

Animal Models in CRS and Pathophysiologic Insights Gained: A Systematic Review

Hyun-Woo Shin, MD, PhD

Objective: Chronic rhinosinusitis (CRS) is a multifactorial inflammatory disease. In particular, CRS with eosinophilic features and/or nasal polyps (NPs) is often recalcitrant to current treatment; thus, appropriate animal models are mandatory to elucidate the pathogenesis of CRS and develop novel and efficient treatment modalities. The author reviewed the recently proposed animal models in CRS and discussed the pathophysiologic insights gained.

Data Sources: Articles in the English language referenced in MEDLINE/PubMed from the year 2006 onward (for last 10 years).

Review Methods: Review of the literature regarding animal models and related pathologic insights in CRS.

Results: Mouse, rabbit, and sheep models of CRS have been used for studying the pathogenesis of CRS. Most of researchers adopted animal models of CRS to prove any molecular mechanisms or therapeutic efficacy. In vitro or human findings and related hypothesis were evaluated in vivo using these models. In addition, novel therapeutic candidates for CRS with or without NP have been applied to animal models.

Conclusion: Animal models have elicited insights into the pathogenesis of CRS and also have been useful in testing new treatment modalities. Although there are still clear limitations in the animal studies, newly proposed or revised animal models would be helpful to understand the exact pathophysiology of CRS

INTRODUCTION

Chronic rhinosinusitis (CRS) is characterized by chronic inflammation of the sinonasal mucosa and is related to mucosal alterations ranging from epithelial thickening to nasal polyp (NP) formation.^{1,2} Chronic rhinosinusitis is frequently categorized into two groups according to the absence or presence of NP: CRS with nasal polyps (CRSwNP), and CRS without nasal polyps (CRSsNP).¹ Chronic rhinosinusitis affects approximately 5% to 15% of the general population, both in Europe and the United States, and causes the tremendous medical costs.² The precise etiology and pathogenesis of CRS and

NPs are largely unknown. Possible etiological factors of CRS include superantigens, abnormal inflammatory cytokine cascade, and biofilms. The presence of NPs, which is associated with TH2-skewed inflammation—particularly in Western countries—implies a greater burden of illness with refractory clinical features.^{1,3} The complexity of CRS and/or NP make the clinical and experimental study very difficult. Recently, several animal models were developed^{4–7} and applied to diverse basic and translational research. These CRS animal models have helped gain a comprehensive and precise understanding of the pathogenesis in CRS and nasal polyposis.

In this review, the author presents an overview of the progress made in CRS animal models and managing patients afflicted with diseases in the most inaccessible and variable of the paranasal sinuses.

METHODS

Study Selection

The electronic database of PubMed was systematically searched for studies using CRS animal models published in English from January 1, 2006, to May 15, 2016 (the articles published electronically during this period were included). The following search terms were used: animal models and chronic rhinosinusitis. The references of relevant publications were also reviewed manually to identify additional studies. The study was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.⁸

Data Items and Summary Measures

The selected articles were classified into two groups: 1) model development and 2) application of the previously established animal models related to CRS. For last 10 years, 10 reports introduced CRS animal models, for which three species of animals were

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From the Obstructive Upper Airway Research Laboratory, Department of Pharmacology (H-w.s.); the Ischemic/Hypoxic Disease Institute (H-w.s.), Seoul National University College of Medicine; Cancer Research Institute, Seoul National University (H-w.s.); the Department of Biomedical Sciences, Seoul National University Graduate School (H-w.s.); and the Department of Otorhinolaryngology–Head and Neck Surgery, Seoul National University Hospital (H-w.s.), Seoul, Republic of Korea

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Send correspondence to Hyun-Woo Shin, M.D., Ph.D. Department of Pharmacology, Seoul National University College of Medicine, Department of Otorhinolaryngology–Head and Neck Surgery, Seoul National University Hospital, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of Korea. E-mail: charlie@snu.ac.kr

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used: mice, rabbit, and sheep. The species of animal, period needed for model establishment, types of stimulants (e.g., allergens or adjuvants), and formation of NP were assessed.

RESULTS

Characteristics for Selected Studies of CRS Animal Models

The characteristics of included studies related to the development of novel or modified animal models for CRS were summarized in Table I. These recently developed in vivo protocols include two *Staphylococcus aureus* biofilm models, six CRS models, and two CRS models with NP lesions. The biofilm models and some CRS models were generated by surgical procedures, but most of the CRS with or without NP models were induced by allergic stimulation and their adjuvants. A detailed description of each model follows.

Sinusitis Biofilm Models

Australian researchers presented an animal model using sheep experimentally infected with *Staphylococcus aureus* to study the possible association between biofilm and sinusitis.⁴ Because bacterial biofilms were detected on the sinus mucosa of human subjects with CRS,⁹ diverse kinds of studies of the role of biofilms in CRS have been published.¹⁰ Jia et al. also proposed a rabbit model of *S. aureus* biofilms by inoculating bacterial suspension in the maxillary sinus after drilling to the sinus cavity.¹¹ In addition to *S. aureus*, fungi have been considered as one of the etiologic factors in CRS pathogenesis. Fungal biofilms have been discovered in CRS patients; thus, Boase et al. developed a sheep model to investigate the role of fungal biofilms in sinusitis.¹² In this model, significant fungal biofilm only occurred when *S. aureus* was the co-inoculum, indicating the possibility of fungal and bacterial synergism.

Mouse and Rabbit CRS Models

Lindsay et al. developed a mouse model of chronic eosinophilic rhinosinusitis using *Aspergillus fumigatus* (Af) extract with intraperitoneal injection and subsequent nasal challenges.⁷ In fact, the original airway model for inflammation used ovalbumin (OVA), a protein found in chicken egg whites, as the allergen to produce asthma in mice. However, Lindsay et al. selected Af as a study antigen because Af has been implicated in the pathophysiology of chronic hyperplastic eosinophilic rhinosinusitis and in allergic fungal sinusitis. Subsequently, Tansavatdi et al. developed a murine model for wound healing in CRS using Af extracts.¹³ This model mimicked the sinus wound healing process rather than the pathogenesis of sinusitis. Considering that sinus surgery is often complicated by adhesions and scarring that can compromise the success of the procedure, an acceptable animal model for normal wound-healing processes in chronically inflamed sinus mucosa is quite meaningful. Liang et al. reported the rabbit model using phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C that stimulates a vigorous inflammatory response with nasal cavity blockage

using a Merocel (Merocel, Medtronic Xomed, Jacksonville, FL) sponge. Upon successfully producing CRS in this model, they further tested their model by investigating the effect of treatment with intravenous antibiotics. Seven of nine treated CRS sides were clear of opacification after treatment; however, all nontreated CRS sides had persistent diseases at week 16.¹⁴ Contrary to other CRS models using allergic or chemical stimulants, Migliaiavacca et al. showed a novel rabbit model for CRS with transmaxillary sinus occlusion without bacterial inoculation.¹⁵

Murine Nasal Polyp Models

In 2011, for the first time, the murine model for CRS with NP was introduced by a South Korean group.⁵ Accumulating evidences support that *Staphylococcus aureus* enterotoxin B (SEB) plays a critical role in the pathogenesis of nasal polyposis. Considering this point, the group investigated the histological and immunologic effects of SEB on the formation of nasal polypoid lesions in an allergic rhinosinusitis murine model. After induction of an OVA-induced allergic rhinosinusitis, OVA with SEB (5 or 500 ng) was instilled into the nasal cavity of mice for 8 weeks. The group examined polyp formation and epithelial disruption microscopically from three coronal sections. Morphologically, polyp lesions were characterized with edematous connective tissue stroma, with eosinophilic infiltration and invasive growth of epithelial cells including the microcavities, which were reported as the characteristic features of NPs in previous reports.^{16,17} The exudate with crystal formation and surrounding eosinophils was also observed in the sinonasal lumen. The criteria for NP included 1) a more elevated lesion than surrounding mucosal folds, 2) the presence of eosinophilic infiltration, and 3) inner microcavities (intraepithelial growth with a differentiated and ciliated lining).

Basically, the initial NP model using OVA (3%, three times a week) plus SEB (5 or 10 ng, once a week) was generated in BALB/c mice; thus, higher level of OVA (6%, three times a week) and more frequent stimulation of SEB (10 ng, three times a week) was needed to induce polyp formation in the following study using the transgenic mice of C57BL/6 strain.¹⁸ The C57BL/6 mice have attenuated allergic airway hyperresponsiveness when compared with Balb/c mice, although the underlying mechanisms remain unclear.¹⁹ Because OVA is not an airborne allergen but a food allergen, some researchers doubted whether OVA was suitable for allergic induction in respiratory disease model. In fact, house dust mite (HDM) is the more common allergen, influencing respiratory allergic diseases including allergic rhinitis and bronchial asthma. On this account, Khalmuratova et al. developed the modified murine NP model for C57BL/six mice with HDM.⁶ They showed slightly weaker polyp formation than the BALB/c polyp model but a very prominent mast cell recruitment commonly observed in human NP tissues.²⁰ In fact, the number of mast cells in the OVA plus SEB model in both BALB/c and B6 mice was around 5 cells/high power field (HPF) and in the HDM plus SEB model was nearly 20 mast

First Author, Publication Year	Country	Animal	Materials	Need Surgery	Develop Period	Diseases	Established Model
Khalmuratova, 2016 ⁶	South Korea	Mouse (B6)	HDM and SEB	No	103 days	CRS with NP	Develop modified polyp model using aeroallergen (HDM)
Kim, 2014 ⁴⁴	South Korea	Mouse (BALB/c)	Aspergillus protease and OVA	No	5 weeks	CRS	Develop modified eosinophilic CRS model with Aspergillus protease
Migliavacca, 2014 ¹⁵	Brazil	Rabbit (New Zealand)	–	Yes	12 weeks	CRS	Develop the animal model of CRS in rabbits without bacterial inoculation
Jia, 2014 ¹¹	China	Rabbit (New Zealand)	<i>S. aureus</i>	Yes	2–8 weeks	Sinusitis (biofilms)	Develop the animal model of <i>Staphylococcus aureus</i> biofilm in CRS
Kim, 2011 ⁵	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Develop a murine polyp model
Boase, 2011 ¹²	Australia	Sheep	Aspergillus fumigatus/Alternaria alternata/ <i>S. aureus</i>	Yes	38 days	CRS	Develop modified CRS sheep model for fungal biofilms in sinusitis
Tansavatdi, 2010 ¹³	United States	Mouse (BALB/c)	Aspergillus fumigatus	No	12 weeks	CRS	Develop an animal model for wound healing in CRS
Liang, 2008 ¹⁴	Taiwan	Rabbit (New Zealand)	PMA and Mero-cel Medtronic	Yes	12 weeks	CRS	Develop an animal model for rhinogenic CRS
Ha, 2007 ⁴	Australia	Sheep	<i>S. aureus</i>	Yes	7 days	Sinusitis (biofilms)	Demonstrate bacterial biofilms in an animal model of sinusitis
Lindsay, 2006 ⁷	United States	Mouse (BALB/c)	Aspergillus fumigatus	No	12 weeks	CRS	Develop an animal model for CRS

CRS = chronic rhinosinusitis; HDM = house dust mite; NP = nasal polyp; OVA = ovalbumin; PMA = phorbol 12-myristate 13-acetate; SEB = *Staphylococcus aureus* enterotoxin B.

cells per HPF in nasal mucosa.^{5,6} Most recently, Kim et al. showed that this NP mouse model demonstrated enhanced B-cell responses reminiscent of B cell responses in human NP.²¹

Although this murine polyp model can provide a very useful tool for studying the pathogenesis of NP, it should be noted that there are some anatomical differences between rodents and humans. The maxillary sinuses in mice and rats are not completely enclosed by the upper jaw bone (maxilla). For this reason, maxillary sinuses in rodent and many nonhuman animals are often referred to as maxillary recesses in the literature.^{22,23} In rodents, the mucus from the anterior maxillary sinus drains toward the anterior nares, but the mucus from the posterior maxillary sinus drains toward the nasopharynx.²³ In addition, the submucosa of the posterior maxillary sinus in mice and rats is more densely occupied by submucosal glands than the anterior maxillary sinus,⁷ whereas maxillary sinus cavities are lined with respiratory epithelium containing few or no goblet cells.^{24,25}

As a result, these different anatomic and physiological features should be considered when using the rodent model of CRS and NPs. Despite the anatomical differences in these animal models, the epithelial remodeling, inflammatory cell infiltration, and collagen deposition (excluding polyp formation) could be evaluated in diverse experimental conditions. Considering the invaluable information from the in vivo system, the development and application of experimental animal models such as those mentioned above are quite helpful to overcome the limitations imposed on the study of human subjects, that is, restriction of sampling and manipulation due to ethical problems.

DISCUSSION

Applications of Sheep CRS Models for Testing Novel Therapeutic Candidates

Sheep CRS models have been continuously used for determining the roles of several etiologic factors and the effects of therapeutic candidates (Table II). Sheep have a similar pattern of diseases to humans, including allergic rhinitis, sinusitis, and nasal polyposis.^{26,27} Other advantages include their tolerance to long surgical procedures and their large nasal cavity that renders them suitable for repeated endoscopic sinus surgery (ESS).⁴ Based on these, many researchers in Australia developed and utilized sheep CRS models. Thomas et al. investigated an eosinophilic response in sheep chronically infected with *O. ovis*.²⁷ The effect of chitosan-dextran derivative gel on mucosal wound healing in CRS was investigated using the sheep model.²⁸ In particular, the sheep biofilm model has been actively applied for testing novel anti-biofilm materials by the Wormald group. Singhal et al. showed that NVC-422, a potent, fast-acting, broad-spectrum nonantibiotic antimicrobial, was an effective topical agent against *S. aureus* biofilms, with dose-dependent efficacy in this animal model of biofilm-associated sinusitis.²⁹ Thereafter, Boase et al. studied the influence of bacterial-induced epithelial damage on *Aspergillus fumigatus* biofilm

formation in sinusitis.³⁰ Manuka honey (MH) and its active component methylglyoxal (MGO) were evaluated for the safety and efficacy of these agents by the same group.³¹ The authors concluded that sinus irrigation with MH/MGO at MGO concentrations between 0.9 and 1.8 mg/mL was both safe to mucosa and efficacious against *S. aureus* biofilm; thus, MH/MGO irrigation could represent a viable treatment option for recalcitrant CRS in an in vivo model. The Wormald group also assessed the safety and efficacy of topical colloidal silver solution and topical liposomal nitric oxide donor for the treatment of *S. aureus* biofilms in sheep models.^{24,32} They showed that both topical agents had effective anti-biofilm activity in *S. aureus* CRS; thus, further investigations are needed.

Applications of CRS Models for Elucidating the Roles of Molecular Targets

Allergic mice models for CRS provided diverse pathophysiological features in CRS (Table II). Wang et al. compared histological and immunological features of bacterial CRS (BCRS) and allergic CRS (ACRS) using BALB/c mice.³³ In this study, the bacterial CRS was established by *Streptococcus pneumoniae* inoculation plus Merocel (Medtronic) ostiomeatal obstruction for 12 weeks. Allergic CRS was developed by OVA sensitization and subsequent multiple OVA intranasal challenge for 12 weeks. The authors reported that the Th1/Th2 ratio in BCRS mice was significantly higher than that in ACRS mice, and overall histological and immunologic features of BCRS and ACRS mouse models were similar to those of human noneosinophilic and eosinophilic CRS, respectively. Another in vivo study using a murine CRS model showed the time-dependent changes in tissue-remodeling cytokine expression corresponding to the inflammatory tissue changes during CRS induction by *Aspergillus fumigatus*.³⁴ The authors suggested further study on the association between BMP, FGF, and MMP regulation and tissue remodeling changes resulting from chronic inflammation.

Applications of Nasal Polyp Models for Underlying Molecular Mechanisms

Although several kinds of CRS animal models were introduced and applied, the in vivo model for NP was quite scarce (Table II). In 2011, Kim et al. developed the murine polyp model using OVA and SEB,⁵ which subsequently was utilized in diverse studies. Using the NP model, Shin et al. first demonstrated that hypoxia-inducible factor-1 (HIF-1)-induced epithelial-to-mesenchymal transition (EMT) contributed to nasal polyposis and then suggested that HIF-1 α inhibitors could be novel therapeutic candidates.³⁵ Epithelial-to-mesenchymal transition, a cellular process whereby epithelial cells acquire mesenchymal properties and loose cell-cell interactions and apicobasal polarity, is known to play fundamental roles in organ development and tumor invasion.³¹ In fact, the authors utilized the murine NP model and showed that several HIF-1 inhibitors could suppress polyp formation in vivo. Recently, the same

TABLE II.
Basic Information for Included Studies of CRS Animal Model Application.

First Author, Publication Year	Country	Animal	Materials	Need Surgery	Study Period	Diseases	Pathophysiologic Insights Gained
Kim, 2016 ²¹	United States	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Demonstration of the activation of B cells in CRSwNP murine model
Kim, 2016 ⁴⁵	South Korea	Mouse (BALB/c)	OVA and SEB	No	24 weeks	CRS with NP	Increased thymic stromal lymphopoietin expression and Th2-skewing after prolonged allergen exposure
Lee, 2016 ¹⁸	South Korea	Mouse (B6)	OVA and SEB	No	103 days	CRS with NP	Elicit the novel role of histone deacetylase Sirtuin 1 in polyposis
Jardeleza, 2015 ²⁴	Australia	Sheep	S. aureus	Yes	2 weeks	Sinusitis (biofilms)	Test the effect of topical liposomal nitric oxide donor on biofilm-associated rhinosinusitis
Shin, 2015 ⁴⁰	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Elicit the novel role of IL-25 in nasal polyposis of CRS
Rajiv, 2015 ³²	Australia	Sheep	S. aureus	Yes	2 weeks	CRS	Test the effect of topical colloidal silver solution on biofilm <i>Staphylococcus aureus</i> CRS
Chang, 2015 ⁴¹	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Test the effect of topical cyclosporine on CRSwNP
Lee, 2014 ²	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Cigarette smoke aggravated eosinophilic inflammation in CRSwNP model
Paramasivan, 2014 ³¹	Australia	Sheep	S. aureus	No	34 days	CRS	Test the safety and effect of methylglyoxal-augmented Manuka honey on biofilm of CRS
Gocea, 2013 ⁴⁶	Romania	Rabbit (New Zealand)	PMA and Mero-cel Medtronic insertion	Yes	12 weeks	CRS	Investigate the effects of cryotherapy on the maxillary antrotomy in CRS
Jin, 2014 ³⁹	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Elicit the expression pattern of IL-17 in CRSwNP
Kim, 2013 ³⁶	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Test the effect of resveratrol on eosinophilic CRSwNP
Boase, 2013 ³⁰	Australia	Sheep	<i>Aspergillus fumigatus</i>	Yes	2 weeks	CRS	Investigate the influence of bacterial-induced epithelial damage on fungal biofilm formation
Kim, 2013 ³⁷	South Korea	Mouse B6	OVA and SEB	No	103 days	CRS with NP	Elicit the expression of periostin in nasal polyposis of CRS

TABLE II.
(Continued)

First Author, Publication Year	Country	Animal	Materials	Need Surgery	Study Period	Diseases	Pathophysiologic Insights Gained
Shin, 2012 ³⁵	South Korea	Mouse (BALB/c)	OVA and SEB	No	103 days	CRS with NP	Elicit the novel role of HIF-1 α and EMT in nasal polypogenesis of CRS
Singhal, 2012 ²⁹	Australia	Sheep	<i>S. aureus</i>	Yes	1 weeks	Sinusitis (biofilms)	Test the effect of NVC-422 against <i>Staphylococcus aureus</i> biofilm
Sautter, 2012 ³⁴	United States	Mouse (BALB/c)	<i>Aspergillus fumigatus</i>	No	3 months	CRS	Investigate the gene expression related to tissue remodeling in CRS
Wang, 2008 ³³	China	Mouse (BALB/c)	<i>S. pneumoniae</i> or OVA	No	12 weeks	CRS	Elucidate histological and immunologic features of bacterial and allergic CRS
Athanasiadis, 2008 ²⁸	Australia	Sheep	Mucosal injury	Yes	112 days	CRS	Test the effect of chitosan gel on mucosal wound healing in CRS
Thomas, 2007 ²⁷	Australia	Sheep	<i>O. ovis</i>	Yes	–	CRS	Investigate an eosinophilic response in sheep chronically infected with <i>O. ovis</i>

CRS = chronic rhinosinusitis; CRSwNP = CRS with nasal polyps; EMT = Epithelial-to-mesenchymal transition; HIF-1 α = hypoxia-inducible factor-1; IL = interleukin; NP = nasal polyp; OVA = ovalbumin; PMA = phorbol 12-myristate 13-acetate; SEB = *Staphylococcus aureus* enterotoxin B.

research group showed that SIRT1—a histone deacetylase—could play a defensive role in CRS; it seems that SIRT1 loss aggravates sinonasal mucosa inflammation, finally leading to epithelial remodeling, including polypogenesis.¹⁸ Mechanistically, SIRT1 inhibited the transcriptional activity of HIF-1 α by acetylating it and suppressed HIF-1–induced EMT in human nasal epithelial cells. The murine NP model was also used for probing the therapeutic efficacy of resveratrol, a well-known SIRT1 activator against NP formation. Interestingly, the anti-polyp effect of resveratrol was also found in earlier in vivo study in which resveratrol was considered as an antiinflammatory agent to inhibit the lipoxygenase pathway.³⁶

Similarly, many investigators have utilized the NP mouse model using OVA and SEB to show the effects of their candidate drugs or target molecules. Kim et al. showed that loss of periostin appeared to enhance polyp-like lesion formation and mast cell infiltration in a mouse model of eosinophilic rhinosinusitis with NPs.³⁷ They induced the NP formation in both periostin-null and wild-type mice by the repeated nasal administration of OVA and SEB. Periostin, a component of the extracellular matrix, was identified in the periosteum and periodontal ligament in adult mice and was presumed to play a role in the recruitment and attachment of osteoblast precursors in the periosteum.³⁸ Jin et al. found that epithelial expression of interleukin (IL)–17C was significantly higher in experimental NP mice compared to control mice.³⁹ They observed that SEB-induced IL-17C expression in nasal epithelial cells was mediated by reactive oxygen species production. Recently, Shin et al. reported that IL-25 secreted from the sinonasal epithelia and infiltrating mast cells play a crucial role in the pathogenesis of CRS with NPs in Asian patients.⁴⁰ Human NPs exhibited higher levels of both IL-25 protein and mRNA. The NP lesions in the mice model also showed the prominent IL-25 expression and were reduced by anti-IL-25 therapy. Beside the number of polyps, anti-IL-25 treatment reduced mucosal edema thickness, collagen deposition, and infiltration of inflammatory cells such as eosinophils and neutrophils. This treatment also inhibited expression of local inflammatory cytokines, including IL-4 and IFN- γ . Other research groups investigated the effects of chronic exposure to cigarette smoke or topical cyclosporine on CRSwNP using this murine NP model.^{41,42}

Advantages and Pitfalls of CRS Animal Models for Studying Its Pathogenesis

Animal models for CRS and/or NPs have many benefits for practicing scientists. We can test a specific hypothesis using animal models, which cannot be proven in clinical studies. Mice, rabbits, and sheep currently used in CRS models have their own strengths and weaknesses. Mice are inexpensive and easy to handle, and many murine specific reagents are commercially available.⁷ Transgenic or knockout mice are readily available, offering a meaningful advantage over rabbit or sheep models of sinusitis. Transgenic manipulation has

provided remarkable advances in uncovering the basic pathophysiologic mechanisms of disease.^{18,35} However, some authors mentioned that the murine model is limited because mice have very small sinus cavities and different immunologic reactions from human.¹⁴ Rabbits have sinus cavities that are well pneumatized, and both their sinonasal anatomy and immunologic reactions are very similar to those of humans, which is considered to be superior to mice. Sheep also have a similar pattern of sinonasal diseases to human.^{26,27} Gardiner et al. showed that the sinonasal anatomy and structure of the nasal cavity, turbinates, frontal, and maxillary sinuses of sheep are analogous to humans.⁴³ In addition, their tolerance to long operative procedures and their large nasal cavity render them suitable for repeated ESS.⁴ These animal models for CRS could bring new insights into the pathogenesis and treatment modality in terms of the ostial obstruction or biofilm formation.

However, there are some pitfalls in the application of the animal models. Investigators should note the anatomical characteristics of each animal, which are quite different from human. For example, mice do not have true sinuses like a human, as mentioned above. However, the rabbit or sheep sinus can be well served when the pathophysiologic mechanisms related to ostial obstruction are explored and a true sinus is necessary.⁷ Immunologic responses also vary with the species and the environment; thus, the traits or phenotypes observed in animal studies should be confirmed in human tissues or cases.

CONCLUSION

Here, this review summarized the previously reported animal CRS models and discussed the pathophysiologic meanings from their applications published for last 10 years. The classical animal models for CRS have been modified, and several novel models for NP were developed. Although there still are clear limitations in the animal studies, newly proposed or revised animal models would be helpful to understand the exact pathophysiology of CRS.

BIBLIOGRAPHY

1. Bachert C, Van Bruaene N, Toskala E, et al. Important research questions in allergy and related diseases: 3-chronic rhinosinusitis and nasal polypoidosis - a GALEN study. *Allergy* 2009;64:520–533.
2. Fokkens WJ, Lund VJ, Mullol J, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology* 2012;50:1–12.
3. Banerji A, Piccirillo JF, Thawley SE, et al. Chronic rhinosinusitis patients with polyps or polypoid mucosa have a greater burden of illness. *Am J Rhinol* 2007;21:19–26.
4. Ha KR, Psaltis AJ, Tan L, Wormald PJ. A sheep model for the study of biofilms in rhinosinusitis. *Am J Rhinol* 2007;21:339–345.
5. Kim DW, Khalmuratova R, Hur DG, et al. Staphylococcus aureus enterotoxin B contributes to induction of nasal polypoid lesions in an allergic rhinosinusitis murine model. *Am J Rhinol Allergy* 2011;25:e255–e261.
6. Khalmuratova R, Lee M, Kim DW, Park JW, Shin HW. Induction of nasal polyps using house dust mite and Staphylococcal enterotoxin B in C57BL/6 mice. *Allergol Immunopathol (Madr)* 2016;44:66–75.
7. Lindsay R, Slaughter T, Britton-Webb J, et al. Development of a murine model of chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 2006;134:724–730; discussion 731–732.
8. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* 2009;62:1006–1012.
9. Sanderson AR, Leid JG, Hunsaker D. Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. *Laryngoscope* 2006;116:1121–1126.

10. Długaszevska J, Leszczynska M, Lenkowski M, Tatarska A, Pastusiak T, Szyfter W. The pathophysiological role of bacterial biofilms in chronic sinusitis. *Eur Arch Otorhinolaryngol* 2015;273:1989–1994.
11. Jia M, Chen Z, Du X, Guo Y, Sun T, Zhao X. A simple animal model of *Staphylococcus aureus* biofilm in sinusitis. *Am J Rhinol Allergy* 2014; 28:e115–e119.
12. Boase S, Valentine R, Singhal D, Tan LW, Wormald PJ. A sheep model to investigate the role of fungal biofilms in sinusitis: fungal and bacterial synergy. *Int Forum Allergy Rhinol* 2011;1:340–347.
13. Tansavatdi KP, McGill L, Riggs S, Orlandi RR. Development of an animal model for wound healing in chronic rhinosinusitis. *Arch Otolaryngol Head Neck Surg* 2010;136:807–812.
14. Liang KL, Jiang RS, Wang J, et al. Developing a rabbit model of rhinogenic chronic rhinosinusitis. *Laryngoscope* 2008;118:1076–1081.
15. Miglia vacca Rde O, Piltcher OB, Kliemann LM, et al. An experimental model of chronic rhinosinusitis in rabbits without bacterial inoculation. *Acta Cir Bras* 2014;29:313–319.
16. Norlander T, Westrin KM, Fukami M, Stierna P, Carlsoo B. Experimentally induced polyps in the sinus mucosa: a structural analysis of the initial stages. *Laryngoscope* 1996;106:196–203.
17. Norlander T, Fukami M, Westrin KM, Stierna P, Carlsoo B. Formation of mucosal polyps in the nasal and maxillary sinus cavities by infection. *Otolaryngol Head Neck Surg* 1993; 109:522–529.
18. Lee M, Kim DW, Yoon H, et al. Sirtuin 1 attenuates nasal polypogenesis by suppressing epithelial-to-mesenchymal transition. *J Allergy Clin Immunol* 2016;137:87–98.e7.
19. Albegger K, Huber EG. [Inflammatory diseases of the nose and nasal sinuses in children]. [Article in German]. *Pediatr Padol* 1985; 20:315–323.
20. Takabayashi T, Kato A, Peters AT, et al. Glandular mast cells with distinct phenotype are highly elevated in chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol* 2012;130:410–420.e5.
21. Kim DY, Lee SH, Carter RG, Kato A, Schleimer RP, Cho SH. A recently established murine model of nasal polyps demonstrates activation of B cells, as occurs in human nasal polyps. *Am J Respir Cell Mol Biol* 2016. Epub ahead of print.
22. Norris AM, Laing EJ. Diseases of the nose and sinuses. *Vet Clin North Am Small Anim Pract* 1985;15:865–890.
23. Phillips JE, Ji L, Rivelli MA, Chapman RW, Corboz MR. Three-dimensional analysis of rodent paranasal sinus cavities from X-ray computed tomography (CT) scans. *Can J Vet Res* 2009;73:205–211.
24. Jardeleza C, Thierry B, Rao S, et al. An in vivo safety and efficacy demonstration of a topical liposomal nitric oxide donor treatment for *Staphylococcus aureus* biofilm-associated rhinosinusitis. *Transl Res* 2015;166: 683–692.
25. Harkema JR, Carey SA, Wagner JG. The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. *Toxicol Pathol* 2006;34:252–269.
26. Rings DM, Rojko J. Naturally occurring nasal obstructions in 11 sheep. *Cornell Vet* 1985;75:269–276.
27. Thomas DC, Wormald PJ. Standardization of diagnostic criteria for eosinophilic chronic rhinosinusitis in the Oestrus ovis infected sheep model. *Am J Rhinol*. 2007;21:551–555.
28. Athanasiadis T, Beule AG, Robinson BH, Robinson SR, Shi Z, Wormald PJ. Effects of a novel chitosan gel on mucosal wound healing following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis. *Laryngoscope* 2008;118:1088–1094.
29. Singhal D, Jekle A, Debabov D, et al. Efficacy of NVC-422 against *Staphylococcus aureus* biofilms in a sheep biofilm model of sinusitis. *Int Forum Allergy Rhinol* 2012;2:309–315.
30. Boase S, Jervis-Bardy J, Cleland E, Pant H, Tan L, Wormald PJ. Bacterial-induced epithelial damage promotes fungal biofilm formation in a sheep model of sinusitis. *Int Forum Allergy Rhinol* 2013;3:341–348.
31. Paramasivan S, Drilling AJ, Jardeleza C, Jervis-Bardy J, Vreugde S, Wormald PJ. Methylglyoxal-augmented manuka honey as a topical anti-*Staphylococcus aureus* biofilm agent: safety and efficacy in an in vivo model. *Int Forum Allergy Rhinol*. 2014;4:187–195. doi: 10.1002/alr.21264.
32. Rajiv S, Drilling A, Bassiouni A, James C, Vreugde S, Wormald PJ. Topical colloidal silver as an anti-biofilm agent in a *Staphylococcus aureus* chronic rhinosinusitis sheep model. *Int Forum Allergy Rhinol* 2015;5: 283–288.
33. Wang H, Lu X, Cao PP, et al. Histological and immunological observations of bacterial and allergic chronic rhinosinusitis in the mouse. *Am J Rhinol* 2008;22:343–348.
34. Sautter NB, Delaney KL, Hausman FA, Trune DR. Tissue remodeling gene expression in a murine model of chronic rhinosinusitis. *Laryngoscope* 2012;122:711–717.
35. Shin HW, Cho K, Kim DW, et al. Hypoxia-inducible factor 1 mediates nasal polypogenesis by inducing epithelial-to-mesenchymal transition. *Am J Respir Crit Care Med* 2012;185:944–954.
36. Kim SW, Kim DW, Khalmuratova R, et al. Resveratrol prevents development of eosinophilic rhinosinusitis with nasal polyps in a mouse model. *Allergy* 2013;68:862–869.
37. Kim SW, Kim JH, Jung MH, et al. Periostin may play a protective role in the development of eosinophilic chronic rhinosinusitis with nasal polyps in a mouse model. *Laryngoscope* 2013;123:1075–1081.
38. Horiuchi K, Amizuka N, Takeshita S, et al. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res* 1999;14:1239–1249.
39. Jin J, Rha KS, Kim DW, Kim YM. IL-17C expression in nasal epithelial cells of chronic rhinosinusitis with nasal polyposis. *Eur Arch Otorhinolaryngol* 2014;271:1097–1105.
40. Shin HW, Kim DK, Park MH, et al. IL-25 as a novel therapeutic target in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 2015;135:1476–1485 e1477.
41. Chang DY, Joo YH, Kim SJ, et al. Therapeutic effects of intranasal cyclosporine for eosinophilic rhinosinusitis with nasal polyps in a mouse model. *Am J Rhinol Allergy* 2015;29:e29–e32.
42. Lee KI, Kim DW, Kim EH, et al. Cigarette smoke promotes eosinophilic inflammation, airway remodeling, and nasal polyps in a murine polyp model. *Am J Rhinol Allergy* 2014;28:208–214.
43. Gardiner Q, Oluwale M, Tan L, White PS. An animal model for training in endoscopic nasal and sinus surgery. *J Laryngol Otol* 1996;110:425–428.
44. Kim JH, Yi JS, Gong CH, Jang YJ. Development of *Aspergillus* protease with ovalbumin-induced allergic chronic rhinosinusitis model in the mouse. *Am J Rhinol Allergy* 2014;28:465–470.
45. Kim DW, Eun KM, Jin HR, Cho SH, Kim DK. Prolonged allergen exposure is associated with increased thymic stromal lymphopoietin expression and Th2-skewing in mouse models of chronic rhinosinusitis. *Laryngoscope* 2016. doi: 10.1002/lary.26004. Epub ahead of print.
46. Gocea A, Tulescu M, Trombitas V, Albu S. Effects of cryotherapy on the maxillary anastomosis patency in a rabbit model of chronic rhinosinusitis. *Biomed Res Int* 2013;2013:101534.