Eosinophils

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Key Points

- Eosinophilia is an indicator of severe CRS with a high chance of recurrence.
- Caucasian nasal polyps are mostly associated with eosinophilia, but the rate of eosinophilic nasal polyp is increasing in Asia.
- Eosinophils use different mechanisms to attack and kill bacteria in CRS, but can also have immunomodulatory functions.
- Glucocorticoids and novel biologics directly target eosinophils in a very effective way.

8.1 Clinical Manifestations and Diagnosis of Eosinophilic CRS

Eosinophilic CRS patients represent a subtype of CRS that is typically characterized by symptoms such as loss of smell, thick mucus production, secondary bacterial infections, long-term nasal congestion, and a poor treatment response [1, 2]. From clinical point of view CRS is nowadays subdivided by CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). In the Caucasian population, a minority of the CRSsNP patients has tissue eosinophilia, but the majority of the CRSwNP patients (about 80%) are characterized by tissue eosinophilia. This is substantially higher than the percentage of CRSwNP patients in Asian populations, where percentages with tissue eosinophilia range from 20 to 60%. However, the percentage of type 2 signature disease in patients with CRS is dramatically increased over the last 20 years, implying an ongoing "eosinophilic shift" in several Asian countries. To determine the role of the different environmental and/or lifestyle factors in the observed eosinophilic shift will require additional research [3, 4].

While Chinese patients have in general a more moderated degree of eosinophilia than Caucasian patients, higher recurrence rates and appearance of comorbid asthma is associated with eosinophilia in both populations. Other studies have linked eosinophilia with more extensive sinus disease and higher post-operative symptom scores [5, 6]. In addition, tissue eosinophil counts are found directly associated with loss of olfactory function in CRSwNP, independent of disease severity [7]. In general, the manifestation of eosinophils is troublesome for the course of the disease with multiple studies indicating it as a risk factor for disease recurrence hampering the improvement in both general and disease-specific quality of life of the patient [5–9]. These features imply that eosinophils are either biomarkers of the disease or the key responsible in driving the disease.

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Some studies have reported that blood eosinophilia is correlated with their infiltration in the polyps and the severity of paranasal cavity computed tomography (CT) findings of CRSwNP patients [6, 8–11]. These studies suggest that blood eosinophil counts or determination of eosinophilic specific markers (like ECP concentrations) in the blood could be a diagnostic maker for eosinophilic CRSwNP. However, it is important to realize that this approach rather indicates an ongoing type 2-driven disease, such as asthma and allergy, and might therefore not unambiguously identify eosinophilic CRS. This is in contrast to the identification of tissue eosinophilia which can be diagnosed by histopathology (Fig. 8.1) or via quantification of eosinophilic proteins like eosinophilic cationic protein (ECP) or Major Basic Protein (MBP) in the tissue. However, there is a lack of clear guidelines and cut-off values to discriminate between eosinophilic and non-eosinophilic CRSwNP. For diagnostic purposes and the introduction of precision medicine in CRS, there is a clear need for validated and continent/country- specific eosinophil related biomarkers in the future [12].

Several environmental stimuli have been proposed to play a role in the pathophysiology and the recruitment of eosinophils in CRS. Eosinophils have been recognized as a central feature in the response to infection with large and multicellular parasites like helminths.

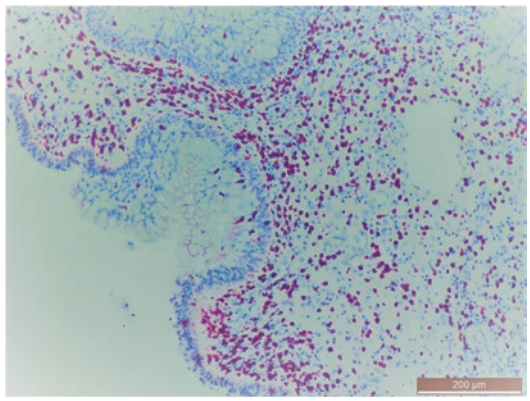

Fig. 8.1 Immunohistochemistry stain for MBP (pink) and DNA (purple) in nasal polyps

Along these lines, the fungal hypothesis, implying a prominent role for fungi or at least fungal allergens at the basis of eosinophilic CRS pathology, was proposed. Some groups observed the intranasal presence of fungi along with eosinophil and eosinophil-degraded products and mucus [13, 14]. Further evidence in favor of this hypothesis was provided by experiments showing that nasal mucus or tissue from patients could trigger eosinophil migration, that blood mononuclear cells of CRS patients responded to fungal antigens with increased IL-5 and IL13-production in vitro, and the observation that *Alternaria* fungi are able induce eosinophil degranulation via activation of the protease-activated receptor (PAR) [15–17]. However, others reported the absence of hyper-responsiveness to fungal antigens in CRS and topical antifungal treatment failed to show any efficacy in a clinical trial setting [18–21]. Despite the fact that the fungal hypothesis is potentially valid in some specific patients, it is rather controversial and doubtful that it is a the base of eosinophilic CRS pathology.

Another hypothesis points to *S. aureus* and its produced toxins. Colonization of the nasal mucosa with *S. aureus* is far more prominent in CRSwNP patients than in healthy controls with reported frequencies up to 90% of the patients. Staphylococcal super antigens can directly drive a type2 inflammatory response with eosinophilic inflammation as a consequence [22–24]. In addition, it was shown that exposure of nasal epithelium to *S. aureus* can induce eosinophil migration and that *S. aureus* can activate specific defense mechanisms in eosinophils as discussed below [25]. Staphylococcal super antigens can also act as allergens, demonstrated by the finding of functional IgE antibodies directed against *S. aureus* antigens in the nasal polyp tissue tissue [26, 27]. Bacterial infections are prominent in eosinophilic CRS patients, and it is clear that other factors like (innate/temporary) defects in the immune barriers, possibly cause by eosinophils, could further contribute to the pathophysiology as it could make patients more susceptible to infection in general.

8.2 Eosinophil Development, Chemotaxis, and Activation in CRS

In 1879, Paul Ehrlich was the first to describe the existence of eosinophils as "cells with granules having an affinity for eosin and other acid dyes." Eosinophil-like cells are found in all vertebrates and are thus highly evolutionary conserved. For this reason, eosinophils must be more than troublemakers and are likely to play crucial, and possibly yet unidentified role in important processes. They originate from CD34+ hematopoietic stem cells in the bone marrow and develop from a common myeloid progenitor to an eosinophil lineage-committed progenitor. The latter exclusively gives rise to eosinophils and IL-3, IL-5 and Granulocyte Macrophage colony-stimulating factor (GM-CSF) are particularly important in regulating eosinophil development, differentiation, and maturation [28–30]. However, the crucial factors seems to be IL-5, a it was found necessary and sufficient for the development of eosinophilia [31]. Other cytokines including IL-3 and GM-CSF synergize with IL-5 in this process [32–34]. In humans, IL-5 receptor expression is unique to eosinophils and basophils, which enables IL5 to work very specifically on those cells to promote maturation, activation, and survival [35, 36]. Once eosinophils have entered the blood, also mediated by IL-5, they have a short half-life, ranging from 8 to 18 h [35].

After circulating in the blood, eosinophils migrate into the nasal mucosa, which is a process mediated by the synergistic influence of cellular adhesion and chemotaxis. Eosinophil adhesion to the endothelium in a type 2 inflammatory context is mediated by VCAM1 and P-selectin in polyp tissue [37, 38]. The type 2 cytokines IL-4 and IL-13 seem of crucial importance for induction of these proteins [39]. Proof for the role of other adhesion molecules in the specific recruitment of eosinophils like L-selectin, MadCAM1, and I-CAM in CRS is rather implicit [40, 41].

The chemotaxis of eosinophils into the tissue is mainly mediated by ligands of the C-C chemokine receptor 3 (CCR3). The importance of this receptor was demonstrated by a study showing that polyp tissue fluid exhibited strong chemotactic activity for eosinophils that was significantly inhibited by blocking CCR3 [42]. While many endogenous ligands are identified for this receptor, eotaxin 1–3, RANTES, monocyte-chemotactic protein (MCP) 1–4 are of particular interest for directing eosinophil chemotaxis. In nasal polyps, eotaxin 1–2, MCP-1, MCP-4, and RANTES levels were found significantly increased [42–46]. A crucial role in guiding eosinophil chemotaxis is attributed to the epithelium, as it is the main source of many of these factors. This role of the epithelium in the chemotaxis is further illustrated by the subepithelial localization of eosinophil often observed in polyps [25, 47]. A link between *S. aureus* colonization and eosinophil accumulation has been proposed. Indeed, *S. aureus* and its super antigen SEB can induce eosinophil migration by inducing eotaxin 1–3 expression. In addition SEB can also induce RANTES and MCP-1 in epithelial cells [42, 48]. However, in nasal polyps it appears that eotaxin, rather than RANTES, in cooperation with IL-5, plays a key role in chemoattraction and activation of eosinophils in NP tissue [49].

Many other factors like complement factors C5a and C3a, platelet-activating factor (PAF), eicosanoids like the CysLTs, SCF, and IL33 might contribute to the chemotaxis, priming, and activation of eosinophils in the nasal polyps [43, 50]. In addition, to their role in eosinophil development, IL-5 and GM-CSF also play a crucial role in eosinophil priming, maturation, and increasing their survival in the tissue. As a consequence, the life span of eosinophils is extended and ranges from 2 to 5 days once migrated in the tissue [51]. *S. aureus* also contributes to the prolonged survival as supernatant of SEB treated epithelial cells was shown to increase eosinophil survival in vitro [44]. Another factor contributing to increased eosinophil survival could be an impairment of NK cell-mediated eosinophil apoptosis in chronic rhinosinusitis likely attributed to deregulated prostaglandin D2 production [52].

It was hypothesized that initial eosinophil recruitment occurs in response to the release of one or more small molecule mediators of inflam-

mation (e.g. DAMPs) due to localized bursts of cell death. The tissue immune microenvironment would subsequently determine the downstream immune consequences mediated by eosinophil effector functions. As a consequence, this would lead to exacerbations of local immune responses (Type 2 -Polarized Microenvironment), suppression of these site-specific immune responses (Type 1/Type 17-Polarized Microenvironment), or essentially little to no modulations of local immune responses (Immune-Neutral Microenvironment). As a consequence, the immune microenvironment present upon eosinophil recruitment would be key as to direct the predominance of specific eosinophil activities and would define the final functional roles of eosinophils. From this perspective the released mediators, action and consequences of eosinophils would be highly dependent on the environment [53]. Additionally the existence of innate eosinophil heterogeneity and tissue resident, immune-regulatory eosinophils have been proposed but more research is required in humans, and to date it is unclear as to what extend this is relevant in CRS [54].

8.3 Functions of Eosinophils in CRS

8.3.1 Effector Functions

A key role for eosinophils in the development of nasal polyps has been proposed by a study on early-phase polyps showing subepithelial presence of eosinophils at the upper surface of the early polyp outgrowth. The polyp formation was associated with the deposition of fibronectin, albumin (pointing to a vascular leak), and extracellular matrix protein and the process was found to correlate to IL-5 and eotaxin-2 tissue levels. This study pointed to a possible central role for eosinophils in polyp formation in the very early stages [51]. Apart from their possible key role in polyp development, eosinophils are also known to play multiple roles in the chronic and established polyps. As described in the previous part, eosinophils accumulate rapidly in polyps where they are primed, activated, synthesize and release lipid mediators, enzymes and proteins that can exert a wide variety of actions.

Eosinophil are characterized by the bilobed nucleus and the cytoplasmic storage of granules (Fig. 8.2). These granules store and secrete cationic proteins and an array of cytokines and chemokines. Eosinophilic granules are not simply storage depots of preformed proteins. It is well-established that differential release of cytokines occurs in response to specific stimuli. Eosinophils can secrete mediators via de novo synthesis following the classical pathway of secretion or can secrete their preformed granule content by exocytosis (or "degranulation"), piecemeal degranulation or cytolysis. In nasal polyps, it was found that 30.7% of the eosinophils seems inactive, 27.5% of the eosinophils undergo cytolysis, and 41.7% of the eosinophils undergo piecemeal degranulation [55, 56].

The most reported role of eosinophils in polyps is associated with the degranulation and release of the highly basic and cytotoxic granule proteins such as ECP, MBP, and eosinophil-derived neurotoxin (EDN) that are released during degranulation or cytolysis. While they play an

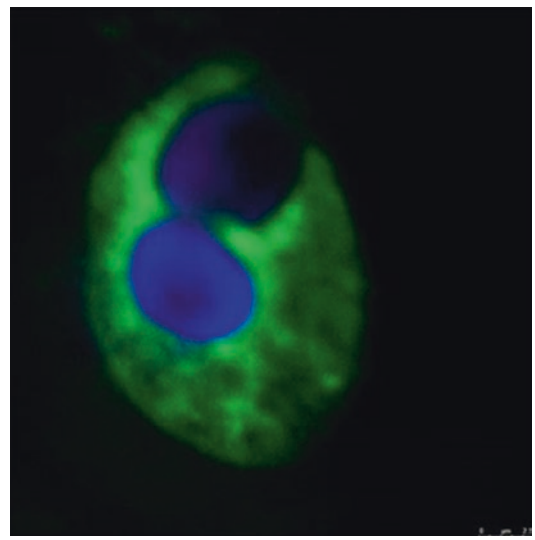

Fig. 8.2 Eosinophil in CRSwNP. Immunofluorescent stain for MBP (green) and DAPI (blue) shows the characteristic bilobed nucleus and granular cytoplasmatic structures

important role in innate immune defense and pathogen elimination, they can be very harmful to the host when they are excessively released. In mucus and CRSwNP tissue the deposition of MBP is described and found associated with epithelial damage [51, 57]. Major basic protein is toxic and causes erosion of the epithelium at concentrations less than 10 μg/ml. Because concentrations of MBP are merely exceeding this concentration in the mucus, it was suggested that epithelial damage might arise from the mucus, rather than the tissue [51]. ECP is a cytotoxic ribonuclease and often used as a marker of eosinophil activity and to monitor disease progression. Interestingly, ECP is dependent on its RNAse activity to exert neurotoxic and antiviral actions, while their antibacterial and antihelminthic effects are independent of this activity. Another RNAse and powerful neurotoxin is EDN. This protein can promote an allergic reaction via dendritic cell activation and it may play an important role in allergic disease [58]. One study showed that EDN enhances airway remodeling in chronic rhinosinusitis and correlates with disease severity [59]. However, only few studies have shown a clear association between EDN and CRSwNP. Beside their well-known antibacterial properties, additional functionalities have been attributed to these eosinophilic proteins. For example, eosinophilic granule proteins such as ECP and EDN have been shown to suppress T cell proliferation in vitro [60]. The toxicity of MBP was shown to be regulated by crystallization and to stimulate histamine and leukotriene C4 release from basophils and to activate mast cells [61]. How and if these mechanism are also important in the pathophysiology of CRS is unclear.

Eosinophils are also an important source of lipid mediators. Nasal polyp tissue-derived eosinophils were shown to possess a specific phenotype with a dysregulated fatty acid metabolism [62]. In addition eicosanoid metabolism is found increased and correlated with ECP and IL5 in CRS [63]. In CRS, eosinophils are an important source of 5-LO and LTC4 synthase. Especially in patients with aspirin hypersensitivity this could play a role where the 5-lipoxygenase pathway is found activated [64].

8.3.2 Extracellular Trap Formation and Charcot–Leyden Crystal Deposition

In addition to degranulation, eosinophils contribute to antibacterial defense by the formation of the so-called eosinophilic extracellular traps (EETs). These extracellular traps can be generated by both viable eosinophils and by eosinophils undergoing extracellular formation associated cell death (EETosis) [65, 66]. While both viable EET formation and EETosis are regulated via different pathways, they are both dependent on NADPH activity and ROS production. In vitro, EET formation is evoked by a sequence of stimuli like adhesion molecules, IL-5, and interferon (IFN)-ɣ, complement factor 5a (C5a), LPS, TSLP, and eotaxin [65, 67]. While the exact pathways of EET formation are unknown, it is clear that EETs can bind and kill bacteria like *S. aureus*, *S. epidermidis*, and *E. coli*. In vitro, eosinophils generate EETs immediately after co-culture with *S. aureus* and without additional stimuli, while EET formation evoked with S. epidermidis required priming with TSLP [27, 67]. It seems that a different additional stimulus (like IL5, C5a, TSLP) is needed to cause EET formation, depending on the type of bacteria [15, 59]. Caucasian CRSwNP patients have elevated, IL-5, eotaxin, IL-33, and TSLP levels; and a consistent colonization with *S. aureus*. Interestingly, these are all possible triggers for EET formation.

A study in Caucasian CRSwNP patients showed that eosinophils are specifically recruited to sites of epithelial damage and form EETs to protect the host from infections with *S. aureus* and possibly other microorganisms [25]. Another study reported EETs in secretions of eosinophilic CRS patients contributing to the increased viscosity of the secretions [68]. Chinese patients with CRSwNP of the type 2 endotype (IL5+ polyps) displayed similar patterns with subepithelial recruitment of eosinophils and EET formation. In addition, EETs correlated positively with the presence of S aureus, but not with Pseudomonas aeruginosa, pan-fungi or Escherichia coli colonization pointing to a prominent role of *S. aureus*

[4]. These results show that the same mechanisms play a role in patients with CRSwNP with a dominant TH2 profile. Later, EETs were found associated with disease severity regardless of polyp status in Asian patients [64].

Beside their role in antibacterial defense, EETs can contribute to the properties of highly viscous eosinophilic mucin and impair its clearance in CRS patients. In addition, EETs can contain intact granules. These granules can cause long-lasting inflammation but could also have immune-regulatory roles [69]. Recently, the process of EETosis was linked to the formation of Charcot–Leyden crystals (CLCs) [70, 71]. CLCs are composed of galectin-10, a major autocrystallizing granule protein of human eosinophils. CLCs were abundantly found in CRSwNP patient mucosa and mucus and have also been found frequently in the tenacious eosinophil-rich mucus of allergic fungal sinusitis patients [72]. In vivo, crystallization of endogenous proteins is often associated with pathological conditions that trigger an inflammatory response. In nasal polyp tissue, it was shown that CLCs, as a result of EETosis, cause a pro-inflammatory response, a secondary neutrophilic inflammation and NETosis [73]. Via various ways (e.g. intact granules or CLCs), eosinophils may thus possess ways to have post-postmortem impacts on innate immunity, local immune response, sterile inflammation, and tissue damage. An overview of the most important mechanisms and effector functions is depicted in Fig. 8.3.

8.3.3 Other Roles of Eosinophils

In addition to their granule proteins, eosinophils produce a remarkable number of pro-inflammatory cytokines and chemokines. The mediators can have pro-inflammatory (e.g. TNFα), anti-inflammatory (e.g. IL-10), tissue remodeling (e.g. TGFβ) or immunomodulatory effects (e.g. IL-4). In addition, they might damage epithelial cells, stimulate epithelial-to-mesenchymal transition, activate or suppress sensory nerves, modulate the activity of stem cells and plasma cells, and alter the mechanical response of airways [67, 68].

Eosinophilic indoleamine 2, 3 dioxygenase (IFNϒ inducible enzyme) was shown to act on the production of kynurenines (KYN) which is reported to induce apoptosis and inhibition of proliferation mainly of Type 1 cells, actively causing a Type 2 bias [74]. Eosinophils can also sustain their own survival and recruitment via the autocrine production of IL5, eotaxin, and GM-CSF. In addition, eosinophils were found to serve as antigen presenting cells in allergic upper airway disease and to express MHCII, costimulatory molecules, and to traffic to regional lymph nodes [75]. However, it is unknown as to what extend these effects are relevant in CRS patients. As previously described, the exact role of the eosinophil and the mediators released are likely dependent on the microenvironment and the specific context of the CRS endotype.

8.4 Treatment Considerations

Eosinophils are implicated in the pathogeneses of a large fraction of the CRSwNP patients. For these patients, the induction of their apoptosis and efficient clearance is crucial in the resolution of inflammation. Treatment with doxycycline can significantly reduce the polyp size and the level ECP in nasal secretions of CRSwNP patients [76]. In contrast to neutrophils, eosinophils are also an important target of glucocorticoids. Glucocorticoids can decrease eosinophilia in multiple ways. For example, they interfere with the recruitment by inhibiting expression for VCAM1, eotaxin, eotaxin-2, and MCP-4 [42]. Further, glucocorticoids can interfere with eosinophil adhesion, chemotaxis, activation and induce apoptosis [77].

Despite the multiple effects of steroid on eosinophils, current FDA approved treatment of intranasal steroids does not provide significant relief for many patients. For these patients monoclonal antibodies bring hope for an exciting new treatment option. In CRSwNP IL-5 is a key cytokine with a possible autocrine role for this cytokine in the activation of eosinophils, and a strong correlation with eosinophilic cationic protein (ECP). The key role of IL-5 was supported by the finding that treatment of eosin-

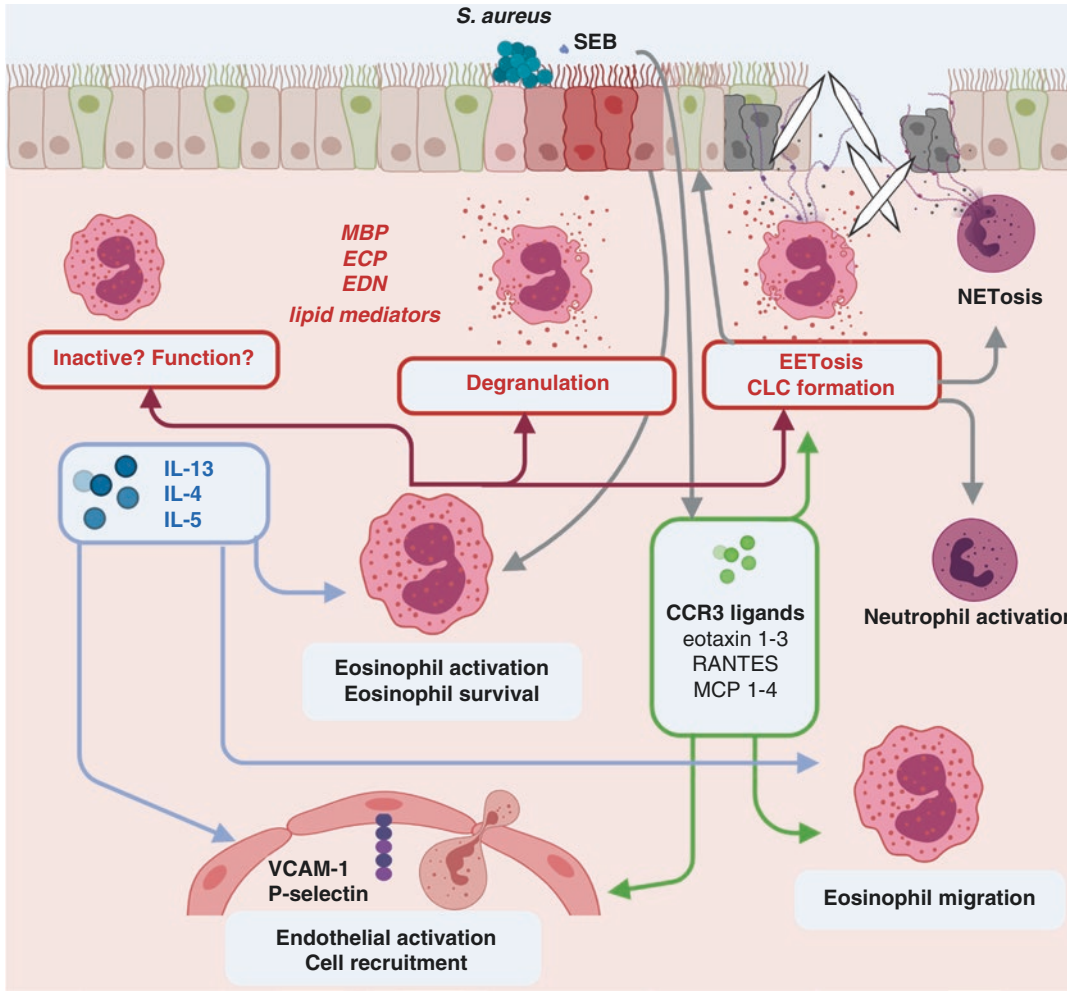

Fig. 8.3 Eosinophil chemotaxis, activation and effector functions in CRS

ophil-infiltrated polyp tissue with neutralizing anti-IL-5 monoclonal antibody, but not anti-IL-3 or anti-GM-CSF antibodies in vitro, resulted in eosinophil apoptosis and decreased tissue eosinophilia [49]. The monoclonal antibodies targetting IL5 signaling including reslizumab and mepolizumab (both anti IL-5), and benralizumab (anti IL-5Rα) directly target IL-5 in the pathophysiology of nasal polyposis (NP). Antibodies against IL5 or IL4/IL13 recepter alpha chain were shown to reduce eosinophils and shrink polyps, supporting a role of eosinophils in the pathogenesis [75, 76]. These drugs also restore olfactory function supporting the hypothesis that eosinophils mediate anosmia. Recently the FDA approved Dupilumab for the treatment of CRSwNP. Blocking IL4 and IL13 simultaneously affects a broad range of type 2 effector cells and affect eosinophil recruitment, chemotaxis and activation far upstream. These effects are likely to account for their great success.

8.5 Translation into Future Daily Practice

Eosinophilia is a key factor in Caucasian CRSwNP patients, but to date, it is not entirely clear if they are the main cause or rather a marker of the disease. While many reports point to a key role of these cells in the pathogenesis, targeting solely eosinophils seems less effective that

targeting key type 2 modulating cytokines. Taking into account their post-mortem effects, but also their possible anti-inflammatory and immune-modulatory role it is a possibility that targeting eosinophils is not always beneficial. The microenvironment is likely key for the effector functions of eosinophils and gaining more insight by endotyping patients is therefore crucial to determine if and how eosinophils should be targeted and to allocate the right patient to the right treatment.

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