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IMMUNOLOGICAL STUDIES OF POLLINOSIS

V. The Enhanced Response in Hay Fever[1]

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If thermostable antibody plays a protective role in hay fever, as would appear to be the case (1), then it becomes important to determine optimal means of stimulating its formation.

With this in view, the author attempted to induce for the first time the so-called enhanced or anamnestic response in ragweed-sensitive patients. It has been shown in an earlier publication (2) that *normal* individuals react more quickly and strongly to a secondary, or "booster" dose of pollen-extract than to an initial course of subcutaneous injections. It is common knowledge that revaccination with typhoid bacilli leads to a relatively greater immune response, and Peshkin (3) has described this phenomenon in children after "repeat"-doses of tetanal toxoid. Animals resemble man in that they too become "educated" to the more efficient production of antibodies after a primary series of inoculations (Beard, Finkelstein, and Beard (4)).

It was the purpose of the present investigation to learn whether pollen-sensitive patients could be made to increase their production of thermostable antibody if they were given booster-courses of pollen-extract some months after their primary experience with this antigen.

METHODS

Fifty-two previously untreated, ragweed-sensitive adults were given 3 to 5 annual courses of pollen-extract under the skin as rapidly as their individual tolerances allowed. The amounts of thermostable antibody present in their sera before, during, and after these courses were gauged by a modification of the Prausnitz-Küstner technic which has been previously described in detail (5). The antibody-titer is expressed in terms of the number of protein-nitrogen units of ragweed-extract which can be neutralized by one ml of heated serum. Preliminary comparison was made among the various sera of a given patient, all of whose specimens had been titrated in the same passive-transfer experiments in order to avoid variations introduced by the test-subject. Data obtained under equivalent circumstances from various individuals were then grouped together for final comparisons.

In order to determine whether the anamnestic or "recall"-phenomenon could be induced in hay-fever subjects, it was necessary to titrate the blood antibody from time to time during the primary course and then to repeat these titrations

[1] Presented before the Eighteenth Meeting of the Society for the Study of Asthma and Allied Conditions, in New York City on December 5, 1942.

after the same doses had again been reached in one or more secondary[2] courses. Since normal individuals had been found in earlier studies (2) to reach an immunological "ceiling" during the course of their immunization with ragweed-extract, this tendency was looked for in the present investigation. Note was also made of the possible influence on the secondary response of such factors as the interval of rest allowed between courses, the retardation in antibody-production which had occurred by the time the booster dose was given, and the speed with which the course was completed. It seemed important to determine if the magnitude of the primary dose might prejudice the patient's response to subsequent courses. Finally, a few patients with perennially treated hay fever were studied immunologically so their behavior could be compared with that of the 52 preseasonally injected patients.

TABLE 1

*Showing the accelerated and enhanced immune response to secondary injections of pollen-extract (52 patients)**

NUMBER OF PATIENTS TREATED	TOTAL DOSE GIVEN		PERIOD OF TREATMENT		ANTIBODY-TITER†	
	Primary series	Secondary series	Primary series	Secondary series	Primary series	Secondary series
			days	*days*		
12	1,000	1,000	40	22	350	1000
21	4,500	3,750	100	44	700	1000
14	10,000	8,000	100	75	1000	1250
21	50,000	25,000	220	70	900	1375

*Some are included in more than one group.

†Expressed in terms of the protein-nitrogen units of pollen-antigen neutralized by 1 ml of serum.

FINDINGS

Relative immune responses to primary and secondary stimuli of equal strength

When serological titrations were performed after equal doses of extract had been given in a primary and in one or more secondary courses, it was found that a given dosage could be taken in less time and would give rise to more thermostable antibody, *after* the patient had been subjected to a primary course, as the following data show. Since most patients were bled several times during each course at predetermined dosage-levels, many of them are included in more than one of the following groups.

The first group was comprised of 12 patients who were studied after a total of 1000 units had been given in an initial course and again in some later year. These individuals were able to take their 18 secondary doses of 1000 units in about half the average time that was needed for their primary treatment with the same amount of antigen, only 22 days being consumed by the later course whereas 40 had been required initially. Three times as much antibody resulted after the later courses, as shown in table 1.

[2] The term, secondary, refers in this paper to any injection given after the completion of the first year's treatment. Some patients received three or four secondary courses, usually a year apart.

In 21 instances, titrations were performed after an average dose of 4500 units had been given in a primary series covering 100 days. The average antibody-titer which resulted was 700. A somewhat smaller dose, averaging 3750 units, was injected into these patients in 31 subsequent courses. Less than half the time was consumed for its administration and nearly 50 percent more antibody was formed, as the table indicates.

Similar comparisons after 14 primary courses of 10,000 units revealed that 50 percent more time was needed and less antibody was developed after the first courses than after 18 subsequent ones.

Finally, in 21 instances the antibody-production was estimated after a primary course of 50,000 units had been completed in 220 days. Later stimulation with about half this amount of antigen required about one-third the treatment-time. The thermostable antibody formed during the initial course averaged 900 units per ml of serum, whereas the concentration following the secondary series was 1375 units.

Other observations pertaining to the secondary response

Early in the course of these investigations, it became apparent that patients tended to reach an immunological ceiling, above which additional extract usually failed to push their antibody-production. The phenomenon was observed during primary courses but seemed to occur more frequently during secondary stimulation. Twenty-four patients who illustrate this are listed in table 2. Only 4 of the 25 instances were found in primary courses, these being starred in the table. It will be seen that the maximal titers had been reached by the time an average dose of 6750 units had been administered these subjects. No increase in antibody-production occurred when the total dose was raised to 20,000 units by additional injections.

Other factors which appeared to affect the immune response of previously treated patients were 1) the length of time the patient had gone without injections and 2) the retardation which had occurred in his antibody-production. There were 17 patients who developed greater humoral immunity during one secondary series than during another of equal size. Analysis of the circumstances revealed that the more effective course had been preceded by a longer period of rest and a greater drop in antibody-concentration. It had been administered in a shorter period of time than the less successful course. Its titer averaged 50 per cent more, as shown in table 3.

No consistent difference could be found between one booster-course and another of the same size given in the same period of time. In other words, after education by the primary injections, one secondary stimulus was as effective as another of the same strength, providing the preceding titers and rest-periods were comparable. Whether the course was chronologically nearer or farther from the primary series did not seem to be of any importance. The education seemed to reach its peak after the first series and to change but little in the ensuing three years.

It was thought that the size and effectiveness of the *primary* stimulus might prejudice the subsequent responsiveness. In order to test this possibility,

TABLE 2

Showing tendency of humoral antibody to reach a ceiling

PATIENT	TITER	DOSE ASSOCIATED WITH TITER	
		Minimal dose (PN units)	Larger doses failing to increase titer
BEH	600	2,000	16,000
BE	3000	1,000	4,500 5,500
BER	1000	15,000	32,000 50,000
	1000	10,000	68,000
CI	750	1,000	3,000 17,000 27,000
CR	1750	1,000	13,000
DEM	1000	1,500	19,000* 28,000*
	1000	1,000	10,000
EV	2000	15,000	21,000
FA	900	800	4,000
HE	1000	2,000	7,000
IT	1000	1,000	5,000 16,000 19,000 34,000
KA	1000	10,000	25,000
KL	1500 2000	4,000 10,000	10,000* 13,500 18,000
KO	1000 850	20,000 10,000	27,000* 18,500
KU	1500	1,750	3,000 9,000
LEW	1500	1,000	10,000
LI	2000	3,500	10,000
LEV	800	1,000	6,000 18,000

TABLE 2—*Concluded*

PATIENT	TITER	DOSE ASSOCIATED WITH TITER	
		Minimal dose (PN units)	Larger doses failing to increase titer
McN	500	1,000	6,500 10,000
MALL	1000	1,500	20,000
MAN	450	1,000	6,000 9,000
PO	400 450	10,000 15,000	17,000* 50,000
REI	500	1,000	2,700 7,700
STR	700	50,000	110,000
SHA	500	4,000	10,000 20,000
Average (24 patients).....		6,750	20,000

*Primary course.

TABLE 3

Showing the influence of the preceding titer and rest-period on the secondary response of 17 patients who received the same-sized dose in two secondary courses with greater antibody-formation after one

	DAYS OF REST BEFORE COURSE	TITER BEFORE COURSE	TREATMENT PERIOD	RESULTING TITER
			days	*Pn units/ml*
More successful course...	300	475	25	1200
Other course.....	200	600	40	800

patients who had registered at the Allergy Clinic too late in the summer to take more than a few thousand units as their introductory course were compared with others who had received intensive primary therapy. Table 4 summarizes the results of this analysis. (In judging these figures, it must be remembered that comparisons are being made among 63 different serum-specimens obtained from 25 patients and that it was impossible to titrate more than a small proportion of these on any one test-subject. The influence of the test-subject on the endpoints has been reduced, however, by grouping of the data.)

The table indicates that 12 patients, who took a primary course of only 3500 units in all, developed definitely less antibody in their sera than did 13 others whose primary injections averaged 50,000 units. The clinical benefits were in keeping with these antibody-titers, a smaller proportion of group 1 reporting good and excellent relief than was the case for group 2. The serological and

clinical effects of the primary course are apparently more favorable when a relatively large dose is attained.

The secondary reactions of these two groups also appeared to be influenced by the size of the primary course. Patients who had received a large initial dosage developed more antibody and greater clinical resistance to atmospherical pollen after a small booster dose than did those who had received a small introductory series. The intensive primary therapy seemed to extend its favorable influence beyond the first season.

It was interesting to find that the larger primary dose elicited so satisfactory an immune response that the titer was only slightly improved when these patients were again stimulated in subsequent years. The average amount of antibody formed by group 2 during their first injections was 1060 units per ml of

TABLE 4

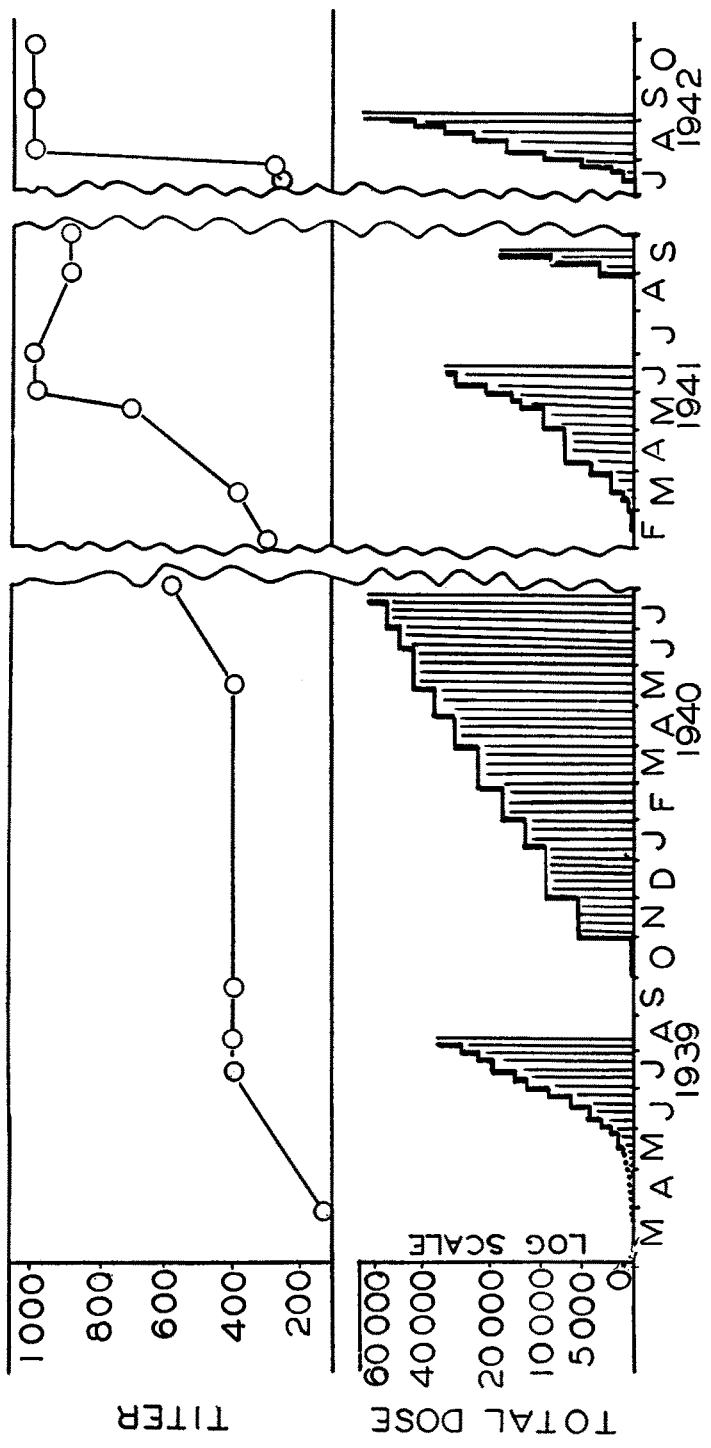
Showing the influence of the size of the total primary dosage on the serological and clinical responses of the hay-fever patient during the first and subsequent years of treatment with pollen-extract

GROUP	NUMBER OF PATIENTS	NUMBER OF COURSES	AVERAGE TOTAL DOSE	RESULTING TITER (MAXIMAL)	RESULTING CLINICAL IMPROVEMENT				
					Poor	Fair	Good	Excellent	Sum of excellent + good cases
					Per cent of cases	Per cent of cases	Per cent of cases	Per cent of cases	Per cent of cases
Primary series of injections									
1	12	12	3,500	575	17	42	25	17	42
2	13	13	50,000	1060	8	23	31	38	69
Secondary series of injections									
1	12	21	5,500*	925	30	13	17	38	55
2	13	17	3,500*	1090	12	6	41	41	82

*The smallest total dose observed to produce the maximal antibody-response. Additional dosage, given in many instances, failed to increase the antibody-production.

serum, as indicated in table 4. In response to secondary stimuli, these patients produced an average of 1090 units of antibody. These titers stand in contrast to those of the group given but little treatment the first year. Their antibody titers rose to 575 the first year, increasing decidedly to 925 upon secondary stimulation but still failing to reach the average for group 2. Both groups illustrate the anamnestic response. Group 1 shows it in the heightened immune reaction of the secondary courses. Group 2 required only $\frac{1}{14}$ of the primary dose to develop an equal, or slightly superior, concentration of thermostable antibody.

The immunological behavior of perennially treated cases was investigated in three instances. None of the patients exhibited any tendency toward enhancement. After the first year, one man maintained his ceiling-level of humoral immunity and an excellent clinical resistance. Another had fair relief his first



SHOWING THE RELATIVE IMMUNE RESPONSE TO PRIMARY AND SECONDARY STIMULI (PATIENT BER)

season of therapy, and poor results the next two years in conjunction with a slight decrease in titer. The third member of the group had excellent resistance his first season, only fairly satisfactory results the next two. His antibody-production fell somewhat after his initial course had been completed and the customary monthly injection throughout the year was instituted. The annual dosage of these patients was in the region of 150,000 units, many times greater than any used to elicit the recall-phenomenon in the 52 patients discussed above.

The doses and antibody-titers of one representative patient, BER, will now be presented in graphic form to illustrate the difference between primary and secondary reactions. After the first four months of subcutaneous injection, an antibody-ceiling was encountered. This persisted despite a long and intensive secondary course which extended well into 1940. Finally, after the dose had reached a total of 65,000 units, an increase in antibody-production occurred. The secondary reaction was on the whole, however, unsatisfactory. This could be referred to the fact that only 49 days of rest had been allowed after the first injections. No diminution in the antibody-output occurred during the rest-period.

A long interval of freedom from injection was permitted before the 1941 stimuli were begun. In these six and one-half months, the titer fell to half its previous maximum and reached 300. Secondary stimulation with 15,000 units administered fairly rapidly (111 days) led to a rapid increase in the activity of the antibody-forming tissues for a neutralizing power of 1000 units per ml of serum was found in the blood. Additional injections bringing the total dose to 33,000 units failed to increase the immunity. The antibody diminished slightly when treatment was interrupted for two and one-half months. When 18,000 units were injected during the pollinating season, no augmentation occurred in the already excellent output of immune bodies.

Previous to his 1942 season, the patient received no injections for 300 days, during which time his antibody-level fell to 300 again. Ten thousand units administered very rapidly (in 11 days only) gave rise to an almost vertical ascent in titer to the earlier maximal level. Additional dosage up to 68,000 units—the same total as was given in 1940—administered in the next 26 days, proved to be of no immunological advantage, for the titer remained at its ceiling. The associated clinical improvement of BER for the years 1939 to 1942 was as follows: 80 per cent, 85 per cent, 60 per cent (unusually high pollen-count), and 98 per cent, respectively.

Apparently, 10,000 units administered over a two-week period ought to afford this patient good protection in future seasons. Treatment will be begun the first of August.

SUMMARY AND DISCUSSION

In attempting to elicit the enhanced immunological response in hay-fever patients, it was found that a primary series of injections with ragweed-extract so educated its recipients that they responded to later doses more quickly and vigorously, providing an adequate period of freedom from treatment had been

allowed. In general, longer periods of rest and lower resting titers were followed by better immune responses than were shorter periods and higher titers. Secondary stimuli given rapidly proved to be more effective than the same-sized doses administered over a longer treatment-period. A primary course of 50,000 units produced higher antibody-concentrations and better clinical results than did an initial series of only 3500 units. Patients receiving the larger dosage their first year appeared to respond more favorably to *secondary* stimuli than did the other group.

These findings suggest that the average patient will fare better if he is treated intensively for several months his first year. An extensive primary course will offer a good opportunity for protective amounts of thermostable antibody to be acquired by his blood and shock-tissues previous to the season of pollination. Periodical tests for immunity, performed at intervals during treatment and at the peak of the pollinating season, will indicate the patient's responsiveness and approximately at what level of immunity he will be clinically comfortable when there is pollen in the air. The goal of secondary stimulation can then be this degree of antibody-formation. As previously suggested, the serum, skin, or shock-tissue may be tested for immunity, the conjunctival test being the simplest.

A subsequent publication will show that this approach to the management of hay fever will often make it possible to give a satisfactory degree of clinical immunity in a few days or weeks, once the patient has been "educated" by an initial series of injections and a rest-period.

CONCLUSIONS

1. Following their education with a primary series of subcutaneous injections with ragweed-extract, pollen-sensitive patients regularly responded to secondary doses, given after months of freedom from treatment, with an enhanced and accelerated production of thermostable antibody.

2. The longer the period of rest, the lower the resting titer, and the faster the administration of the secondary stimulus, the greater was the immune response to a given dose.

3. A relatively large primary dosage elicited a better serological and clinical immunity than did a small one. The responses to secondary stimuli were also more favorable in the group given large initial dosage.

4. The phenomenon of the immunological ceiling was encountered. It occurred more frequently during secondary stimulation than during the first year's therapy.

5. Three patients given perennial treatment for hay fever showed no tendency toward an enhanced response.

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Erratum

On page 33, Vol. 47, No. 1 (July 1943), third line from top: for "direct" read "indirect".