Mast Cell Dependent Airway Hyperresponsiveness (AHR)

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[Abstract] Asthma is a complex phenotype that involves multiple mechanisms, including adaptive and innate immunity as well as physiological and mechanical changes in the airways. In the models of asthma induced by sensitization and aerosolized allergen exposure in the absence of adjuvant, mast cells facilitate the development of in ammation and airway hyper-responsiveness. This model is useful to analysis of function of mast cells in AHR.

Materials and Reagents

1. Mice (C57BL/6)
   Note: BALB/c is also appropriate to this experiment.
2. Ovalbumin (OVA) (grade V) (Sigma Aldrich, catalog number: A5503)
3. Pentobarbital (Sigma-Aldrich, catalog number: P3761)
4. Acetylcholine chloride (Sigma-Aldrich, catalog number: A6625)
5. Phosphate buffered saline (PBS) (Life Technologies, Gibco®, catalog number: 20012)
6. Wright–Giemsa stain (Muto Pure, catalog number: 15022)
7. Saline

Equipment

1. Syringe (1 ml) (Terumo Medical Corporation, catalog number: SS-01T2525)
2. Needle (26G1/2)
3. Ultrasonic nebulizer (Nihon Kohden, catalog number: TUR-3200)
4. Differential pressure transducer (Nihon Kohden, catalog number: TP-602T)
5. Light microscope (Olympus, model: CX-41)
6. Tracheal cannula (Natume, catalog number: SP31)
7. Cytospin (Shandon, model: Cytospin 3)
8. Centrifuges

Procedure

A. Priming and challenge

1. Mice were immunized by intraperitoneally injection of OVA (10 μg/mouse) eight times at 2-day intervals (priming) for 2 week.
2. On day 26 after the last injection, mice were treated with aerosolized OVA (1%) for 20 min per day (challenge).
3. Repeat an aerosol challenge on day 27 and 28.

B. Measurement of airway responsiveness

4. On day 30, 36 h after the last aerosol challenge, mice were anesthetized with a pentobarbital (50 mg/kg) intraperitoneally.
5. Cannula was inserted by opening a direct airway through an incision in the trachea (see Figure1).
6. Stepwise increases in acetylcholine dose (0.6 to 20 mg/ml) were given with an ultrasonic nebulizer.
7. Airway opening pressure was recorded continuously.
   *The data were expressed as the provocative concentration 150 (PC150), the concentration at which airway pressure was 150% of its baseline value. PC150 was calculated by log-linear interpolation for individual animals.

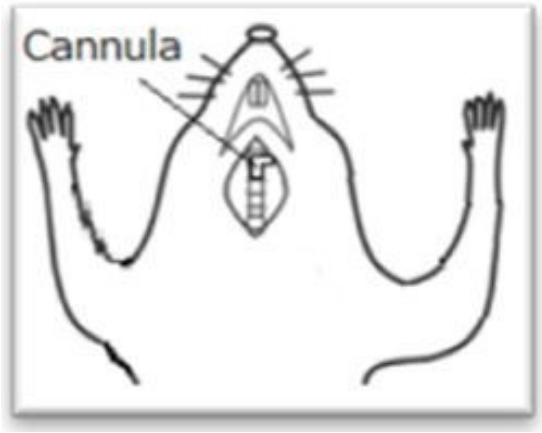

Figure 1. Cannula was inserted into a direct airway through an incision in the trachea after opening

C. Bronchoalveolar lavage and cell counting

8. Mice were given a lethal dose of pentobarbital (120 mg/kg, i.p.), and the lungs were gently lavaged three times with PBS at a pressure of 25 cm H_2O via the tracheal cannula.

9. Total cell counts were determined by light microscopy.
10. The lavage fluid was centrifuged at 800 rpm for 5 min at 4 °C.
11. The cell pellet was resuspended in saline, and cytospin preparations were made.
12. Differential counts on 200 cells were performed by light microscopy using Wright-Giemsa stain.

Acknowledgments

This protocol was developed and implemented by Dr. Masato Kubo at Division of Molecular Pathology, Tokyo University of Science, Chiba, Japan and Dr. Hiromasa Inoue at Department of Pulmonary Medicine, Kagoshima University, Kagoshima, Japan. This work was supported by a Grant-in-Aid-of-Scientific Research in Priority Areas of the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation.

References

1. Sawaguchi, M., Tanaka, S., Nakatani, Y., Harada, Y., Mukai, K., Matsunaga, Y., Ishiwata, K., Oboki, K., Kambayashi, T., Watanabe, N., Karasuyama, H., Nakae, S., Inoue, H. and Kubo, M. (2012). Role of mast cells and basophils in IgE responses and in allergic airway hyperresponsiveness. *J Immunol* 188(4): 1809-1818.
2. Taube, C., Wei, X., Swasey, C. H., Joetham, A., Zarini, S., Lively, T., Takeda, K., Loader, J., Miyahara, N., Kodama, T., Shultz, L. D., Donaldson, D. D., Hamelmann, E. H., Dakhama, A. and Gelfand, E. W. (2004). Mast cells, Fc epsilon RI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. *J Immunol* 172(10): 6398-6406.