EDITORIAL

EVIDENCE AND ROLE OF AUTOANTIBODIES IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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In this study, we review our current knowledge of the autoimmune etiopathogenesis of chronic rhinosinusitis with nasal polyps including bacterial infections, viral infections and immunomediated mechanisms and to discuss pathogenesis with relevance for pharmacotherapy. Relevant publications on the etiopathogenesis and treatment of chronic rhinosinusitis with nasal polyps (CRSwNP) from 1977 to 2013 were analyzed. The characteristic signs and symptoms include appearance of relapsing nasal polyps, with typical symptoms such as nasal obstruction, nasal discharge and, usually, loss of the sense of smell. The etiology and pathogenesis remain unknown. Proposed theories of causation include bacterial or viral infections and immunomediated mechanisms. The autoimmune aetiology of of unknown origin or failure to respond to classic pharmacological treatments with nasal and oral steroids is now suspected. At present, the nature of the antigen trigger, the exact role played by B/T cells and anti-dsDNA autoantibodies in the pathogenesis of nasal polyposis remains unclear. Corticosteroids and surgery are the first line of treatment in CRSwNP. In the case of corticosteroid treatment failure, other drugs can be used such as rituximab, belimumab or omalizumab which have demonstrated clinical efficacy in the treatment of nasal polyposis with comorbid asthma. Immunosuppressive drugs such as methotrexate, and cyclophosphamide have also been used with varying degrees of success.

Chronic rhinosinusitis (CRS) is a clinical syndrome associated with persistent inflammation of the nasal and paranasal sinus mucosa in which symptoms persist for more than 12 weeks without complete resolution. CRS, which exists worldwide, is one of the most common chronic diseases in adults and affects more than 10% of the European population and is often associated with asthma, acetylsalicylic acid syndrome (ASA syndrome), Kartagener syndrome and cystic fibrosis (1). The condition is referred to as "rhinosinusitis" because inflammation of both the nasal and sinus mucosa is often present. The definition of "chronic rhinosinusitis" encompasses two common variants: chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). Although these conditions are clinically and morphologically distinct (2), surgery and medical therapies, such as antibiotics and corticosteroids, are effective in treating both forms of the disease (3, 4).

CRSwNP is clinically characterised by the appearance of polyps in the nasal cavity, with typical symptoms such as nasal obstruction, nasal discharge and, usually, anosmia or loss of the sense of smell.

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Symptoms of CRS can be differentiated based on mucosal remodelling and inflammatory patterns. The aetiology and pathogenesis of CRS remain controversial and poorly understood. However, recent studies have implicated Alternaria, fungi, biofilms and toxins secreted by Staphylococcus bacteria as key pathogens that may initiate the symptomatic mucosal inflammation (5, 6).

Radiographic studies using cranial computed tomography (CT) and magnetic resonance imaging (MRI) typically show inflammation of the sinonasal cavity and various extents of polipoid formation in the nasal fossa. Bone structures are often normal, although partial resorption of bone without true infiltration is occasionally observed.

Differential diagnoses to consider in respect to CRSwNP are inverted papilloma, sinonasal cancer, Kartagener syndrome, and cystic fibrosis. It is often difficult to distinguish between CRSwNP and inverted papilloma; however, the characteristic aspect of inverted papilloma on MRI (brain- like) and a simple biopsy can guide the diagnosis.

The histology of CRSwNP shows intense oedematous stroma with albumin deposition, formation of pseudocysts, a germinative centre and subepithelial and perivascular inflammatory cell infiltration with significant tissue eosinophilia. CRSsNP shows fibrosis, basement membrane thickening, goblet cell hyperplasia, subepithelial oedema, and mononuclear cell infiltration. The subsequent remodelling is a dynamic process in both diseases, with progressive extracellular matrix production and degradation that is regulated by various mediators. Among these, TGF-beta plays a central role in the attraction and proliferation of fibroblasts and the production or deficit of T-lymphocytes (7).

Histologic studies have demonstrated significant tissue eosinophilia in a high proportion of CRS cases, most prominently in CRSwNP (8). The actual factors inducing this mucosal eosinophilia remain uncertain, but many studies have reported that IL-5 (an eosinophil survival and differentiation factor), eosinophil cationic protein and eotaxins (eosinophil chemoattractants) are significantly increased in polyp tissue (9). Several researchers have studied the relationship between the matrix metalloproteinase mRNA expression, a Zn^{2+} endopeptidase responsible for extracellular matrix degradation and its progressive histologic changes, and nasal polyps. These studies have demostrated an increased expression of matrix metalloproteinases (Type 9) in stroma of patients with nasal polyps and even more so in patients with recurrent nasal polyps (10).

Aetiology

The aetiology and pathogenesis of chronic rhinosinusitis with nasal polyps are not well understood. Initially, the disease was thought to be triggered by infection, however, chronic rhinosinusitis with nasal polyps can be an autoimmune disorder.

Infectious hypothesis

Staphylococcus aureus is a frequent coloniser of the nasal cavity and upper airway. This bacterium is associated with an average persistent carrier rate of 20-30% of adults (7). In two studies, an increased colonization rate of S. aureus was demonstrated in patients with CRSwNP (63.6%) but not in patients with CRSsNP (27.3%) (11). Sachse et al. (12) reported that, regardless of its intracellular or extracellular localisation in the epithelium, S. aureus is capable of inducing IL-6 synthesis in vitro and may contribute to the TH2 cytokine pattern in patients with CRSwNP.

Massive inflammatory reactions most likely result from the polyclonal activation of T and B lymphocytes, which is caused by a protein secreted from S. aureus and biofilm, the S. aureus enterotoxins (SEs) known as superantigens. SEs have been reported to further shift the cytokine pattern in nasal polyps toward TH2 cytokines. This might amplify eosinophilic inflammation by disfavouring the Treg cytokines IL-10 and TGF-Beta1 (13). S. aureus superantigens can also induce the formation of polyclonal IgE directed against multiple inhalant allergens, which was suggested to be implicated in maintaining a continuous activation of mast cells as part of the pathomechanism by which SEs affect mucosal inflammation (14). Another important trigger protein derived from S. aureus is S. aureus-derived protein A, which induces a significant increase in histamine, leukotriene, and prostaglandin D2 levels, indicating mast cell activation (13).

Fungi have also frequently been hypothesised to be involved as disease generators/modulators in

patients with nasal polyps. Fungi have been shown to be present in the nasal passages and sinuses of nearly all patients with CRS, although some studies reported no significant difference in fungal isolation in comparison with healthy controls (15). Okano et al. recently showed that fungal antigens from Aspergillus, Alternaria, and Candida species are less capable of inducing eosinophilia-associated cellular responses in nasal polyps, but no significant correlations with the amount of pro-phlogogenic factors such as IL-5 or IL-13 were found. At the same time, no correlations were detected between the presence of fungi and nasal polyps, peripheral blood eosinophilia, or the radiologic severity of sinusitis (16).

The most important role of bacterial biofilms and the presence of fungi is in inducing the epithelial damage observed in patients with CRSwNP, together with genetic deficiencies or epithelial repair mechanisms, or both. Alterations in mucosal macrophages located at the epithelial barrier on the interface with the external environment might contribute to the immune barrier dysfunction in patients with CRSwNP. Moreover, the increased presence of S. aureus in patients with CRSwNP may be partly explained by an inefficiency of the phagocytic system in the sinomucosal tissue of these patients (17).

It is likely that bacterially-induced damage to epithelia that results in the exposure of the basal stratus can trigger an autoimmune process. In fact, the presence of IL-5 and of IgE antibodies to S. aureus enterotoxin specific IgE (SE-IgE positivity) in human nasal polyps was shown to be associated with an increased risk of asthma comorbidity, indicating the decisive role of Staphylococcal superantigens in amplifying and aggravating airway disease. The rate of asthma comorbidity was approximately 6-fold higher when IgE antibodies to SEs were present in mucosal tissue of patients with CRSwNP (7). Human papilloma virus and human herpes virus types 1-7 have also been suggested as possible infections causing CRS, but a role for these agents in the disease has not been directly demonstrated (18).

Immunologic theory

The results of at least one recent study indicate an autoimmune pathogenesis for CRSsNP. Bruce

et al. (19) hypothesised that the local mucosal inflammatory microenvironment and chronic inflammation associated with CRSwNP are caused by the expansion of autoreactive B-cell clones that might play a role in perpetuating inflammation. In this study, the authors examined nasal polyps and other nasal tissue (uncinate process and inferior turbinate) for the presence of class-switched autoantibodies using an ELISA approach. The results of the study demonstrated the presence in nasal polyp tissue of measurable IgG and IgA autoantibody levels (more positive results for IgG autoantibodies than for IgA autoantibodies) against many of the antigens tested.

Kato et al. observed that a large number of the autoantibodies found were reactive against nuclear antigens (anti-dsDNA). Autoantibodies of this type play a central pathogenic role in many autoimmune diseases such as systemic lupus erythematosus (SLE). The study showed there was no correlation between anti-dsDNA autoantibody levels and total IgG levels; furthermore, the increased levels of autoantibodies were confined to nasal polyp tissue extract and were not observed in other nasal extracts or in the inferior turbinates of patients with CRSwNP (20).

Increased levels of IgG anti-dsDNA autoantibodies were found more frequently in patients with a more aggressive clinical course requiring revision surgery. However, none of the patients who underwent revision surgery for CRSsNP had increased levels of anti-dsDNA IgG antibodies. Highly increased levels of autoantibodies were found locally in nasal polyps, but the levels of autoantibodies were not high in other nasal anatomical sites such the uncinate process and the inferior turbinate. In particular, the uncinate process tissue of patients with CRSsNP, which is frequently involved in chronic inflammation, showed no increase in anti-dsDNA antibody levels. This suggests that chronic inflammation alone does not increase autoantibody levels (19).

The use of multiple revision surgeries was suspected of playing a role in inducing autoantibody levels in patients with CRSsNP, indicating that multiple surgeries themselves may be the trigger of this response.

It is likely that memory B cells reactive to self-antigens might persist after surgical removal of the inflamed tissue and potentially play a role in

triggering recurrent inflammation. It can be stated that multiple revision surgeries are not a risk factor for autoimmune process; in fact, in patients who underwent revision surgery for CRSsNP, increased levels of anti-dsDNA IgG antibodies have never been demonstrated (19).

Recent evidence strongly suggests a potentially important pathogenic role for B lymphocytes and levels of the B-cell activating factor (BAFF) and IL-6 in nasal polyp tissue, two important cytokines associated with autoimmunity (21). B-cell activating factor (also known as BLyS, TNFSF13B, TALL-1, and THANK) with a proliferation-inducing ligand called APRIL, are recently identified members of the TNF superfamily that play important roles in B-cell generation, survival, proliferation, and maturation (22, 23). BAFF are highly increased in nasal polyp tissue from patients with CRSwNP in comparison with patients with CRSsNP or healthy subjects.

Some authors have also found that nasal polyps contain increased levels of the cytokine IL-6 and chemokines such as B-lymphocyte chemoattractant (CXCL13) and stromal cell-derived factor 1α, which are known to play a role in B-cell recruitment and plasma cell differentiation (20)

Several studies have reported that BAFF is expressed by myeloid cells and especially from T cells (24). BAFF expression in T cells has been suggested to be elevated in autoimmune diseases such as SLE. Although normal B cells do not usually make BAFF, it has been reported in other autoimmune diseases such as malignant B cells and salivary gland B cells in Sjogren syndrome (21, 25).

Kato et al. suggested the possibility that synthesis of BAFF is made by infiltrating eosinophils in the submucosa of nasal polyps. Data from immunohistochemical data show that some eosinophil-like cells from patients with CRSwNP were stained for BAFF (26).

Antinuclear antibodies and BAFF expression in T cells, a polyclonal class-switched autoantibody response against nuclear components and some epithelial antigens, are found in nasal polyps, suggesting that immune mechanisms are involved (19, 21).

Therapeutic considerations

B cells are increasingly recognised as important therapeutic targets in the treatment of many autoimmune diseases. The importance of B cells in linking innate and adaptive immunity and their contribution to long-term immune memory are evident, based on the success of rituximab in treating multiple autoimmune diseases. The use of rituximab in autoimmune diseases with an antibody-mediated aetiology has a strong rationale and is increasingly reported in the literature. Rituximab is a chimeric human–mouse monoclonal antibody against the lymphocyte CD20 surface antigen, and treatment with this agent induces depletion of B lymphocytes by various mechanisms. Clinical evidence with rituximab therapy in patients with autoimmune disease demonstrates that levels of autoantibodies, (e.g., anti-dsDNA), decrease more rapidly than total immunoglobulin levels after therapy (24).

Belimumab, an anti-BAFF agent, was recently approved as a novel treatment for autoimmune diseases. Belimumab demonstrates a selective target on BAFF and has clinical activity in reducing anti-dsDNA antibody levels secondary to depletion of both naïve and transitional B cells without affecting traditional memory B cells (19).

In patients with CRSwNP and severe asthma, omalizumab, a monoclonal humanised anti-IgE antibody, has been used with good results. Omalizumab is an anti-IgE antibody that has demonstrated clinical efficacy in the treatment of nasal polyposis with comorbid asthma. It is only indicated for patients with an allergen-induced disease and had beneficial effect on upper and lower airway symptoms and on the asthma related quality-of-life score (25).

There are also concerns that the therapeutic use of anti-TNF-alpha agents or other immunosuppressive drugs, often used in course of treatment for other comorbidity, can have good clinical results but the effects were not studied in the long term and may be associated with the development of cancer and lymphomas.

There are no data showing a real efficacy in the prevention of polyp recurrence, in the use of oral or local corticosteroids in patients with CRSsNP, especially in those patients with a history of multiple surgeries. In these patients, the use of local or oral steroids contribute to improvements in maxillary ostial clearance, mucociliary clearance, airway

peak nasal inspiratory flow (27), and effects on T cells, eosinophils, and messenger RNA for IL-4 and IL-5 (28). Side effects of topical corticosteroids in nasal polyps are rare. Minor nose bleeding has been reported in some subjects and is attributed to the vasoconstrictor activity of corticosteroids. Septal perforation is extremely rare (29). The systemic bioavailability of intranasal steroids is variable, and depending on the steroid, potential side effects include effects on growth, ocular effects, and effects on bone and on the hypothalamic-pituitary-adrenal axis. Thus, steroids such as mometasone furoate and fluticasone furoate, which also have systemic bioavailability, would be considered safer, particularly if higher doses are used than the doses of intranasal steroids used for treating uncomplicated allergic rhinitis (30).

Platelet activating factor (PAF) inhibitors as rupatadine, have been considered a good therapy because rupatadine (10 mg once daily) can inhibit the stimulatory effects of PAF on mast cells and provides additional protection against inflammation cascade with good results (31).

Although we have much to learn about the anti-dsDNA and producing B cells in patients with CRSwNP, their presence within the most therapeutically refractory patients provides new potential avenues for targeted therapy in patients with this disease. We recommend a four-week division of the overall drug-dosing cycle, as it appears to be particularly safe, even though we do not recommend the use of this drug as a first-line therapy.

The positive effects of immunosuppressive drugs such as methotrexate and cyclophosphamide have been occasionally observed in patients undergoing pharmacological treatment for other autoimmune diseases, but to date there have been no clinical trials or publications in merit.

CONCLUSIONS

Chronic rhinosinusitis with nasal polyps is a significant health problem. The causation of the disease is multifactorial, and no single factor can explain the pathogenesis of polyps; however, published data on autoimmunity in CRSwNP are limited.

The autoimmune aetiology of CRSwNP of unknown origin or failure to respond to classic pharmacological treatments with nasal and oral steroids is now suspected. Experimental models and some studies suggest that autoimmunity results from the initial activation of naïve T cells through microbial molecular mimicry or superantigens and is subsequently amplified by the enhanced processing and presentation of autoantigens. B cells are likely to play an important role in this process. The importance of B cells in linking innate and adaptive immunity and their contribution to long-term immune memory are evident based on the success of rituximab in treating multiple autoimmune diseases. Clinical experience with rituximab therapy in patients with autoimmune disease demonstrates that levels of autoantibodies, such as anti-dsDNA, decrease after therapy.

At present, the nature of the antigen trigger, the exact role played by B/T cells and anti-dsDNA autoantibodies in the pathogenesis of nasal polyposis remain unclear. Corticosteroids and surgery are the first line of treatment in CRSwNP. Immunosuppressive drugs such as methotrexate, and cyclophosphamide have also been used with varying degrees of success. However, in the absence of controlled trials, no definitive therapeutic recommendations are currently available. Although we have much to learn about the role of anti-dsDNA autoantibodies in patients with CRSwNP, their presence in the most therapeutically refractory patients provides a new potential avenue for targeted therapy in patients with this disease.

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