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IgE MEDIATED AND NON-IgE MEDIATED ALLERGIC-TYPE REACTIONS TO ASPIRIN

By

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Allergic-type responses to aspirin (ASA) include the occurrence of generalized pruritis, erythema, urticaria, angioedema, rhinorrhoea, asthma and anaphylactoid shock (8). These clinical manifestations suggest the presence of reaginic antibodies mediating the anaphylactic-type reactions; however, until recently (5), no convincing evidence of the presence of these antibodies in the sera of patients with allergic reactions to aspirin has been described (1, 6). In addition, patients with the frequently observed triad of asthma, nasal polyps and ASA sensitivity have been studied, and no immunological basis for the adverse reaction to ASA has been found (6, 24, 26).

In a preliminary report (16), evidence was presented suggesting a role for reaginic antibodies in certain allergic-type responses to ASA. This report is an extension of that study. The Rat Mast Cell Test (RMCT) (21) was used to analyze the sera of 18 patients with allergic-type reactions to ASA, 10 of whom had the ASA triad, whereas the other 8 had anaphylactic-type reactions after ASA ingestion.

De Weck (5) recently used chemical techniques to demonstrate the presence of aspirin anhydride impurities in commercial ASA samples. He suggested that aspirin anhydride is much more likely than ASA itself to react with proteins *in vivo*, and to be responsible for the appearance of anti-salicyloyl and

anti-aspiryl IgE-type antibodies in patients ingesting ASA. He also provided evidence of the immunogenicity of the aspiryl anhydride in both animals and man. The presence of anti-aspiryl antibodies in humans has recently been confirmed by Amos et al. (1). Since skin testing with ASA has been found to be dangerous (3), it was decided to use the Aspiryl-polylysine (ASP-PLL) conjugates which previous investigators have safely used (5, 26). Thus direct skin tests utilizing ASP-PLL were performed on most of the patients studied.

In addition, four of the patients giving both positive RMCT responses to ASA and positive skin tests to ASP-PLL had the RMCT repeated after adsorption of the IgE immunoglobulins from their sera.

MATERIALS AND METHODS

(a) Fasting sera of the 18 patients and six normal human sera were obtained and stored in small aliquots at -20°C until used.

(b) *Antigens*

(1) Aspirin (plain Acetylsalicylic Acid) was obtained from Charles E. Frost & Co., Montreal, Quebec. The ASA was dissolved in Medium 199 at a concentration of 2 mgm/ml, pH 6.2 and four 10-fold dilutions of the above concentration were prepared. ASA was the antigen used in the RMCT.

(2) Aspiryl-polylysine was provided through the kindness of Dr. Alain de Weck, Bern, Switzerland. ASP-PLL is a conjugate of aspirin anhydride with polylysine (it averages 20 lysine residues per molecule). The polylysine backbone is substituted to about 50 per cent with aspiryl groups, and then succinylated in order to block the remaining free amino groups. Details of the method of preparing ASP-PLL have already been published by Dr. de Weck (5). The concentration of the stock solution is 1 micromole ASP-PLL/ml, and the concentrated preparation was diluted 1:5, 1:10, 1:100 and 1:1000 for scratch and intradermal skin tests.

(c) *Antisera*

The IgG fraction of a goat antiserum monospecific for human IgE and a normal goat IgG globulin fraction were provided through the kindness of Dr. Brian Underdown of the University of Toronto. The antiserum to IgE was prepared by immunization of a goat with purified IgE myeloma protein P.S. The antiserum was made specific for IgE by passing it over a column of Agarose to which Normal Human Serum (NHS) had been covalently coupled

by the cyanogen bromide technique (2, 18). The antiserum did not react in immunodiffusion tests with NHS, but did precipitate with both purified E myeloma P.S. and N.D. Using a double-antibody technique with rabbit-anti goat IgG as the precipitating antibody system, the goat anti-IgE bound radioactively labelled IgE protein, but did not bind radioactively labelled purified IgA, IgG and monoclonal IgM protein.

(d) *Rat Mast Cell Test (RMCT)*

The RMCT was performed as outlined previously (14). Briefly, equal volumes of 0.05 ml of allergic serum, antigen, normal rat serum and mast cell suspension were incubated at 37° C for 3 min. After the incubation period, a 2 per cent glutaraldehyde solution in 0.2 M. Sodium phosphate buffer pH 7.3 was added to the cell mixture. After 60 min at 4° C the cell mixture was spun on a PR- International Centrifuge at 300 g for 5 min at 4° C. A 0.05 ml aliquot of the resultant pellet was placed on a siliconized slide, air dried, and then covered with a coverslip that had been stained with Wright's dye. The mast cell changes were observed under the microscope, and the results expressed as a percentage of morphologically altered cells compared to the total mast cell population. The same procedure was carried out for the 5 controls:

- (1) Mast cell suspension (includes buffer and rat serum);
- (2) Mast cell suspension + ASA;
- (3) Mast cell suspension + normal human serum (NHS);
- (4) Mast cell suspension + NHS and ASA;
- (5) Mast cell suspension + allergic serum without ASA.

Finally the Experimental-Mast Cell suspension plus allergic serum with ASA.

All the sera were examined in double-blind fashion on three separate occasions over a range from two to five ASA concentrations, and a serum was considered positive if the affected mast cells were double the control values.

(e) *Preparation of Immunoabsorbents*

Beaded Agarose (Sephacrose® 4B) (obtained from Pharmacia Fine Chemicals, Uppsala, Sweden), was activated using Cyanogen Bromide (CNBR) (Eastman Kodak Co., Rochester, N.Y.) at alkaline pH using the method introduced by Axen et al. (2, 18) and detailed by Cuatrecasas (4). The IgG fraction of goat antiserum monospecific for human IgE was coupled to the activated Agarose at a ratio of 10 mg of Protein to 5 ml of Agarose. The antibody-CNBR activated Aagrose mixture was maintained at a pH of 8.5 using 0.1 M. NaHCO₃, and stirred at 4° C overnight. The mixture was then spun down at 1000 RPM for 5 min at the same temperature; the supernatant was removed; the mixture was washed with Phosphate Buffered Saline (PBS) until the Optical Density (O.D.) was less than 0.01; and it was next washed with 1 M acetic acid to remove any non-covalent binding protein. The antibody-agarose suspension was then washed

with PBS until the pH of the eluate was 7 and the O.D. was less than 0.01. Utilizing the same procedure, the normal goat IgG globulin fraction was coupled to the CNBR activated Agarose beads for use as the experimental control. 1 ml of the allergic serum was added to 0.1 ml of the Agarose-anti IgE adsorbent; it was stirred at room temperature for one h; then at 4° C for 2 h; and it was finally spun down at 2500 RPM for 10 min at 4° C. The same procedure was carried out using the Agarose-goat IgG globulin adsorbent.

(f) *Quantitation of the Immunoglobulins*

The levels of immunoglobulins G, A, M and D were measured by the single radial immunodiffusion technique (Mancini) (12) utilizing the Quanti-Plate Immunoglobulin test kits supplied by Kallestad Laboratories. Normal values for our laboratory are: IgG = 600-1600; IgA = 75-420; IgM = 50-200, and IgD = 0-6 mg/100 ml.

The IgE levels were measured by the radioimmunosorbent technique utilizing Phadebas radioimmunoassay kit supplied by Pharmacia.

Selection of Patients

The 18 patients were selected from among about 1000 patients investigated in the Allergy Clinic in 1971 and 1972. No attempt was made to study the incidence of ASA intolerance in this group, but it was apparent that the ASA triad patients were much more prevalent, as a large number were available for this study. Thus, the first 10 patients seen who fulfilled the clinical criteria for the ASA triad were included in the study. But only nine patients were found who had anaphylactic responses to ASA, and one was excluded because of excessive cytotoxic activity of his sera on the mast cells; the other eight were included in the study.

RESULTS

This group of patients, listed in Table 1, included five males and five females from all age groups. All of the patients had at least two documented asthmatic responses after ASA ingestion. Many of these patients also had sinusitis, perennial rhinitis and most had had polypectomies performed on several occasions. Three of the patients were considered to have perennial allergic rhinitis. The direct skin test to ASP-PLL was negative in the seven patients tested. The RMCT was also negative in the 10 patients studied, and two examples of the typical negative responses obtained in this group, as well as the Normal Human Serum (NHS) control responses, are

TABLE 1
Description of ASA Triad Patients and Laboratory Results.

Patient	Age & sex	Clinical disease	RMCT ASA	Direct skin tests Aspiryl- polylysine
D.H.	12 M	A, CS, P, PR	Negative	Not done
R.C.	25 M	A, AR, CS, P, S	Negative	Negative
N.C.	50 M	A, P, PR	Negative	Not done
M.K.	60 M	A, CS, P, PR, S	Negative	Negative
E.F.	40 M	A, CS, P, S	Negative	Not done
A.B.	52 F	A, P	Negative	Negative
G.B.	45 F	A, AR, P	Negative	Negative
A.P.	24 F	A, CS, P, PR, S	Negative	Negative
A.S.	20 F	A, AR, P	Negative	Negative
D.B.	68 F	A, CS, P, PR, S	Negative	Negative

A, Asthma; AR, Allergic Rhinitis; CS, Corticosteroid Therapy; P, Nasal Polyps; PR, Perennial Rhinitis; S, Sinusitis.

shown in Table 3. The case histories of these two patients are described in more detail below.

Mrs. A.B. A 52-year-old female who has for the past 10 years had intermittent asthma associated with respiratory infections. She was first seen in the Emergency Department of the Hospital in 1971 in status asthmaticus that appeared to be precipitated by ASA ingestion. She was found to have bilateral nasal polyps and was negative on allergy skin testing. In 1972, she came to Emergency again because of a painful laceration of her hand, and was given Darvon compound propoxyphene plus ASA) for the pain. She reappeared in Emergency 2 h later stating that 30 min after taking one tablet she had developed asthma. She responded to epinephrine and aminophylline therapy.

Mr. R.C. A 25-year-old male who was first seen in the Hospital in February 1971 in consultation regarding the management of his bronchial asthma prior to the surgical removal of bilateral nasal polyps. He stated that he noted aspirin tablets had precipitated attacks of asthma on several occasions during the past 2 years. His asthma worsened during the winter of 1972, requiring increasing doses of corticosteroids, and he was found to have bilateral maxillary sinusitis with fluid levels and large bilateral polyps obstructing the nasal airways. Surgery was performed and 3 h after the operation, he inadvertently received Darvon compound (propoxyphene plus ASA); within 30 min or less, he was

TABLE 2

Description of Patients with Anaphylactic Type Responses to ASA and Laboratory Results.

Patient	Age & sex	Atopic state*	ASA response	RMCT ASA	Direct skin test Aspiryl-polylysine
A.U.	43 F	No	Urticaria	Positive	Not done
G.Z.	35 M	S.A.R.	Urticaria & Angioedema	Positive	Not done
P.C.	28 F	No	Pruritis & Erythema	Positive	Positive
G.P.	43 F	S.A.R.	Anaphylactic shock, urticaria, angioedema.	Positive	Positive
W.G.	40 F	S.A.R. P.A.R.	Generalized Pruritis	Positive	Positive
S.P.	40 F	No	Urticaria & Angioedema	Positive	Positive
B.S.	41 F	S.A.R.	Pruritis & Erythema	Positive	Positive
J.J.	24 M	S.A.R.	Urticaria	Positive	Positive

* P.A.R., Perennial Allergic Rhinitis; S.A.R., Seasonal Allergic Rhinitis.

found cyanotic and in status asthmaticus. Prompt therapy, including a tracheal intubation, saved his life.

The Anaphylactic Group

Table 2 gives a description of the eight patients who developed anaphylactic-type responses after ASA ingestion. These patients were selected on the basis of clear-cut clinical evidence that anaphylactic-type responses occurred on at least two occasions after the ingestion of ASA. Five of the eight patients in this group had seasonal allergic rhinitis, and none of these patients had nasal polyps or chronic sinusitis. There were six females and two males in this group and their ages ranged from 25 to 43. The RMCT was positive in all eight patients, as was the direct skin test to ASP-PLL in the six patients

TABLE 3
Rat Mast Cell Test (% Cytopathic Activity).

Patients	Time interval	Concentration of ASA					
		0*	0.2	2.0	20	200	2000 µg/ml
ASA Triad							
A.B.	6 months	10%	9%	8%	9%	8%	8%
Control A		8	10	8	8	9	8
R.C.	1 month	5	4	3	5	3	5
Control B		4	3	3	4	3	5
Anaphylactic group							
A.U.	6 months	17	—	—	—	53	28
G.Z.	6 months	22	—	—	—	30	61
P.C.	1 month	14	65	63	50	76	55
G.P.	3 years	5	19	32	24	85	78
W.G.	9 months	3	48	90	86	85	85
S.P.	2 months	15	28	45	80	60	30
B.S.	2 years	3	18	22	40	21	31
J.J.	3 years	4	35	38	45	32	10
Control C		6	7	5	6	6	5

The RMCT on the sera of A.U. and G.Z. were performed at only two antigen concentrations of ASA.

* The 0 control refers to the patient's serum plus mast cells without ASA. Controls A, B, and C represent different normal human sera.

tested. Illustrative case histories on four of these patients are detailed below.

P.C. A 28-year-old non-atopic female who had a lumbar discectomy, after which she took an ASA compound frequently for several months. Later she noted marked generalized pruritis after taking the medication and, in addition to the pruritis, a marked generalized erythematous rash also began to occur. When she was advised that these reactions might be due to codeine she took plain ASA, and the rash and pruritis promptly reappeared. She was able to take codeine without untoward reaction.

G.P. A 43-year-old female who has had recurrent tension headaches for many years for which she took plain ASA without noticeable adverse effects. She then developed intermittent urticaria of undetermined cause for a period of 6 months. Finally, one day she took two ASA tablets and within 20 min she developed urticaria, massive generalized angioedema and wheezing respirations; she also

felt weak and fainted. She recovered only after several injections of epinephrine. Since then she has noted that she develops angioedema of the eyes after just handling ASA for administration to her children. She had had hay fever due to sensitivity to grass and tree pollen for many years, but has responded to hypsensitization to these antigens. She has had no recurrence of the urticaria or angioedema since she discontinued either handling or ingesting ASA.

B.S. A 41-year-old female who has both seasonal and perennial allergic rhinitis. In 1963, after ASA ingestion, she developed a macular erythematous rash which was quite pruritic. She avoided ASA until 1969 when she took the drug again and in 1 h developed the same reaction. She has avoided ASA since then and has had no recurrence of the above symptoms.

J.J. A 24-year-old male with seasonal allergic rhinitis (grass) who had a generalized urticarial reaction to ASA intake 5 years ago and then again 3 years ago. He thus avoids ASA and has had no recurrence of the urticaria in the past 3 years.

RMCT Results

Table 3 also indicated the RMCT cytopathic activity in the eight patients of the anaphylactic group; the time interval between the occurrence of the clinical response and the performance of the test; and the concentration of the ASA used in producing the RMCT responses. The control responses represent mast cells alone, or with the varying concentrations of ASA, NHS plus mast cells alone or with the five concentrations of ASA used. All these control tests were negative, and the NHS control responses of 3–10 per cent of the three sera, A, B, C (shown in Table 3), represent the range of RMCT cytopathic activity found in the normal control sera, with or without ASA in this study. The mast cells alone or with ASA also gave similar responses.

All the patients in the anaphylactic group had positive RMCT responses. It may also be noted that some of the serum samples of the anaphylactic group produced high serum control cytotoxic responses; this has been observed occasionally in assays done on some highly allergic sera. It may be due to immune complexes or aggregated IgE as, when the IgE is adsorbed out of these sera, the serum control responses revert to low control values. An example of this response may be noted in Table 5 (Patient S.P.).

TABLE 4
Skin Tests to ASP-PLL.

	Scratch		Intradermal		1:5	RMCT
	1:100	1:1000	1:100	1:10		
P.C.	-	+	++	N.D.	N.D.	90%
G.P.	-	±	+	++*	N.D.	85
W.G.	-	+	++	N.D.	N.D.	90
S.P.	-	+	++	N.D.	N.D.	87
B.S.	-	-	-	+	++	40
J.J.	-	-	-	+	++	45
ASA						
Triad (7)	-	-	-	-	-	

The RMCT cytopathic activity values are the highest percent activity recorded in each of the 6 patients of the anaphylactic group. + = approximately a 5 mm wheal larger than saline control; ++ = approximately a 10 mm wheal larger than saline control.

- = negative; N.D. = not done; * mild systemic reaction.

The results of another RMCT assay done on additional serum samples obtained from three patients in the anaphylactic group are shown in Fig. 1, and the results indicate the varying types of dose response curves that have been found. The bi-modal curve of patient P.C. suggests an immune response to more than one ASA antigenic determinant.

Aspiryl-Polylysine Skin Tests

Table 4 indicates that the ASA triad patients all had negative ASP-PLL skin tests, whereas the six patients of the anaphylactic group who were tested, all had positive responses. The results showed that the patients who had the highest RMCT responses to ASA also had their positive intradermal reactions at a higher dilution of the ASP-PLL antigen. The skin test using ASP-PLL appeared to be a safe procedure, but caution is necessary in patients with a good history of anaphylactic shock as our only adverse reaction occurred in patient (G.P.) whose history of anaphylactic shock to ASA was described earlier. She had a positive intradermal reaction (5 mm

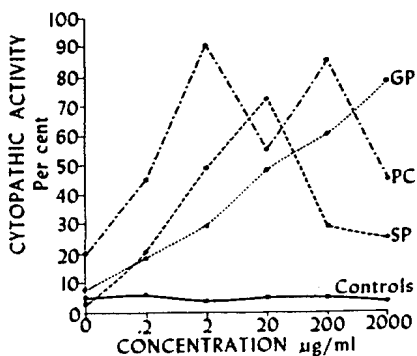


Fig. 1.

The RMCT cytopathic activity on three patients with anaphylactic reactions to ASA, plus the negative responses to a NHS control.

wheel, larger than the saline control) at a 1:100 dilution of the ASP-PLL. The test was repeated at the next dilution (1:10), and the wheal was slightly larger (8 mm, but with pseudopods). She then developed pruritis of the hands and face, tightness in the chest and hoarseness. The reaction promptly subsided without therapy. Thus, it is suggested that in patients with a history of anaphylactic shock, the skin tests starting with the scratch be repeated only at 30 min intervals, and that a minimal 1 + reaction be taken as the end point of the procedure.

Adsorption Studies

Table 5 indicates the maximal RMCT responses, the antigen concentration used and the immunoglobulin levels in the four anaphylactic-type patients before and after the specific adsorption of their IgE immunoglobulins. The results indicate that using the monospecific IgE antiserum adsorbent, all or most of the IgE was removed from the four sera. There was also a minimal decrease in the serum levels of the other immunoglobulins. The IgD globulins were qualitatively present before and after both adsorption procedures, but at too low a

TABLE 5

The RMCT Results before and after the IgE and Control Goat IgE Globulin adsorptions.

Patient	RMCT % control	Positive maximal	Conc. ASA μg/ml	IgE μ/ml	mg/100 ml		
					IgE	IgA	IgM
<i>S.P.</i>							
Before ads.	20	87	2	100	1760	290	120
After IgE ads.	2	2	2	0	1520	265	97
After control ads.	8	48	2	50	1320	180	97
<i>B.S.</i>							
Before ads.	6	35	20	170	420	115	56
After IgE ads.	2	2	20	0	350	84	45
After control ads.	6	30	20	100	350	97	45
<i>J.J.</i>							
Before ads.	4	30	20	100	1200	180	150
After IgE ads.	5	5	20	27	1200	135	120
After control ads.	4	29	20	80	1050	155	110
<i>W.G.</i>							
Before ads.	4	61	2	150	1200	235	145
After IgE ads.	4	6	2	0	900	220	115
After control ads.	3	38	2	100	1000	220	125
The IgE levels are in units/ml.							
ads. = adsorption.							

The 4 NHS controls had RMCT cytopathic activity alone or with all concentrations of ASA at 2-6 per cent in the four assays.

level to be quantitated. The results also show that the control goat globulin adsorbent removed varying proportions of IgE as well as the other immunoglobulins from the four sera, indicating some non-specific loss of protein occurred with the method used. Although these agarose polymers have the

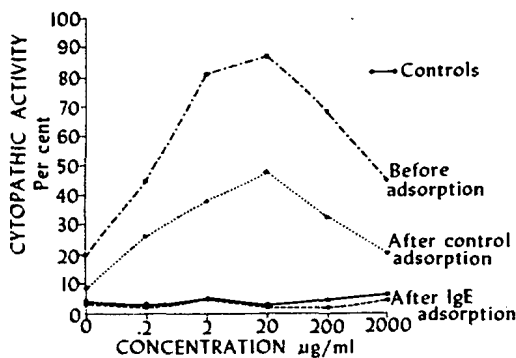


Fig. 2.

The RMCT on patient S.P. before and after the specific IgE and control adsorption studies along with the NHS control RMCT responses to the ASA antigen. IgE levels before adsorption = 100 μ /ml, after specific IgE adsorption = 0 μ /ml, after control goat globulin adsorption = 50 μ /ml.

greatest capacity to bind antigen, they have a propensity to trap antigen non-specifically in the interstices of the gels, and it is difficult to wash these gels free of the unbound protein (25). It seems likely that this mechanism accounts for the non-specific loss of some IgE protein by the control goat globulin adsorbent.

Thus, with the partial removal of some IgE globulin by the control adsorbent, there was some loss of RMCT activity in the four cases. However, the results clearly demonstrate that with removal of almost all the IgE immunoglobulin in the four cases, the RMCT cytopathic activity to ASA was completely abolished.

Figs. 2 and 3 illustrate the complete RMCT assay of the two anaphylactic-type sera, S.P. and W.G., and the NHS controls utilizing the full range of ASA concentrations used in this study. The results, again, clearly demonstrate that removal of all the detectable IgE immunoglobulins from the sera of both patients was associated with the complete loss of

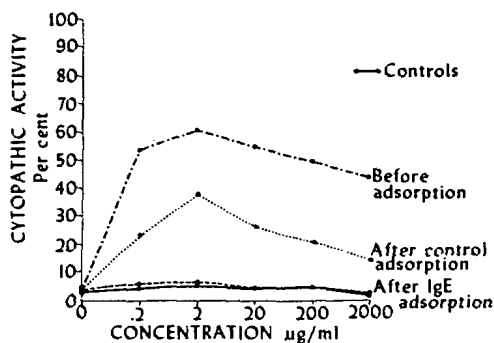


Fig. 3.

The RMCT on patient W.G. before and after the specific IgE and control adsorption studies along with the NHS control RMCT responses to the ASA antigen. IgE levels before adsorption = 150 μ /ml, after specific IgE adsorption = 0 μ /ml, after control goat adsorption = 100 μ /ml.

RMCT cytopathic activity to all the concentrations of ASA used in this assay.

DISCUSSION

Analysis of the results of this study indicate that allergic-type reactions to ASA may be divided into those mediated by reagenic and non-reagenic pathways: the patients with anaphylactic-type responses mediated by an IgE reagenic pathway, and the patients with the ASA triad mediated by an as yet uncharacterized mechanism.

The evidence presented in this paper indicating the participation of reagenic IgE antibodies in the patients with anaphylactic type responses to ASA includes:

- (1) the patients tested had positive intradermal skin tests to Aspiryl-polylysine;
- (2) the patients' sera had positive rat mast cell responses on incubation with ASA;
- (3) after specific adsorption of IgE from four patients with positive RMCT's, the rat mast cell test became negative.

There is much experimental evidence to suggest that IgE immunoglobulin specifically binds to rat peritoneal mast cells (9, 15), and the IgE adsorption experiments performed in these studies provide further supportive evidence for the previous reports cited. Most previous studies of patients with aspirin intolerance have not found evidence of reaginic activity, but most of these studies have had an almost exclusive population of ASA-induced asthma patients (1, 6, 20, 22, 26). Therefore, our results would correlate with these previous studies in which no reaginic activity could be demonstrated in the ASA triad patients. However, one of the main purposes of this study was, in addition, to select a group of patients who had only anaphylactic-type responses to ASA; and the results indicated that only in this group of patients was evidence of IgE reaginic activity found. This finding is not unique, as de Weck has already described a number of patients with anaphylactic-type responses to ASA who had positive immediate skin hypersensitivity reactions when tested with the ASP-PLL antigen (5, 11).

A possible explanation for the negative results in the ASA triad patients is that the asthma is induced by a non-immunological pathway. It has been shown that bradykinin produced marked bronchoconstriction in guinea-pigs, and that this effect may be suppressed by ASA or epinephrine (10). Aerosolized Kinin can also produce bronchospasm in human asthmatics (10). Samter & Beers (19) in their extensive review suggested that the cause of the ASA-induced asthma may be due to an alteration of bronchial receptors. Their hypothesis suggests that the normal receptors, usually protected from kinin stimulation by ASA, are so altered by the pre-existing disease process that they are stimulated rather than protected by ASA. The presence of altered bronchial activity in asthma is supported by the findings of Parker & Smith (13) who reported that a decreased cyclic AMP response to B-adrenergic agents was observed in subjects with symptomatic bronchial asthma. ASA has also been shown to have an effect on the synthesis or release of the slow reacting substance of Anaphyl-

axis, SRS-A (17) and prostaglandins (23). Thus ASA can influence the activity of a number of enzyme systems (7) that control the release of chemical mediators that have a potential role in the activation of the asthmatic attack.

None of the mechanisms described above require antibody participation, and one of these, or an as yet uncharacterized pathway, may mediate the allergic-type reactions in patients with the ASA triad.

SUMMARY

Eighteen patients with allergic-type reactions to aspirin (ASA) ingestion were studied. Ten had the ASA triad: asthma, nasal polyps and ASA sensitivity. Eight had anaphylactic-type responses to ASA, including generalized pruritis, erythema, urticaria, angioedema and anaphylactic shock. To determine the participation of IgE reagins in these reactions, the following studies were performed:

- (1) Direct skin tests to Aspiryl-polylysine;
- (2) The rat mast cell test (RMCT) to ASA on all sera;
- (3) IgE immunoglobulin adsorption of four sera giving both positive RMCT responses and positive direct skin test to Aspiryl-polylysine, and repetition of the RMCT after adsorption.

The findings suggested that the ASA triad patients had their asthmatic response to ASA mediated by a non-reaginic pathway, as both direct skin tests and RMCT responses were negative. The anaphylactic group had positive direct skin tests and positive RMCT's; and removal of IgE from the sera of four of these patients abolished the positive RMCT responses to ASA. These results indicate a participation of IgE-type reagins in anaphylactic-type reactions to ASA in man.

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