NON-ANAPHYLACTIC FOOD ALLERGIES MEDIATED BY IMMUNE COMPLEXES:

I. EVIDENCE FOR IMMUNE COMPLEX MEDIATED VASCULAR INFLAMMATION

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INTRODUCTION

A great deal of interest has recently been focused on food allergies. IgE mediated, anaphylactic food allergies are recognized and accepted by the allergy community; however, much controversy surrounds the area of non-anaphylactic food reactions. The symptoms of non-anaphylactic food allergies often involve behavioral changes associated with vague gastrointestinal and respiratory complaints which many physicians discount as psychosomatic. The literature contains reports of double blind controlled studies which both refute (1,2,3) and confirm (4,5) the concept that a large population of individuals may have non-IgE mediated food hypersensitivities. Proponents of this concept argue that patients with food and chemical allergies suffer from small vessel inflammatory disease (6,7). Additionally several recent reports describe food associated antigen/antibody complexes (8,9,10) and complement activation (11) in patients with food allergies. Another recent report describes a patient with coexisting immune complex disease and IgE mediated atopy (12). The purpose of this report is to present evidence for immune complex mediated, complement consuming vascular phenomena in patients with food allergies.

MATERIALS AND METHODS

1. Patient Selection

One hundred twelve allergy referral patients were selected whose symptomatic complaints and intradermal testing reactions identified them as having aberrant immunologic reactivity to foods and petrochemicals. The symptoms include abdominal pain, alternating diarrhea and consitpation, along with dizziness, blurred vision, headache, photophobia, forgetfulness, irritability, depression, facial edema, and headaches associated with the ingestion of certain foods or exposure to certain petrochemical vapors. The inciting foods were varied and the petrochemical offenders generally included perfumes, scented hair sprays, and vapors of formaldehyde or phenol. These patients ranged in age from 27 to 77 with a mean age of 49. The ratio of male to female was approximately 1:3. All patients were screened for possible diseases which are known to be associated with immune complexes. Antinuclear antibodies, lupus erythematosus preparation tests, rheumatoid factor assays, complete blood counts, urinalysis, immunoglobulin levels, as well as liver function, renal function, and electrolyte blood chemistry panels were performed on every patient. Thorough physical examinations with complete neurologic evaluation were performed on every patient. Patients in whom any of the laboratory or clinical parameters suggested systemic lupus erythematosus (SLE), rheumatoid arthritis, adrenal malfunction, myocardial disease, renal disease, malignancy, or acute viral infection were rejected from the study. All venipunctures were performed after a 14 hour overnight fast between 9:00 and 10:00 AM to minimize possible circadian effects. Parallel age and sex matched controls were performed such that a minimum of 100 controls were performed for each parameter tested.

2. Control Population

Age and sex matched controls were selected from a population of asymptomatic medical office workers, laboratory personnel, and medical students who denied a history of chronic disease or symptoms of food allergy. A past history of mild pollinosis did not exclude the individual from this population. At least 100 such controls were tested for each biochemical parameter.

3. Prostaglandin F_2A (PGF_2A)

PGF_2A was assessed by a commercially available radioimmunoassay from Clinical Assays, Inc. Cambridge, Mass. 02139. The technique involves chromatographic separation of plasma and double antibody incubations.

4. Immune Complexes

Immune complexes were determined by a modification of the method of Nadelson, et.al. (13) in which complexes are separated from serum using polyethyleneglycol (PEG) precipitation. Serum (0.5ml.) is diluted with an equal volume of phosphate buffered saline (PBS) (0.1 M Phosphate, pH 7.4). One ml of 5% polyetheleneglycol (MW 6000) is added and the mixture is vortexed for 1 minute. The mixture is allowed to stand stand for 10 minutes at room temperature. It is then subjected to 20 minutes of centrifugation at 3000 rpm. The supernatant is then decanted and the tubes are allowed to drain for 2 minutes. The residue is then dissolved in 0.1 ml of PBS. IgG concentration of the residue is then determined using a commercially available electroimmunodiffusion system (AEID-Antibodies Incorporated, Davis, Calif. 95616). Results are corrected for the 5 fold concentration.

5. C3 and C4

Serum levels of C3 and C4 were determined using commercially available radialimmunodiffusion plates (Kallestadt Laboratories, Inc. Minneapolis, Minn.).

6. Statistical Analysis of Data

Patients were ranked and divided into groups according to their immune complex measurements. In each group, means, medians and standard deviations were computed. Significance tests were carried out using a criterion of Karl Pearson which estimates the ratio of intergroup to intragroup variabilities. Its distribution is closely approximable by a $X^2/6$ distribution for a X^2 with six degrees of freedom. Similar computations were carried out using PGF_2A and C3 for ranking and classification.

Formula: $\Sigma\{x_j - (\Sigma\gamma p_j x_j)\}^2\sigma_j^{-2}$

where x_j is mean in jth IC-class, $_j$estimated

s.d. of mean for that class: $\gamma_j = \frac{1}{\sigma_j^2}(\Sigma\frac{1}{\sigma_j^2})^{-1}$

This is about X_6^2

RESULTS

Control reference ranges were established for each of the biochemical parameters measured. The range for immune complexes in the control population was 0 - 10 micrograms of immune complex associated IgG per deciliter (DL) of serum. The coefficient of variation (CV) of this assay was 22%. The range for PGF_2A in the control population was 50-359 picograms per DL with a CV of 19.8%. The control range for C3 was

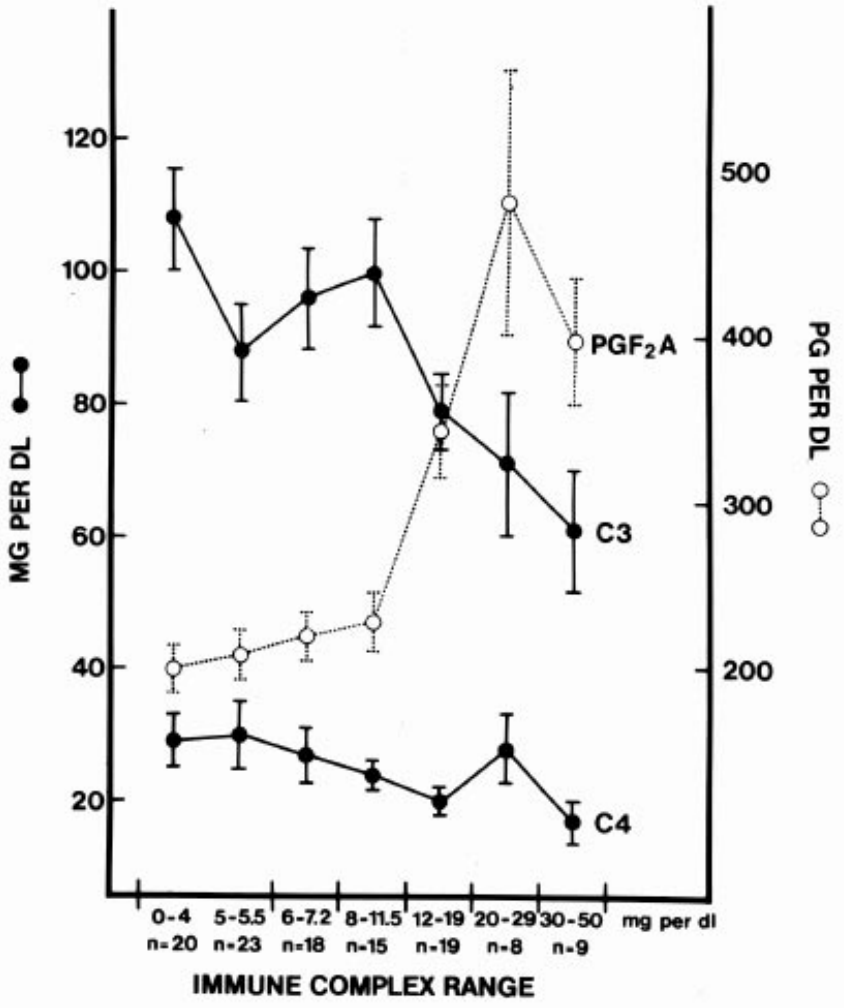
70 to 176 milligrams per DL with a CV of 6.3% while that of C4 was 14 to 51 milligrams per DL with a CV of 11.2%. Analysis of the data derived from the control population demonstrated no significant relationship between levels of any of the parameters measured.

Figure 1 shows the relationship of PGF_2A, C3, and C4 levels to immune complex concentrations (IC) in the patient population. A non-linear relation between immune complexes and PGF_2A is noted. IC levels are directly related to PGF_2A and inversely related to C3 and C4 levels. Table I shows the statistical analysis of the relationship between immune complexes and the various other parameters. The relation IC - PGF_2A and IC - C3 are highly significant while IC C4 is marginally significant. Similar computations were carried out using PGF_2A and C3 for ranking and classification. It was found that once the IC level had been taken into account, no other significant relations existed between the other measurements. IC levels closely approximated the severity of subjective symptoms reported by the patients.

DISCUSSION

Recent evidence suggests that the generation of immune complexes in response to showers of antigen introduced through the respiratory tract, the gastrointestinal tract, or the skin is a normal phenomenon (14). The concentration, biological activity, pathogenicity, and duration of circulation of these complexes are regulated by a number of unrelated factors. These factors include the structural class and complement fixing nature of the antibody as well as the rate of antibody synthesis, the amount of antigen introduced, and the integrity of the fixed phagocytic cells in the reticuloendothelial system. Generally the circulation of

Figure 1


MG PER DL
PG PER DL
120
100
80
60
40
20
500
400
300
200
PGF2A
C3
C4
0-4 n=20
5-5.5 n=23
6-7.2 n=18
8-11.5 n=15
12-19 n=19
20-29 n=8
30-50 n=9
mg per dl
IMMUNE COMPLEX RANGE

immune complexes in normal individuals is a transient phenomenon and is associated with little or no symptoms. Several diseases have been recognized in which immune complex circulation is prolonged and symptoms result which are associated with their deposition in vascular basement membranes. Examples of such diseases include serum sickness, systemic lupus erythematosus, and acute post streptococcal glomerulonephritis. When immune complexes deposit in vascular basement membranes, they often activate the complement system. This can be detected as a reduction in the various components of circulating complement. The most frequently measured of these components are C3 and C4. The activation of complement is followed by a variety of inflammatory events including the release of anaphylatoxins and chemotactic factors. The anaphylactoxins promote the release of various mediators from basophiles and mast cells while the chemotactic factors attract cellular elements of inflammation . These cells aggregate and mount a locally destructive attack. The overall outcome of this inflammatory phenomenon is transient or permanent damage to capillary endothelial cells. Prostaglandin F_2A is a hormonal regulator of inflammation which is released from activated cells of inflammation (15 - 18) and damaged endothelial cells (19-21).

It is well recognized that immune complex mediated disease can cause vague, multisystem symptoms and behavioral changes. For example, patients with systemic lupus erythematosus and central nervous system involvement often complain of vague multisystem symptoms and can manifest behavioral changes such as uncharacteristic hyperactivity, disorientation, aggression, headache, visual changes, and frank psychosis (22). These

changes have been shown to be reversible with appropriate immunosuppressive therapy. The central nervous system disease is related to immune complexes in the peripheral circulation which either cross the blood brain barrier or cause reactions in adjacent vascular structures which result in changes in complement components in the cerebrospinal fluid (23,24). The immune complexes in lupus are generally made up of native DNA and a complement fixing IgG anti-native DNA antibody(25-28). A constellation of similar symptoms and behavioral changes has often been reported in patients who suffer from food allergies (6,7, 29-34). Several recent reports (8,9,10) show clear biochemical evidence of circulating immune complexes associated with food antigens in normal and atopic people after oral food challenge. Clinical evidence for immune complex mediated vasculitis has been reported in patients with food and petrochemical allergies (6,7).

Analysis of the data from this study shows that the trends of change in IC levels were statistically significantly related to PGF_2A, C3, and C4. None of the other parameters measured had significant relationship with anything other than IC. This is consistant with the concept that the process is initiated by the generation of IC and that the prostaglandin and complement changes are a result of these complexes. Although the classical pathyway of complement activation appears to be most likely involved in this phenomenon, activation of the alternate pathway cannot fully discounted.

This study presents compelling biochemical evidence for immune complex mediated vascular inflammation. It is possible that the symptoms associated with the diagnosis of food and petrochemical allergies are caused by this immune complex mediated vascular inflammation. The pathophysiology, mechanism of action and genetics of this phenomenon are presently under study.

LEGEND FOR FIGURE I

Levels of PGF_2A, C3, and C4 plotted against the level of IC in food allergy patients.

TABLE I

Statistical Analysis of the Relationship Between IC and Other Parameters

Relation	Significance
IC - PGF_2A	$X^2/6 = 8.91$ $p < 0.001$
IC - C3	$X^2/6 = 4.11$ $p < 0.001$
IC - C4	$X^2/6 = 2.12$ $p < 0.05$

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