

A novel model of invasive fungal rhinosinusitis in rats

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ABSTRACT

Background: Invasive fungal rhinosinusitis (IFRS) is a life-threatening inflammatory disease that affects immunocompromised patients, but animal models of the disease are scarce. This study aimed to develop an IFRS model in neutropenic rats.

Methods: The model was established in three consecutive steps: unilateral nasal obstruction with Merocel sponges, followed by administration of cyclophosphamide (CPA), and, finally, nasal inoculation with *Aspergillus fumigatus*. Fifty healthy Wistar rats were randomly divided into five groups, with group I as the controls, group II undergoing unilateral nasal obstruction alone, group III undergoing nasal obstruction with fungal inoculation, group IV undergoing nasal obstruction with administration of CPA, and group V undergoing nasal obstruction with administration of CPA and fungal inoculation. Hematology, histology, and mycology investigations were performed.

Results: The changes in the rat absolute neutrophil counts (ANCs) were statistically different across the groups. The administration of CPA decreased the ANCs, whereas nasal obstruction with fungal inoculation increased the ANCs, and nasal obstruction did not change them. Histological examination of the rats in group V revealed the hyphal invasion of sinus mucosa and bone, thrombosis, and tissue infarction. No pathology indicative of IFRS was observed in the remaining groups. Positive rates of fungal culture in tissue homogenates from the maxillary sinus (62.5%) and lung (25%) were found in group V, whereas groups I, II, III, and IV showed no fungal culture in the homogenates.

Conclusion: A rat IFRS model was successfully developed through nasal obstruction, CPA-induced neutropenia, and fungal inoculation. The disease model closely mimics the pathophysiology of anthropic IFRS.

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Invasive fungal rhinosinusitis (IFRS) is a serious disease with a high mortality rate that has increased over the last 10 years because of the growing number of immunocompromised patients.^{1–3} To explore the pathogenesis of and treatment strategies for IFRS, a well-documented animal model is needed to address questions concerning fungal infections and to further our understanding of the occurrence, progression, control, and elimination of fungal infections.⁴

Animal experiments in the study of fungal sinus disease date back to the last century,⁵ when several studies on noninvasive fungal sinusitis were conducted. Dufour *et al.*⁶ established an experimental noninvasive fungal sinusitis model in rabbits caused by *Aspergillus fumigatus*. Byung *et al.*⁷ developed a mouse model of *Aspergillus*- and *Alternaria*-induced noninvasive fungal rhinosinusitis. Boase *et al.*⁸ reported a sheep model and found that significant fungal biofilm occurred when *Staphylococcus aureus* and a fungus were the co-inoculums. However, to our knowledge, the only model of IFRS is that developed by Rodriguez *et al.*⁹ through the depletion of neutrophils using anti-Gr-1 monoclonal antibody and the administration of *A. fumigatus* intranasally (although this is not the only available method). For IFRS, animal models that mimic the pathophysiology of infection are necessary but scarce. We thus attempted to develop a novel IFRS model in rats to meet this research need.

The IFRS rat model is based on studies on the development of rat models for bacterial rhinosinusitis and invasive fungal pneumonia.^{10,11}

METHODS

Animals and Strains

Fifty healthy female Wistar rats weighing 250.3 ± 14.1 g were obtained from the Animal Breeding Center of Shanxi Medical University. They were 10–12 weeks old and were allowed free access to food and water. The rats were housed in groups of five animals per cage, and were monitored three times daily after the intervention. All of the animal experiments were conducted in compliance with national and local regulations and institutional ethical guidelines on the humane use of animal research.

A. fumigatus (AF293 wild strain from the Fungi and Fungal Diseases Research Center of Peking University) was used in the study. The *A. fumigatus* was grown on Sabouraud agar at 37°C for 7 days, after which the conidia were harvested and counted with a hemocytometer. Centrifugation was performed and the conidia were resuspended in normal saline and filtered through sterile gauze to obtain a 10^7 conidia/mL suspension.

Study Groups and Interventions

Table 1 and Fig. 1 show the interventions for the study groups. The model was established in three consecutive steps: unilateral nasal obstruction with Merocel sponges (Medtronic Xomed, Jacksonville, FL), followed by the administration of cyclophosphamide (CPA), and, finally, nasal inoculation with *A. fumigatus*. The 50 rats were randomly divided into 5 groups of 10.

On the 1st day, the synthetic sponge (Merocel) was cut into $1.5 \times 1.5 \times 10$ -mm sticks and shaped. The rats were anesthetized by the i.p. injection of 10% chloral hydrate at 3 mL/kg and a piece of the sponge was inserted into the left nasal cavity of each rat in groups II, III, IV, and V.

On the 6th day, the rats in groups IV and V were given an i.p. injection of CPA (Sigma-Aldrich, St. Louis, MO) at a dose of 90 mg/kg. Subsequent injections of CPA at a dose of 60 mg/kg were then given every 4 days for a total of 3 doses.¹²

On the 11th day, the rats in groups III and V were anesthetized by inhalation of ether, and 50 μ L of the *A. fumigatus* suspension was slowly administered into the left nasal cavity. All of the rats fully recovered within 1 minute of the procedure. The rats were then inoculated with *A. fumigatus* conidia suspension every day for a week.

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Table 1 Study groups and intervention

Group	Intervention
I	Without an intervention
II	Unilateral nasal obstruction alone
III	Nasal obstruction with fungal inoculation
IV	Nasal obstruction with administration of CPA
V	Nasal obstruction with administration of CPA and fungal inoculation

CPA = cyclophosphamide.

On the 18th day, all of the surviving rats were killed under anesthesia with 100 mg/kg of ketamine administered i.p.

Evaluation

Rat Hematology. For the blood count analysis, whole blood samples (0.5 mL) were collected from all of the rats on the 1st, 6th, 10th, 14th, and 18th days. The blood counts were analyzed (in duplicate) with an MS4s 5-part differential automatic hematology system (Melet Schloesing Laboratories, Osny, France).

Histological Evaluation. The animals' snouts and the anterior margin of the intraocular plane defined the limits of sectioning. Slides were stained with hematoxylin-eosin for regular visualization, with periodic acid-Schiff stain for visualization of the fungi.

Fungal Cultures. The tissues of interest in this experiment were the nasal sinus and lung tissue. The tissues to be used for the fungal culture were removed using a sterile technique and mechanically homogenized in sterile saline. The homogenates were cultured on Sabouraud dextrose agar plates for 7 days at 37°C. Visible colonies on the plates were identified morphologically to determine whether the tissue had a fungal burden.

Statistical Analysis

Statistical analyses were performed using SPSS 13.0 (SPSS, Inc., Chicago, IL). Comparisons across interventions were evaluated using repeated measures analysis of variance (REP ANOVA). Statistical significance was taken to be $p < 0.05$.

RESULTS

General Observation

Compared with the control rats, the rats with nasal blockage showed increased frequency of sneezing and nasal rubbing. From the 6th day onward, the rats injected with CPA (groups IV and V) exhibited dull fur, reduced food and water intake, and an emaciated appearance. The rats (groups III and V) inoculated with *A. fumigatus* from day 11 displayed bloody nasal discharge. In group V, one of the rats had symptoms of cerebral infarction, mainly hemiplegia of left forelimb, and another two showed shortness of breath and cyanosis. During the experiment period, two rats in group V died.

Table 2 and Fig. 2 show the changes in rat body weight over time. The data were analyzed with REP ANOVA, and the results showed that the body weight changed over time but that the trends differed between the five groups ($F = 6.179$; $p < 0.01$). Multiple comparisons between the groups indicated statistically significant differences in the body weights in groups IV and V compared with groups I, II, or III ($p < 0.05$). The remaining group's pair wise comparisons did not reveal any differences. These results indicated that neither nasal obstruction nor nasal obstruction with fungal inoculation had a significant effect on the body weight of the rats but that administration of CPA did lead to body weight loss.

Absolute Neutrophil Count

The rat absolute neutrophil counts (ANCs) changed over time in the groups (see Table 3 and Fig. 3). The data were analyzed with REP ANOVA, and the results showed that the rat ANCs changed over time with different trends in the five groups ($F = 53.29$; $p < 0.01$). Multiple comparisons between the groups revealed no differences in rat ANCs between groups I and II or between groups IV and V. There were statistically significant differences in the rest of group pair wise comparisons. The results indicated that nasal obstruction had no significant effect on the rat ANCs, but that the administration of CPA greatly decreased the rat ANCs and nasal obstruction with fungal inoculation caused the ANCs to increase when CPA was not administered.

Histological Examination

The histological analyses were confined to the maxillary sinuses and the areas of the nasal cavity nearest the sinuses. In groups II and IV, there were edema and sporadic lymphocytes in the connective tissue, without epithelial erosions. In group III, *Aspergillus*-exposed rats had no pathological indications of IFRS, although epithelial erosions and infiltration of inflammatory cells in the subepithelial space were observed.

However, the rats in group V had hyphal masses with lesions centered predominantly in the left-side maxillary sinus cavities. The nasal mucosa contained a large number of fungal components, which caused fungal vasculitis, vascular embolism, hemorrhage, and coagulative necrosis. Simultaneously, bone resorption (caused by the direct intrusion of hyphal masses) was observed without obvious infiltration of the inflammatory cells or osteoclast cells near seriously necrotic tissues (Fig. 4). The hyphae were red and bamboo shaped with acute-angled branches, which are morphological features of *A. fumigatus*. Large numbers of fungal components and pyogenic granulomas were also seen in the pulmonary tissues of two rats in group V (Fig. 5). In one rat in group V, fungi lumped in the cerebrum, causing hemorrhage and coagulative necrosis (Fig. 6).

Fungal Cultures

After culturing the samples at 37°C for 7 days, *A. fumigatus* appeared as grayish-green colonies on the Sabouraud dextrose agar plates. The positive rates of this fungus in the tissue homogenate cultures from the nasal sinus and lung in group V were 62.5 and 25.0%, respectively, whereas all of the cultures from groups I, II, III, and IV showed negative results.

DISCUSSION

Using the protocol shown in Fig. 1, we successfully established an IFRS model. Because repeatability and mimicry are key features of a successful animal model, we suggest that four basic issues encountered in our study may be helpful in standardizing an animal model of IFRS: (1) the initiation of nasal obstruction by Merocel sponges; (2) neutropenia induced by the systemic use of CPA; (3) the intranasal inoculation of *A. fumigatus*, and (4) establishing suitable criteria for a standard repeatable animal model.

Nasal Obstruction by Merocel Sponges

In human beings, blockage of the sinonasal osteomeatal complex is thought to impair sinus drainage, reduce ventilation, and thus generate an acute inflammatory response. Jacob *et al.*¹³ reported the successful establishment of a rhinosinusitis model in mice by giving both the infecting organism and the obstruction of the sinus drainage (Merocel sponge). Liang *et al.*¹⁴ similarly developed a rabbit model of chronic rhinosinusitis using Merocel sponges. In our study, nasal obstruction had no effect on the body weight or ANCs of the rats, but

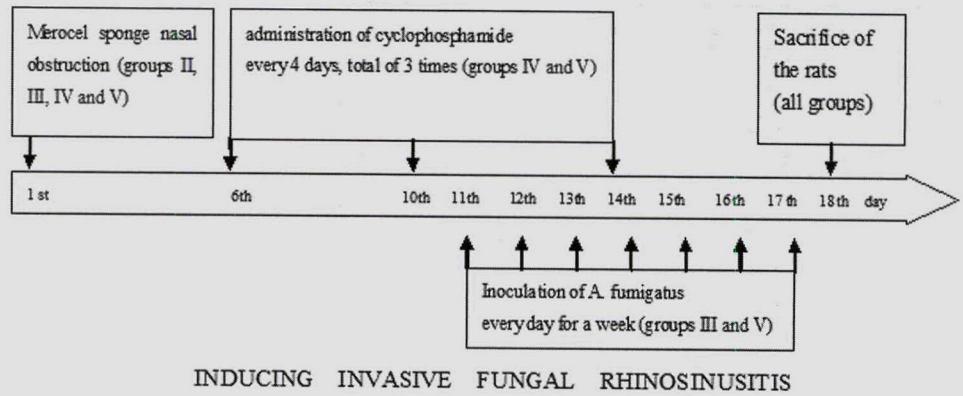


Figure 1. Procedures for developing the model.

Table 2 Changes of rat body weight in groups (g)

Group	n	1 Day	6 Days	10 Days	14 Days	18 Days
I	10	252.6 ± 13.23	260.7 ± 12.50	266.6 ± 12.39	271.9 ± 14.59	276.3 ± 14.36
II	10	249.7 ± 15.46	254.5 ± 13.83	257.6 ± 13.53	264.7 ± 16.57	269.6 ± 14.42
III	10	248.3 ± 13.08	252.7 ± 12.59	257.7 ± 12.75	260.5 ± 13.60	266.5 ± 13.41
IV	10	245.8 ± 15.04	251.9 ± 14.94	248.6 ± 14.27	233.4 ± 16.13	218.9 ± 17.49
V	8	253.0 ± 16.03	256.8 ± 14.02	253.9 ± 16.27	236.5 ± 14.82	215.3 ± 17.27

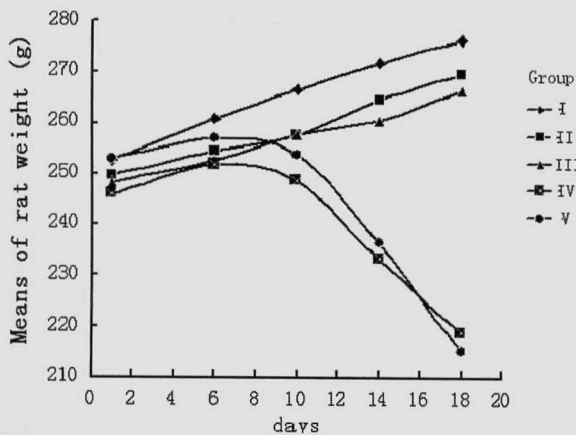


Figure 2. Changes in rat body weight across groups (g). In groups I, II, and III, the rat body weight showed a gradually increasing trend throughout the course of the experiment. The rats in groups II and III underwent nasal obstruction or nasal obstruction with fungal inoculation. The rats in groups IV and V showed weight gain before the additional administration of cyclophosphamide (CPA) on the 6th day, followed by a steady decline in weight thereafter.

did induce edema in local tissue. Collectively, these results support the use of Merocel sponges to develop expected sinusitis in sequence.

Nasal obstruction by Merocel sponges closely mimics the natural course of anthropic IFRS (without surgical damage to local bone or mucosa integrity). Furthermore, the technique is easy to manipulate, which helps with the standardization of the method.

Neutropenia Induced by CPA

CPA is a nitrogen mustard alkylating agent. It has a bimodal effect on the immune system, depending on the dose and schedule of administration.^{15,16} High doses of CPA (which are required for effective chemotherapy) may lead to immunosuppression, and especially the suppression of bone marrow, resulting in decreased production of all types of blood cells made from bone marrow and to conditions such as neutropenia and anemia.^{17,18} However, low doses of CPA

cause the inhibition of CD4⁺25⁺T regulatory cell function, which can lead to enhanced immune responses against a variety of antigens.¹⁹

To establish IFRS animal models with CPA, two critical factors—dose and time—must be established. Other scholars have tried and failed to use CPA to develop animal models of IFRS.⁹ These failures may be related to an inappropriate dose, schedule or both. During our pilot study, we selected the CPA dose in accordance with the changes in ANC. Prolonged severe neutropenia (<500 cells/mm³ consecutively for >7 days) is a risk factor for developing IFRS.^{20,21} The results of our study show that the ANCs of all of the rats injected with CPA decreased soon after the injection and was maintained at <500 cells/mm³ until the 18th day. Thus, an appropriate dose of CPA in accordance with the ANCs is critical for the successful development of an IFRS model in rats.

The model of invasive fungal pneumonia requires the administration of CPA before the nasal inhalation of *Aspergillus* spores.¹¹ However, in IFRS, nasal packing is also required. There is little research on whether nasal packing or the i.p. injection of CPA should come first. In our experience, CPA should be administered after the nasal blockage has been initiated, because the Merocel nasal obstruction often triggered sinusitis on the obstructed side over the 3 days and provided local lesions for invasive fungal infection in the immunocompromised host.^{13,22} The rats with neutropenia also suffered adverse drug reactions of CPA, including body weight loss and increased mortality,²³ and could not bear the effects of nasal packing under general anesthesia. An appropriate schedule of CPA administration is another critical factor in the successful development of an IFRS model.

Intranasal Fungal Inoculation and Detection

In general, *Aspergillus* is reported to be the main causative organism of fungal sinusitis.^{24,25} Indeed, *A. fumigatus* is one of the most common *Aspergillus* species causing disease. An estimated several hundred of its spores are inhaled by an individual each day. Typically, these spores are quickly eliminated by the immune system in healthy individuals. However, in immunocompromised individuals, *A. fumigatus* can cause aspergillosis, including IFRS via sporulation, hyphal growth, overrunning of the host's weakened defenses, and invasion of the nasal sinus mucosa. In this study, *A. fumigatus* was used to induce fungal inflammation and was inoculated in the rats every

Table 3 Changes of rat ANC in groups (cell/mm³)

Group	n	1 Day	6 Days	10 Days	14 Days	18 Days
I	10	2762 ± 563.2	2694 ± 808.2	2768 ± 790.3	2741 ± 706.0	2427 ± 791.3
II	10	2575 ± 447.7	3084 ± 902.6	3115 ± 959.6	2989 ± 781.7	2844 ± 995.1
III	10	2976 ± 443.9	3215 ± 816.5	3140 ± 903.2	4735 ± 1207	5666 ± 1297
IV	10	2832 ± 292.6	3102 ± 676.1	344.0 ± 101.3	275.0 ± 89.23	302.0 ± 98.30
V	8	3093 ± 701.3	3166 ± 872.6	343.8 ± 100.8	263.8 ± 82.28	263.8 ± 91.33

ANC = absolute neutrophil count.

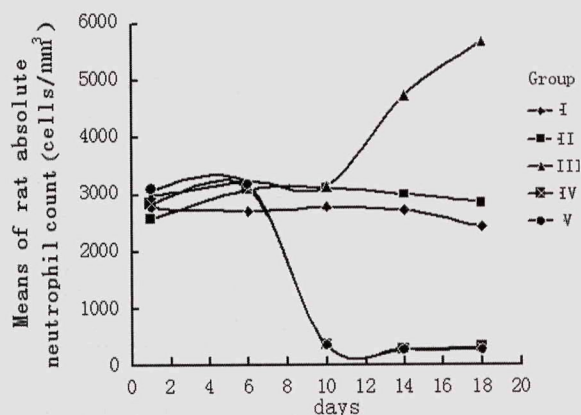


Figure 3. Changes in the rat absolute neutrophil counts (ANCs) across groups (cell/mm³). In groups I and II the rat ANC were relatively stable throughout the course of the experiment. In group III the ANCs were stable from days 1 to 10 when the rats had nasal obstruction only, but increased from the 11th day when the rats underwent additional fungal inoculation. In groups IV and V, the rat ANCs were stable until the 6th day when cyclophosphamide (CPA) was administered and rapidly dropped below 500 cells/mm³ thereafter.

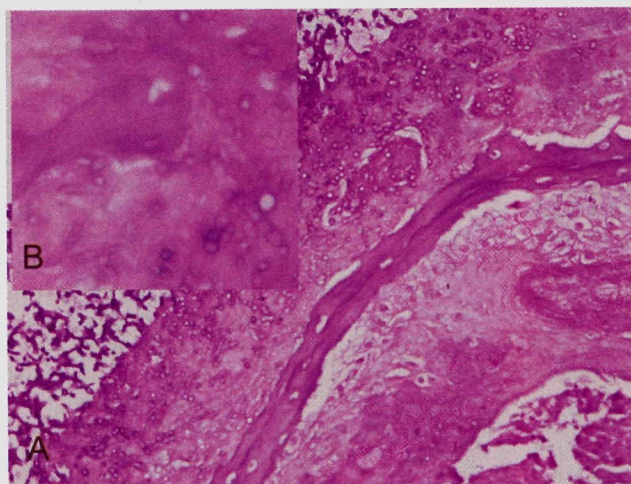


Figure 4. (A) Hyphal invasion of the sinus mucosa, with tissue infarction (periodic acid-Schiff, ×400). (B) Bone resorption caused by the intrusion of hyphal masses (periodic acid-Schiff, ×1000).

day for a week to continuously enhance its virulence. A similar procedure was used by Byung *et al.*⁷ in a noninvasive fungal rhinosinusitis model. The rats in group V with persistent *Aspergillus* exposure under CPA-induced neutropenia had pathology indicative of IFRS.

It has been reported that nasal lavages (rather than standard swab techniques) can be used to obtain specimens for fungal

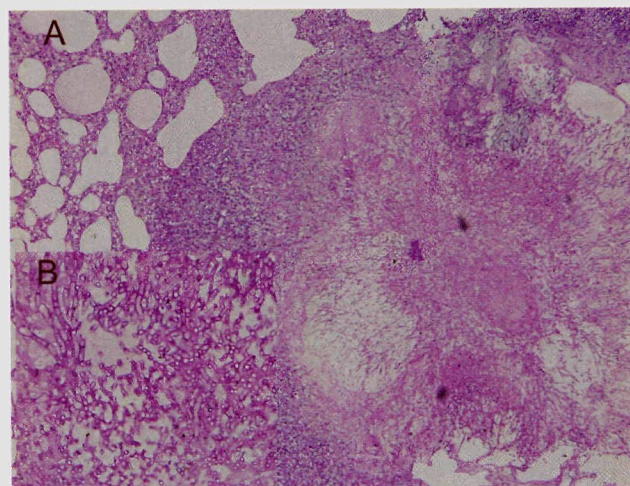


Figure 5. (A) abundant fungal components and pyogenic granulomas in pulmonary tissues (hematoxylin-eosin, ×100). (B) Hyphal masses distributed in necrotic tissues. The hyphae are red and bamboo shaped, with acute-angled branches (periodic acid-Schiff, ×400).

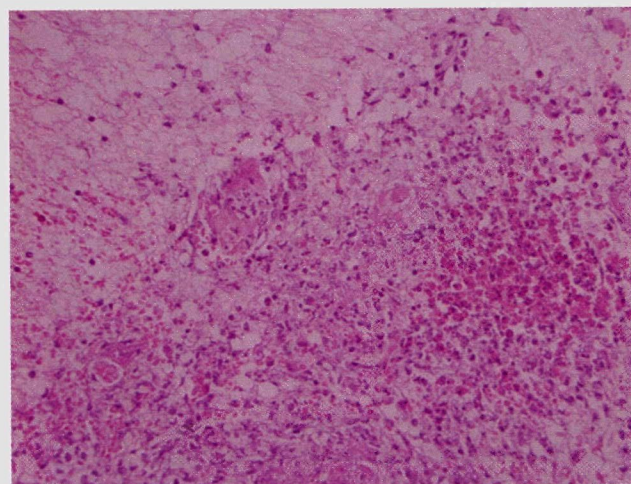


Figure 6. Fungi lumped in the cerebrum with hemorrhage and coagulative necrosis (hematoxylin-eosin, ×400).

detection. This modification of the sample acquisition process was reported to yield positive culture results in 50–96% of patients with chronic rhinosinusitis.²⁶ The rate of fungus detection is usually influenced by the detection techniques used. However, patients with chronic sinusitis who only yield positive sinus cultures (but have no organisms visualized histologically) are not universally regarded as having IFRS.²⁷ In invasive pulmonary aspergillosis studies, tissue homogenates rather than lavages are often used for

fungal detection, and thus positive results indicate that the fungi has invaded the tissue.^{28,29}

Based on these previous studies, our study used tissue homogenates to obtain specimens to test fungal invasion. The positive rates of fungal culture in the tissue homogenates from the maxillary sinus and lung in group V were 62.5 and 25%, respectively, whereas the remaining groups showed negative results. The positive rates of fungal culture are relatively lower than those obtained from nasal lavages. Five rats in group V had fungi-invaded sinus tissue and two rats had fungi-invaded lung tissue. However, the rats in the remaining groups could not be determined whether fungi had invaded sinus and lung tissue.

Criteria for a Standard IFRS Animal Model in Rats

The clinical diagnosis of IFRS is based on the patient's symptoms, immune status, physical examination (including nasal endoscopy), sinus radiology, histopathological findings, and mycological study.^{30–32} According to the consensus on the diagnosis of invasive fungal infections in immunocompromised patients established by the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group of the National Institute of Allergy and Infectious Diseases in 2008,³³ proven IFRS is defined by the presence of fungi-associated tissue damage on the histopathological examination of a biopsy specimen or the positive culture result (consistent with infection) from a sample obtained aseptically from a clinically or radiologically abnormal site.

With reference to the standards for the clinical diagnosis of IFRS and definition of proven IFRS according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group of the National Institute of Allergy and Infectious Diseases criteria,³³ the following parameters were used in our study to determine whether the model was successful: general response (body weight), immune status (ANCs), and histological and mycological examination. Collectively, our results show that five of the eight rats in group V showed signs that accorded with the IFRS model criteria. Two of the rats in group V suffered simultaneously from invasive fungal pneumonia, and another developed rhinocerebral mycosis as ascertained by histology.

CONCLUSION

Based on a pilot study, we successfully developed a repeatable rat model of IFRS by initiating nasal blockage with Merocel sponges, followed by the CPA-induced neutropenia and the intranasal inoculation of *A. fumigatus*. Histology and mycology proved that fungal invasion occurs with this model. The model closely mimics the pathophysiology of anthropic IFRS and should thus be of great value in future studies.

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REFERENCES

- Kasapoglu F, Coskun H, Ozmen OA, et al. Acute invasive fungal rhinosinusitis: Evaluation of 26 patients treated with endonasal or open surgical procedures. *Otolaryngol Head Neck Surg* 143:614–620, 2010.
- Li Y, Li P, and Zhang G. Diagnosis and endoscopic surgery of chronic invasive fungal rhinosinusitis. *Am J Rhinol Allergy* 23: 622–625, 2009.
- Thompson GR III, and Patterson TF. Fungal disease of the nose and paranasal sinuses. *J Allergy Clin Immunol* 129:321–326, 2012.
- Capilla J, Clemons KV, and Stevens DA. Animal models: An important tool in mycology. *Med Mycol* 45:657–684, 2007.
- Chakrabarti A, Jatana M, and Sharma SC. Rabbit as an animal model of paranasal sinus mycoses. *J Med Vet Mycol* 35:295–297, 1997.

- Dufour X, Kauffmann-Lacroix C, Goujon JM, et al. Experimental model of fungal sinusitis: A pilot study in rabbits. *Ann Otol Rhinol Laryngol* 114:167–172, 2005.
- Ahn BH, Park YH, and Shin SH. Mouse model of *Aspergillus* and *Alternaria* induced rhinosinusitis. *Auris Nasus Larynx* 36:422–426, 2009.
- Boase S, Valentine R, Singhal D, et al. A sheep model to investigate the role of fungal biofilms in sinusitis: Fungal and bacterial synergy. *Int Forum Allergy Rhinol* 1:340–347, 2011.
- Rodriguez TE, Falkowski NR, Harkema JR, et al. Role of neutrophils in preventing and resolving acute fungal sinusitis. *Infect Immun* 75:5663–5668, 2007.
- Cingi C, Esikizmir G, Burukoglu D, et al. The histopathological effect of thymoquinone on experimentally induced rhinosinusitis in rats. *Am J Rhinol Allergy* 25:e268–e272, 2011.
- Zhao Y, Park S, Warn P, et al. Detection of *Aspergillus fumigatus* in a rat model of invasive pulmonary aspergillosis by real-time nucleic acid sequence-based amplification. *J Clin Microbiol* 48:1378–1383, 2010.
- Van de Sande WW, van Vianen W, ten Kate MT, et al. Caspofungin prolongs survival of transiently neutropenic rats with advanced-stage invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 52:1345–1350, 2008.
- Jacob A, Faddis BT, and Chole RA. Chronic bacterial rhinosinusitis: Description of a mouse model. *Arch Otolaryngol Head Neck Surg* 127:657–664, 2001.
- Liang KL, Jiang RS, Wang J, et al. Developing a rabbit model of rhinogenic chronic rhinosinusitis. *Laryngoscope* 118:1076–1081, 2008.
- Matar P, Rozados VR, Gervasoni SI, et al. Th2/Th1 switch induced by a single low dose of cyclophosphamide in a rat metastatic lymphoma model. *Cancer Immunol Immunother* 50:588–596, 2002.
- Su YC, Rolph MS, Cooley MA, et al. Cyclophosphamide augments inflammation by reducing immunosuppression in a mouse model of allergic airway disease. *J Allergy Clin Immunol* 117:635–641, 2006.
- Soong D, Haj R, Leung MG, et al. High rate of febrile neutropenia in patients with operable breast cancer receiving docetaxel and cyclophosphamide. *J Clin Oncol* 27:e101–e102, 2009.
- Nagasaki Y, Eriguchi Y, Uchida Y, et al. Combination therapy with micafungin and amphotericin B for invasive pulmonary aspergillosis in an immunocompromised mouse model. *J Antimicrob Chemother* 64:379–382, 2009.
- Lutsiak ME, Semnani RT, De Pascalis R, et al. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood* 105:2862–2868, 2005.
- DelGaudio JM, and Clemson LA. An early detection protocol for invasive fungal sinusitis in neutropenic patients successfully reduces extent of disease at presentation and long term morbidity. *Laryngoscope* 119:180–183, 2009.
- Ruhnke M, Bohme A, Buchheidt D, et al. Diagnosis of invasive fungal infections in hematology and oncology—Guidelines from the Infectious Diseases Working Party in Haematology and Oncology of the German Society for Haematology and Oncology (AGIHO). *Ann Oncol* 23:823–833, 2012.
- Fernandez IJ, Stanzani M, Tolomelli G, et al. Sinonasal risk factors for the development of invasive fungal sinusitis in hematological patients: Are they important? *Allergy Rhinol (Providence)* 2:6–11, 2011.
- Long JM, Lee GD, Kelley-Bell B, et al. Preserved learning and memory following 5-fluorouracil and cyclophosphamide treatment in rats. *Pharmacol Biochem Behav* 100:205–211, 2011.
- Montone KT, LiVolsi VA, Lanza DC, et al. In situ hybridization for specific fungal organisms in acute invasive fungal rhinosinusitis. *Am J Clin Pathol* 135:190–199, 2011.
- Challa S, Uppin SG, Hanumanthu S, et al. Fungal rhinosinusitis: A clinicopathological study from South India. *Eur Arch Otorhinolaryngol* 267:1239–1245, 2010.
- Polzehl D, Weschta M, Podbielski A, et al. Fungus culture and PCR in nasal lavage samples of patients with chronic rhinosinusitis. *J Med Microbiol* 54:31–37, 2005.

27. Taxy JB. Paranasal fungal sinusitis: Contributions of histopathology to diagnosis: A report of 60 cases and literature review. *Am J Surg Pathol* 30:713–720, 2006.
28. Leleu C, Menotti J, Meneceur P, et al. Efficacy of liposomal amphotericin B for prophylaxis of acute or reactivation models of invasive pulmonary aspergillosis. *Mycoses* 56:241–249, 2013.
29. Sheppard DC, Marr KA, Fredricks DN, et al. Comparison of three methodologies for the determination of pulmonary fungal burden in experimental murine aspergillosis. *Clin Microbiol Infect* 12:376–380, 2006.
30. Soler ZM, and Schlosser RJ. The role of fungi in diseases of the nose and sinuses. *Am J Rhinol Allergy* 26:351–358, 2012.
31. Groppo ER, El-Sayed IH, Aiken AH, et al. Computed tomography and magnetic resonance imaging characteristics of acute invasive fungal sinusitis. *Arch Otolaryngol Head Neck Surg* 137:1005–1010, 2011.
32. Deshazo RD. Syndromes of invasive fungal sinusitis. *Med Mycol* 47(suppl 1):S309–S314, 2009.
33. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 46:1813–1821, 2008. □

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