

Role of IgE in Hyperresponsiveness Induced by Passive Sensitization of Human Airways

NIKKI WATSON, KRISTINA BODTKE, ROBERT A. COLEMAN, GORDON DENT, BENITA E. MORTON, ELKE RÜHLMANN, HELGO MAGNUSSEN, and KLAUS F. RABE

Krankenhaus Grosshansdorf, Zentrum für Pneumologie und Thoraxchirurgie, Grosshansdorf Germany; and Glaxo Wellcome Research and Development, Respiratory Diseases Division, Stevenage, Herts, United Kingdom

Incubation of airways from nonatopic patients with serum from patients with high IgE levels confers responsiveness to "specific" (allergen) and hyperresponsiveness to "nonspecific" (histamine) stimuli. We have tested the hypothesis that the level of IgE determines the degree of specific and nonspecific responsiveness. Bronchial rings from nonatopic patients were sensitized overnight with serum containing high levels of allergen-specific IgE, or with an allergen-specific chimeric IgE (JW8) in physiologic buffer. *In vitro* responsiveness to allergen and histamine was evaluated and compared with nonsensitized tissues from the same patients. Responses to specific allergen were demonstrated in all tissues sensitized with atopic serum or chimeric IgE, but not in nonsensitized tissues. Allergen responses were specific, since tissues sensitized using serum containing high *Dermaohagoides farinae*-specific IgE only, did not respond to either horse or dog allergens. The potency and magnitude of the maximal contraction to histamine was significantly ($p < 0.05$) increased in tissues sensitized using atopic serum with high total IgE concentrations compared with nonsensitized preparations, but was unchanged in tissues sensitized using chimeric IgE or serum with low total IgE levels. Therefore, specific IgE determines allergen responsiveness in passively sensitized human airways, but histamine hyperresponsiveness is independent of specific IgE and appears to be related to some other factor associated with serum containing high concentrations of total IgE. **Watson N, Bodtke K, Coleman RA, Dent G, Morton BE, Rühlmann E, Magnussen H, Rabe KF. Role of IgE in hyperresponsiveness induced by passive sensitization of human airways.**

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Passive sensitization of human airway smooth muscle has been described previously (1-9). It is evident from these studies that incubation of airways from nonatopic individuals with serum from atopic patients produces *in vitro* responsiveness to specific allergen (1-9) and nonspecific hyperresponsiveness to bronchoconstrictor agents (histamine and neuropeptides) (5, 8, 9). The nature of the specific responsiveness (the contractile response to allergen) has been extensively studied and it is widely accepted that cysteinyl leukotrienes, and to a lesser extent histamine, mediate this response in sensitized tissue (2, 3, 6, 10-12). The nature of the nonspecific hyperresponsiveness induced by passive sensitization of human airways is less well understood. Several studies have investigated potential changes in the smooth muscle (post-junctional factors) which might account for the hyperresponsiveness, including changes in the velocity and magnitude of smooth muscle cell shortening (13), alterations in Ca^{2+} mobilization (5), and changes in protein kinase C-mediated responses (14).

The role of IgE present in the sensitizing serum in the induc-

tion of specific responsiveness and nonspecific hyperresponsiveness is, however, largely uninvestigated. It is not known whether the degree of responsiveness (specific and nonspecific) is in some way related to the concentration of allergen-specific IgE in the sensitizing serum, whether IgE alone is sufficient to confer specific (allergen) and nonspecific (histamine) responsiveness, or whether allergen responsiveness and histamine hyperresponsiveness are interdependent. These questions may have clinical relevance, since Burrows and colleagues (15) demonstrated a close relationship between the concentrations of circulating IgE in the serum and the frequency of reported asthma both in patients with and without positive skin prick tests. Additionally, nonspecific airway hyperresponsiveness can be demonstrated in a number of other conditions where allergen-specific IgE is not implicated, such as chronic obstructive bronchitis (16), during viral infections (17), and in cigarette smokers (18).

We hypothesized that in passively sensitized human airways, IgE represents the major determinant for the induction of both the specific and nonspecific responsiveness. Therefore, in the present study we have investigated the role of IgE in the induction of allergen responsiveness and histamine hyperresponsiveness. This has been achieved using bronchial rings from nonatopic patients, sensitized with serum from individuals containing high concentrations of allergen-specific IgE and a range of levels of total IgE, or with a chimeric, hapten-specific IgE (JW8) directed against the hapten/carrier complex 4-hydroxy-3-iodo-5-nitrophenylacetic acid/bovine serum albumin (NIP-BSA).

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Correspondence and requests for reprints should be addressed to K. F. Rabe, M.D., Krankenhaus Grosshansdorf, Zentrum für Pneumologie und Thoraxchirurgie, LVA Hamburg, Wöhrendamm 80, D-22927 Grosshansdorf, Germany.

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TABLE 1
PROFILE OF TOTAL AND SPECIFIC IgE OF SERUM DONORS
USED FOR TISSUE SENSITIZATION*

No.	Donor	Total IgE (U/ml)	Specific				
			D ₁ (U/ml)	D ₂ (U/ml)	E ₁ (U/ml)	E ₂ (U/ml)	E ₃ (U/ml)
1	J.F.	636	28.50	41.50	< 0.20	< 0.20	< 0.20
2	C.A.	> 3,000	15.69	19.62	17.40	20.30	2.02
3	C.K.	505	1.77	17.90	< 0.20	0.20	0.20
4	P.S.	1,835	2.61	17.70	34.30	5.98	nd
5	K.S.	2,586	> 44	7.97	14.35	32.74	1.64
6	M.S.	> 3,000	0.96	0.67	2.41	> 44	< 0.20

* For clinical purposes, IgE levels 0–100 U/ml are considered normal, between 100 and 150 U/ml elevated but not pathologic, whereas concentrations in excess of 150 U/ml are considered pathologic. FAST class determinations are based on the following criteria: FAST 0 = < 0.35 U/ml, FAST 1 = 0.35 to 0.75 U/ml, FAST 2 = 0.76 to 2.99 U/ml, FAST 3 = 3.00 to 17.50 U/ml, and FAST 4 > 17.50 U/ml. The specific allergens are: D₁ = *Dermatophagoides pteronyssinus*; D₂ = *Dermatophagoides farinae*; E₁ = cat epithelium; E₂ = dog epithelium; E₃ = horse epithelium, as defined by Allergopharm KG.

METHODS

Serum Donors

Serum was prepared from whole blood of six individuals demonstrating high total IgE (> 500 U/ml to > 3,000 U/ml) and a range of levels of specific IgE for five common aeroallergens (Table 1). Sera were not pooled but were frozen individually in 200 to 250- μ l aliquots until required.

Tissue Donors

Macroscopically normal bronchial tissue was obtained from 22 patients undergoing surgery for lung cancer (18 male, 4 female; mean age 62 yr; range: 51 to 72 yr). At the time of operation 15 were active smokers, five were ex-smokers, having not smoked for at least 2 yr, and two were nonsmokers. Preoperative lung function parameters and medications are given in Table 2. In addition to their negative history of atopy, serum IgE levels of all tissue donors were measured immediately after surgery (see Table 3).

Immediately after resection, peripheral airways (2 to 5 mm internal diameter) were dissected free of alveolar tissue and cut into rings (2 to 4 mm length) ready for sensitization. Tissues were rotated overnight at room temperature in tubes containing modified Krebs buffer (composition mM: NaCl 118.4, KCl 4.7, MgSO₄ 0.6, CaCl₂ 1.3, KH₂PO₄ 1.2, NaHCO₃ 25.0, glucose 11.1) in the absence (nonsensitized controls) or presence of sensitizing serum (10% vol/vol) or the chimeric, hapten-specific IgE, JW8 (10 μ g/ml). The next morning rings were transferred to 10 ml organ baths containing oxygenated (95% O₂: 5% CO₂) modified Krebs buffer (pH 7.4, 37° C). Tissues were equilibrated for at least 60 min at a resting tension of 250 to 300 mg, before the commencement of experimental protocols. All concentration–effect curves were constructed in a cumulative manner, using incremental concentrations spaced at half log₁₀ intervals. At the end of experiments, nonsensitized tissues were exposed to a single concentration of spasmogen (carbachol; 0.1 mM or histamine 0.3 mM) to ensure that negative responses to allergen were not the result of deterioration of contractile responses in these tissues.

Specificity of the Sensitization Process

Using six tissues taken from the same individual, three tissues were sensitized with serum from a donor with a modest concentration of total IgE (< 636 U/ml), high specific IgE to *Dermatophagoides farinae* (FAST 4), and low specific IgE to horse and dog allergens (Donor 1, Table 1) and three tissues acted as nonsensitized controls (see Table 3a for tissue donor IgE concentrations).

Cumulative concentration–effect curves to histamine (0.1 μ M to 0.1 mM) were constructed in all tissues. After completing histamine concentration–effect curves, tissues were washed and reequilibrated for 60 min before construction of cumulative concentration–effect curves to the allergens, *D. farinae*, dog or horse epithelium. A single allergen concentration–effect curve was constructed in a sensitized and nonsensitized pair of tissues. Sensitivity to *D. farinae* was checked in tissues receiving dog or horse allergens by the application of a single concentration of *D. farinae* (10 U/ml) at the end of the allergen concentration–effect curve. Tissue wet weights were recorded at the end of the experiment.

Effect of High IgE Serum Sensitization on Responses to Allergen and Histamine

Using two tissues taken from the same individual, one tissue was sensitized with serum from a donor with specific IgE for *D. farinae* (FAST

TABLE 2
INDIVIDUAL DATA OF TISSUE DONORS

No.	Patient	Sex	Age	SS	Histology	VC	FEV ₁	% Pred	Drugs [†]
1.	D.B.	M	71	Ex*	SqCA	4.5	2.6	83	1, 2, 4
2.	W.K.	M	68	No	SqCA	2.5	1.8	55	none
3.	D.U.	F	62	No	AdCA	3.5	2.8	116	none
4.	R.R.	M	56	Ex	AlvCA	3.7	2.1	59	none
5.	I.W.	F	61	Yes [†]	AdCA	3.7	1.9	87	4
6.	H.J.	M	61	Yes	GrCA	4.9	3.2	92	none
7.	P.G.	M	60	Ex	AdCA	4.0	3.1	98	none
8.	F.P.	M	51	Yes	SqCA	4.5	2.7	81	none
9.	E.B.	M	63	Yes	SqCA	3.6	2.2	71	none
10.	A.G.	M	68	Yes	AdCA	4.5	2.7	78	3
11.	H.F.	M	53	Yes	AdCA	5.4	3.8	98	none
12.	H.M.	M	54	Yes	AdCA	5.0	3.8	97	none
13.	W.S.	M	66	Yes	SqCA	6.3	3.6	96	none
14.	G.S.	M	69	Yes	AdCA	4.1	2.7	96	none
15.	K.-H.M.	M	62	Yes	SqCA	4.1	2.7	80	none
16.	K.K.	F	52	Yes	AdCA	2.7	2.0	78	1, 4
17.	W.F.	M	72	Yes	SqCA	4.0	3.2	114	none
18.	W.S.	M	57	Ex	SqCA	5.6	3.8	99	none
19.	J.M.	M	65	Ex	AdCA	5.4	3.8	119	none
20.	R.H.	M	65	Yes	AdCA	4.4	2.1	61	none
21.	H.K.	M	52	Yes	AdCA	3.8	2.9	85	none
22.	I.F.	F	71	Yes	AdCA	3.4	1.8	91	2, 3

Definition of abbreviations: SS = smoking status; VC = vital capacity; FEV₁ = forced expiratory volume in one second; % pred = % predicted; AdCA = adenocarcinoma; SqCA = squamous cell carcinoma; AlvCA = alveolar cell carcinoma; GrCA = great cell carcinoma.

* Stopped smoking at least 2 yr ago.

[†] Smoking up to the time of the operation.

[‡] Drug classes: 1 = anticholinergics; 2 = theophylline; 3 = corticosteroids; 4 = β -adrenoceptor agonists.

TABLE 3
IgE LEVELS ASSOCIATED WITH SERUM OF TISSUE DONORS*

No.	Patient	Sex	Total (U/ml)	D ₂ (U/ml)	E ₂ (U/ml)	E ₃ (U/ml)	Sensitizing Serum
(a) Specificity of sensitization							
1	D.B.	M	13	< 0.20	0.39	< 0.20	Donor 1
2	W.K.	M	18	0.27	0.38	0.27	Donor 1
3	D.U.	F	29	0.29	0.24	0.24	Donor 1
4	R.R.	M	55	0.30	1.84	0.28	Donor 1
5	I.W.	F	< 10	0.27	0.24	0.30	Donor 1
(b) Effects of high IgE serum sensitization on response to allergen and histamine							
5	I.W.	F	< 10	0.27	0.24	0.30	Donor 2
6	H.J.	M	12	0.28	0.30	0.81	Donor 2
7	P.G.	M	17	0.24	0.38	0.20	Donor 2
8	F.P.	M	73	< 0.2	< 0.2	0.22	Donor 2
9	E.B.	M	16	0.31	0.88	0.34	Donor 2
(c) Effects of chimeric IgE (JW8) sensitization on response to allergen (NIP-BSA) and histamine							
10	A.G.	M	26	< 0.20	nd	nd	na
11	H.F.	M	21	< 0.20	nd	nd	na
12	H.M.	M	120	< 0.20	nd	nd	na
13	W.S.	M	< 10	< 0.20	nd	nd	na
14	G.S.	M	< 10	< 0.20	nd	nd	na
15	K-H.M.	M	28	< 0.20	nd	nd	na
(d) Comparison of the effects of high serum IgE versus chimeric IgE (JW8) sensitization on responses to histamine							
16	K.K.	F	60	nd	nd	nd	Donor 3
17	W.F.	M	46	nd	nd	nd	Donor 2
18	W.S.	M	13	nd	nd	nd	Donor 4
19	J.M.	M	17	nd	nd	nd	Donor 5
20	R.H.	M	29	nd	nd	nd	Donor 2
21	H.K.	M	101	nd	nd	nd	Donor 3
22	I.F.	F	36	nd	nd	nd	Donor 6

Definition of abbreviations: D₂ = *Dermatophagoides farinae*; E₂ = dog epithelium; E₃ = horse epithelium; nd = not determined; na = not applicable.

* See Table 1 for IgE profile of sensitizing serum and Table 2 for individual patient data.

4) and total IgE concentrations in excess of 3,000 U/ml (Donor 2, Table 1) and the other acted as a nonsensitized control (see Table 3b for tissue donor IgE levels). A cumulative concentration-effect curve to histamine (0.1 μ M to 0.1 mM) was constructed in each tissue, after which tissues were washed and reequilibrated for 60 min before construction of cumulative concentration-effect curves to *D. farinae*. Tissue wet weights were recorded at the end of the experiment.

Effect of Chimeric, Hapten-specific IgE (JW8) Sensitization on Responses to Allergen and Histamine

Using two tissues taken from the same individual, one tissue was sensitized using the chimeric, hapten-specific IgE antibody JW8 (10 μ g/ml) while the other acted as a nonsensitized control (see Table 3c for tissue donor IgE levels). The concentration of JW8 was selected to approximate to the concentrations of total IgE used; therefore, since 1 U IgE approximates to 2.4 ng IgE, 10 μ g/ml represents approximately 4,000 U/ml.

Cumulative concentration-effect curves to histamine (0.1 μ M to 0.1 mM) were constructed and once completed, tissues were washed and reequilibrated for 60 min before construction of a cumulative concentration-effect curve to the specific allergen NIP-BSA (1 ng/ml to 1 μ g/ml).

Effect of High IgE Serum or Chimeric, Hapten-specific IgE (JW8) Sensitization on Responses to Histamine

Using three tissues taken from the same individual, one tissue was sensitized using serum containing high *D. farinae*-specific IgE, one tissue was sensitized using the chimeric, hapten-specific IgE antibody, JW8 (10 μ g/ml), and one tissue acted as a nonsensitized control (see Table 3d for sensitizing serum and IgE levels of tissue donors).

Cumulative concentration-effect curves to histamine (0.1 μ M to 0.1 mM) were constructed in all tissues and, after washing and reequilibrating for a further 60 min, sensitization was confirmed by a positive response to the appropriate allergen (*D. farinae* 30 U/ml or NIP-BSA 1 μ g/ml).

Measurement and Analysis of Data

All responses were recorded as changes in isometric tension (mg). The potency of histamine was determined by an iterative curve-fitting procedure (Kaleidagraph; Synergy Software, Reading, PA) for each individual tissue and is expressed as the pEC₅₀ value (i.e., $-\log_{10}$ of the concentration of histamine giving a half maximal effect). Statistical analysis of the data was performed using an unpaired Student's *t* test. Repeated measures analysis of variance was performed where appropriate and when significance was found, pairwise comparisons between groups were performed by Newman-Keuls multiple comparisons test. *P* < 0.05 was considered significant and all values quoted are the mean $\pm$ SEM of at least 5 experiments with tissues derived from different individuals.

Materials

Histamine and carbachol were obtained from Sigma Chemical Company (Deisenhofen, Germany). *D. farinae* and horse and dog allergens were obtained from Allergopharm KG (Reinbek, Germany). JW8, a chimeric hapten-specific IgE, λ I antibody whose heavy chain is composed of a human C ϵ constant region fused to a mouse variable (V_H) region, and the specific hapten for this antibody, NIP-BSA, were generously provided by Ciba-Geigy, Basel, Switzerland. For further details regarding the generation of JW8 see Neuberger and colleagues (19). All drug solutions were prepared using distilled water, with the exception of allergen and antibody solutions which were prepared using 0.9% saline.

RESULTS

Specificity of the Sensitization Process

In tissues sensitized with serum containing high specific IgE for *D. farinae* (FAST 4), but low specific IgE for horse and dog allergens, there was a concentration-related contraction to *D. farinae*, but no significant response to either dog or horse epithelium (Figure 1). Histamine caused concentration-dependent

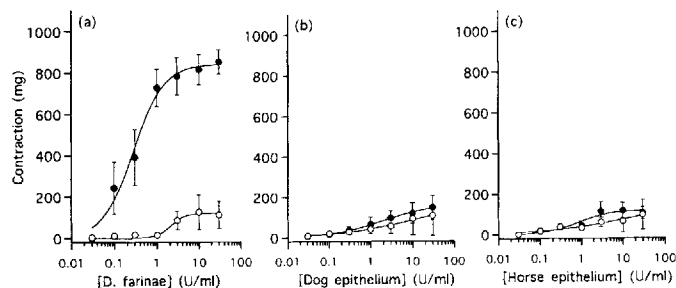


Figure 1. Contractile concentration-effect curves to (a) *D. farinae*, (b) dog and (c) horse allergens in nonsensitized human bronchus (open circles) and human bronchus sensitized (closed circles) with serum containing modest total IgE (636 U/ml) high (FAST 4) *D. farinae*-specific IgE and low (FAST 0) horse- and dog-specific IgE (see Tables 1 and 3a for details). Contractions are expressed as mg changes in tension. Data are the mean $\pm$ SEM of 5 experiments, using tissue derived from different individuals.

contractions in both serum-sensitized and nonsensitized preparations (Figure 2). The potency of histamine was slightly higher in sensitized tissues ($pEC_{50} = 5.99 \pm 0.09$) compared with nonsensitized tissues ($pEC_{50} = 5.73 \pm 0.07$), but the difference did not achieve statistical significance ($p = 0.07$). There was no significant difference in the maximal response to histamine in sensitized tissues (669 ± 53 mg or 58 ± 4 mg/mg wet weight) when compared with nonsensitized tissues (632 ± 81 mg or 50 ± 6 mg/mg wet weight). Tissue wet weights were not significantly different between sensitized (11.9 ± 1.1 mg) and nonsensitized tissues (12.8 ± 0.8 mg).

Effect of High IgE Serum Sensitization on Responses to Allergen and Histamine

Allergen, *D. farinae*, caused concentration-related contractions in serum-sensitized tissues, but not in nonsensitized control tissues (Figure 3a). Histamine caused concentration-dependent contractions in both serum-sensitized and nonsensitized preparations (Figure 3b). The potency of histamine was significantly increased in serum-sensitized tissues ($pEC_{50} = 6.05 \pm 0.11$) compared with

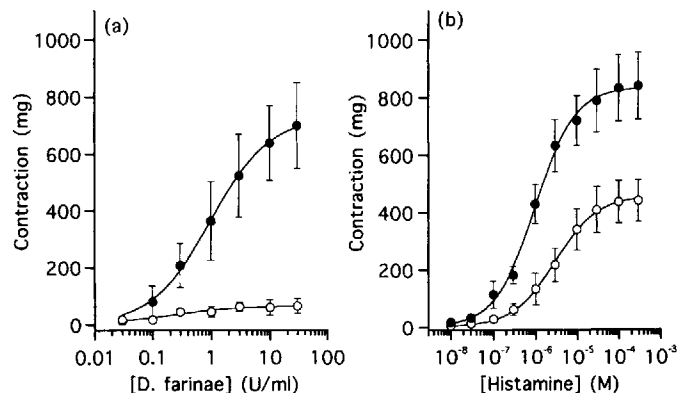


Figure 3. Contractile concentration-effect curves to (a) *D. farinae* and (b) histamine, in nonsensitized (open circles) human bronchus and bronchus sensitized (closed circles) with serum containing very high total IgE ($> 3,000$ U/ml) and high specific (FAST 4) IgE for *D. farinae* (see Tables 1 and 3b for details). Contractions are expressed as mg changes in tension. Data are the mean $\pm$ SEM of 5 experiments, using tissue derived from different individuals.

nonsensitized tissues (5.46 ± 0.18) and the magnitude of maximal contractions was also significantly greater in serum-sensitized tissues (840 ± 116 mg or 93 ± 13 mg/mg wet weight) when compared with nonsensitized tissues (440 ± 73 mg or 55 ± 10 mg/mg wet weight) from the same individual. Tissue wet weights were not significantly different between sensitized (9.2 ± 0.6 mg) and nonsensitized tissues (8.3 ± 0.8 mg).

Effect of Chimeric, Hapten-specific IgE (JW8) Sensitization on Responses to Allergen and Histamine

NIP-BSA caused concentration-related contractions in JW8-sensitized preparations, but was without effect in nonsensitized tissues (Figure 4a). Histamine caused concentration-dependent contractions in both JW8-sensitized and nonsensitized preparations, but neither the potency ($pEC_{50} = 5.28 \pm 0.19$, JW8-sensitized; 5.26 ± 0.22 , nonsensitized) nor the magnitude of maximal contractions (903 ± 123 mg, JW8-sensitized; 832 ± 221 mg, nonsensitized) were significantly different in JW8-sensitized tissues when compared with nonsensitized tissues from the same individual (Figure 4b).

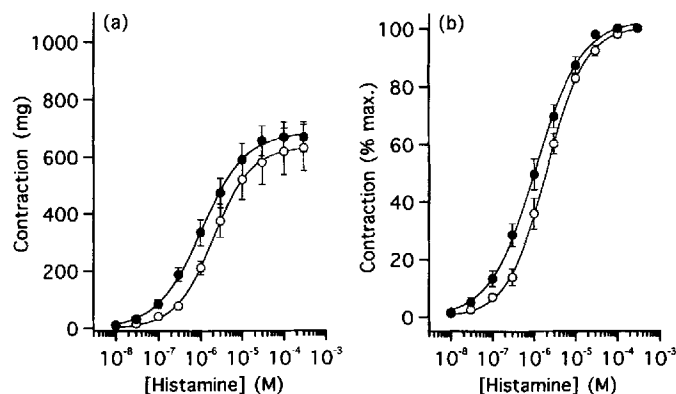


Figure 2. Contractile concentration-effect curves to histamine in nonsensitized human bronchus (open circles) and bronchus sensitized (closed circles) with serum containing modest total IgE (636 U/ml) high (FAST 4) *D. farinae*-specific IgE and low (FAST 0) horse- and dog-specific IgE (see Tables 1 and 3a for details). Contractions are expressed as (a) mg changes in tension and (b) percentage of maximal contraction. Data are the mean $\pm$ SEM of 5 experiments, using tissue derived from different individuals.

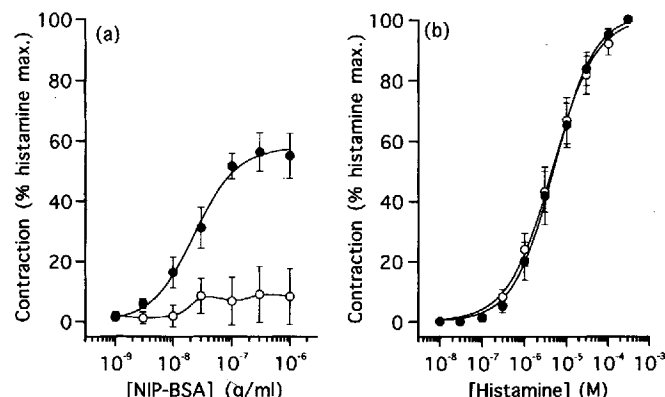


Figure 4. Contractile concentration-effect curves to (a) allergen, NIP-BSA, and (b) histamine, in JW8-sensitized (closed circles) and non-sensitized (open circles) human bronchus (see Table 3c for details). Contractions are expressed as mg changes in tension. Data are the mean $\pm$ SEM of 6 experiments, using tissue derived from different individuals.

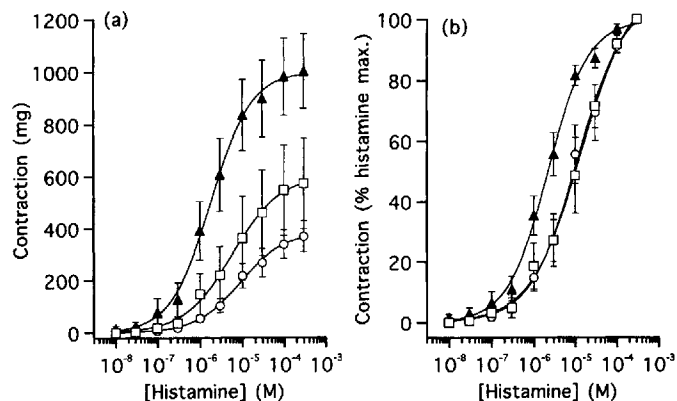


Figure 5. Contractile concentration-effect curves to histamine in high *D. farinae*-specific IgE-sensitized (closed triangles), JW8-sensitized (open squares), and nonsensitized (open circles) human bronchus (see Tables 1 and 3d for details). Contractions are expressed as (a) changes in mg tension, and (b) percentage of maximal contraction. Data are the mean $\pm$ SEM of 7 experiments, using tissue derived from different individuals.

Effect of High IgE Serum or Chimeric, Hapten-specific IgE (JW8) Sensitization on Responses to Histamine

The effect of histamine, while consistent among tissues from the same individual, can vary between individuals both in potency and in the magnitude of the maximal contraction. Therefore, it was considered necessary to compare histamine responsiveness in tissues taken from the same individuals, sensitized (or not) with either high IgE containing serum or chimeric IgE, JW8.

There was a significant increase in the potency and magnitude of the maximal contractions to histamine in the serum-sensitized tissue when compared with nonsensitized and JW8-sensitized tissues (Figure 5). The potency (pEC_{50}) of histamine was 5.67 ± 0.14 in serum-sensitized tissues, which was significantly greater than that in either JW8-sensitized (4.97 ± 0.20) or nonsensitized (5.07 ± 0.16) tissues. The magnitude of the maximal contraction to histamine was $1,003 \pm 142$ mg in serum-sensitized tissues, which was substantially and significantly greater than in JW8-sensitized (572 ± 174 mg) and nonsensitized (369 ± 60 mg) tissues. Sensitization was confirmed by a positive response to the appropriate sensitizing allergen (i.e., NIP-BSA; 440 ± 152 mg or $78 \pm 21\%$ of histamine maximum and *D. farinae*; 494 ± 126 mg or $58 \pm 21\%$ histamine maximum).

DISCUSSION

The present study shows that "specific" hyperresponsiveness to allergen can be induced in nonatopic human airways by passive sensitization with either allergen-specific IgE present in atopic serum or a chimeric hapten-specific IgE. In addition, the specificity of allergen responses in these tissues is related to the concentration of allergen-specific IgE in the sensitizing serum. However, the "nonspecific" hyperresponsiveness to histamine does not appear to be directly associated with IgE, but may be related to some other factor or factors present in the sensitizing serum, concentrations of which may be influenced by the amount of total IgE. Therefore, allergen-specific IgE appears to be the only component of serum required for the induction of "specific" (allergen) responsiveness, but is not sufficient for the induction of increased "nonspecific" responsiveness to a bronchoconstrictor agent (histamine).

The present data confirm previous reports that passive sensitization of human bronchial smooth muscle leads to increased

responsiveness to histamine (5) and induces responsiveness to the specific sensitizing allergen (*D. farinae*) compared with nonsensitized tissues. However, the demonstration of a leftward shift in the concentration-effect curve to histamine, along with an increase in the magnitude of maximal contraction, is a novel finding of this study. Additionally, the lack of responses to the common allergens, dog and horse epithelium, in tissues that were sensitized with serum containing high concentrations of *D. farinae*-specific IgE, demonstrates for the first time the specificity of the *in vitro* sensitization process. These observations are in keeping with the concept that exposing airways from nonatopic individuals to serum from atopic donors containing high allergen-specific IgE results in the loading of high-affinity IgE receptors (Fc ϵ R1) on tissue mast cells and basophils with the specific IgE (20-22). Subsequent exposure of these airways to the allergen results in Fc ϵ R1 crosslinking and cell activation, with the release of bronchoconstrictor mediators such as cysteinyl leukotrienes and histamine (6, 11, 12, 21, 22). The IgE-dependence of this effect has been demonstrated previously by Tunon de Lara and colleagues (7), who showed that IgG was unable to elicit such responses.

Exposing tissues to serum containing approximately equivalent concentrations of allergen-specific IgE, but high versus modest ($> 3,000$ versus 636 U/ml) total IgE, resulted in approximately equivalent responses to specific allergen. However, an increase in the potency and magnitude of maximal contractions to histamine was observed in high IgE-sensitized tissues which was not observed in the low IgE-sensitized tissues. This observation suggests that the nonspecific hyperresponsiveness to histamine is unrelated to the concentration of specific IgE in the sensitizing serum but seems to be related to the level of total IgE. In tissues sensitized with the chimeric IgE antibody, JW8, sensitivity to its specific hapten/carrier complex, NIP-BSA, could be demonstrated in the absence of histamine hyperresponsiveness. This finding again suggests that nonspecific hyperresponsiveness is unrelated to the concentration of specific IgE. Interestingly, although there was no significant change in histamine responses in tissues sensitized with low-IgE-containing serum compared with nonsensitized tissues, there was a leftward shift in the response to histamine that was absent in tissues sensitized with JW8. This may suggest that the development of hyperresponsiveness, although related to total IgE, requires the presence of some other serum factor or factors.

These present observations suggest a number of possibilities regarding the induction of nonspecific hyperresponsiveness by passive sensitization: (1) high IgE alone is sufficient to induce hyperresponsiveness, (2) high IgE along with some other serum factor is required, or (3) IgE itself is not required, but the concentration of IgE is related to the amount of the serum factor that induces the hyperresponsiveness. The first possibility seems unlikely because no change in histamine responsiveness was observed in JW8-sensitized tissues despite the fact that concentrations of chimeric IgE were equivalent to total IgE levels in sensitizing serum (4,000 U/ml). However, the possibility of poor affinity of this antibody or decreased capacity to induce mast cell activation cannot be excluded. The involvement of a serum factor, the concentrations of which may be related to IgE, seems a more likely possibility, but whether this factor alone or in combination with IgE induces hyperresponsiveness cannot be determined from the present studies.

To more conclusively define the role of IgE in changes in airway responsiveness it would be necessary to remove this immunoglobulin from the sensitizing serum or prevent its interaction with the tissue. We are currently investigating both of these experimental approaches and the preliminary results support the concept presented here (Watson and Rabe, personal communication).

It is important to realize that this study describes a model of sensitization using macroscopically normal airways taken from patients with various lung diseases. We cannot rule out the possibility that the individual condition of a patient may have influenced our results but, since nonsensitized control tissues from the same individuals were always run in parallel, we are confident that the results are valid on a comparative basis and provide insight into the mechanisms of induced hyperresponsiveness.

Therefore, the present study has demonstrated both specific (allergen) and nonspecific (histamine) hyperresponsiveness associated with passively sensitized human airways, in keeping with previous reports (5, 8), and has demonstrated specificity of the allergen hyperresponsiveness. However, our data suggest that while the specific allergen responsiveness is entirely IgE-dependent, the nonspecific histamine hyperresponsiveness is either independent of IgE or requires IgE in the presence of some other factor or factors present in the sensitizing serum. Further studies are required to determine the nature of the serum factor and whether histamine hyperresponsiveness is conferred by the factor alone or in combination with IgE.

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