Passive sensitization of human airways induces myogenic contractile responses in vitro

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Mitchell, R. W., K. F. Rabe, H. Magnussen, and A. R. Leff. Passive sensitization of human airways induces myogenic contractile responses in vitro. J. Appl. Physiol. 83(4): 1276–1281, 1997—We assessed effects of passive sensitization on human bronchial smooth muscle (BSM) response to mechanical stretching in vitro. Bronchial rings were sham (control) or passively sensitized overnight by using sera from donors demonstrating sensitivity to Dermatophagoides farinae and having immunoglobulin E (IgE) concentrations of 2,600 ± 200 U/ml. Tissues were fixed isometrically to force transducers to measure responses to electrical field stimulation (EFS) and quick stretch (QS). The myogenic response to QS was normalized to the maximal response to EFS (%EFS). The myogenic response of sensitized BSM was 47.9 ± 10.9 %EFS to a QS of ~6.5% optimal length (Lo); sham-sensitized tissues had a myogenic response of 13.5 ± 6.4 %EFS (P = 0.012 vs. passively sensitized). A QS of ~13% Lo in sensitized BSM caused a response of 82.8 ± 20.9 %EFS; sham-sensitized tissues developed a response of 38.2 ± 17.3 %EFS (P = 0.004). BSM incubated with serum from nonallergic donors did not demonstrate increased QS response (4.6 ± 1.4 %EFS, P = not significant vs. tissue exposed to atopic sera). However, tissues incubated in sera from nonatopic donors supplemented with hapten-specific chimeric IgE (JW8) demonstrated augmented myogenic response to QS of ~6.5% Lo (21.9 ± 6.2 %EFS, P = 0.027 vs. nonatopic sera alone). We demonstrate that passive sensitization of human BSM preparations causes induction and augmentation of myogenic contractions to QS; this hyperresponsiveness corresponds to the IgE concentration in sensitizing sera.

airway smooth muscle; bronchial smooth muscle; quick stretch; electrical field stimulation; serum immunoglobulin E spontaneous tone compared with sham-sensitized animals (10). This increased, prostanoid-derived spontaneous tone was associated with increased responsiveness to muscarinic agonists and decreased responsiveness to β-adrenergic-receptor agonists (10). After ablation of spontaneous tone in these animals, either through cyclooxygenase inhibition or removal of the epithelium, tissues from sensitized and sham-sensitized guinea pigs demonstrated similar muscarinic and β-adrenergic receptor responsiveness (10). In a dog model of allergic bronchospasm, spontaneous mechanical activity of the ASM also was demonstrated (8); canine tracheal smooth muscle from nonsensitized animals has been shown normally to be quiescent (6). Spontaneous activity, stretch-induced myogenic contractions, and increased phasic electrical activity also could be induced in this tissue in the presence of the potassium channel blocker tetraethylammonium (6). This compound also has been demonstrated to increase the number of nexuses in canine ASM (2); these low-impedance cell-cell connections are associated more with single-unit types of smooth muscle (14).

We tested the hypothesis that passive sensitization of human bronchi augments spontaneous mechanical activity. We assessed the effect of quick stretch (QS) on myogenic responsiveness of passively sensitized and sham-sensitized human bronchial smooth muscle (BSM). We also assessed the effect of immunoglobulin E (IgE) concentrations in human serum on responsiveness of BSM preparations to electrical field stimulation (EFS) and QS. We show in vitro for the first time that passive immune sensitization, by using both endogenous and exogenous IgE, induces and augments myogenic contractile activity of the smooth muscle from seventh-generation human airways. We also demonstrate that increased serum IgE concentration corresponds to the induction and augmentation of this myogenic hyperresponsiveness.

METHODS

Preparation of human airways. Tissues were obtained from 13 patients undergoing thoracotomy for lung cancer at the Krankenhaus Grosshansdorf (LVA Freie and Hansestadt Hamburg, Germany). All patients gave informed consent for surgery consistent with both German law and the Declaration of Helsinki; none was chronically treated with theophylline, β-adrenoceptor agonists, corticosteroids, or anticholinergic drugs. None of the patients had a history of atopy or respiratory allergies. Immediately after they were surgically excised, lung sections were placed in ice-cold Krebs-Henseleit...
Passive sensitization of bronchial ring preparations. Sensitizing buffer was prepared by adding 1 ml of serum to 9 ml of KH that had been gassed with a 95% O2-5% CO2 mixture to maintain a pH of 7.35–7.45. For 12 bronchial rings from 6 patients, sham-sensitized (control) tissues were maintained in KH buffer alone or sensitized in 1:9 serum to KH buffer overnight (9, 11, 16). For 42 bronchial rings from an additional 7 patients, tissues were either 1) incubated in sera (1:9 KH buffer) from atopic donors (IgE concentrations from 505 to >3,000 U/ml), 2) incubated in a 1:9 dilution of serum from nonatopic individuals with serum IgE levels ≤115 U/ml (control serum), or 3) incubated in a 1:9 dilution of serum from the same nonatopic individuals supplemented with a hapten-specific chimeric IgE, JW8 (16). The concentration of JW8 was selected to approximate the concentrations of total IgE in the sera from atopic donors (2). Control and sensitized bronchial rings were allowed to recover for 10 min between stretches. These rings were allowed to equilibrate for 90 min. A QS of 0.25 mm was then elicited randomly as described above for each tissue. Chart speed was set at 25 mm/min, and myogenic responses were measured for 2 min, during which time contractile force induced by the QS began to wane. Bronchi were allowed to recover for 10 min between stretches. These QSs were ~6.5 and 13.0% of bronchial ring diameter, respectively. Myogenic responses to QS were measured as a transient contraction above baseline after the QS and normalized to the maximal response of each tissue to EFS (%EFS). The myogenic contraction induced by QS (mg) was compared with the maximal EFS response (mg).

Analysis of data. All data were expressed as means ± SE. Where paired comparisons were made, data were analyzed for statistical significance by paired two-tailed Student's t-test. Where data from more than two experimental groups were analyzed, a difference among the groups was first determined by a one-way analysis of variance. When a difference among groups was detected by an analysis of variance, statistical significance was assessed by Fisher's test for multiple comparisons. Statistical significance was claimed whenever P < 0.05.

RESULTS

Tissue response to antigen challenge and EFS. Bronchial ring preparations from six patients were randomly assigned to either antigen-sensitized (outside diameter 3.92 ± 0.15 mm) or sham-sensitized (outside diameter 3.67 ± 0.17 mm) groups (P = 0.203). Additional pairs of sensitized and sham-sensitized bronchial rings from these donors were challenged with D. farinæae antigen. Sham-sensitized tissues did not respond to antigen challenge; D. farinæae induced contractions in all sensitized bronchial rings (148 ± 20 %EFS; P = 0.0001 vs. sham-sensitized bronchial rings).

There was no significant difference in the response to EFS between groups during the initial equilibration period. Sham-sensitized tissues demonstrated contractions of 280 ± 40 mg compared with 383 ± 89 mg [P = 0.204; not significant (NS)] for bronchial rings that were passively sensitized.

Myogenic contractile response of human bronchial rings with QS. Human bronchial rings demonstrated amplitude-dependent myogenic contractile responses to QS of 0.25 and 0.50 mm (Fig. 1). These stretches corresponded to 6.9 ± 0.34 and 13.8 ± 0.68% of optimal ring diameter for sham-sensitized and 6.4 ± 0.25% (P = 0.235 vs. sham-sensitized) and 12.9 ± 0.50% (P = 0.238 vs. sham-sensitized) for passively sensitized preparations. For a QS of 0.25 mm, all six bronchial rings exposed to passive sensitization responded with a myogenic contraction of 47.9 ± 10.9 %EFS. By contrast, only three of six sham-sensitized preparations contracted to 13.5 ± 6.4 %EFS (P = 0.012 vs. sensitized; Fig. 2). For a QS of 0.50 mm, sensitized bronchial rings responded with a myogenic contraction (6 of 6 tissues responding) to 82.8 ± 20.9 %EFS; sham-sensitized
preparations (5 of 6 tissues responding) contracted to 38.2 ± 17.3 %EFS (P = 0.004 vs. sensitized; Fig. 2).

Myogenic contractile response of control serum-incubated human bronchial rings with QS. From seven additional patients, bronchial rings passively sensitized by using sera from atopic donors demonstrated myogenic contractile responses (14 of 14 tissues) to QS of 0.25 mm (37.5 ± 6.5 %EFS) (Fig. 3). By contrast, bronchial rings from these same patients that were sham sensitized overnight in sera from nonatopic donors (IgE concentrations ≤11 U/ml) had no appreciable QS response (4.6 ± 1.4 %EFS, 8 of 14 tissues; P < 0.0001 vs. passively sensitized bronchial rings). Tissue exposed to sera containing ≤11 U/ml IgE demonstrated similar responsiveness to tissues incubated overnight in KH buffer alone (P = NS; see above). To test the hypothesis that the IgE concentration in the serum corresponds to the hyperresponsiveness to QS observed in these bronchial ring preparations, we assessed the effect of addition of a hapten-specific chimeric IgE, JW8, to sera from nonatopic donors (≤11 U/ml IgE) on myogenic responsiveness. An augmented QS response

Fig. 1. Representative myograms in response to a 0.5-mm quick stretch (QS) of human bronchial ring preparations. A pair of contiguous 7th-generation bronchial rings from a resected human lung were dissected; 1 ring was passively sensitized overnight, and the other served as a sham-sensitized control (see METHODS). After equilibration to a stable resting tone, rings were activated by electrical field stimulation (EFS); all myogenic responses were normalized to response of each tissue to EFS (%EFS). Rapid upstroke of myogram denotes QS of 0.5 mm. For this representative pair of bronchial rings, only the sensitized tissue demonstrated a myogenic contractile response to QS.

Fig. 2. Myogenic responses of sensitized and sham-sensitized human bronchial rings. In response to QS of 0.25 and 0.50 mm, tissues passively sensitized to sera from atopic donors demonstrated significantly greater myogenic contractile responses than paired, sham-sensitized tissues. All data are expressed as %EFS. *P < 0.05.

Fig. 3. Myogenic responses of passively sensitized and sham serum-sensitized human bronchial rings. In response to QS of 0.25 mm, tissues passively sensitized to sera from atopic donors demonstrated significantly greater myogenic contractile responses than did contiguously dissected bronchial rings incubated in sera from nonatopic individuals. However, when a hapten-specific chimeric immunoglobulin E (JW8) was mixed with sera from nonatopic donors, contiguously dissected tissues demonstrated augmented responsiveness to QS. All data are expressed as %EFS. *P < 0.05, **P < 0.001.
(21.9 ± 6.2 %EFS; P = 0.027 vs. sham-sensitized control, P = 0.046 vs. passively sensitized bronchial rings) was elicited from bronchial rings (14 of 14 tissues) incubated with nonatopic sera plus exogenous IgE (JW8).

In contrast to the QS response, passive sensitization did not affect parasympathetic contraction caused by EFS (Fig. 4). Maximal response to EFS was similar in tissues incubated with sera from nonatopic (449 ± 65 mg) and atopic donors (412 ± 77 mg) and in tissues incubated in sera containing J W8 (473 ± 74 mg) (P = NS for all comparisons). The QS was not significantly different among sham-sensitized (6.1 ± 0.3 %L0), passively sensitized (6.4 ± 0.3 %L0), or J W8-exposed (6.7 ± 0.3 %L0) bronchial rings (P = NS for all comparisons).

All passively sensitized tissues demonstrated a significant Schultz-Dale contraction (497 ± 126 mg; Fig. 5) to D. farinae (30 U/ml); sham-sensitized tissues did not contract in response to the antigen. Tissues incubated in sera from nonatopic donors supplemented with JW8 all demonstrated contractile response (576 ± 94 mg) to the specific hapten NIP-BSA (10 µg/ml).

**DISCUSSION**

The purpose of this study was to assess the effect of passive immune sensitization on intrinsic contractile activity of the smooth muscle of conducting airways. We found that ~50% of seventh-generation human bronchial ring preparations demonstrate modest contractile response to a QS of ~6.5% of L0 with sensitization, all bronchi tested elicited a myogenic contractile response to this QS that was greater in magnitude than responses induced for sham-sensitized tissues (Figs. 1–3). We also found that the induced myogenic contractile response depended on the magnitude of the QS; a QS of ~13% of L0 caused a greater intrinsic contraction, and sensitized bronchi demonstrated significantly greater myogenic response than sham-sensitized ring preparations (Fig. 2).

To test the hypothesis that a serum component other than IgE was responsible for the tissue hyperresponsiveness to QS, we assessed responses of bronchial rings from three separate groups: 1) passively sensitized, using sera from atopic donors, 2) sham sensitized, using sera from nonatopic donors with serum IgE levels ≤11 U/ml, and 3) passively sensitized, using sera from these same nonatopic donors but supplemented with a hapten-specific chimeric IgE (JW8). We found that the presence of serum alone did not augument subsequent myogenic responses compared with tissues incubated overnight in buffer only. However, tissues incubated with either sera from atopic donors or sera from nonatopic donors supplemented with JW8 demonstrated augmented myogenic contractile response to QS (Fig. 3). These data suggest the presence of increased IgE is necessary for the induction and augmentation of myogenic contractile response to QS in human bronchial rings in vitro.

Previous studies have demonstrated increased electrical activity in airways of asthmatic subjects compared with nonasthmatic volunteers (1). Increased electrical activity (spontaneous action potentials) of ASM has been demonstrated to be consonant with the induction of myogenic contractions in response to QS in canine airway tissues (5, 6). We have shown previously in a canine model of allergic bronchospasm that the normally quiescent tracheal smooth muscle demonstrates spontaneous contractile activity (8). Microelectrode studies of BSM strips from the same canine model demonstrated significant spontaneous electrical activity of the sarcolemma of the smooth muscle myocyte compared with tissues from sham-sensitized animals (12), which normally show no action potentials (6, 12). These studies suggested alterations in excitation, induced by immune sensitization, may cause the hyperresponsiveness observed in asthmatic individuals and in a canine model of allergic bronchospasm (5, 6, 8, 12).

We have also demonstrated previously augmentation of intrinsic contractile activity of passively sensitized human bronchi (9). Sensitized tissues contracted with...
greater velocity and shortened to a greater extent than paired control bronchial rings. The previous study suggested alterations in contraction coupling may also contribute to the ASM hyperresponsiveness observed with passive immune sensitization. Jiang et al. (4) have demonstrated a twofold increase in actomyosin adenine nucleotide and myosin light chain kinase activity in the immune-sensitized canine model of allergic bronchospasm. This increased parameter of contraction coupling could be attributed to a 30% increase in myosin light chain kinase content and activity found in sensitized tissues (4).

The above-mentioned previous studies suggest that the increased myogenic response observed on QS of human bronchi that we observed may be due to increased contraction coupling, perhaps through an alteration in membrane excitability with passive sensitization by IgE. The increased sensitivity of the smooth muscle to mechanical stretch could be a consequence of an alteration in potassium channel activity (6). Alternatively, the attachment of the Fc fragment of IgE to mast cell or smooth muscle membranes may alter calcium flux through voltage-dependent channels, with a concomitant change in membrane potential (13).

It is important to specify some limitations of our findings. Our data are limited to passively sensitized bronchial rings from patients undergoing lung resection for carcinoma. The effect of long-term tobacco consumption on either the general or myogenic responsiveness of BSM in humans is not known. However, we used contiguous tissue preparations from the same individuals in these studies, and comparisons of data were made between and among bronchial rings from similar airways. Using contiguous rings also ensured similar airway dimensions so that a QS of 0.25 or 0.50 mm was not significantly different between groups of tissues.

Our experimental design also considered predominantly the relationship between airway responsiveness and atopy as marked by serum IgE concentrations. It is nonetheless possible that other serum factors could be responsible for the induction of hyperresponsiveness and that these occurred in concert with induction of increased IgE concentrations in sera from atopic donors. However, sera from nonatopic donors that were supplemented with J W8 induced greater responses to QS of human bronchi compared with tissues incubated with sera containing low IgE concentrations (Fig. 3). Therefore, it appears that addition of IgE alone is sufficient to induce this response and the specific contractile response to antigen (either D. farinae or NIP-BSA; Fig. 5).

We did not observe nonspecific augmentation to EFS in these bronchial rings (Fig. 4), whereas Ichinose et al. (3) demonstrated that incubation of human airways with IgE increased cholinergic neurotransmission in vitro. However, Ichinose and colleagues elicited EFS responses by using frequencies between 1 and 8 Hz on bronchial strips compared with 40 Hz on bronchial rings in the present study. It is possible that a frequency of 40 Hz is supramaximal for both sham- and passively sensitized tissues and that all tissues respond similarly to high-frequency EFS. In contrast to our studies, Watson et al. (16) have demonstrated in human tissues that IgE (J W8) is not sufficient to induce nonspecific contractile hyperresponsiveness to histamine. They concluded that nonspecific histamine hyperresponsiveness may be independent of IgE or may require IgE in the presence of some other factor(s) present in sensitizing serum (16). A separation between specific and nonspecific hyperresponsiveness also has been demonstrated in an IgE-deficient mouse model (7). Both wild-type and IgE-deficient mice were sensitized to specific allergens, and, whereas IgE-deficient mice did not produce IgE in response to antigen challenge, both wild-type and IgE-deficient strains demonstrated bronchial hyperreactivity and eosinophilia. These data further suggest that IgE may not be necessary for nonspecific airway hyperresponsiveness. It is important to note that, in our study, J W8 did induce a myogenic response that was significantly different from that in sham-sensitized control bronchial rings; however, the myogenic response to QS induced by sera from atopic individuals was significantly greater than that induced by J W8 (Fig. 3). This significantly greater response could be because of additional serum factors (16).

We demonstrate in vitro for the first time that sensitization induces and augments myogenic contractile activity of the smooth muscle from human seventh-generation airways. We also demonstrate that the presence of serum alone does not affect myogenic contractile responses but that the presence of significant concentrations of IgE (either endogenous or exogenously added) induces passive sensitization of human bronchial rings in vitro. Myogenic responses were induced and augmented in the presence of either IgE-rich sera from atopic donors or in the presence of J W8, a hapten-specific chimeric IgE mixed with sera from nonatopic individuals. Tissues incubated in sera from nonatopic donors or in the absence of serum (KH buffer alone) demonstrated similar, minimal response to QS. Our data suggest that allergic bronchospasm in vivo may be manifested, in part, through augmentation of myogenic responsiveness of the smooth muscle from intrapulmonary, conducting airways.

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