

Allergic and idiopathic rhinitis: an ultrastructural study

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Abstract Nasal hyperreactivity is one of the most important underlying mechanisms in both allergic (AR) and idiopathic rhinitis (IR). In order to study the pathomorphological changes in this entity, tissue samples from patients with AR, IR, and from patients without chronic inflammation were taken during nasal surgery. Primary antibodies against Substance P (SP), calcitonin gene-related peptide (CGRP), and endothelial nitric oxide synthases (NOS III) were applied and the immunocomplexes were visualized by immunocytochemistry. The nasal mucosa of patients with AR and IR showed similarities on the ultrastructural level. Neurogenic inflammation was indicated by a strong innervation pattern with sensory nerve fibers containing SP and CGRP. We could show that extensive edema and cellular infiltration might be characteristic for AR. On other hand there was no evidence of eosinophilic or NO involvement

in IR. Finally, on the ultrastructural level, AR and IR showed many similarities. Based on these findings anti-inflammatory therapy modalities could be recommended for both types of rhinitis.

Keywords Allergic rhinitis · Nasal mucosa · Idiopathic rhinitis · Electron microscopy · Immunoelectron microscopy

Introduction

While the pathophysiology of allergic rhinitis (AR) has largely been solved, many aspects of pathogenesis and terminology of idiopathic rhinitis (IR) remain still unclear. However, nasal hyperreactivity is a cause of both diseases [1, 2]. In the United States, 32 million patients are affected by AR, while 19 million patients show symptoms of IR. In addition, 26 million patients have a mixed rhinitis which is caused by both specific and unspecific hyperreactivity [3–6].

With a prevalence of 15–25%, AR is the most common allergic disease in adults in Germany [7, 8]. According to the WHO ARIA criteria, it is differentiated between an intermittent and a persistent type of AR [1, 4]. AR is caused by an IgE-mediated specific hyperreactivity of the nasal mucosa [2].

As a consequence, patients suffer from symptoms like watery rhinorrhea, nasal obstruction as well as itching and sneezing that altogether considerably impair the quality of life [1, 7].

In addition to Th2-type cytokine interactions neurogenic inflammatory mechanisms have been described [4, 9]. So, neuropeptides like CGRP and SP can act as mediators in sensory nerve fibers and axon reflex glandular secretion,

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inflammatory cell chemotaxis, and vascular permeability. As a result of that, neurogenous edema may occur. Symptoms of nasal hyperreactivity are triggered by stimulation of sensory neuropeptides containing neurons.

IR belongs to noninfectious, nonallergic rhinitis (NINAR), formerly known as vasomotor rhinitis, which is caused by an unspecific hypersensitivity of the nasal mucosa with symptoms identical to those of AR [1, 2, 4, 7, 10]. The diagnosis of IR can be made by excluding allergies, infections, structural disorders, and chronic rhinosinusitis or cases of unclear etiology [4]. The affected patients either have considerable nasal obstruction (“blockers”) or hypersecretion (“runners”) [4]. IR is characterized by nasal hypersensitivity to unspecific stimuli that are not based on immunological mechanisms [1, 4].

Studies on the underlying morphological changes in both types of rhinitis are rare [2, 11]. Giannessi et al. merely performed ultrastructural analyses of the epithelium in “vasomotor rhinitis” [12]. Moreover, studies are difficult to compare due to the non-uniform definitions of IR [13, 14]. According to the recently published GA²LEN paper from Bousquet et al. [2] further studies should be performed to find common as well as different characteristics in nasal biopsies of patients with IR and AR.

The aim of this study therefore was to determine pathomorphological changes in the nasal mucosa and to compare the ultrastructural and immunoelectron microscopic findings in patients with AR and IR in correlation to a control group.

Materials and methods

Patients

From May 2004 to June 2006 a total of 51 biopsies (36 male, 15 female patients; age ranging from 20 to 63 years; mean age: 35.3 years) were taken 10 mm behind the head of the lower nasal concha during endonasal interventions (septo- or septorhinoplasty with partial turbinectomy) due to chronically obstructed nasal breathing.

Inclusion criteria

All patients were examined preoperatively by nasal endoscopy, skin tests for a standard battery of common allergens and a radioallergosorbent test (RAST) for total and specific IgE.

AR

A total of 20 patients (15 men, 5 women; age ranging from 22 to 55 years; mean age: 32.4 years) with persistent AR

had a positive history for the typical year-round symptoms of moderate severity. In addition to rhinoscopy, AR was confirmed by positive prick tests, provocation testing and detection of allergen-specific IgE (RAST).

IR

In 16 patients (nine men, seven women; age ranging from 25 to 61 years; mean age: 35.1) with at least an 1-year history of rhinitis without a detectable cause, specific hyperreactivity was excluded by prick tests and IgE determination (RAST) and an eosinophilic inflammation by nasal cytology.

Controls

Biopsies from inferior turbinates of 15 patients were taken during septorhinoplasty with partial turbinoplasty as control. These control group consisted of twelve males and three females without chronic inflammations of nasal mucosa; age ranging from 20 to 63 years; mean age was 34.1 years. Allergy in patients of the control group was excluded by negative prick tests as well as negative detection of allergen-specific IgE (RAST).

Exclusion criteria

Patients with topic or systemic glucocorticoid therapy, a specific immunotherapy, oral or topic application of antihistamines in the month prior to surgery, smokers and patients with acute and chronic forms of rhinosinusitis were excluded.

Methods

The tissue samples were fixed in 3.5% phosphate-buffered glutaraldehyde (according to Schultz–Karlsson [15]), 4% paraformaldehyde or a paraformaldehyde–glutaraldehyde mixture. In all biopsies, a preliminary histological examination of the entire mucosa was performed by hematoxylin–eosin staining.

Electron microscopy

The samples were rinsed with phosphate buffer, fixed in 1% osmium acid, and rinsed again before dehydration in an ascending acetone series. Following a saturation phase with Durcupan, the samples were heat-polymerized and hardened. The samples were subsequently cut into semi-thin slices, stained with toluidine blue, and examined under light microscopy to select suitable regions for electron microscopy.

After double contrasting with uranyl acetate and lead citrate, the ultrathin sections (70 nm; Reichert–Jung Ultracut

E, Vienna, Austria) were subjected to electron microscopic evaluation (EM 902 A Zeiss, Jena, Germany) and photo-documentation.

Immunoelectron microscopy

The samples were embedded and polymerized for immunoelectron microscopy after dehydration in Unicryl. They were subsequently incubated with the primary antibodies anti-Substance P (Affiniti, Exeter, UK) and anti-CGRP (Chemicon, Temecula, USA) in a dilution of 1:500–1:1,000 under refrigeration overnight. To detect endothelial nitric oxide synthase (NOS) anti-NOS (Transduction Laboratories, Lexington, USA) was used in a dilution of 1:400. A streptavidin-gold marker (Auroprobe EM Streptavidin G10, AMERSHAM, Buckinghamshire, UK) was performed following the application of the biotinylated secondary antibody (VECTOR, Burlingame, USA; dilution 1:200). The procedure was completed after several rinses with a double contrast medium.

The study has been approved by the Institutional Review Board of the Martin Luther University Halle/Wittenberg and is in accordance with the Declaration of Helsinki.

Results

Allergic rhinitis

The hyperplastic and partially disintegrated epithelium showed numerous goblet cells. The lamina propria mucosae of all examined patients demonstrated extensive edema. In the subepithelial region, there were dilated capillaries with highly active, elevated endothelial cells containing numerous pinocytotic vesicles (Fig. 1). Around the capillaries we observed concentrically proliferated pericytes. The nasal mucosa offered a dense infiltration with lymphocytes, macrophages, mast cells, and plasma cells. In contrast to IR, a large number of activated plasma cells with a marked endoplasmatic reticulum were found in allergic mucosa (Fig. 2). The mast cells showed partly emptied vesicles and typical thickening of their granules depending on the stage of maturation (Fig. 3a, b). There was particularly massive infiltration with eosinophilic granulocytes (Fig. 4) forming characteristic oval acidophilic granules. Considerably fewer non-myelinated nerve fibers with neuropeptide-containing dense core vesicles were detected around the arterial vessels and the seromucosal glands (Fig. 5). Strong accumulation of endothelial NOS was identified in the seromucosal glands, the endothelium of subepithelial and periglandular capillaries, and endothelium of arterioles and arteries (Fig. 6).

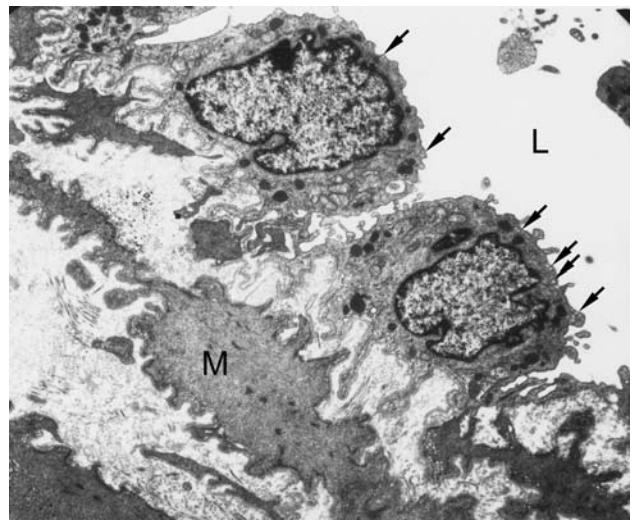


Fig. 1 AR, dilated capillary with highly active, elevated endothelial cells with numerous pinocytotic vesicles (arrows). L lumen, M muscle cells. Electron microscopy. Original magnification: $\times 7,000$

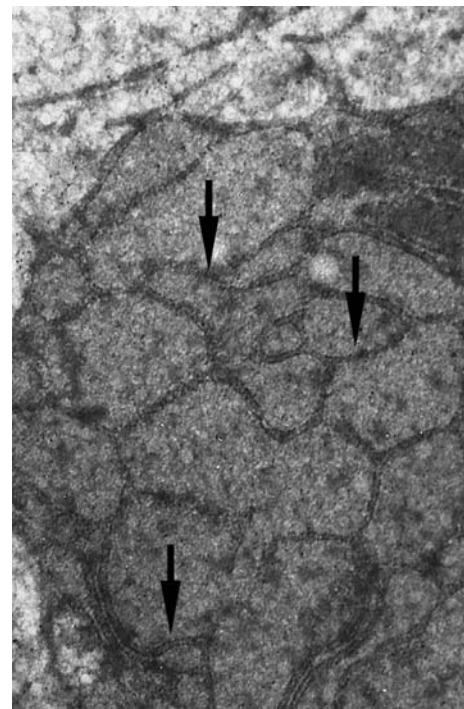


Fig. 2 AR, activated plasma cell with a marked endoplasmatic reticulum (arrows). Electron microscopy. Original magnification: $\times 12,000$

Idiopathic rhinitis

Under a pseudostratified epithelium, there was a lamina propria mucosae only slightly infiltrated with inflammatory cells showing negligible edema formation as well as areas with partial collagen accumulation. Pathologic infiltration with eosinophils, plasma cells, or mast cells was not observed. In both AR and IR, the expression of the neuropeptides CGRP

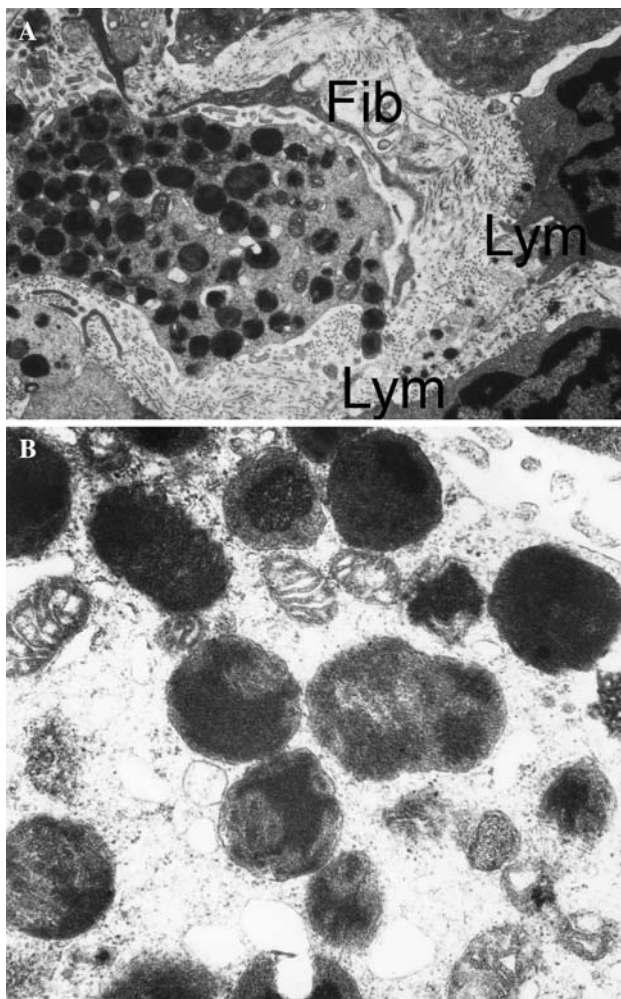


Fig. 3 **a** AR, mast cell in subepithelial mucosa with lymphocytes (*Lym*) and fibrocytes (*Fib*). Electron microscopy. Original magnification: $\times 7,000$. **b** AR, partly emptied vesicles and typical thickening of granules of a mast cell in a higher magnification. Electron microscopy. Original magnification: $\times 12,000$

and Substance P was increased intra-axonally, although CGRP was regularly found in dense core vesicles (Fig. 7), and Substance P was existing both in the axoplasm and bound to the dense core vesicles. In seromucous glands, we found an extensive perinuclear endoplasmic reticulum as well as increased mitochondria numbers. Furthermore, the capillaries were abnormally dilated, and the activated endothelial cells showed increased pinocytotic vesicles and so-called stress filaments (Fig. 8). Endothelial NOS were detectable to a clearly lower degree comparable to the control group. Again, a dense supply with non-myelinated nerve fibers was detected like in AR. See Table 1 for results of semiquantitative analysis.

Control group

Where no signs of chronic inflammatory disease of nasal mucosa could be observed, a highly prismatic ciliated

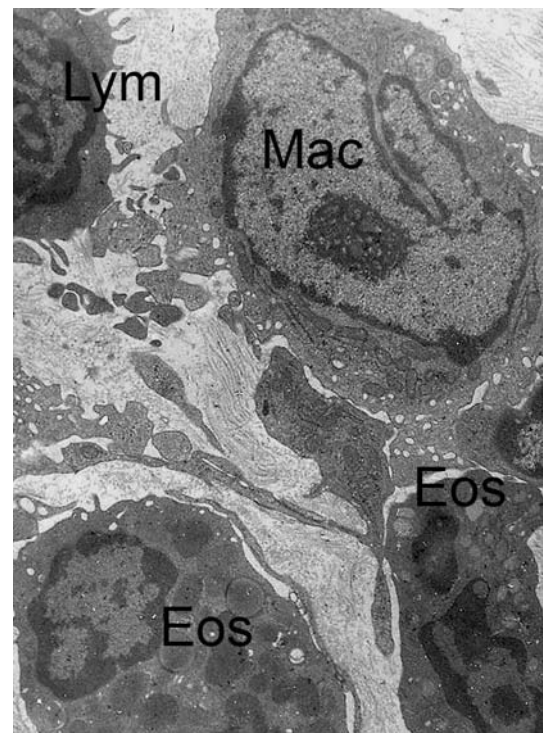


Fig. 4 AR; dense infiltration with lymphocytes (*Lym*), macrophages (*Mac*) and eosinophilic granulocytes (*Eos*). Electron microscopy. Original magnification: $\times 4,400$

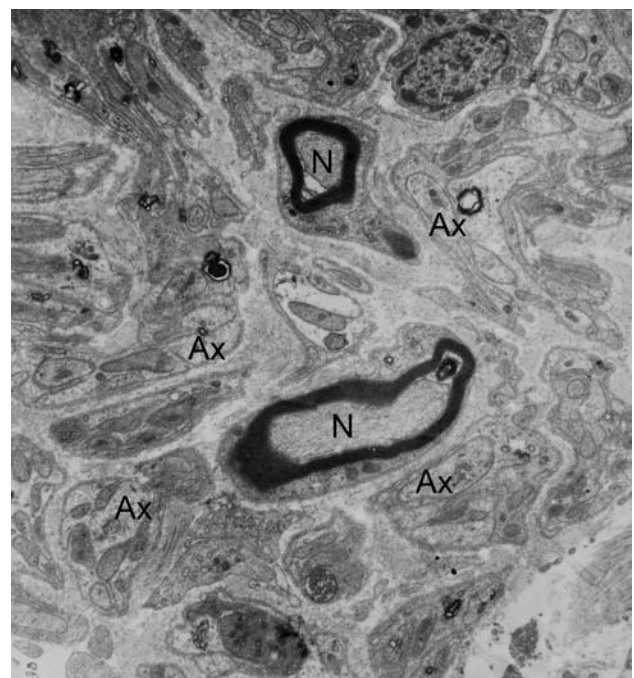


Fig. 5 AR, dense supply of axons of non-myelinated nerve fibers (*Ax*) and myelinated nerves (*N*) around arterial vessels and seromucosal glands. Electron microscopy. Original magnification: $\times 7,000$

epithelium with typical 9 + 2 pattern of microtubule was present. The vessels and seromucosal glands showed normal morphology and tissue infiltration with inflammatory

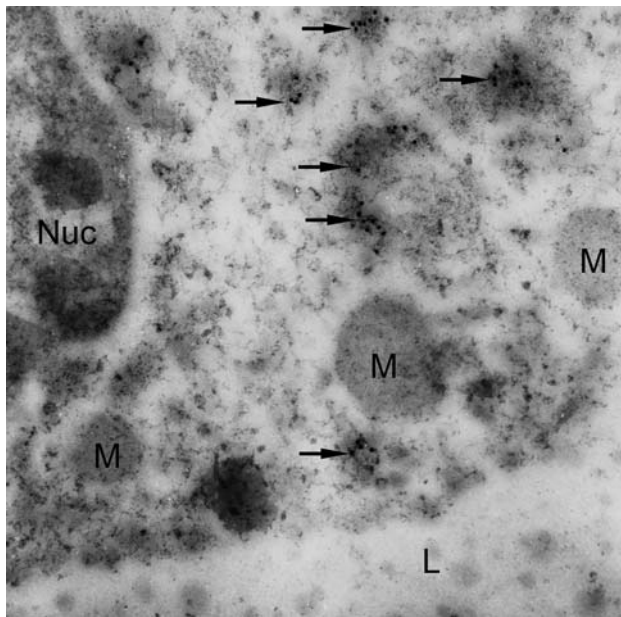


Fig. 6 AR, detection of endothelial NOS in the cytoplasm (arrows) of a periglandular capillary (L lumen). M mitochondria, Nuc nucleus. Immunoelectron microscopy. Original magnification: $\times 50,000$

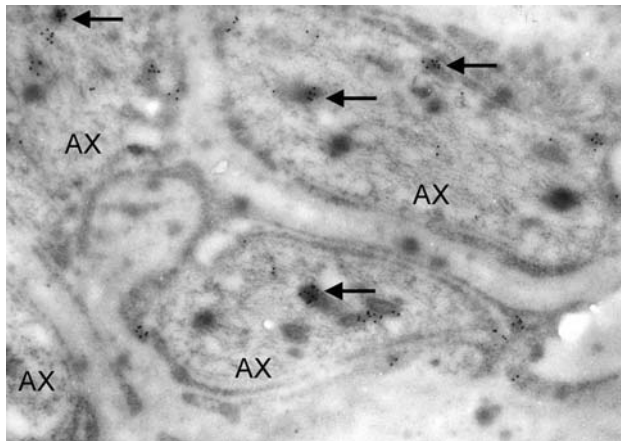


Fig. 7 IR, axons (Ax) of a nonmyelinated nerve fiber with immunoelectron microscopic marking of CGRP (arrows) in dense core vesicles. Immunoelectron microscopy. Original magnification: $\times 30,000$

cells was minimal in the lamina propria. No evidence for edema could be found. CGRP and SP were marked in non-myelinated nerve fibers. However, there was endothelial NOS—immunoreactivity in endothelial cells of capillaries near the glands as well as in arterial vessels, whereas the seromucous acinus cells and the glandular ducts showed endothelial NOS immunoreactions in the cytoplasm.

Discussion

Epidemiological data on the incidence of AR and IR were mainly recorded in the US, but a similar prevalence of 15%

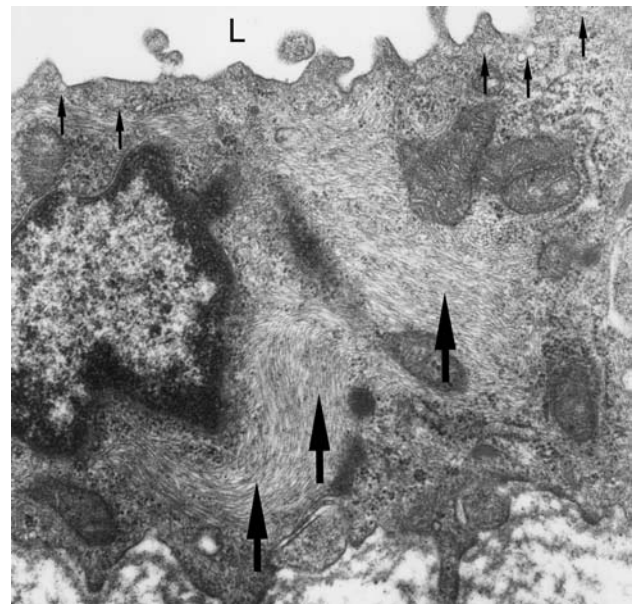


Fig. 8 IR, activated endothelial cells of capillary (L lumen) showed increased pinocytotic vesicles (small arrows) and stress filaments (big arrows). Electron microscopy: Original magnification: $\times 20,000$

can also be assumed for the European population [2–4]. We are dealing with two very common diseases of the nasal mucosa and the associated socioeconomic problems here.

The cause of IR is an unspecific hyperreactive rhinopathy with mucosal reactions to irritants (e.g. smoking) from the environment, physical strain, and conditional changes. According to ARIA documentation items like “vasomotor rhinitis” have to be replaced by IR [1, 4, 7]. The symptoms hypersecretion, nasal obstruction, itching, and sneezing are identical to those of AR [2–5]. The definition of IR as a nonallergic rhinitis is largely based on exclusion criteria, i.e., the absence of specific IgE to allergens [2].

The causes and pathogenesis of AR are known in detail up to the exact determination of cytokine patterns. After sensitization to specific stimuli, there is an IgE-mediated release of inflammatory mediators like histamine, leucotrienes, and bradykinin. The nasal mucosa of patients with persistent AR thus also shows massive infiltration with eosinophilic granulocytes, mast cells, and active plasma cells that are not detected in IR [2, 4, 13]. Activated plasma cells may have an extensive endoplasmic reticulum. However, the involvement of inflammatory cells like mast cells and eosinophils in IR as described by Powe’s study group [16] can neither be confirmed by our pathomorphological examinations nor by literature [4, 14]—a fact that is mainly due to patient selection and the corresponding inclusion criteria. According to definition, patients with confirmed eosinophilic rhinitis like in aspirin sensitivity (NARES) are not included in IR [4, 8, 13, 17].

The histamine originating in mast cells as well as kinins, prostaglandins, and leucotriene activate the central,

Table 1 Ultrastructural and immunoelectron microscopic findings in patients with AR, IR, and of the control group

	Changes in epithelium	Edema	Dilatation of capillaries	Inflammatory cells	Hyperinnervation	SP	CGRP	eNOS
Allergic rhinitis	++	+++	+++	+++	+++	+++	+++	+++
Idiopathic rhinitis	—	+	++	+	+++	+++	+++	+
Control group	—	—	—	+	—	+	+	+

Averaged semiquantitative scores: negative —, moderate +, strong ++, very strong +++

vasomotor, and secretory centers via hypersensitivity of the sensory, SP- and CGRP-containing nerve fibers and parasympathetic nerve endings. This leads to classical symptoms like hypersecretion, impaired nasal respiration, sneezing, and itching [1, 10, 18, 19].

In contrast to the noninflamed nasal mucosa of the control group, AR and IR demonstrated a clear hyperinnervation with increased expression of the neuropeptides CGRP and Substance P in periglandular nerve fibers—a sign of neuronal hyperactivity—as common, overlapping pathomechanism [4, 20]. In the abundant visible nerve fibers, there were increasing numbers of so-called dense core vesicles containing sensory neuropeptides like SP and CGRP [21]. The involvement of these neurotransmitters in both types of nasal hyperreactivity has been detected on the ultrastructural level in our examinations.

In addition to kinins as essential mediators of persistent AR, the neuropeptides CGRP and Substance P released by histamine stimulation also play an important role in antidromic reflex arc, in neurogenic inflammation, and in mast cell activation [1, 8, 18–20, 22].

Neurogenic inflammation is, in turn, subject to modulation via mediators like leucotrienes, kinins, and tachykinins [4, 22]. In conjunction with neural plasticity, SP is highly stimulated by chronic excitation of the sensory nerves [4, 5, 23]. Tai and Baraniuk [20] attribute the pain sensitivity and itching to the stimulation of sensory, neuropeptide-containing nerve fibers. The release of SP and CGRP leads to stimulation of glandular secretion and increased plasma extravasation [14, 20].

Using radioimmunoassay, Fang [9] found significant increased concentrations of SP and a higher concentration of CGRP in allergic nasal mucosa, whereas Heppt [10] demonstrated greater innervation density of SP-containing sensory nerves in IR. In AR, SP has a proliferation-promoting effect on eosinophilic granulocytes [10, 24].

The abnormally dilated capillaries are the morphological correlate for nasal hypersecretion in both types of rhinitis. The increase in transport and metabolic exchange processes and the higher extravasation of serum components or inflammatory cells can be confirmed by the numerous pinocytotic vesicles detected [11], the highly activated and damaged endothelial cells and the stress filaments in the

capillary endothelium. The concentric proliferations of pericytes presented here point to a chronic inflammation. Especially in AR, there is considerable mucosal edema, while partly collagenated regions were found in IR.

As already described by Elwany [11], the seromucosal glands showed elevated mitochondria numbers and an expanded endoplasmatic reticulum as signs of increased production of glandular secretion. Here there are morphological similarities with allergic rhinitis. Neuropeptides like SP and CGRP lead to an increased glandular secretion [9, 17].

The cytokines released in AR also induce NO synthase and lead to a boosted NO production combined with consecutive vasodilatation and edema formation that cause an increase in allergic symptoms [25]. In agreement with the findings of Kang and Olthoff, a strong expression of endothelial NOS in the glands and vessels was found in patients with AR [26, 27]. Incubation with lipopolysaccharides led to an increased expression of inducible NOS. After cytokine stimulation, NOS seems to be involved in the inflammatory hypersecretory processes of AR via higher NO production [1, 26, 28]. However, although it is in contrast to the studies by Giannessi, neither we nor others [4, 12, 27] were able to detect an increased expression of endothelial NOS in IR.

Therapeutically, nasal hyperreactivity in both types of rhinitis can be treated with anti-inflammatory drugs. Topical glucocorticosteroids as highly potent nasal anti-allergic agents to decrease the hyperreactivity and vascular permeability with a reduction of intercellular edema, have been successfully used in IR [3, 4, 5, 10, 29]. Treatment should be continuously applied for at least 6 weeks [4].

The anti-allergic agent Azelastin also has anti-inflammatory effects by inhibiting leucotriene synthesis and can be recommended for IR [30]. The application of saline solutions may also improve the symptoms [6].

New therapeutic approaches consist of the development of substances that intervene in the neurogenic inflammatory processes and inhibit the synthesis of neuropeptides and nitric oxide.

Repeated application of alkaloid capsaicin (8-methyl-N-vanillyl-6-nonenamid) consecutively leads to the depletion of neuropeptides, nociceptive, and sensory nerve endings

(type C), to the loss of sensitivity to different chemical irritants (e.g. cigarette smoke) and thus, to the suppression of neuropeptide effects [4, 31, 32]. The administration of capsaicin together with local anesthetics to alleviate the initial pain was successfully applied especially in patients with IR and led to a significant reduction of symptoms [32]. The fact that IR patients on capsaicin therapy reacted better than AR patients is considered a sign of underlying peptide-neurogenic inflammation. In AR, anti-inflammatory treatment is, however, more effective due to the dominance of cellular-inflammatory changes [4].

There are different hypotheses about the pathophysiology of IR. According to the pathomorphological changes demonstrated in this study, typical differences in AR and IR can be found, which, however, support the theory that underlying neurogenic inflammation causes nasal hyperactivity.

Based on these findings anti-inflammatory treatment can be recommended for both types of rhinitis.

Conclusion

Allergic and IR can be attributed to nasal hyperreactivity. The comparable pathophysiological findings of the nasal mucosa also explain the similarity in symptoms. Allergic rhinitis is based on immunological inflammatory processes, while IR can be attributed more to neurogenic inflammatory mechanisms. Both types of rhinitis can be successfully treated with anti-inflammatory therapy.

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