

An Analysis of the Role of IgE in Intolerance to Aspirin and Tartrazine

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Total serum IgE and eosinophil count were determined for 30 patients with intolerance to aspirin. Total IgE levels in the aspirin intolerant patients were similar to those expected in a non-atopic population. In contrast, total eosinophil count (TEC) tended to be elevated in the aspirin intolerant group. Elevated TEC was observed both in bronchospastic (57%) and in urticarial (25%) aspirin intolerance. Specific anti-aspiryl and anti-tartrazyl antibodies of the IgE class were assayed by the galactosidase immunosorbent test (GIST)*. IgE anti-aspiryl antibodies were possibly detected in one patient, but did not correlate with clinical intolerance to aspirin. It is unlikely that the clinical symptoms and the eosinophilia of intolerance to aspirin and tartrazine are mediated by antibodies of the IgE class.

Key words: antibodies; aspirin; bronchospasm; eosinophilia; IgE; intolerance; tartrazine; urticaria

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There are about one million people in the United States with intolerance to aspirin (20). Aspirin intolerance is characterized by bronchospasm, generalized urticaria/angioedema, shock or severe rhinitis (6). This symptom complex was first termed "aspirin allergy". The term "allergy" implies an altered state induced by an antigen and mediated by IgE antibodies. Samter & Beers (16) proposed that the adverse reactions to aspirin were not due to antigen-antibody reactions because aspirin intolerance was commonly found in asthmatics with negative allergy skin tests and no anti-aspiryl antibodies were found in these individuals; thus, the term "intolerance" was introduced as a substitute for "allergy" to aspirin. However, Minden & Farr (14) reported that the sera of patients receiving aspirin contained antibodies that react with acetylated human serum albumin, but that the presence of these antibodies did

not correlate with clinical intolerance to aspirin. Utilizing phage neutralization and hemagglutination techniques, Amos et al. (1) reported finding anti-aspiryl antibodies in human sera, but as in the study of Minden & Farr, there was no clinical correlation with intolerance to aspirin.

None of the studies referred to in the preceding paragraph were designed to detect anti-aspiryl antibodies of the IgE class and there are data which suggest that some cases of aspirin intolerance may be mediated by such antibodies. For example, Phillips & Perelmutter (15) reported that all six of their patients with urticarial aspirin intolerance who received intradermal tests with aspiryl-polylysine demonstrated positive immediate tests. DeWeck also reported positive immediate skin tests to aspiryl-polylysine in three patients with urticarial-type intolerance to aspirin (8).

The research described here is an analysis

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*Abbreviations: ASA, aspirin; Y5, tartrazine (FDC Yellow Dye No. 5); GIST, galactosidase immunosorbent test (28).

of the possible role of specific IgE antibodies in aspirin and tartrazine intolerance.

MATERIAL AND METHODS

Subjects were adult outpatients and were not acutely ill at the time of the studies. Intolerance to aspirin was determined by clinical history (21). Intolerance to tartrazine was determined by double-blind crossover oral challenge with at least 0.22 mg of tartrazine, as previously reported (22). Serum total IgE (RIST, Pharmacia) and total eosinophil count (TEC) were determined as described (5, 7). Allergy skin tests were performed by the scratch test method with a set of common pollen, mold and animal dander inhalent allergens.

The galactosidase immunosorbent test (GIST) for specific IgE antibodies against aspirin and tartrazine was performed by adaptation of the method previously reported (28). Immunosorbent discs for the GIST were prepared by first insolubilizing rabbit immunoglobulin (RIg) on to filter paper discs by the periodate method (18, 27) to provide a "lawn" to which to couple aspirin or tartrazine. To this end, circular discs, 6.3 mm in diameter, of Whatman No. 541 filter paper were oxidized by treatment with 0.01 M NaIO₄ (sodium m-periodate, Sigma Chemical Co.) for 16 h at room temperature. The oxidized discs were washed five times with H₂O and three times with 0.01 M phosphate buffered saline, pH 7.2. Two hundred milligrams of RIg (Cohn Fraction II prepared from normal rabbit serum) were reacted with 1700 oxidized discs in 200 ml phosphate buffered saline for 16 h at room temperature. The unreacted RIg was decanted and the discs were washed three times with phosphate buffered saline. The washed discs were reacted with 0.1 M ethanolamine, pH 7.8, for 2.5 h at room temperature. The discs were then washed three times with phosphate buffered saline, three times with 0.1 M CH₃COONa, and twice again with phosphate buffered saline. Aspirin and tartrazine were each coupled to

the RIg immunosorbent discs, with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (4). Fifty RIg immunosorbent discs were suspended in 40 ml H₂O with 200 mg of carbodiimide and 200 mg of either aspirin (ASA) or tartrazine. After 2 days in the dark at room temperature, the reaction mixture was decanted and the discs were washed exhaustively and sequentially with H₂O, 6 M urea and E-buffer (28), until the wash solution possessed no detectable ASA or tartrazine as determined from fluorescence emission at 514 nm (λ excitation=305 nm) and absorbance at 420 nm, respectively. The resulting ASA-discs fluoresced when excited at 366 nm with a Woods lamp and the tartrazine discs possessed a yellow color. The ASA-discs and tartrazine-discs were utilized in the GIST for IgE antibodies as previously described (28). Galactosidase activity in the GIST was assayed fluorometrically by hydrolysis of umbelliferylgalactopyranoside (29). Each GIST for specific anti-aspiryl and anti-tartrazyl IgE antibodies was conducted with 0.1 ml of the patient's serum. Tests for total IgE conducted concomitantly by means of the GIST (28) were sensitive to as little as 0.5 ng total IgE per test. Sera from Balb/C mice immunized with covalent conjugates of aspirin and Busycon hemocyanin gave a positive GIST reaction with ASA-discs ($P < 0.02$) but not with Tartrazine-discs ($P > 0.05$); for these control studies in mice the GIST was conducted with goat anti-mouse Ig instead of sheep anti-human IgE.

RESULTS

Serum IgE concentration and total eosinophil count were obtained for 30 patients with aspirin intolerance, 19 of the bronchospastic type and 11 of the urticaria/angioedema type. Data for the bronchospastic patients are given in Table 1. The range of serum IgE was from 11 to 1250 Units/ml; The range of TEC was from 22 to 840 eos/mm³. The geometric mean of serum IgE was 157 Units/ml; the geometric mean of TEC was 376 eos/mm³. Allergy skin tests to

Table 1
Characterization of patients with bronchospastic intolerance to aspirin

Patient number	Serum IgE (units/ml)	TEC (eos/mm ³)	Allergy skin tests	Steroid therapy
1	525	840	—	+
2	1150	519	+	+
3	650	600	+	—
4	19	700	—	—
5	198	300	+	+
6	11	281	—	+
7	305	580	+	—
8	165	530	+	+
9	115	22	+	+
10	80	700	—	—
11	84	120	+	+
12	1250	144	+	—
13	75	70	+	+
14	64	220	+	+
15	120	208	+	+
16	54	99	—	—
17	68	666	+	—
18	1140	165	+	—
19	720	388	—	+

pollens, animal dander, and mold, by the scratch test and, when appropriate, by the intracutaneous test were positive in 68% (13/19) of the bronchospastic patients and 58% (11/19) were receiving steroid therapy. Corresponding data for the aspirin intolerant patients with urticaria/angioedema are given in Table 2. The range of serum IgE was from 48 to 510 Units/ml; the range of TEC was from 50 to 680 eos/mm³. The

geometric mean of serum IgE was 177 Units/ml; geometric mean TEC was 325 eos/mm³. Allergy skin tests were positive in 82% (9/11) and 18% (2/11) of the patients with urticaria/angioedema were receiving steroid therapy. Comparison of the IgE and TEC data in Tables 1 and 2 by parametric and non-parametric statistical techniques showed no significant differences, either in serum IgE concentration or in TEC, between

Table 2
Characterization of patients with urticaria/angioedema intolerance to aspirin

Patient number	Serum IgE (units/ml)	TEC (eos/mm ³)	Allergy skin tests	Steroid therapy
20	95	160	+	—
21	105	462	+	+
22	99	488	+	—
23	270	231	+	—
24	355	157	+	—
25	48	368	+	—
26	510	260	+	—
27	92	242	+	—
28	325	680	—	+
29	194	50	+	—
30	290	472	—	—

the bronchospasm and urticaria/angioedema groups. Regression analysis of the data in Tables 1 and 2 showed that IgE concentration was not linearly correlated with eosinophil count, in either the bronchospastic or urticarial groups (linear correlation coefficient = 0.01, $P > 0.05$).

The serum IgE concentrations given in Tables 1 and 2 were compared with 150 U/ml, the value reported to be two standard deviations above the normal geometric mean IgE concentration (3). As shown in Table 3, about one-half of the patients in both the urticarial (54.5%) and bronchospastic (47.4%) groups had IgE concentrations above the normal cut-off of 150 U/ml. As shown in Table 4, if elevated serum

IgE associated with positive skin tests to common allergens were excluded, only 21.1% of the remaining patients had elevated IgE, i.e. two of seven patients in the urticarial group and two of 12 with bronchospasm.

The TEC given in Tables 1 and 2 were compared with the normal cut-off of 300 eos/mm³ (7). As shown in Table 5, almost one-half of the urticaria/angioedema (45.5%) and bronchospasm (47.4%) groups had eosinophilia. Exclusion of data in which eosinophilia could be associated with positive skin tests and in which normal eosinophil count could be accounted for by steroid therapy produced the results shown in Table 6. One-quarter (25.0%) of the urticarial group and over half (57.1%) of the

Table 3
Frequency of elevated serum total IgE in aspirin intolerance

Type of aspirin intolerance	No. of cases	No. with elevated IgE	Frequency of elevated IgE (%)
Urticarial	11	6	54.5
Bronchospastic	19	9	47.4
Total	30	15	50.0

Table 4
Corrected frequency of elevated serum total IgE in aspirin intolerance*

Type of aspirin intolerance	No. of cases	No. with elevated IgE	Frequency of elevated IgE (%)
Urticarial	7	2	28.6
Bronchospastic	12	2	16.7
Total	19	4	21.1

* Data for patients with positive skin tests for common inhalent allergens and elevated IgE levels were not included in this Table.

Table 5
Frequency of elevated eosinophil count (TEC) in aspirin intolerance

Type of aspirin intolerance	No. of cases	No. with elevated TEC	Frequency of elevated TEC (%)
Urticarial	11	5	45.5
Bronchospastic	19	9	47.4
Total	30	14	46.7

Table 6
Corrected frequency of elevated total eosinophil count (TEC) in aspirin intolerance*

Type of aspirin intolerance	No. of cases	No. with elevated TEC	Frequency of elevated TEC (%)
Urticarial	8	2	25.0
Bronchospastic	7	4	57.1
Total	15	6	40.0

* Data for patients with positive allergy skin tests and elevated TEC were not included in this Table. Data for patients on steroid therapy and with normal TEC also were not included.

Table 7
Galactosidase immunosorbent test (GIST) for specific IgE antibodies against aspirin (ASA) and tartrazine (Y5)

Patient	Bronchospasm	Urticaria	Anti-ASA*	Anti-tartrazine*
31	—	ASA, Y5	640 620	550 750
32	—	ASA, Y5	510 430	420 440
33	—	Y5 only	1185 1110	1110 1245
34	—	—	660 675	825 750
35	—	ASA, Y5	510 630	690 690
36	Y5	ASA	660 600	705 750
37	—	ASA, Y5	660 450	720 585
38	—	ASA	630 450	675 720
39	—	ASA	675 495	645 780
40	ASA, Y5	—	585 510	780 630
41	ASA	—	420 390	510 480
42	ASA	—	660 615	720 570
43	—	—	630 390	525 510
44	—	—	600 495	750 720
45	—	—	555 585	699 810
46	—	—	510 555	690 735

* Numbers represent fluorescence of hydrolysis product in GIST.

bronchospastic group had eosinophilia uncomplicated by positive skin tests and by steroid therapy. Thus, in 40.0% of the aspirin intolerant patients there was otherwise unexplained eosinophilia.

The sera of 16 individuals were studied for anti-aspiryl and anti-tartrazyl IgE antibodies. As shown in Table 7, five of these individuals are tolerant to aspirin and to tartrazine (No. 34 and Nos. 43-46). Two patients (No. 41 and No. 42) have bronchospasm in response to aspirin, one (No. 40) has bronchospasm in response either to aspirin or tartrazine, and one (No. 36) has bronchospasm induced by tartrazine and urticaria induced by aspirin. The rest of the patients have urticaria in response either just to aspirin or to tartrazine also, except for patient No. 33. Patient No. 33 is definitely able to take aspirin with impunity as confirmed by a challenge test, but has an urticarial response to challenge with tartrazine. The numbers in the columns of Table 7 give the intensity of fluorescence due to hydrolysis of the fluorogenic substrate used in the GIST for IgE antibodies (27, 29). The GIST results for patient No. 33 are higher than the rest. Patient No. 33 may thus possibly possess IgE antibodies against both aspirin and tartrazine but he is clinically sensitive only to tartrazine. As shown in Table 7, the remaining 10 aspirin-intolerant patients studied were indistinguishable from the five normal controls both with respect to anti-aspiryl and anti-tartrazyl IgE antibodies.

DISCUSSION

Although aspirin is an extremely useful analgesic and antipyretic drug, some individuals have adverse responses to aspirin characterized by acute bronchospasm, generalized urticaria/angioedema, shock or severe rhinitis (20). The frequency of aspirin intolerance in various diseases and conditions varies. Aspirin intolerance was reported to be 0.9% in otherwise normal individuals, 1.4% in chronic rhinitis, 3.8% in asthmatics, and 23% in chronic urticaria

(6, 20). Fifteen percent of the group intolerant to aspirin are also intolerant to tartrazine (22).

Serum IgE and TEC varied over a very wide range in both bronchospastic and urticarial aspirin-intolerant patients (Tables 1 and 2). The geometric mean IgE concentration and TEC were elevated compared with results reported for normal individuals. In the case of bronchospastic aspirin intolerance the geometric mean IgE concentration and TEC was 157 Units/ml and 376 eos/mm³, respectively. In order to permit analysis of the relationship between serum IgE, total eosinophil count, and aspirin intolerance, data from Tables 1 and 2 for patients whose laboratory findings could be explained either by atopy, steroid therapy, or by both, were excluded. As shown in Table 4, only 21.1% of the aspirin-intolerant patients without atopy had serum IgE concentration greater than the normal cut-off value. However, at least 20% of the apparently normal population also has serum IgE concentration greater than a cut-off two standard deviations above the mean (23). Thus, there is no unexplained elevation in serum IgE in our aspirin-intolerant patients. In contrast, in 40.0% of the aspirin-intolerant patients there is otherwise unexplained eosinophilia (Table 6). Isolated and unexplained eosinophilia is rare (7). Therefore, the data in Tables 4 and 6 indicate that eosinophilia is a frequent occurrence in aspirin intolerance but elevated serum total IgE is not. However, it appeared possible that although total IgE serum concentration is normal, specific IgE antibodies against aspirin or tartrazine might be present in case of intolerance to aspirin. For this reason, we determined whether specific anti-aspiryl and anti-tartrazyl antibodies of the IgE class were associated with intolerance to aspirin and tartrazine. The results in Table 7 show that intolerance to aspirin and to tartrazine does not appear to be associated with specific antibodies of the IgE class. The one possible instance of anti-aspiryl antibodies was the case of a patient

(No. 33) who is able to tolerate aspirin, but not tartrazine. Thus, it appears that IgE antibodies have nothing to do with either bronchospastic or urticarial aspirin intolerance, the two most common forms of aspirin intolerance.

There is published evidence which suggests a role for IgE as mediator of aspirin intolerance. For example, Phills & Perelmutter (15) reported that all six patients with urticarial aspirin intolerance who received intradermal tests with aspiryl-polylysine demonstrated positive immediate tests. Also, DeWeck reported positive immediate intradermal tests in three patients with urticarial sensitivity to aspirin (8). In contrast to these two reports, however, none of the aspirin-intolerant patients skin-tested with aspiryl-polylysine by Schlumberger et al. (19) gave positive immediate reactions. The results presented here do not support the hypothesis that intolerance to aspirin and positive skin tests to aspiryl-polylysine are mediated by IgE antibodies. Perhaps skin-sensitizing antibodies of a class other than IgE are involved in intolerance to aspirin and positive skin tests to aspiryl-polylysine. In addition, aspirin and other salicylates may exert a direct effect on lymphocytes. This notion is supported by the finding that ingestion of aspirin decreases the *in vitro* blastogenic response to phytohemagglutinin (10). It is well established that aspirin and other analgesics inhibit the prostaglandin synthetase system (9, 25), but tartrazine has no such inhibitory effect. Since 15% of the group intolerant to aspirin are also intolerant to tartrazine (22), the prostaglandin system cannot be directly implicated in the adverse reactions to tartrazine, aspirin and many other compounds (Table 1 in ref. 19). Recently, however, a possible relationship between prostaglandin and SRS-A synthesis has been elucidated, through studies of arachidonic acid metabolism (30), and the potential role of SRS-A as a mediator of adverse drug reactions has not yet been investigated.

We found eosinophilia to be a frequent

concomitant of bronchospastic and urticarial aspirin intolerance (Table 6); these results are in agreement with several previous reports (2, 17, 26, 31). In contrast, Stevenson et al. (24) found no eosinophilia, but only a statistically insignificant trend towards eosinophilia, in their seven aspirin-intolerant asthmatic patients. The reason for this discrepancy may be the relatively small number of patients in the study of Stevenson et al. (24) and the fact that their subjects were selected because they were known to have sustained only mild to moderate asthmatic reactions to aspirin; patients who gave a history of severe asthmatic response to aspirin were excluded from the study. In the present study, however, intolerance to aspirin were excluded from the study. In the than by challenge because of the severity of most of the adverse responses in our patients.

The pathogenetic mechanisms of intolerance to aspirin remain to be elucidated, but the data presented here support the concept that those mechanisms do not involve IgE antibodies, in spite of the "allergic" nature of some of the symptoms. Kallós was the first to ascribe these adverse reactions to histamine release, through direct drug action, rather than through antibody mediation, due to some inborn biochemical abnormality of aspirin-intolerant individuals (11). Indeed, Stevenson et al. (24), have clearly demonstrated an increase in plasma histamine during adverse responses to aspirin induced *in vivo*. However, Stevenson et al. were unable to demonstrate histamine release from the basophilic leukocytes of aspirin-intolerant asthmatics by aspirin, aspirin-rich plasma and other salicylate preparations *in vitro*.

Schlumberger et al. (19) have shown that in the serum of 17 aspirin-intolerant patients, no IgE antibodies with salicyloyl or acetylsalicyloyl specificity could be found by passive transfer to the skin of rhesus monkeys; furthermore, these authors also found that the total IgE values in the same sera were within normal limits, as were all

other antibody classes. Consistent with these results, Schlumberger et al. (19), found negative skin tests to aspiryl-polylysine in aspirin-intolerant individuals. The "positive" skin tests to aspiryl-polylysine, published in the literature, are most probably due to effects of polylysine itself, which is a membrane-active polycation eliciting non-specific articular reactions in many people. No controls with polylysine were reported by the authors who obtained "positive" results with aspiryl-polylysine.

Although specific biochemical lesion or lesions have not been uncovered, the hereditary nature of some cases of aspirin intolerance has been demonstrated in several studies (12, 13, 22). It is an important aim for future research to search for possible inborn or acquired errors of metabolism underlying adverse responses to aspirin and tartrazine.

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