

# BRIEF COMMUNICATION

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## Loss and recovery of leukocyte sensitivity to antigenic histamine release during intensive injection therapy with allergen extract

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*During allergen injection therapy with a relatively intensive dosage regimen, the leukocytes of the peripheral blood lost sensitivity to antigenic histamine release not only specifically to the allergen injected but also to another allergen not included in the injections. After discontinuance of injections, leukocyte unresponsiveness persisted for 3 months, but then sensitivity was recovered. A second course of injections failed to decrease the leukocyte sensitivity again. Serial measurements of other pertinent immunologic responses are presented.*

In our studies<sup>1</sup> with 33 allergic children given a customary prolonged series of injections of allergen extracts, providing 1,460 to 2,840  $\mu$ g of protein nitrogen (PN)\* in 2 to 3 years, complete loss of leukocyte sensitivity to antigen as measured by release of histamine was never observed. In an effort to achieve complete desensitization, more intensive rapid dosage regimens were used. The present report concerns an unusual occurrence in one boy of complete loss of leukocyte sensitivity to histamine release by the allergen injected and also by another allergen to which he was sensitive but which was not included in the injections.

### CASE REPORT

The observations were made during the treatment of a 14-year-old boy of Puerto Rican descent who was born and lived in New York city. Since early childhood he had had allergic rhinitis characterized by nasal obstruction and clear nasal discharge. Symptoms had been continuous, without seasonal fluctuations, and over the years had become more

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\*One microgram of PN = 100 protein nitrogen units (PNU).

TABLE I. Injections of *Alternaria* extract

| First course |                       |                             | Second course |                       |                             |
|--------------|-----------------------|-----------------------------|---------------|-----------------------|-----------------------------|
| Day          | Dose<br>( $\mu$ g PN) | Cumulative<br>( $\mu$ g PN) | Day           | Dose<br>( $\mu$ g PN) | Cumulative<br>( $\mu$ g PN) |
| 1            | 0.05                  |                             | 125           | 5                     |                             |
|              | 0.1                   | 0.15                        |               | 10                    |                             |
| 2            | 0.2                   |                             |               | 20                    | 35                          |
|              | 0.4                   | 0.75                        | 126           | 40                    |                             |
| 3            | 0.6                   |                             |               | 60                    |                             |
|              | 1                     | 2.35                        |               | 80                    | 215                         |
| 4            | 2                     |                             | 127           | 100                   |                             |
|              | 4                     | 8                           |               | 100                   | 415                         |
| 5            | 6                     |                             | 128           | 100                   |                             |
|              | 7                     | 21                          |               | 100                   | 615                         |
| 6            | 8                     |                             | 129           | 100                   |                             |
|              | 9                     | 38                          |               | 100                   | 815                         |
| 7            | 10                    |                             | 130           | 100                   |                             |
|              | 10                    | 58                          | 131           | 50                    |                             |
| 8            | 20                    |                             |               | 50                    | 1,015                       |
|              | 30                    | 108                         | 132           | 100                   |                             |
| 9            | 40                    |                             | 133           | 100                   |                             |
|              | 50                    | 198                         | 134           | 100                   |                             |
| 10           | 50                    |                             | 136           | 100                   |                             |
|              | 50                    | 298                         | 138           | 100                   |                             |
| 11           | 50                    |                             | 140           | 100                   |                             |
|              | 50                    | 498                         | 142           | 100                   |                             |
| 12           | 60                    | 558                         | 144           | 100                   | 1,815                       |
| 13           | 70                    | 628                         | 146           | 50                    |                             |
| 14           | 70                    | 698                         |               | 50                    |                             |
| 15           | 80                    | 778                         | 148           | 100                   |                             |
| 16           | 90                    | 868                         | 149           | 100                   |                             |
| 17           | 90                    | 958                         | 150           | 100                   | 2,215                       |
| 18           | 50                    | 1,008                       |               |                       |                             |
| 19           | 70                    | 1,078                       |               |                       |                             |
| 20           | 90                    | 1,168                       |               |                       |                             |
| 21           | 100                   | 1,268                       |               |                       |                             |
| 22           | 100                   |                             |               |                       |                             |
| 23           | 100                   |                             |               |                       |                             |
| 24           | 100                   | 1,568                       |               |                       |                             |

severe. No environmental factor or food had been identified as aggravating the condition. Antihistamine and decongestant drugs afforded some relief. Conjunctivitis occurred frequently, but he never had experienced asthma or skin disorders. A brother suffers from asthma. Nasal mucous membranes were pale and boggy and almost completely blocked the passages; secretions contained numerous eosinophils. Intracutaneous skin tests with allergens at a concentration of 1  $\mu$ g of PN per milliliter gave 7 to 8 mm. wheals and 15 to 30 mm. of erythema with *Alternaria*, house dust, cotton, feathers, ragweed, and cockroach.

## PLAN OF STUDY

After the subject was found to have a strongly positive reaction to skin test and abundant release of histamine from leukocytes with *Alternaria* extract, he consented to undergo immunochemical studies during intensive injection therapy with this allergen. The dosage regimen achieved is given in Table I. The response to the injections was followed by repeated determinations of leukocyte histamine release, antigen-neutralizing capacity of serum, and skin tests. Prausnitz-Küstner tests and passive sensitization of normal leukocytes were to be carried out with sera from various phases of the treatment. No drugs were administered during the entire study.

Observations were made between August 24, 1970 (Day 0), and January 25, 1971 (Day 150).

## METHODS AND MATERIALS

Antigenic histamine release from leukocytes of peripheral blood and antigen-neutralizing capacity of serum were determined by reported procedures.<sup>2</sup>

Skin tests were performed with disposable needles and syringes, about 0.01 ml. extract being injected intradermally.

Prausnitz-Küstner tests were carried out in conventional manner<sup>4</sup> in a single subject with dilutions of serum stored at  $-20^{\circ}$  C.; all sites to be compared were prepared at the same time and tested with antigen 48 hours later.

Leukocytes of a nonallergic person were passively sensitized for histamine release by addition of the subject's serum diluted with buffer to the entire cell mass from centrifuged heparinized blood, incubating at  $37^{\circ}$  C. for 40 minutes and subsequently separating leukocytes from erythrocytes with the aid of dextran; histamine release by antigen was then determined by the procedure already mentioned.<sup>2</sup>

Allergen extracts were purchased from Center Laboratories, Port Washington, N. Y.

## RESULTS

*Alternaria* extract injected in the amount and frequency shown in Table I caused no generalized reactions or symptoms and only moderate diffuse swelling and tenderness sometimes at the site of injection. The changes in leukocyte sensitivity (LS), revealed by percentages of histamine in leukocytes released by a fixed concentration of antigen, and in antigen-neutralizing capacity (ANC) of serum<sup>2</sup> are depicted in Figs. 1 and 2. Before treatment 52 per cent of histamine in leukocytes was released by *Alternaria* at  $0.003\text{ }\mu\text{g}$  of PN per milliliter, and the ANC was 12. On Day 10, when the dose had reached  $100\text{ }\mu\text{g}$  of PN daily and a total of  $298\text{ }\mu\text{g}$  of PN, LS began to decrease and ANC had risen to 435. By Day 21,  $1,268\text{ }\mu\text{g}$  of PN had been injected, the ANC had reached 1,300, and leukocytes released no histamine with *Alternaria* even at  $10\text{ }\mu\text{g}$  of PN per milliliter. Injections were discontinued on Day 25. Leukocytes remained unresponsive to histamine release by *Alternaria* for another 91 days. During this unresponsive period, leukocytes also failed to release histamine with house dust extract at concentrations from 0.2 to  $20\text{ }\mu\text{g}$  of PN per milliliter on repeated examinations, although the skin test with house dust had been positive before the injections. We had not measured histamine release with dust before therapy but, in our experience histamine release regularly occurs with house dust extract in skin test-positive subjects.<sup>3</sup> On Day 115, leukocyte responsiveness to antigenic histamine release was found to have begun to recover; 26 per cent was released by *Alternaria* at  $0.1\text{ }\mu\text{g}$  of PN per milliliter (30 times the concentration required to release 52 per cent before therapy) and 30 per cent was released by dust at  $3\text{ }\mu\text{g}$  of PN per milliliter. ANC had remained between 1,300 and 1,600 throughout the period of leukocyte unresponsiveness (determined for sera collected in this period with his leukocytes when sensitivity had recovered).

On Day 125 a second series of injections with *Alternaria* extract was begun (Table I). The daily dose was brought to  $200\text{ }\mu\text{g}$  of PN in 2 days without generalized reactions and only moderate local swelling around the sites of injections. By Day 150,  $2,115\text{ }\mu\text{g}$  of PN of *Alternaria* extract had been injected without halting the increase in leukocyte sensitivity to *Alternaria* (which reached 50 per cent at  $0.1\text{ }\mu\text{g}$  of PN per milliliter) or to house dust (63 per cent at  $3\text{ }\mu\text{g}$  of PN per milliliter).



**TABLE II.** Results of intradermal tests (0.01 ml. of 1  $\mu$ g of PN per milliliter)

| Day | Alternaria<br>(wheal/erythema, mm.) | House dust<br>(wheal/erythema, mm.) |
|-----|-------------------------------------|-------------------------------------|
| 0   | 9/20                                | 4/6                                 |
| 31  | 2/3                                 | 0                                   |
| 85  | 8/20                                | 4/8                                 |
| 110 | 10/20                               | 3/5                                 |
| 129 | 8/30                                | 8/30                                |
| 150 | 8/30                                | 6/25                                |

**TABLE III.** Results of passive transfer to skin (Prausnitz-Küstner test)

| Day serum obtained | Dilution | Reaction to Alternaria (10 $\mu$ g PN/ml.)<br>(wheal/erythema, mm.) |
|--------------------|----------|---------------------------------------------------------------------|
| 0                  | 1:10     | 9/30                                                                |
| 0                  | 1:100    | 7/20                                                                |
| 0                  | 1:1000   | 5/7                                                                 |
| 36                 | 1:10     | 10/30                                                               |
| 36                 | 1:100    | 10/30                                                               |
| 36                 | 1:1000   | 6/9                                                                 |
| 68                 | 1:10     | 8/30                                                                |
| 68                 | 1:100    | 9/25                                                                |
| 68                 | 1:1000   | 8/10                                                                |
| 116                | 1:10     | 11/30                                                               |
| 116                | 1:100    | 8/25                                                                |
| 116                | 1:1000   | 8/12                                                                |
| 150                | 1:10     | 9/30                                                                |
| 150                | 1:100    | 6/30                                                                |
| 150                | 1:1000   | 8/15                                                                |

Controls: Recipient was shown to give a negative skin test to Alternaria. No reaction in prepared sites at 48 hours, just prior to testing.

The intensity of reaction to intradermal injections of Alternaria and dust extract decreased during the period of leukocyte unresponsiveness and increased as leukocyte sensitivity was recovered (Table II).

Results of passive transfer to skin with the subject's serum (Table III) clearly show detectable specific skin-sensitizing antibody to Alternaria in sera throughout the observations in at least a 100-fold dilution, and reactions of nearly the same intensity in periods of leukocyte responsiveness and unresponsiveness.\* In other passive-transfer tests with the same recipient, dust at 10  $\mu$ g of PN per milliliter gave reactions of equal intensity (5 mm. wheal/10 mm. erythema) with sera from Days 0, 36, 68, and 116, while controls with antigen and sera were negative. It was also shown that the extracts of Alternaria and house dust had no significant cross-reactivity by demonstrating that P-K sites exhausted by the one antigen subsequently still gave strong reactions with the other.

\*Quantitative determinations of total IgE in sera were performed by Dr. Richard Newcomb of the Children's Asthma Research Institute and Hospital, Denver, who kindly supplied these data: Sera from Days 0, 11, 38, 116, 129, and 150 had IgE contents of 150 to 170 ng. per milliliter (in the normal range), indicating remarkably little fluctuation during both courses of injections and a normal level of IgE in serum during the period of leukocyte unresponsiveness.

Aliquots of leukocytes from a nonallergic person were exposed simultaneously to sera from the subject obtained on Day 58 (during the leukocyte unresponsive period) and on Day 140 (after recovery of leukocyte sensitivity). The leukocytes were passively sensitized so that *Alternaria* at 1  $\mu$ g of PN per milliliter released a maximum of 44 per cent histamine from leukocytes sensitized with serum from Day 58 and a maximum of 28 per cent with serum from Day 140.\*

The histamine content of the leukocytes never decreased and actually tended to increase during the period of leukocyte unresponsiveness and both courses of injections.

Symptoms were noticeably less during the period of leukocyte unresponsiveness and seemed to remain at the same level during the second course of injection therapy.

Urine and blood were examined repeatedly, and no evidences of renal abnormality, hemolysis, or inflammatory response were detected.

### COMMENT

Especially noteworthy is the finding in this subject that during the unresponsive period the leukocytes released no histamine to dust extract as well as none to *Alternaria* (the allergen injected). Release with dust was not measured before injections, but release with both *Alternaria* and dust was abundant in the recovery period. Thus, the leukocytes (basophils) were rendered unresponsive nonspecifically, perhaps generally, to antigenic histamine release. This occurred even though a 1:100 dilution of serum obtained during the unresponsive period contained enough specific skin-sensitizing antibody to permit ready detection by P-K tests. In addition, leukocytes of a nonallergic subject were passively sensitized to histamine release by *Alternaria* with serum from the unresponsive period, indicating that the serum did not contain factors which could have either prevented fixation of IgE antibody to the basophils or blocked combination between cell-fixed antibody and antigen. Specific reagin in sera of subjects with unresponsive leukocytes has also been reported.<sup>11</sup> Total histamine content of the leukocytes was not diminished at any time but tended to increase after the injections. The unresponsiveness of leukocytes was observed to persist for 95 days from the onset of unresponsiveness and 91 days after injections were discontinued.

Antibody responsible for the ANC of the serum remained at the same high levels during unresponsiveness and recovery of the leukocytes and thus appeared to play no role, and they may be viewed simply as an expression of conventional antibody response to antigenic material. The leukocyte unresponsiveness seemed to be only a phase in the effects of the intensive dosage regimen of injections in a previously untreated subject and did not occur with the second course given 95 days after the first course.

Considering the estimated life span of 8 to 12 days for basophils,<sup>5</sup> the unresponsive stage seemed too long to be easily explained by exhaustion of energy-producing biochemical systems in basophils necessary for histamine release. Some

\*Procedure carried out by Dr. Michael Schumacher who kindly contributed these data.

persistent effect of injected allergen or of the immune response on the basophil population may have continued to disturb: (1) fixation of IgE antibody, or (2) antigen-antibody interaction with constituents of the cell membranes.

This much seems clear: The intensive program of injection therapy did not "desensitize" this allergic individual or induce "immunological paralysis." He continued to produce specific IgE (skin-sensitizing) and IgG (antigen-neutralizing) antibodies. The depression of antigenic release of histamine from leukocytes was transitory. These considerations do not provide an immunologic basis for recommending this form of injection therapy with *Alternaria* extract for general use.

For the sake of brevity, consideration of the rare occurrence in our experience of leukocyte unresponsiveness to antigenic histamine release during injection therapy, in contrast to reports by others<sup>6-11</sup> that this is more frequently observed, will be deferred to another communication.

## REFERENCES

- 1 Lyman, M., May, C. D., Alberto, R., and Aduna, N.: Effect of intensive regimens of injection with allergen extracts in children on leukocyte sensitivity to antigenic histamine release, *J. ALLERGY* 47: 119, 1971. (Abst.)
- 2 May, C. D., Lyman, M., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood, *J. ALLERGY* 46: 12, 1970.
- 3 May, C. D., Lyman, M., Alberto, R., and Cheng, J.: Immunochemical evaluation of antigenicity of house dust extract, *J. ALLERGY* 46: 73, 1970.
- 4 Sherman, W. B.: Hypersensitivity, Philadelphia, 1968, W. B. Saunders Company, p. 70.
- 5 Osgood, E. E., et al.: Duration of life and of the different stages of maturation of normal and leukemic leukocytes, *Rev. Hematol.* 9: 543, 1954.
- 6 Pruzansky, J. J., and Patterson, R.: Histamine release from leukocytes of hypersensitive individuals. II. Reduced sensitivity of leukocytes after injection therapy, *J. ALLERGY* 39: 44, 1967.
- 7 Lichtenstein, L. M., Norman, P. S., and Connell, J. T.: Comparison between skin-sensitizing antibody titers and leukocyte sensitivity measurements as an index of the severity of ragweed hay fever, *J. ALLERGY* 40: 160, 1967.
- 8 Pruzansky, J. J., and Patterson, R.: Immunologic changes during hyposensitization therapy, *J. A. M. A.* 203: 289, 1968.
- 9 Sadan, N., et al.: Immunotherapy of pollinosis in children, *N. Engl. J. Med.* 280: 623, 1969.
- 10 Melam, H. L., Pruzansky, J. J., and Patterson, R.: Correlations between clinical symptoms, leukocyte sensitivity, antigen-binding capacity, and Prausnitz-Küstner activity in a longitudinal study of ragweed pollinosis, *J. ALLERGY* 46: 292, 1970.
- 11 Levy, D. A., Lichtenstein, L. M., Goldstein, E. O., and Ishizaka, K.: Immunologic and cellular changes accompanying the therapy of pollen allergy, *J. Clin. Invest.* 50: 360, 1971.