

Cytotoxic testing for food allergy: Evaluation of reproducibility and correlation

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Cytotoxic food tests still present conflicting opinions concerning their validity. Nine atopic patients with or without a history of food allergy were studied along with 5 nonatopic patients. All tests were conducted in a double-blind fashion with 6 determinations for each of 10 food antigens. Reproducibility of the test (5/6 positive or negative) was demonstrated with wheat, milk, yeast, chocolate, and orange. In the nonatopic group, reproducible results were obtained for wheat, egg, yeast, chocolate, and chicken. Clinical correlation with 11 foods in 7 patients was demonstrated. However, there were 46 positive tests without correlation and 2 negative tests with positive histories. Therefore, there appears to be reproducibility of the tests to only 3 foods but no apparent correlation with clinical symptoms. At the present time, cytotoxic tests offer no reliable help in establishing the diagnosis of food allergy.

The presentation and wide variety of symptoms related to food ingestion have been an area of controversy for many years. In recent years allergic symptoms due to foods, with the exception of a few clear-cut situations, have been diagnosed by scientifically unproved methods.

Frequently, the patient is aware of the food which causes symptoms and either avoids it or uses it knowing it will cause symptoms. Beyond this, the diagnostic ability of the physician becomes much more hampered and insecure. Rowe and Rowe,¹ as recently as 1972, described a method of food avoidance which, in their hands, was stated to be very reliable in establishing the presence of food sensitivity. Other investigators^{2, 3} have subsequently tried their approach to dietary manipulation with varying degrees of success. Food challenge methods have been described with disagreement in the manner of carrying out the procedure. Provocative methods, including subcutaneous,⁴ sublingual,^{5, 6} and intradermal^{7, 8} administration of antigen to subjects, are still in use, although such methods are still controversial. An in vitro cytotoxic method^{9, 10} of establishing food sensitivity has also been utilized with conflicting opinions regarding its reliability. It is this test procedure we evaluated with the intent of observing

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its reproducibility and clinical correlation in regard to detecting food sensitivities.

Specific reactions of leukocytes have been a source of interest for many years. In 1947 Squire and Lee¹¹ reported a reduction in the number of leukocytes of untreated ragweed-sensitive subjects in response to exposure to ragweed extract. They performed their study using blood from untreated patients sensitive to ragweed clinically and by skin test. When blood from these patients was mixed with 1:20 saline extract of short ragweed, the total white blood count (WBC) was reduced 43% after a 1-hr incubation. No such reduction was observed when the same procedure was performed with blood from nonsensitive subjects. Subsequently, Black¹² reported an *in vitro* method testing the effect of foods using the viability of leukocytes as a sign of allergic reaction. However, Chambers¹³ repeated this work and was unable to duplicate Black's results. Following these studies, Bryan and Bryan⁹ modified Black's method and indicated a good clinical correlation. Nevertheless, the test is still not generally regarded as useful,^{14, 15, 16} and no assessment of validity or reproducibility of its results has been carried out except by Lieberman and associates.¹⁶

MATERIALS AND METHODS

The procedure was carried out according to the technique described by Bryan. One of the authors (T. B.) became familiar with the technique by visiting Dr. Bryan's laboratory and subsequently instituted it in an office practice. The food antigens used were dry powdered material obtained commercially (Greer Laboratories). Five mg of antigen was mixed with 5 ml of sterile distilled water and allowed to stand at room temperature for 24 hr. Fifteen ml of sterile distilled water was then added; 3 drops of the mixture were placed in 6 different locations on each set of slides and allowed to dry. The locations of the food antigens were randomized in such a way that they never appeared in the same order in any of the 14 sets.

All slides, coverslips, pipettes, and antigen bottles were prepared by first washing in liquid detergent and rinsing in running tap water. This was followed by serial rinsing in sodium hydroxide, tap water, and finally alcohol. The slides, coverslips, and serology tubes were coated with silicone and finally rinsed with absolute alcohol.

Three petroleum jelly rings, 8 mm in diameter, were placed on each slide. The slides were then numbered 1 through 20, with each slide having 3 food sites and identified with a letter. Sixty cards were prepared for each of the 60 locations and labeled to correspond to a single antigen site. The cards were mixed and withdrawn one at a time, 6 cards per food. The record of the location of the foods was not reviewed until all of the tests had been completed.

A total of 14 subjects were studied. Nine were atopic patients being seen in the Allergy Clinic who were all receiving hyposensitization for inhalant allergy. The 5 in the nonatopic group were hospital personnel with no known history of allergic respiratory symptomatology. In addition, they completed a questionnaire to ensure that they had no symptoms that could be interpreted as due to foods, e.g., gastrointestinal, gastrourinary, or neuromuscular complaints. Seven of the 9 atopic subjects gave a history of symptoms related to ingestion of 1 or more of the 10 foods included in our study. These included asthma, skin rash, vomiting, abdominal distention, nasal itching, dysuria, and a dislike for the food.

All subjects were on a regular diet prior to testing. Eighteen ml of venous blood was drawn in the morning or after an 8 to 10 hr fast and was prepared as described by Bryan and Bryan.⁹ The blood was mixed with 2 ml of transfusion citrate and then divided into specially prepared tubes with narrowed centers and centrifuged at 250 rpm for 90 min. The plasma and buffy coat were separated and the plasma was mixed with sterile distilled water in a ratio of 7 drops of water in 42 drops of serum. The buffy coat was added from 3 of the specially prepared tubes described previously. Two drops of this suspension were then placed on each of

TABLE I. Reproducibility of cytotoxic tests in atopic patients

Subject	Wheat	Egg	Milk	Beef	Yeast	Tomato	Chocolate	Orange	Tea	Chicken
K. W.	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP
R. P.	RP	RP	RP	N	RP	RP	N	RP	RN	N
J. B.	RP	N	RP	N	RP	N	RP	RP	RN	N
L. H.	RP	RN	RP	N	RP	N	RP	N	RN	N
M. B.	RP	N	RP	RP	RP	N	RP	RP	N	N
B. N.	RP	RN	RP	RN	RP	RP	RP	RP	RN	N
I. W.	RP	N	RP	RP	RP	RP	RP	RP	N	N
H. E.	RP	RN	RP	N	RP	RP	RP	N	RN	N
A. R.	RP	N	RP	RP	RP	RP	RP	RP	N	RP
Total										
R	9	5	9	5	9	6	8	7	6	2

R: reproducibility (95% confidence level).

N: non-reproducible.

RP: positive.

RN: negative.

TABLE II. Reproducibility of cytotoxic tests in nonatopic patients

Subject	Wheat	Egg	Milk	Beef	Yeast	Tomato	Chocolate	Orange	Tea	Chicken
M. M.	RP	RN	RP	N	RP	RN	RP	N	RN	RP
B. B.	RP	RN	N	RP	RP	N	RP	RP	N	RN
T. S.	RP	RN	N	RN	N	RN	N	N	RN	RN
M. I. B.	N	RN	RN	RP	RP	RN	RP	N	RN	RN
C. M.	RP	RN	N	N	RP	N	RP	N	N	RN
Total										
R	4	5	2	3	4	3	4	1	3	5

R: reproducibility.

N: non-reproducible.

RP: positive.

RN: negative.

the locations on the slides, covered with coverslips, and sealed with the petroleum jelly rings. The slides were then allowed to stand for 2 hr, after which time they were read as negative or positive by the method described by Bryan and Bryan.⁹ The neutrophils were observed for cessation of pseudopod formation giving the appearance of rounding. This was compared with the control, which had no antigen on the slide. Each subject served as his own control. According to Bryan and Bryan,⁹ the test was read as 1+ (50% reduction in cell motility) to 4+ (all cells round and nonmotile with some disintegration). We read the tests as positive or negative with no attempt to grade them as zero to 4+ based on the 50% reduction in motility. When all subjects had been completed, the code was broken and the results were tabulated. For a test to be reproducible at the 95% confidence level, 5 or 6 of six tests done at the same time had to be the same—either positive or negative.

RESULTS

Tables I and II summarize the results of reproducibility in the atopic and nonatopic groups, respectively. In the atopic group, a total of 66 reproducible results, or 73% of the tests performed, were obtained. Eighty-six percent of the reproducible tests in this group were positive. All of the tests for wheat, milk, and yeast were reproducible, and they were positive in all subjects. Seven and 8 of 9 tests for orange and chocolate, respectively, were positive. In evaluating the results for individual subjects, all of the tests for Subject K. W. were reproducible and positive.

TABLE III. Correlation of reproducible positive tests with positive food tests

Subject	Number of foods associated with symptoms	Number of foods with positive tests	Foods with both positive tests and symptoms
<i>Atopic group</i>			
K. W.	1	10	1
R. P.	2	6	1
J. B.	0	5	0
L. H.	1	4	1
M. B.	1	6	1
B. N.	0	6	0
I. W.	3	7	3
H. E.	6	5	3
A. R.	1	8	1
<i>Nonatopic group</i>	0	17	0

In the nonatopic group, egg and chicken were reproducible in each of the 5 tests, and wheat, yeast, and chocolate had reproducible results in 4 or 5 subjects. All tests for egg were negative, and 3 tests for chicken were negative and 2 were positive. Overall, there were 68% reproducible results with 53% of those being positive. As seen in Table III, symptoms due to the foods being tested were implicated a total of 15 times by the atopic patients. There was a total of 57 positive tests in this group. However, there were 4 instances in which the test did not correlate with the history, which leaves 11 instances in which the history does correlate with the test. In 2 of these 4 instances in which the patient associates the ingestion of food with a symptom, the test was reproducible but negative. In the other two, the test was not reproducible and therefore would not be considered reliable. If the 23 tests in the atopic group considered not significantly reproducible are eliminated, this results in 46 (or 70%)* of the 66 reproducible results being "falsely positive." In the nonatopic group there were 17 reproducible positive tests, which would give a 57% incidence of positive tests in this group.

In one subject (R. P.), in whom the test was not significantly reproducible, the patient had not eaten the food for over 48 hr. He purposely limits his intake to twice weekly since more frequent ingestion results in asthma. The other subject, in whom the test was not significant, ingested the food at least daily. This food was orange, and her only complaint was nasal itching while peeling the orange.

The two situations with negative tests but positive histories involved tea and egg. The tea was totally avoided, but the egg was tolerated in foods.

DISCUSSION

In both the atopic and nonatopic groups of subjects studied, there were a significant number of reproducible cytotoxic tests. However, these included a large number of "false positives" in which there was no correlation with the history. In

*Total positive tests (57) minus number of times food symptoms correlate with the result (11) = number of "false positives" (46) $\frac{46}{66} \times 100 = 69.7\%$.

only 2 instances were there false negatives, and in 1 of the 2 cases the food had been completely eliminated from the diet. In the other, the food was only used as an ingredient in cooking, and symptoms were avoided in this way. Having avoided the food for a prolonged period of time may, theoretically, have resulted in a negative test just as it may result in loss of clinical sensitivity.

The occurrence of 86% positive reproducible tests in the atopic group and 53% positive reproducible tests in the nonatopic group suggests the possibility of a nonspecific effect of the plasma. In addition, it may be that the foods themselves exert a toxic effect on the cells, and lesser concentration may not produce this effect. It is known that skin tests to inhalants are sometimes positive without clinical evidence of symptoms associated with them. A similar situation may explain our obtaining the large number of "false positives" in this series.

In one study recently reported,¹⁸ the test was repeated as many as 8 times in the same individual; however, it was not done on one specimen as in our procedure. They used 70 items per test and found several positive tests, which they correlated with a leukocyte response to a feeding challenge with the positive food. In a subsequent report, these same authors noted a high percentage of positive tests in subjects who were randomly selected without regard to history of atopy.¹⁹ Others¹⁶ have also noted a substantial incidence of false-positive reactions in their studies.

The factor of technician error must be taken into consideration since the test is highly subjective. This may have been a factor in our case, although T. B., having had previous experience, read the tests and personally carried out all of the preparations described.

That technician error may be a factor affecting results is implied by the fact that Bryan (Trans. Am. Soc. O. & O. Allergy)²⁰ noted 18% positive in 53,000 tests in 1,100 subjects and indicated a lower number of positives after the first 700 tests, which may have been due to improved skill with practice. He also included this in his list of factors potentially responsible for adversely affecting the test.¹⁰ Our overall incidence of positive tests was higher than that recorded by Dr. Bryan²⁰ and may reduce the importance of the reproducible test in this case. A communication with another physician²¹ involved in doing the test also emphasized the importance of technique.

CONCLUSION

The results of this study with a limited population of patients demonstrated that the test has a significant degree of reproducibility by these methods; however, clinical correlation was lacking. The reproducibility was demonstrated by one person reading the tests in a blind fashion. Lieberman and associates¹⁶ have shown that reproducibility of interpretation between individuals was poor. In the customary application of this test, the large number of positive tests would require the elimination of many foods. This would severely restrict the patient's diet and possibly lead to nutritional problems. The mechanism of this controversial test remains unclear, and with the failure of clinical correlation in this study, we cannot recommend this test for clinical application.

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