




REVIEW ARTICLE

Which are the best murine models to study Eosinophilic Chronic Rhinosinusitis? A contemporary review



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HIGHLIGHTS

- BALB/c mice seem to produce a more robust eosinophilic response than C57BL/6 mice.
- OVA and SEB protocol was the one that most resembled to ECRS.
- OVA and AP protocol also induced inflammation similar to ECRS, but to a lesser extent.

KEYWORDS

Chronic rhinosinusitis;
Eosinophilic
rhinosinusitis;
Mouse;
Nasal polyps;
Staphylococcus aureus;
Aspergillus oryzae
protease

Abstract

Objective: Mechanisms that lead to Eosinophilic Chronic Rhinosinusitis (ECRS) are not fully established in the literature. It is desirable to assess ECRS in a model that embraces most of the related events. This article reviewed the murine models for ECRS and compared them regarding eosinophilic polypoid formation.

Methods: The authors reviewed the articles that included the terms "chronic rhinosinusitis" OR "chronic sinusitis" AND "animal model". We analyzed articles in English that evaluated both the number of polyps and the number of eosinophils in the sinus mucosa of mouse models.

Results: We identified a total of 15 articles describing different models of ECRS that used BALB/c or C57BL/6 mice, and different triggers/stimulants such as *Staphylococcus aureus* Enterotoxin B (SEB) + Ovalbumin (OVA); House Dust Mite (HDM) ± Ovalbumin (OVA); and *Aspergillus oryzae* Protease (AP) + Ovalbumin (OVA). OVA associated with SEB was the commonest protocol to induce ECRS in both BALB/c and C57BL/6 mice, and it produced a robust response of eosinophilic nasal polyps in both. AP + OVA protocol also led to a good ECRS response. The other models were not considered adequate to produce eosinophilic polyps in mice.

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Conclusion: In conclusion, OVA associated with SEB seems to produce the most robust eosinophilic sinonasal inflammation.

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Introduction

Chronic Rhinosinusitis (CRS) is characterized by inflammation of the sinonasal mucosa lasting longer than 12 weeks, leading to negative impact in the patient's quality of life.¹ CRS poses a significant burden to both the patient and society due to its high prevalence, vast, and costly symptoms, high indirect costs, and not entirely effective treatments.¹

The mechanisms that lead to Eosinophilic CRSwNP (ECRS) are not fully established in the literature. There is a clear need for further studies to understand ECRS physiopathology and to identify any perspective of new treatments.² CRSwNP may be presented with either predominant type 1, type 2, or type 3 immune responses, and several times the inflammation may be mixed.³⁻⁵

Type 2 CRSwNP tend to be much more resistant to current therapies, exhibiting higher rates of recurrence than the other endotypes.⁶ It is characterized by overexpression of the cytokines IL-4, IL-5, and IL-13 and activation and recruitment of eosinophils and mast cells.¹ Moreover, the amount of eosinophilic infiltration and the intensity of the inflammatory response are reported to be closely related to the prognosis and severity of the disease.⁷

The complexity of this pathology makes the clinical and experimental study models very problematic. Therefore, it is reasonable to assess ECRS in such a model that embraces most of the related events in a cost-effective way. The murine model has been helpful to study ECRS, because it allows the evaluation of the pattern of inflammation, epithelial remodeling, and collagen deposition.⁸ The availability of transgenic animals is useful for genetic and pathogenic studies. Finally, the murine model is the *in vivo* model dealing with the smallest animal, being an advantage in both ethical and financial perspectives.

However, there are limitations when transposing animal models findings to humans. The size of mice makes it challenging for studies involving surgical models or drugs involving implants and/or stents. Also, mouse nasal polyps are smaller in size and number, whereas they often occupy a considerable percentage of the nasal cavity⁹ in humans.^{8,9}

Over the years, several murine models for CRSwNP have been developed. In 2011, the first murine model was described in BALB/c animals, with Intranasal (IN) *Staphylococcus aureus* Enterotoxin B (SEB),¹⁰ and Intraperitoneal (IP) and IN Ovalbumin (OVA).¹⁰ Since then, several models have been proposed, in both C57BL and BALB/c animals.⁹⁻³⁵ However, there is no consensus in the literature on which murine model induces a more robust eosinophilic response and a higher production of polyps.

In this review, we pool data and discuss murine models that induced ECRS presented in the literature. We also compare these studies regarding the tissue eosinophilia and the

number of polypoid lesions present in the mucosa of the sinuses, which are fundamental to the evaluation of these studies.

Methods

Study selection

We searched for English studies that described murine models of ECRS at the PubMed database. The following terms were used in the search: "animal models" and "chronic rhinosinusitis" or "chronic sinusitis". A total of fifty-nine articles were achieved at this first research. Additional filtering was performed to this first research, accordingly: an ECRS model in mouse should be fully described; it should assess both the sinuses histology, counting both the number of polyps and the number of eosinophils on the tissue (using 400× magnification analysis); it should be written in English; should have the article available on Pubmed. Because of this assessment, a total of 44 articles were excluded: 23 described neither the number of polyps nor the number of eosinophils; 20 only evaluated the number of eosinophils, and one only evaluated the number of polyps in the sinonasal mucosa (Fig. 1).

At this stage, 15 articles were carefully read. References to relevant publications were also manually reviewed to identify additional studies. The study was conducted following the statement of preferred reporting items for systematic reviews and meta-analyses, according to PRISMA³⁶ (P – Murine; I – Animal Model of ECRS; C – Comparison; O – Tissue eosinophilia and the number of polypoid lesions present in the mucosa of the sinuses).

Data items and summary measures

The selected articles were analyzed according to mice used (BALB/c or C57BL/6) and according to the drugs used to induce eosinophilic polyps (SEB, HDM, and AP) and its combinations (SEB + HDM, SEB + OVA, AP + OVA). The dosage of each drug and the stimulus duration were also assessed. The parameters used to assess the results were the number of eosinophils and polypoid lesions in the sinonasal mucosa at histology.

Local IRB was not requested, as this is a review article.

Results

From the selected articles, 11 induced ECRS with SEB^{10,14,37-45}; 1 with AP²⁴; 1 compared two models, the first induced with HDM and the latter with SEB²⁹; and 2 induced ECRS with SEB in transgenic animals.^{11,13} Additionally, in 5

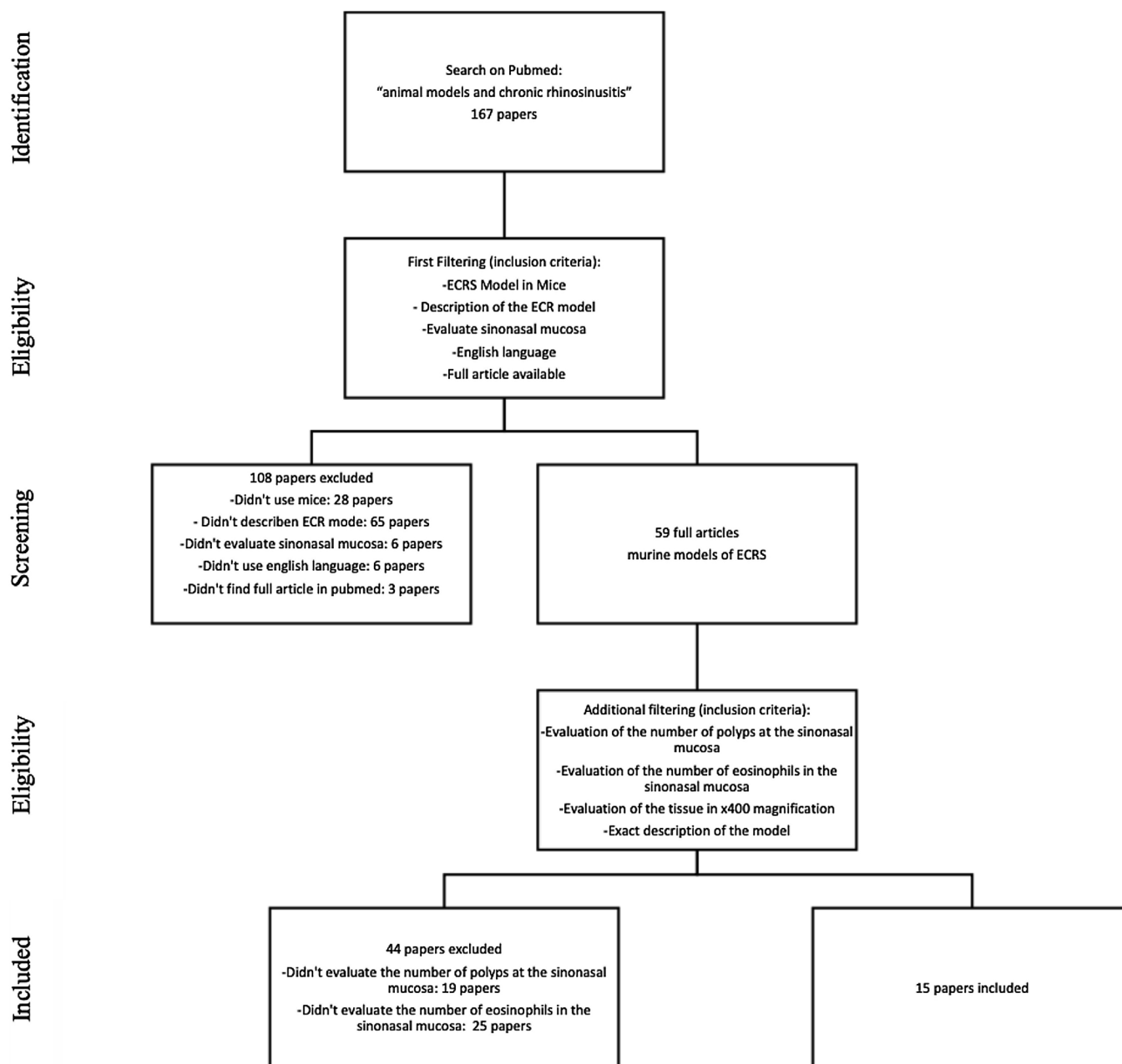


Figure 1 Flow of information through the different phases of the review.

articles, the models were used to assess different therapies to inhibit polyp formation (Resveratrol alone³⁷ or associated with Mucoadhesive Nanostructured Microparticles,¹⁴ Cyclosporine,⁴⁰ Chloroquine,⁴³ and Tofacitinib.⁴⁴

BALB/c mice were used in 13 experimental models (total number: 159 animals), whereas B57BL/6 were chosen in 8 (total number: 61 animals). All the animals were 4–5-week-old at the beginning of the experimental assays.

BALB/c animals

The same drug combination was used in all 13 experiment protocols with BALB/c mice: OVA was administered intraperitoneally, followed by intranasal OVA and SEB. Together, 159 mice were submitted to this protocol (Table 1).

The same dosage of IP OVA (25 µg) and the same concentration of intranasal OVA (3%) was used in all the protocols using BALB/c. Intranasal SEB dosage was defined to be 10 ng in 10 experimental groups (n = 106),^{14,37,38,40,41,43–46} 5 ng in two experimental groups (n = 30)^{10,39} and 500 ng in one study (n = 15).¹⁰ In 12^{10,14,37–41,43–46} of these experimental groups, the experiment lasted 103 days, while in a single experimental group,⁴¹ the mice were stimulated for 186 days.

In the groups that used a dose of 10 ng of SEB for 103 days,^{10,14,37–41,43–46} the number of polyps varied from 1 to 20 per field of ×400 magnification, and the eosinophil count at the tissue ranged from 20 to 200 per field. The decrease of SEB dosage to 5 ng did not considerably change the eosinophil count (which varied from 60 to 150 eosinophils per ×400 magnification field). However, it induced fewer

Table 1 Articles in which eosinophilic nasal polyps were induced with an experimental model in BALB/c mice. Model A, B, and C: Ovalbumin (OVA) was administered Intraperitoneally (IP) followed by Intranasal (IN) OVA and *Staphylococcus aureus* Enterotoxin B (SEB) for 103 days. Model D: Ovalbumin (OVA) was administered Intraperitoneally (IP) followed by Intranasal (IN) OVA and *Staphylococcus aureus* Enterotoxin B (SEB) for 186 days.

Article	Number of BALB/c mice	Protocol used (drug/dosage)	Time of experiment	Number of polyps/HPF	Number of Eosinophils in the tissue
Kim et al., 2011 ¹⁰ (Model A)	15	SEB: 5 ng (IN) OVA: IP: 25 µg and IN: 3%	103	0–4	150
Kim et al., 2011 ¹⁰ (Model B)	15	SEB: 500 ng (IN) OVA: IP: 25 µg and IN: 3%	103	0–3	80
Kim et al., 2013 ³⁹ (Model C)	20	SEB: 10 ng (IN) OVA: IP: 25 µg and IN: 3%	103	1–4	100–200
Lee et al., 2014 ^[47] (Model C)	8	SEB: 10 ng (IN) OVA: IP: 25 µg and IN: 3%	103	3	50
Hong et al., 2015 ⁴¹ (Model C)	15	SEB: 10 ng (IN) OVA: IP: 25 µg and IN: 3%	103	1–2	60–100
Shin et al. 2015 ⁴⁰ (Model C)	10	SEB: 10 ng (IN) OVA: IP: 25 µg and IN: 3%	103	3–4	40–60
Chang et al., 2015 ⁴² (Model C)	10	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	103	1–3	40–70
Kim et al., 2016 ⁴³ (Model C)	11	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	103	2–4	50
Kim et al., 2016 ⁴³ (Model D)	8	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	186	8–12	150
Lee et al., 2017 ¹⁴ (Model C)	7	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	103	4–8	150 ^a
Choi et al., 2020 ⁴⁵ (Model C)	20	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	103	5–20	40–70
Joo et al., 2021 ⁴⁶ (Model C)	10	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	103	2–4	80–150
Wee et al., 2021 ³⁸ (Model C)	10	SEB: 10 ng OVA: IP: 25 µg and IN: 3%	103	1–5	20–30

HPF, High Power Field.

^a Mean Value.

polyps (ranging from 0 to 4).¹⁰ The increase of SEB dosage to 500 ng did not affect the number of polyps (0–3 per field) or the eosinophil count (mean value of 80 eosinophils per magnification field).

One single article compared two groups of BALB/c mice using the combination of OVA and SEB as previously described, but with different times of experimentation (103 vs. 186 days).⁴¹ The authors observed that the increase in time of experimentation led to an increase in both variables (number of polyps: 2–4 vs. 8–12; mean eosinophil count 50 vs. 150; Table 1). A more robust inflammatory response was found in the more extended protocol (186 days) when compared to the shorter one (103 days). Nevertheless, the main tissue changes were already observed after 103 days of stimulation, and that is probably why the shorter protocol was preferred by most of the authors.

In BALB/c animals, the best protocol to induce eosinophilic polyps lasted 103 days, with an intranasal SEB dosage of 10 ng. As a result, this is the most frequent protocol used to induce eosinophilic nasal polyps.^{10,14,37–41,43–46}

C57BL/6 animals and the drugs SEB and OVA

C57BL/6 mice were stimulated with IP OVA, followed by IN OVA and SEB, in three experimental protocols. Together, 15 mice were submitted to this protocol (Table 2).

All three experiments with C57BL/6 used the same dosage of OVA intraperitoneally (25 µg). Intranasal drug concentration was: 3% of OVA and 20 ng of SEB in one experimental group (n = 5)¹³; and 6% of OVA and 10 ng of SEB in two other (total n = 10).^{11,29} In all three groups,^{11,13,29} the experiment lasted 103 days.

The increase in OVA led to an important eosinophil count at the tissue (which ranged from 30 to 150 cells per ×400 magnification field), but to a relatively low impact in polyp formation (varying from 0 to 2).^{11,29} In contrast, the increase in intranasal SEB to 20 ng significantly increased the eosinophil count (from 400 to 600 eosinophils per ×400 magnification field) and the number of polyps (ranging from 1 to 7).¹³

Table 2 Articles in which eosinophilic nasal polyps were induced with an experimental model in C57BL/6 mice. Model E and F: Ovalbumin (OVA) was administered Intraperitoneally (IP) followed by Intranasal (IN) OVA and *Staphylococcus aureus* Enterotoxin B (SEB) for 103 days.

Article	Number of C57BL/6 mice	Protocol used (drug/dosage/time)	Time of experiment	Number of polyps/HPF	Number of Eosinophils in the tissue
Kim et al., 2013 ¹¹ (Model E)	5	SEB: 10 ng OVA: IP: 25 µg and IN: 6%	103	0–2	30–150
Khalmuratova et al. 2016 ³⁰ (Model E)	5	SEB: 10 ng OVA: IP: 25 µg and IN: 6%	103	0.5–1.5	60 ^a
Bae et al., 2020 ¹³ (Model F)	5	SEB: 20ng OVA: IP: 25 µg, IN: 100 µg and 3%	103	1–7	400–600

HPF, High Power Field.

^a Mean Value.**Table 3** Articles in which eosinophilic nasal polyps were induced with an experimental model in C57BL/6 mice. Model G: House Dust Mice (HDM) was administered Intradermic (ID) followed by Intranasal (IN) for 103 days. Model H: House Dust Mice (HDM) was administered Intradermic (ID) followed by Intranasal (IN) HDM and *Staphylococcus aureus* Enterotoxin B (SEB) for 103 days.

Article	Number of C57BL6 mice	Protocol used (drug/dosage/time)	Time of experiment	Number of polyps/HPF	Number of Eosinophils in the tissue
Khalmuratova et al. 2016 ³⁰ (Model G)	5	HDM: 100 µg ID and 20 µg IN	103	0	40
Khalmuratova et al. 2016 ³⁰ (Model H)	5	SEB: 10 ng HDM: 100 µg ID and 20 µg IN	103	0.5–1.2	70

HPF, High Power Field.

C57BL/6 animals and the drugs HDM and SEB

One assay administered ID and IN HDM to induce eosinophilic polyps in C57BL/6 mice (n = 5), while IN SEB was associated in the other group to the protocol (n = 5). Ten mice were included in this study, and the experiment lasted 103 days (Table 3).

The same dosage of HDM intradermic (100 µg) and intranasal (20 µg) OVA was used,²⁹ and intranasal SEB dosage, applied in only one group, was defined to be 10 ng (n = 5).

As pointed out in Table 3, the use of only HDM induced a mean number of 40 eosinophils per ×400 magnification field, and no polyp was observed in this protocol. The association of IN SEB (10 ng) increased both the number of polyps (from 0.5 to 1.2) and the eosinophil count at the tissue (mean of 70 eosinophils per field).

Thus, the assay with only HDM could be considered a good protocol to study allergic rhinitis, but it failed to be a representative protocol to induce eosinophilic nasal polyps.

C57BL/6 animals and the drugs AP and OVA

One study induced eosinophilic polyps in C57BL/6 mice by using the same protocol with three different periods of stimulation: 53-, 67-, and 95-days.²⁴ During the assay, OVA (25 µg) was administered IP, followed by IN OVA (75 µg) and intranasal AP (2U). Together, 36 mice were submitted to this protocol (Table 4).

In the experimental group that lasted 53 days, no polyps were observed, and the eosinophil count at the tissue ranged from 125 to 150 per field. The increase of experimental stimulation to 67 days increased the number of polyps to 0.5–1.5 polyps per field and maintained the number of eosinophil counts (from 125 to 150 eosinophils per ×400 magnification field). In contrast, the experiment that lasted 95 days induced an increased number of polyps (from 2 to 3.5 polyps per ×400 magnification field) and maintained the number of eosinophils (from 100 to 125 eosinophils per ×400 magnification field).

Discussion

Animal models are especially important to study the pathophysiology of a specific disease (in this case, ECRS), and to evaluate the effect of possible new therapies. In this aspect, murine models of ECRS have advantages and disadvantages, like any other animal models. The major advantages of murine models are: 1) They are cheap; 2) They are easy to handle; and 3) Many reagents and antibodies are easily available. Transgenic or knockout mice, important for studies of the pathophysiological mechanisms of the disease, are also available.^{11,13}

In our review, the murine model most used involved BALB/c animals and the combination of IP and IN OVA with IN SEB.^{10,37–41,43–46} The OVA concentration remained constant in all experimental groups: IP: 25 µg and IN: 3%. The ideal SEB concentration to induce eosinophilic polyps was 10 ng. The reduction of SEB concentration to 5 ng consider-

Table 4 Articles in which eosinophilic nasal polyps were induced with an experimental model in C57BL/6 mice. Model I: Ovalbumin (OVA) was administered Intraperitoneally (IP) followed by Intranasal (IN) OVA and *Aspergillus oryzae* protease (AP) for 53 days. Model J: Ovalbumin (OVA) was administered Intraperitoneally (IP) followed by intranasal (IN) OVA and *Aspergillus oryzae* protease (AP) for 67 days. Model K: Ovalbumin (OVA) was administered Intraperitoneally (IP) followed by Intranasal (IN) OVA and *Aspergillus oryzae* Protease (AP) for 95 days.

Article	Number of C57BL6 mice	Protocol used (drug/dosage/time)	Time of experiment	Number of polyps/HPF	Number of Eosinophils in the tissue
Kim et al., 2017 ²⁴ (Model I)	12	AP: 2U OVA: IP: 25 µg and IN: 75 µg	53	0	125–150
Kim et al., 2017 ²⁴ (Model J)	12	AP: 2U OVA: IP: 25 µg and IN: 75 µg	67	0.5–1.5	125–150
Kim et al., 2017 ²⁴ (Model K)	12	AP: 2U OVA: IP: 25 µg and IN: 75 µg	95	2–3.5	100–125

HPF, High Power Field.

ably decreased polyp induction,¹⁰ whereas the increase to 500 ng did not change the number of eosinophilic polyps per field.¹⁰

The duration of the models that used BALB/c animals and the combination of IP and IN OVA with IN SEB ranged from 103 to 186 days.^{10,37–41,43–46} The duration of 186 days produced more robust eosinophilic polyps.⁴¹ It is important to note that the average lifespan of BALB/c mice ranges from 180 days to 365 days, and that mice would end the long-lasting protocol with around 214 days of age. With this, we conclude that the duration of the protocol of 103 days brings satisfactory results with lower chance of animal loss due to natural death.^{10,37–41,43–46}

Using the same protocol with IP and IN OVA and IN SEB, but with C57BL/6 mice, an increase of either the dose of SEB¹³ and/or OVA^{11,29} was necessary to achieve a significant number of eosinophilic polyps in sinonasal mucosa. One possible reason for this is that C57BL/6 mice have attenuated allergic airway hyperresponsiveness compared to BALB/c mice, which has been demonstrated for asthma models.⁸ To achieve the same polyp and eosinophil index, the SEB dose had to be adjusted to 20 ng¹³ or the OVA concentration to 6%.^{11,29} The increase of SEB seemed to be more efficient inducing ECRS in C57BL/6 mice than the increase in OVA.

Another model with C57BL/6 mice used ID and IN HDM either alone or combined with IN SEB.²⁹ HDM alone did not induce polyps but stimulated eosinophilia, whereas the combination of HDM with SEB induced polyps.²⁹ In summary, it seemed that the use of HDM alone can serve as a model for the study of allergic rhinitis, but not for nasal polyps. Moreover, the combination of HDM and SEB was not as efficient as OVA + SEB protocol to induce ECRS.^{10,37–41,43–46}

The combination of IN AP with IP and IN OVA was enough to produce polyps and eosinophilia,²⁴ but the assays that lasted 53 and 67 days failed to produce robust nasal polyps. As the duration of the experiment increased, more polyps were observed. Possibly, if authors used the time of 103 days, they might find the same number of polyps as the combination of OVA and SEB.

In summary, IP and IN OVA associated with IN SEB is the most used protocol in both BALB/c and C57BL/6 mice to induce ECRS, as it also seems to be the most efficient to produce both polyps and eosinophils at nasal tissue. The IP

and IN OVA, associated with IN AP model was also a good model to induce ECRS in C57BL/6 mice. In contrast, the combination of ID and IN HDM and IN SEB was the least efficient model to produce eosinophilic nasal polyps in mice.

Conclusion

IP and IN OVA associated with SEB seems to produce the most robust eosinophilic sinonasal inflammation, especially in BALB/c mice.

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Conflicts of interest

The authors declare no conflicts of interest.

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Nothing to add.

References

1. Deconde AS, Soler ZM. Chronic rhinosinusitis: epidemiology and burden of disease. *Am J Rhinol Allergy*. 2016;30:134–9.
2. Rouyar A, Classe M, Gorski R, Bock MD, Le-Guern J, Roche S, et al. Type 2/Th2-driven inflammation impairs olfactory sensory neurogenesis in mouse chronic rhinosinusitis model. *Allergy*. 2019;74:549–59.
3. Stevens WW, Schleimer RP, Kern RC. Chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol Pract*. 2016;4:565–72.
4. Wang X, Zhang N, Bo M, Holtappels G, Zheng M, Lou H, et al. Diversity of TH cytokine profiles in patients with chronic rhinosinusitis: a multicenter study in Europe, Asia, and Oceania. *J Allergy Clin Immunol*. 2016;138:1344–53.
5. Tomassen P, Vandeplass G, Van Zele T, Cardell LO, Arebro J, Olze H, et al. Inflammatory endotypes of chronic rhinosinusitis

- based on cluster analysis of biomarkers. *J Allergy Clin Immunol.* 2016;137:1449–56.e4.
6. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. *Rhinology.* 2020;58:1–464.
 7. Jiang N, Kern RC, Altman KW. Histopathological evaluation of chronic rhinosinusitis: a critical review. *Am J Rhinol Allergy.* 2013;27:396–402.
 8. Shin H. Animal models in CRS and pathophysiologic insights gained: a systematic review. *Laryngoscope Invest Otolaryngol.* 2016;1:116–23.
 9. 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;55:170–5.
 10. Kim DW, Khalmuratova R, Hur D, Jeon SY, Kim SW, Shin HW, 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, e-255-261.
 11. Kim SW, Kim JH, Jung MH, Hur DG, Lee HK, Jeon SY, et al. Periostin may play a protective role in the development of eosinophilic chronic rhinosinusitis with nasal polyps in a mouse model. *Laryngoscope.* 2013;123:1075–81.
 12. Lee M, Kim DW, Yoon H, So D, Khalmuratova R, Rhee CS, et al. Sirtuin 1 attenuates nasal polypogenesis by suppressing epithelial-to-mesenchymal transition. *J Allergy Clin Immunol.* 2016;137:87–98.e7.
 13. Bae JS, Ryu G, Kim JH, Kim EH, Rhee YH, Chung YJ, et al. Effects of Wnt signaling on epithelial to mesenchymal transition in chronic rhinosinusitis with nasal polyp. *Thorax.* 2020;75:982–93.
 14. Lee M, Park CG, Huh BK, Kim SN, Lee SH, Khalmuratova R, et al. Sinonasal delivery of resveratrol via mucoadhesive nanostructured microparticles in a nasal polyp mouse model. *Sci Rep.* 2017;7:40249.
 15. Khalmuratova R, Lee M, Park JW, Shin HW. Evaluation of neo-osteogenesis in eosinophilic chronic rhinosinusitis using a nasal polyp murine model. *Allergy Asthma Immunol Res.* 2020;12:306–21.
 16. Sautter NB, Delaney KL, Hausman FA, Trune D. Tissue remodeling gene expression in a murine model of chronic rhinosinusitis. *Laryngoscope.* 2012;122:711–7.
 17. Tao Y, Yuan T, Li X, Yang S, Zhang F, Shi L. Bacterial extract OM-85 BV protects mice against experimental chronic rhinosinusitis. *Int J Clin Exp Pathol.* 2015;8:6800–6.
 18. Mulligan JK, Pasquini WN, Carroll WW, Williamson T, Reaves N, Patel KJ, et al. Dietary vitamin D3 deficiency exacerbates sinonasal inflammation and alters local 25 (OH)D3 metabolism. *PLoS One.* 2017;12:e0186374.
 19. Alt JA, Lee WY, Davis BM, Savage JR, Kennedy TP, Prestwich GD, et al. A synthetic glycosaminoglycan reduces sinonasal inflammation in a murine model of chronic rhinosinusitis. *PLoS One.* 2018;25:e0204709.
 20. Selvaraj S, Liu K, Robinson AM, Epstein VA, Conley DB, Kern RC, et al. In vivo determination of mouse olfactory mucus cation concentrations in normal and inflammatory states. *PLoS One.* 2012;7:e39600.
 21. 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;28:465–70.
 22. Nam YR, Lee KJ, Lee H, Joo CH. CXCL10 production induced by high levels of IKK ϵ in nasal airway epithelial cells in the setting of chronic inflammation. *Biochem Biophys Res Commun.* 2019;514:607–12.
 23. Ren X, Wang Z. High chemokine ligand 11 levels in nasal lavage fluid: a potential predictor of and therapeutic target for murine eosinophilic chronic rhinosinusitis. *Life Sci.* 2021;271:119218.
 24. Kim HC, Lim JY, Kim S, Kim JH, Jang YJ. Development of a mouse model of eosinophilic chronic rhinosinusitis with nasal polyp by nasal instillation of an Aspergillus protease and ovalbumin. *Eur Arch Otorhinolaryngol.* 2017;274:3899–906.
 25. Jang YJ, Lim JY, Kim S, Lee YL, Kweon MN, Kim JH. Enhanced interferon- β response contributes to eosinophilic chronic rhinosinusitis. *Front Immunol.* 2018;9:2330.
 26. Zhang L, Jiang LL, Cao ZW. Interleukin-33 promotes the inflammatory reaction in chronic rhinosinusitis with nasal polyps by NF- κ B signaling pathway. *Eur Rev Med Pharmacol Sci.* 2017;21:4501–8.
 27. Morimoto Y, Hirahara K, Kiuchi M, Wada T, Ichikawa T, Kanno T, et al. Amphiregulin-producing pathogenic memory T Helper 2 cells instruct eosinophils to secrete osteopontin and facilitate airway fibrosis. *Immunity.* 2018;49:134–50.e6.
 28. Lee M, Lim S, Kim YS, Khalmuratova R, Shin SH, Kim I, et al. DEP-induced ZEB2 promotes nasal polyp formation via epithelial-to-mesenchymal transition. *J Allergy Clin Immunol.* 2022;149:340–57.
 29. 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.* 2016;44:66–75.
 30. Park SC, Kim SI, Hwang CS, Cho HJ, Yoon JH, Kim CH. Multiple airborne allergen-induced eosinophilic chronic rhinosinusitis murine model. *Eur Arch Otorhinolaryngol.* 2019;276:2273–82.
 31. Lee SB, Song JA, Choi GE, Kim HS, Jang YJ. Rhinovirus infection in murine chronic allergic rhinosinusitis model. *Int Forum Allergy Rhinol.* 2016;6:1131–8.
 32. Tharakan A, Dobzanski A, London NR, Khalil SM, Surya N, Lane AP, et al. Characterization of a novel, papain-inducible murine model of eosinophilic rhinosinusitis. *Int Forum Allergy Rhinol.* 2018;8:513–21.
 33. Ramanathan M Jr, Tharakan A, Sidhaye VK, Lane AP, Biswal S, London NR Jr. Disruption of sinonasal epithelial Nrf2 enhances susceptibility to Rhinosinusitis in a mouse model. *Laryngoscope.* 2021;131:713–9.
 34. He F, Liu H, Luo W. The PI3K-Akt-HIF-1 α pathway reducing nasal airway inflammation and remodeling in nasal polyposis. *Ear Nose Throat J.* 2021;100:NP43–49.
 35. Wang S, Zhang H, Xi Z, Huang J, Nie J, Zhou B, et al. Establishment of a mouse model of lipopolysaccharide-induced neutrophilic nasal polyps. *Exp Ther Med.* 2017;14:5275–82.
 36. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* 2009;6:e1000100.
 37. Kim SW, Kim DW, Khalmuratova R, Kim JH, Jung MH, Chang DY, et al. Resveratrol prevents development of eosinophilic rhinosinusitis with nasal polyps in a mouse model. *Allergy.* 2013;68:862–9.
 38. Shin HW, Kim DK, Park MH, Eun KM, Lee M, So D, et al. IL-25 as a novel therapeutic target in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol.* 2015;135:1476–85.e7.
 39. Hong SL, Zhang YL, Kim SW, Kim DW, Cho SH, Chang YS, et al. Interleukin-17A-induced inflammation does not influence the development of nasal polyps in murine model. *Int Forum Allergy Rhinol.* 2015;5:363–70.
 40. Chang DY, Joo YH, Kim SJ, Kim JH, Jung MH, Kim DW, et al. Therapeutic effects of intranasal cyclosporine for eosinophilic rhinosinusitis with nasal polyps in a mouse model. *Am J Rhinol Allergy.* 2015;29:e29–32.
 41. 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;126:E265–272.

42. Lee M, Kim DW, Khalmuratova R, Shin SH, Kim YM, Han DH, et al. The IFN- γ -p38, ERK kinase axis exacerbates neutrophilic chronic rhinosinusitis by inducing the epithelial-to-mesenchymal transition. *Mucosal Immunol.* 2019;12:601–11.
43. Choi MR, Xu J, Lee S, Yeon SH, Park SK, Rha KS, et al. Chloroquine treatment suppresses mucosal inflammation in a mouse model of eosinophilic chronic rhinosinusitis. *Allergy Asthma Immunol Res.* 2020;12:994–1011.
44. Joo YH, Cho HJ, Jeon YJ, Kim JH, Jung MH, Jeon SY, et al. Therapeutic effects of intranasal tofacitinib on chronic rhinosinusitis with nasal polyps in mice. *Laryngoscope.* 2021;131:E1400–1407.
45. Wee JH, Ko YK, Khalmuratova R, Shin HW, Kim DW, Rhee CS. Effect of lipopolysaccharide and polyinosinic:polycytidylic acid in a murine model of nasal polyp. *Sci Rep.* 2021;11:1021.
46. Lee K Il, Kim DW, Kim EH, Kim JH, Samivel R, Kwon JE, et al. Cigarette smoke promotes eosinophilic inflammation, airway remodeling, and nasal polyps in a murine polyp model. *Am J Rhinol Allergy.* 2014;28:208–14.