TYPE I ALLERGIC REACTIONS OF THE MIDDLE EAR AND EUSTACHIAN TUBE:
AN EXPERIMENTAL STUDY

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To clarify the role of type I allergic reactions in etiology and pathogenesis of otitis media with effusion and to determine whether or not the middle ear is an allergic "shock" organ, we made animal models of nasal allergy in guinea pigs by passive sensitization with serum of homologous animals containing specific IgE antibodies. We also examined the eustachian tube, tympanic cavity (histologically), and tubal function after the induction of type I allergic reactions of the nose. However, the involvement of histologic changes was limited only up to the area near the pharyngeal orifice. The tubal dysfunction evoked by nasal allergic reactions was transient, culminating in no middle ear effusion. Upon direct antigen-challenge into the tympanic cavity, allergic changes were observed in the mucosa lining the tympanic bulla, even though no microscopic effusion was present. Findings of the present study suggest that type I allergic reactions of the nose are not an etiologic factor for otitis media with effusion, although the middle ear is potentially an allergic shock organ.

Although allergy has long received attention as a causative factor of otitis media with effusion (OME), its exact role in the etiology and pathogenesis in the disorder is still unclear. Many previous clinical studies have supported the allergic theory because of a high incidence of OME in allergic patients (Lewis, 1929; Proetz, 1931; Jordan, 1949; Derlacki, 1952). However, other investigators have claimed that allergy does not play a substantial role in the formation of middle ear effusion (MEE) because of a lack of direct scientific evidence to support the supposition that the secretory fluid in the middle ear left is an allergic effusion (Suehs, 1952; Senturia, Gessert, Carr, and Bauman, 1958; Boor, 1962; Sirala, 1964; Lim and Birk, 1971). Since the discovery of IgE by Ishi-
Zaka, Ishizaka, and Hornbrook (1966), the mechanism of atopic allergic reaction (type I immunologic reaction) has been elucidated, resulting in the establishment of accurate diagnostic tests for IgE mediated disorders (Ishizaka et al., 1966). Nevertheless, results of many investigations on IgE in patients with OME did not support the allergic theory of this disease (Mogi, Honjo, Maeda, Yoshida, and Watanabe, 1974; Lewis, Schram, and Lim, 1978; Mogi, Maeda, Yoshida, and Watanabe, 1979; Bernstein, Lee, Conboy, Ellis, and Li, 1983).

In a previous paper (Tomonaga, Kurono, and Mogi, 1988), we evaluated, for both nasal allergy (NA) and OME, 224 pediatric patients with NA, and 102 control subjects (Tomonaga et al., 1988). All patients and control subjects were subjected to routine allergic tests such as history, nasal and blood smear for eosinophilia, allergen skin test, nasal provocation test, and serum IgE. For OME, the presence of MEE was proven by parasentesis after otoscopic examination plus pure tone and impedance audiometries. The results showed that 50% of patients with OME were allergen sensitive, and 21% of patients with NA suffered from OME, as compared to 17 and 6%, respectively, of the control subjects. It was also demonstrated that the incidence of tubal dysfunction is significantly greater in patients with OME sensitive to allergen(s) than in patients with OME sensitive to allergen, and that the incidence of tubal dysfunction is significantly higher in the group of NA patients than control group. Ackerman, Friedman, Doyle, Bluestone, and Fireman (1984), Skoner, Doyle, and Fireman (1978), and Skoner, Doyle, Chamovitz, and Fireman (1986) have demonstrated the induction of eustachian tube obstruction after intranasal provocation by histamine or allergens in allergen sensitive subjects (Ackerman et al., 1984; Skoner et al., 1978, 1986).

In the present investigation, we made animal models of NA and examined the eustachian tube, tympanic cavity (histologically), and tubal function after the induction of type I allergic reactions in the nasal cavity. The aim of this study was to clarify the exact role of NA in the etiology and pathogenesis of OME and to determine whether or not the middle ear is an allergic "shock" organ.

MATERIALS AND METHODS

Animals. Healthy male, hartley guinea pigs, weighing 300–400 grams, were used. Bilateral otoscopic and flexible fiberoscopic observations of the drumhead were performed prior to beginning the study.

Preparation of antiserum for passive sensitization. Five milligrams of 2,4-dinitrophenilated-ascaris (DNP-ascaris) (Kissei Pharmaceutical Co., Matsumoto, Japan) soluble in 0.5 ml of phosphate buffered saline was mixed with 5 mg of aluminum hydroxide-gel as adjuvant. One milliliter of the mixture was injected into the foot pads of guinea pigs. The same dose of antigen was intracutaneously added into the back region twice at 2 and 4 weeks after the initial injection. An-
Fig. 1. Passive cutaneous anaphylaxis (PCA) test. Antiserum obtained from immunized guinea pigs has more than 1:1,024 titer of IgE antibody against DNP. Antiserum was obtained 1 week after the final immunization and proven to have more than 1:1,024 titer of IgE antibody against DNP by the 72 h passive cutaneous anaphylaxis (PCA) test (TADA and OKUMURA, 1971) (Fig. 1).

 Passive sensitization. One milliliter of the antiserum was interavenously injected into the dorsal vein of the foot pads of guinea pigs for passive sensitization.

Local challenge of the antigen into the nasal cavity and tympanic cavity. One week after the passive sensitization, 0.5 ml of DNP-ovalbumin (DNP-OVA) in saline at 5 mg/ml concentration was dropped into the right nasal cavity of guinea pigs. Another group of passively sensitized guinea pigs received 0.1 ml of the same antigen solution into the right tympanic cavity through the tympanic bulla. For each challenge, the animal was anesthetized with pentobarbital sodium solution.

Evaluation of the eustachian tube function. Tubal function was evaluated by inflation and deflation tests (MILLER, 1965) using tympanometry (Amplaid 702, Dana Japan Ltd., Tokyo) after the eardrum was perforated. Inflation and deflation tests (MILLER, 1965) were conducted on the passively sensitized animals before the antigen challenge into the nasal cavity, and 0.5, 1, 2, 3, 4, 5, and 6 h after the challenge. The number of animals tested at each period after the antigen challenge was 5. Therefore, a total of 35 sensitized animals were used for this experiment. Five other passively sensitized guinea pigs were challenged with saline into the nose. These animals served as control.

Histologic examination. Besides the 40 guinea pigs including the 5 control animals tested for tubal function, another 40 passively sensitized animals underwent injection of the antigen into the tympanic cavity. Five of them were killed at periods of 0.5, 1, 2, 3, 4, 5, and 6 h after the antigen challenge. Five animals
which were injected with saline served as control. Animals tested for the eustachian tube function were killed immediately after the test.

Tubotympanum, nasopharynx, and nasal cavity were dissected from each killed guinea pig, fixed with Mota's solution, decalcified by EDTA, and dehydrated by ethanol. Serial tissue sections were stained with hematoxylin eosin (HE) and toluidine blue.

RESULTS

Tubal function tests

Following challenge with DNP-OVA into the nose, all animals which were passively sensitized exhibited moderate to severe sneezing, hypersecretion, and mouth breathing. Sneezing disappeared within 40 min, but the hypersecretion and mouth breathing lasted for 3 h. These nasal allergic reactions gradually became weak 12–13 days after the passive sensitization.

As can be seen in Fig. 2, an increase in the opening pressure (OP) of the eustachian tube is observed in all animals tested at 30 and 60 min after the antigen-challenge into the nose. The mean value of OP was $478 \pm 34.0$ mmH$_2$O ($N=5$) at 30 min and $463 \pm 39.6$ mmH$_2$O ($N=5$) at 1 h. These values were significantly higher ($P<0.01$) than the values ($365 \pm 45.0$ mmH$_2$O, $N=35$) before the antigen-challenge. The mean values of OP 3 h after the antigen-challenge were almost the same as the mean values before the provocation.

Histologic changes

The most remarkable changes seen in mucous membranes lining the nose,
nasopharynx, and tubotympanum, after the antigen-challenge of passively sensitized guinea pigs were eosinophilic and basophilic cells infiltrations, and edema. Upon nasal antigen-challenge, these changes were seen in the mucous membrane of the nose, nasopharynx, and eustachian tube near the pharyngeal orifice (but not in the rest of eustachian tube). Figure 3 presents an exemplification of the histologic changes seen in the mucosa near pharyngeal orifice of the tube. These changes were most intense in animals killed at 30 min after the antigen-challenge. Noticeable infiltration of eosinophils and mast cells was not seen in the mucous membrane of guinea pigs sacrificed at 2, 3, 4, 5, and 6 h after challenge. However, edema lasted 3 h after the challenge. The number of mast cells after the challenge

Fig. 3. Histologic changes of the mucosa near the pharyngeal orifice of the tube 30 min after the nasal antigen-challenge. A: HE staining, ×200. A marked infiltration with eosinophils. B: Tissues section of the mucosa near the pharyngeal orifice obtained from normal control animals. HE staining, ×200.
was greater in the submucosal layer than in the mucosal layer. The intensity of the histologic changes paralleled the increase of OP of the eustachian tube in guinea pigs tested.

When the antigen was challenged into the tympanic cavity, histologic changes were limited to the tympanic cavity and eustachian tube (other than the part near the pharyngeal orifice). Figure 4 exhibits eosinophil and mast cell infiltration in the lining membrane of the tympanic bulla of animals sacrificed at 30 min after the intratympanic antigen-challenge. Mast cell infiltration is also seen in the eustachian tube (Fig. 5A) and tympanic (Fig. 5B) mucosa after the antigen-challenge. Figure 6 is a schematic illustrating the histologic changes 30 min
Fig. 5. Mast cell infiltration in mucosa of the eustachian tube and tympanic bulla after antigen-challenge. A: Mucosa of the eustachian tube, ×200. Thirty minutes after the intranasal antigen-challenge. B: Mucosa of the tympanic bulla, ×200. Thirty minutes after the intratympanic antigen-challenge.

after the challenge into the nose and tympanic cavity. MEE was not seen, by gross appearance, in the tympanic cavity of any animals challenged with the antigen into either the nasal cavity or the tympanic bulla. However, histologically, MEE was noted in the lumen of the eustachian tube in occasional.

DISCUSSION

Many investigators have attempted experimental studies to clarify the role of allergy in the formation of OME (Smirnov, 1938; Koch, 1947; Hopp, Elevitch, Pumphrey, Irving, and Hoffman, 1954; Yamashita, Okazaki, and Kumazawa, 1980; Miglets, 1973; Doyle, Takahara, and Fireman, 1985). However, as
Fig. 6. A schematic drawing presenting the histologic changes 30 min after the antigen-challenge into the nose and tympanic cavity.

Doyle et al. (1985), pointed out, an acute inflammatory process, that culminates in OME in studies by Smirnov (1938), Koch (1947), Hopp et al. (1954), and Yamashita et al. (1980), was not due to IgE mediated immunologic reactions (type I allergy). Since these investigators challenged protein antigens into the tympanic cavity of systemically, actively sensitized animals, and, since the formation of antigen specific IgE antibodies in the sensitized animals was not proven, the development of OME in their studies can be attributed to IgG-mediated antigen-antibody reactions which were later demonstrated by Ryan, Cantanzaro, Wassenman, and Harris (1986) and Suzuki, Kawauchi, and Mogi (1988).

Miglets (1973) passively sensitized squirrel monkeys to ragweed pollen with human serum containing the reaginic antibodies to ragweed pollen. Ragweed pollens were then insufflated into middle ear, three times per day for 4 days, through a blunt 27-gauge needle. Miglets (1973) demonstrated the development of MEE with the predominating cell type being polymorphonuclear leukocytes and the typical histologic changes expected to occur in type I allergic reactions in the tympanic mucosa, within 5 days after the initial antigen-insufflation. The presence of IgE fluorescent mast cells and IgE antibodies was observed in the middle ear mucosa. On the basis of these findings, Miglets (1973) supported the allergic theory for OME and regarded the middle ear as an allergic “shock organ.” Doyle et al. (1985) performed a similar experiment using rhesus monkeys. In their study, pollen was insufflated into the tympanic cavity through the eustachian tube by means of a noninvasive technique. After the sedation, 50 mg of pollen was placed in a small Politzer’s bag, and the velum was manually elevated to close the nasopharyngeal oral part. Then, the pollen was insufflated
into nose. It was proven that the pollens reach the tympanic cavity. The challenge schedules were similar in both duration and timing for the two studies. However, DOYLE et al. (1985) failed to see both effusion and inflammatory changes in the tympanic cavity, both macroscopically and histologically. They disagreed with Miglets’ opinion (MIGLETS, 1973) that the middle ear acts as an allergic “shock organ” and suggested that the inflammatory reactions of the middle ear seen in the study were due to infection which was secondary to the traumatized catheterization of the eustachian tube.

In the two studies, nonhuman primates were passively sensitized with human serum reaginic antibodies to ragweed pollen because PATTERSON, JORDAN, FINK, NISHIMURA, and FINK (1965) demonstrated that human antibodies would be passively transferred to other primates (monkey). In the present investigation, we passively sensitized guinea pigs with homologous IgE antibodies against DNP-ascaris. A high antibody activity of IgE to this antigen was confirmed by PCA test. Upon DNP-OVA challenge into the nose, passively sensitized animals presented moderate to severe sneezing, hypersecretion, and mouth breathing immediately after the local challenge. The symptoms are typical of immediate type I allergic reactions of the nose.

Results of the present investigation showed that the eustachian tube is involved, both functionally and morphologically, in type I allergic reactions of the nose. However, the involvement of histologic changes was limited only up to the area near the pharyngeal orifice. In other words, the antigen-challenge to the nose did not induce any histologic change in the tympanic mucosa. The histologic changes and dysfunction of the tube evoked by allergic reactions of the nose were transient, resulting in no formation of MEE. Although direct antigen-challenge to the tympanic cavity did not evoke a microscopic MEE, the mucosa lining the tympanic cavity exhibited allergic inflammatory changes. Therefore, it is certain that the middle ear is potentially an allergic "shock organ." This evidence is in accord with the opinion (Mogi, MAEDA, and WATANABE, 1980) that the middle ear is an immunologically potential site.

RYAN et al. (1986) and SUZUKI et al. (1988) induced experimental OME in guinea pigs by injection of antigen following active sensitization. The cause for the formation of MEE in these two studies was immune complexes, while in the present investigation type I allergic reactions culminated in histologic changes of the middle ear mucosa.

The discrepancy between results of the study by DOYLE et al. (1985) and of the present study is probably due to the difference in the amount of antigen delivered to the tympanic cavity. Even though DOYLE et al. (1985) confirmed the presence of pollen antigens in the tympanic cavity, the amount was not determined. Monkeys were passively sensitized by human reaginic antibodies in the DOYLE et al.’s study (1985), while in our present study the experiment was performed on guinea pigs passively sensitized with homologous IgE antibodies. This dif-
ference might be another possible reason for the discrepancy.

We believe that, even if the middle ear is an allergic shock organ, it is unlikely that type I allergic reactions are an etiologic factor for OME, because the eustachian tube functions as a "gate keeper," limiting the ability of most substances to access the middle ear (DOYLE et al., 1985). Therefore, IgE-mediated allergic reactions may be a factor in the delay in the clearance of MEE rather than an etiology. In a future study we will report the effect of nasal allergic reactions on the clearance of MEE.

REFERENCES


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