

# Concentration of IgE antibodies, PK titers, and chopped lung titers in sera from children with hypersensitivity to cod

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*Fifteen sera from 13 children with cod-induced bronchial asthma have been investigated regarding their concentration of IgE antibodies to cod allergen as measured with radioallergosorbent test (RAST), and their ability to passively sensitize human skin in vivo and chopped human lung in vitro. Highly significant correlations were found between the results obtained in all 3 tests ( $p < 0.001$ ). However, the direct scratch test reactions did not agree as well with the results of these 3 tests. The sensitivity of the methods seemed to be similar for the RAST and the Prausnitz-Küstner (PK) test and somewhat lower for the chopped human lung test. Storage of sera for some years, followed by thawings and freezings, reduced the PK titers. This suggests that deterioration of IgE antibody activity is more evident in biologic tests than in immunochemical in vitro tests such as RAST.*

The Prausnitz-Küstner (PK) test, described in 1921, is still the most accepted method for quantitation of reagins, and every new method should therefore be compared with this technique. Since it was shown in 1967 that most reaginic antibodies belong to the immunoglobulin class E,<sup>1, 2</sup> methods for quantitation of IgE have been developed and applied to the field of allergy. The concentration of IgE antibodies in sera estimated with the radioallergosorbent test (RAST)<sup>3</sup> have shown good agreement with the results of provocation tests,<sup>4-6</sup> skin tests,<sup>5</sup> and the test for histamine release from passively sensitized chopped human lung (CHLT).<sup>7</sup> In this study a comparison is made between the PK titers and the results of RAST and CHLT when testing sera from 13 skin tested children with a history of hypersensitivity to cod.

## MATERIAL AND METHODS

The clinical material under study consisted of 13 children with bronchial asthma provoked by the cod allergen. In addition they also had other allergic manifestations such as urticaria, angioedema, and allergic rhinoconjunctivitis. The children were investigated during the period from 1964 to 1969 at the Department of Paediatrics, Rikshospitalet, Oslo, by one of us (K.A.) with history and skin and provocation tests.<sup>8</sup> They were all shown to have

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a hypersensitivity to cod in addition to other allergies. Desensitization therapy had not been given in any case when the blood samples were taken. Sera were prepared from venous blood, divided in small portions, and stored at  $-20^{\circ}\text{C}$ . until analyzed.

## Allergen

A partially purified, freeze-dried cod allergen (DS 22)<sup>9</sup> was used in all tests.

*Direct skin tests.* Direct skin tests were performed as scratch tests with DS 22 in a concentration of  $20\text{ }\mu\text{g}$  per milliliter. The tests were made on the volar surface of the forearm, and they were read and recorded after 20 minutes. The size of the wheal was compared with a standard negative and a standard 3+ control. A 3+ reaction was a wheal of the same size as that elicited by a scratch test with a 1 mg. per milliliter histamine solution. Each additional plus indicated a doubling of the wheal size.<sup>8</sup>

*Radioallergosorbent test (RAST).* The RAST analyses were performed as described,<sup>3</sup> with some modifications.<sup>10</sup> One milligram of the fish allergen DS 22 was coupled to 100 mg. CNBr-activated cellulose particles, and 0.5 mg. of this allergen-polymer-complex (APC) was incubated with  $50\text{ }\mu\text{l}$  serum for 18 hours at room temperature. After the serum excess had been washed away,  $100\text{ }\mu\text{l}$  of immunosorbent purified  $^{125}\text{I}$ -labeled rabbit anti-IgE (approximately 1 ng. antibody nitrogen with an activity of 80,000 to 100,000 c.p.m.) was added, and after another incubation period for 18 hours and subsequent washings the remaining radioactivity on the APC was measured in a gamma scintillation counter. All sera were tested at 2 or 3 dilutions. The concentration of IgE antibodies in the sera was expressed as per thousand of that of a reference serum.<sup>10</sup>

*Prausnitz-Küstner test (PK test).* For the PK test, sterile saline with 0.5 per cent phenol was used as diluent. Intracutaneous injections of 0.1 ml. of serial twofold dilutions of sera were made in the volar surface of the forearm on one of the authors (K.A.). Twenty-four hours later 0.1 ml. aliquots of the cod allergen ( $0.2\text{ }\mu\text{g}$  per milliliter and at the lower end point serum dilution also  $20\text{ }\mu\text{g}$  per milliliter) were injected in the passively sensitized skin sites. The resulting wheals were measured 20 minutes later, and the reciprocal of the highest serum dilution that gave a wheal exceeding that obtained with a negative control serum was regarded as the PK titer. The titrations were all performed within a few months after the blood samples were taken. Seven sera were titrated 2 to 4 times during the first year; all other sera were titrated only once. Furthermore, 2 sera were retested after storage for 5 and 7 years, respectively, followed by repeated thawings and freezings.

*Chopped human lung test (CHLT).* The CHLT was performed according to the method of Sheard, Killingback, and Blair,<sup>11</sup> with minor modifications.<sup>7</sup> One hundred milligram samples of chopped and washed fresh human lung were incubated overnight with 2 ml. of serial dilutions of the sera. The tissue samples were then washed, and 2 ml. of the cod allergen was added. The allergen concentration used was  $0.1\text{ }\mu\text{g}$  per milliliter, which gave optimal test conditions in preliminary experiments. The histamine released at the challenge with allergen was measured on an atropinized guinea pig ileum and expressed in per cent of the total histamine of the tissue samples as extracted by boiling. The reaginic activity was expressed as the reciprocal of the serum end dilution titer, defined as the highest dilution giving a histamine release exceeding 150 per cent of that obtained when unsensitized tissue was challenged with allergen. All sera were tested against lung specimens from 2 different donors. The geometric mean of the 2 titers obtained at the separate test occasions was regarded as the mean titer.

## RESULTS

All patients showed strongly positive direct skin test reactions to the cod allergen, but there was no significant correlation between the strength of these reactions and the serum concentration of IgE antibodies to the same fish allergen as measured by RAST ( $r = 0.52$ ;  $0.10 > p > 0.05$ ). However, a better agreement was found between the skin test reaction and the ability of the serum passively

**TABLE I.** Concentration of IgE antibodies and reaginic activity in sera from skin-tested children with hypersensitivity to cod

| No. | Patient initials | Skin test reaction | RAST* | PK titer<br>Geometric mean and range | CHLT titer<br>Geometric mean and range |
|-----|------------------|--------------------|-------|--------------------------------------|----------------------------------------|
| 1   | W. S.            | 4+                 | 4,625 | 1,200                                | 339 (240-480)                          |
| 2   | P. W.            | 4+                 | 2,650 | 1,200                                | 120 (120-120)                          |
| 3   | D. E.            | 5+                 | 1,465 | 1,000 (800-1,600)†                   | 120 (60-240)                           |
| 4   | E. H.            | 4+                 | 1,450 | 400 (400-400)                        | 90 (90-90)                             |
| 5   | B. R.            | 6+                 | 365   | 600                                  | 21 (15-30)                             |
| 6   | T. T.            | 5+                 | 315   | 730 (600-800)                        | 21 (30-15)                             |
| 7   | I. B.            | 3+                 | 250   | 500                                  | 12 (15-10)                             |
| 8   | H. N.            | 5+                 | 231   | 200 (200-200)‡                       | 10 (10-10)                             |
| 9a  | T. N.            | 5+                 | < 2   | 400 (400-400)                        | Neg. (neg.-neg.)                       |
| 9b  | T. N.            | 5+                 | 230   | 20 (10-40)                           | 14 (10-20)                             |
| 9c  | T. N.            | 5+                 | 54    | 40 (40-40)§                          | 20 (40-10)                             |
| 10  | A. R.            | 5+                 | 130   | 600                                  | 69 (60-80)                             |
| 11  | A. A.            | 4+                 | 55    | 20                                   | 3 (10-neg.)                            |
| 12  | L. H.            | 4+                 | 34    | 300                                  | 17 (30-10)                             |
| 13  | E. E.            | 3+                 | 4     | 10                                   | Neg. (neg.-neg.)                       |

\*The concentration of IgE antibodies is expressed as per thousand of that of a reference serum.

†Titers at retesting in duplicate after the RAST and CHLT experiments = 200, 200.

‡Titers at retesting in duplicate after the RAST and CHLT experiments = 80, 60.

§Titers at retesting in duplicate after the RAST and CHLT experiments = 40, 80.

to sensitize human skin in vivo ( $r = 0.77$ ;  $p < 0.001$ ) and lung tissue in vitro ( $r = 0.55$ ;  $p < 0.05$ ).

When the serum concentration of IgE antibodies specific for the cod allergen was estimated with RAST, positive results were obtained with sera from 12 of the patients (Table I). With one serum (No. 13), a doubtful positive value was obtained. The mean concentration of IgE antibodies to the fish allergen in the series was 847 per thousand of that of a reference serum. The error calculated for the RAST values obtained when testing sera at separate dilutions was  $\pm 13$  per cent, the dilution factor ranging from 2 to 10.

Three serum samples were analyzed from Patient 9. The first sample gave negative results both in RAST and CHLT, while the second and third serum samples yielded distinctively positive results in both tests. The latter 2 samples were taken 2 years after the first one, and at the time of these later samplings, the patient had been treated with prednisone for some months (10 mg. daily). All serum samples gave positive PK titers, the first sample 400-400, the second sample 10-40, and the third sample 40-40 (the figures represent reciprocals of end point titers in duplicate tests). The latter serum sample was retested after the serum had been used in the in vitro tests and after repeated sterile filtration. The PK titers then obtained were 40-80.

With the PK test, reagins to the cod allergen were shown in the sera from all patients in the study. The mean titers varied between 10 and 1,200, with a geometric mean of 231. Repeated titrations with 7 sera before the RAST and CHLT experiments and with 3 of these sera after RAST and CHLT showed very little titer variation. In only one case (9b), a variation exceeding one dilution step (10-40) was seen. The allergen concentration of 20  $\mu\text{g}$  per milliliter

did not in any case give a higher PK titer than the standard concentration of 0.2  $\mu\text{g}$  per milliliter.

In CHLT, positive results were obtained with sera from 12 of the patients, but the titers obtained were markedly lower than in the PK test with a geometric mean of 24. However, a good correlation was shown between the titers obtained in the 2 tests ( $r = 0.83$ ;  $p < 0.001$ ). The end dilution titers in the passive transfer tests also showed a highly significant correlation with the serum concentration of IgE antibodies to the cod allergen as measured by RAST. The coefficient calculated for the RAST values and the PK titers was 0.84 ( $p < 0.001$ ) and for the RAST values and the CHLT titers, 0.97 ( $p < 0.001$ ).

As most sera had been stored for several years after the PK titrations and previous to the RAST and CHLT experiments, the question arose whether the PK titers had decreased or not during storage. This was of special interest as one of the 3 serum samples from Patient 9 seemed to have become inactivated, judging from the negative results obtained in RAST and CHLT. Two sera (Patients 3 and 8) taken in 1964 and 1966, respectively, were therefore retested in the PK system after the RAST and CHLT experiments and subsequent sterile filtration (millipore filters, 0.22  $\mu$ ). The results of these passive transfer tests showed a reduction of the geometric mean titer from 1,000 to 200 in one serum and from 400 to 69 in the other. In the third serum sample from Patient 9, which was taken shortly before the series of experiments, no reduction of the PK test activity was found after the *in vitro* tests followed by millipore sterile filtrations.

## DISCUSSION

It has been shown clearly that most reaginic antibodies belong to the immunoglobulin class E.<sup>1, 2</sup> Therefore, one may expect to find good agreement between the concentration of reagins and IgE antibodies in reaginic sera. In this study, the reaginic activity was estimated biologically with an *in vivo* method (PK test) and with an *in vitro* test (CHLT). In both tests the results obtained agreed quite well with the concentration of IgE antibodies estimated immunochemically with RAST. The best correlation was found between the results of RAST and CHLT, while the agreement between the PK titers and the results of the 2 other tests, though still highly significant, was less pronounced. One explanation of the latter findings may be that the PK test does not yield the same precision as the *in vitro* tests. The PK titers were in most cases based on single determinations, and even when the tests are performed on one single recipient, as in this study, the results depend on several biologic variables.<sup>12</sup> Recently, a highly significant correlation has been reported between the PK and RAST results in a study of ragweed-sensitive patients.<sup>13</sup>

In the present study the skin tests were used only for screening skin sensitivity. Scratch tests were therefore used instead of intracutaneous tests, and no attempts were made to find the highest allergen dilution that still gave a positive direct skin reaction. If this had been done, a better correlation would possibly have been obtained between the results of the skin tests and the other tests.

As seen in Table I, the sensitivity of RAST and the PK test was about the

same, while the titers obtained in CHLT were about 10 times lower. In a previous study,<sup>7</sup> RAST and CHLT were found to be almost equally sensitive when sera from patients allergic to horse dandruff, birch, and timothy pollen were investigated. The reason for the apparently low sensitivity of CHLT seen in this study is unclear, but one explanation may be that the reaginic activity had deteriorated during storage and the freezings and thawings necessary for the preliminary experiments. Whole reaginic serum is said to maintain its potency for years at  $-20^{\circ}\text{C}$ ,<sup>14</sup> while diluted serum is much more labile.<sup>15</sup> The ability to passively sensitize a tissue possibly decreases more rapidly upon storage than the antigenicity and antibody activity of the IgE antibodies required for detection with RAST. In passive cutaneous anaphylaxis (PCA) in monkeys, Johansson and Stanworth<sup>16</sup> found that sera stored at room temperature for 2 weeks showed a 2-3-step titer decrease (doubling serial dilutions) compared with fresh sera, while the RAST values were almost unchanged. The low CHLT titers obtained in this study may therefore reflect a decrease of the reaginic activity caused by the storage conditions rather than a low sensitivity of the method as such. The decreased PK titers obtained with the 2 sera that were tested after the RAST and CHLT experiments are in support of this theory. The sterile filtration of the sera using a millipore filter may possibly have contributed to the titer decrease, but according to earlier experiences (K.A.) such treatment does not give any significant reduction of the PK titer. This was also confirmed by the results obtained with the third serum sample from Patient 9.

The results of the experiments performed with the 3 serum samples from Patient T. N. seem somewhat puzzling. The first serum sample had a fairly high PK titer in 2 determinations, but in RAST and CHLT performed 2 years later negative results were obtained. With 2 fresh serum samples taken within an interval of 2 months when the patient had been on steroid treatment for some time, the PK titers were markedly reduced. With these sera, however, distinctively positive results were obtained in RAST and CHLT. These findings may be explained by the assumption that the first serum sample had been inactivated to a higher degree than the other sera in the study. Unfortunately, it was not possible to check the PK titer in the first serum sample as no more serum was left from this bleeding.

The PK titers of the 3 retested sera seemed to be about 4 times higher than the CHLT titers for corresponding sera. This difference in sensitivity between the 2 passive transfer tests is about the same as has been reported for the PK test in relation to the passive leukocyte histamine release test.<sup>17</sup>

The PK test is the most sensitive test hitherto known for estimation of reagins. However, it has many disadvantages, such as a low capacity with reference to the number of sera that can be tested at the same time, difficulty in obtaining standardized test conditions, and, above all, the risk of transferring serum hepatitis. These drawbacks are excluded in RAST, and as both the specificity and the sensitivity seem to be comparable to that of the PK test, RAST appears to be a most useful method for studies of reaginic allergy.

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