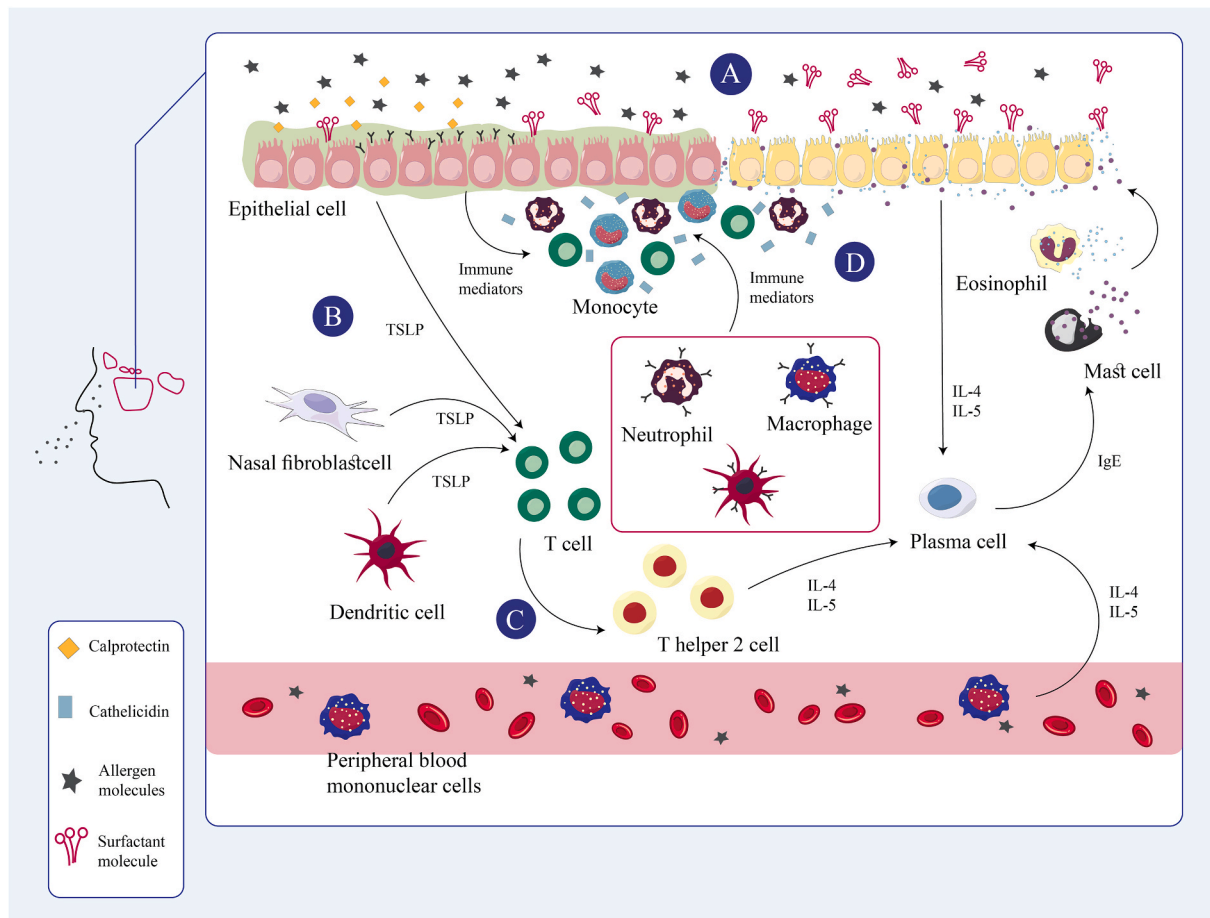


Fig. 1. Possible mechanisms of *Alternaria* in inducing CRS. A) Decreased production of surfactants and mucus hypersecretion can prevent allergen clearance in the sinuses and may lead to chronic inflammation. B) Production of high levels of TSLP by nasal epithelial cells, nasal fibroblast (especially nasal polyp fibroblast), and DCs leads to the differentiation of naive T cells toward the Th2. C) In dealing with *Alternaria* allergens, nasal epithelial cells, Th2, and PBMN produce IL-4 and IL-5 and these cytokines trigger the production of IgE from plasma cells. Afterward, eosinophils covered with IgE could bind to *Alternaria* proteins and lead to eosinophils degranulation, release of cationic proteins, and localized eosinophilic inflammation. D) Higher expression of cathelicidin LL-37 in nasal epithelial cells of patients with CRS after exposure to *Alternaria* induced the innate immune responses by multiple pathways and had a chemotactic effect on a large number of immune cells. Furthermore, higher expressions of TLRs in nasal epithelial cells and different immune cells after challenging *Alternaria* allergens result in the production of immune mediators and higher local inflammations. CRS: Chronic Rhinosinusitis. TSLP: Thymic Stromal Lymphopoietin. DCs: Dendritic Cells. TLRs: Toll-Like receptors.

while Th1, Th2, and Treg immune responses with dominant eosinophil and neutrophil inflammation were observed in animals without allergic background [1]. In this study, culture-filtrated extracts of *Alternaria* were used, and fungal spore might induce different immunological responses [1]. In another study, multiple airborne allergens including *Alternaria*, *Aspergillus*, *Staphylococcus aureus*, and house dust mite were administered intranasally in C57BL/6 mice models for four, eight, 12, or 16 consecutive weeks [2]. Vigorous eosinophilic inflammation was detected after 12 weeks of applying a combination of these four allergens. Allergens significantly increased epithelial thickening and eosinophil infiltration after eight weeks, as well. Moreover, goblet cell hyperplasia, tissue eosinophil aggregates, and polyp-like lesions with the increased level of IL-5 and eotaxin in the nasal lavage fluid, were observed after 12 weeks. However, in contrast to the previous study, Th1 immune responses were undetectable. The authors suggested that 12-week or longer exposure to airborne allergens was sufficient for triggering eosinophilic CRS in a murine model [2]. The role of each allergen in CRS was not investigated separately in this study.

Another research used *Alternaria* for inoculations into the nasal cavity of C57BL/6/J mice to understand the role of these fungi in the pathogenesis of rhinosinusitis [86]. Interestingly and unlike expectations, neutrophils were the major inflammatory cells detected in the sinus and the nasal cavity. The authors hypothesized that fungi acted as

initiators and the bacterial infection followed and played a prominent role in developing CRS, which was responsible for the neutrophil infiltration of the tissue. Moreover, epithelial thickening and disarray, goblet cell hyperplasia, and inflammatory cell infiltration were revealed in histopathological examination [86]. Noteworthy, in a group of mice, inoculation of fungi into the nasal cavity was performed in the presence of mucosal scratch, but compared to other groups, no significant difference was observed in inflammatory cells. The authors proposed that solitary fungi can induce mucosal inflammation without mucosal injury in the animal model [86].

The sheep model was also used to investigate the *Alternaria*-sinusitis interaction [87]. In this study, *Aspergillus* and *Alternaria* with and without *S. aureus* were inoculated into the sinus by an endoscopic sinus surgery. Results revealed no effect in the systemic Type-I hypersensitivity because fungi could not form biofilm without co-inoculation with *S. aureus*. Fungus alone formed biofilm in only one sheep out of 30, while 80% of sinuses showed the evidence of fungal biofilm when *S. aureus* was co-inoculated [87]. Notably, observation of the *Aspergillus* florid biofilm structure showed a higher synergistic effect of this fungus with *S. aureus* than *Alternaria*. This phenomenon results from the rapid growth kinetics of *Aspergillus* at a favorable growth temperature (37 °C), while *Alternaria* has an optimal growth at 28 °C. Therefore, the authors reported *S. aureus* as an essential cofactor in fungal persistence

and proliferation in sheep sinuses [87]. This bacterium might grow and contribute to the biofilm formation of fungus by disrupting the mucosal and immune system, including interfering with mucosal integrity, cilia and mucus motility, and TLR signaling [88]. However, the interactions between bacteria and fungi in the sinuses are more complex, and further studies are needed to characterize the quality of this interconnection.

Overall, in animal models, *Alternaria* and immune system interactions led to increased inflammation and chronic sinusitis. However, due to the application of different animal models and CRS subtypes, as well as various measurement methods, concluding out of these studies is not possible. It is necessary to have a global standard for animal models to accurately assess the role of *Alternaria* in the development and progression of CRS. Moreover, in comparison to *in vitro* studies, different environmental factors such as bacterial infections may be involved in stimulating different immune responses in animal models. Thus, it is evident that for the precise identification of roles of *Alternaria* in CRS development, *ex vivo* reports should be confirmed by clinical and animal studies.

5. Why is it useful to identify the pathogenesis mechanisms of *Alternaria* in causing CRS?

In recent years, various studies have reported *Alternaria* as a major cause of sinusitis in patients with different clinical status. For instance, Lou et al. reported a case of an immunocompromised patient with orbital apex syndrome and invasive fungal sinusitis caused by *Alternaria* spp. [89]. Another study reported a case of an immunocompetent 19-year-old man with *Alternaria*-induced rhinosinusitis that involved cerebral parenchyma [90]. *Alternaria*-associated fungus ball of orbit nose and paranasal sinuses was also reported in immunocompetent women [91]. Therefore, in addition to *Aspergillus* spp. and *Mucorales*, *Alternaria* causes sinus disorders in different patients. In this manner, previous studies have reported *Alternaria* as a potential trigger of CRS, and researchers have tried to identify the pathogenesis mechanisms of this fungus. Understanding *Alternaria* interactions with innate and adaptive immune systems as well as precise mechanisms of pathogenesis of microorganisms can facilitate developing preventive and treatment approaches against CRS.

Accordingly, a recent study has proposed melittin and apamin as a treatment approach against fungi-induced airway inflammatory diseases [92]. Melittin and apamin, two main components of bee venom peptide, have antibacterial, anti-fibrotic, and anti-inflammatory effects in various cells. In this study, nasal fibroblast cells dealt with *Alternaria*, and anti-inflammatory effects of melittin and apamin were evaluated thereafter. The findings showed that apamin could significantly hinder the production of *Alternaria*-induced IL-6 and IL-8, while a similar effect was not detected for Melittin. In addition to the suppression of expression of matrix metalloproteinase-9, tissue inhibitors of matrix metalloproteinase-1, and collagen type I in nasal fibroblast cells, these substances inhibited *Alternaria*-induced phosphorylation of Smad 2/3 and p38 MAPK, as well. This finding proposes a possible role for melittin and apamin in treating fungi-induced airway inflammatory diseases [92]. Another investigation also reported that Superoxide Dismutase (SOD) could reduce the inflammation induced by *Alternaria*, making it a new preventive and therapeutic option for CRS patients [93]. The results of this study showed that SOD significantly hindered the production of IL-6 and IL-8 in sinonasal epithelial cells of CRSwNP patients after exposure to *Alternaria* [93]. Likewise, by further investigating the pathogenesis mechanisms of *Alternaria* in CRS, immune system reactions can be prevented by blocking the inflammatory cascade.

6. Conclusion

Alternaria has been reported as one of the CRS-related pathogens. Although this fungus increases the production of cytokines, and causes chronic inflammation that can lead to CRS and nasal polyposis, the exact

pathogenesis mechanisms of *Alternaria* have not been elucidated yet. Furthermore, most studies have been performed *ex vivo*. Therefore, more animal and clinical studies are needed to better understand the role of *Alternaria*-induced CRS. Finally, *Alternaria* is a ubiquitous fungal allergen, and dealing with this fungus remains a daily routine. Consequently, it seems that genetic and ecological factors can affect *Alternaria*-induced CRS, which should also be considered in future investigations.

Author contribution

AS and ZC conceived and designed the study. AS and SV contributed in comprehensive research. MD, AS and AK wrote the paper. MD, DD and SR participated in manuscript editing. Notably, all authors have read and approved the manuscript.

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The authors declare that they have no competing interests.

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