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THE SENSITIZING EFFECTS OF EMULSIFIED POLLEN ANTIGENS IN ATOPIC SUBJECTS NATURALLY SENSITIVE TO AN UNRELATED ANTIGEN

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IN the course of immunologic and absorption studies with injections of ragweed pollen extract emulsified in Arlacel A-Drakeol, it was noted by us that 5 of 6 nonatopic subjects developed a delayed skin reactivity to this antigen.¹ Subsequently, in another series of 15 nonallergic subjects who received such an emulsion, 9 developed delayed skin reactivity, 7 showed immediate skin reactivity, while 3 had both types.² The delayed reactivity was transferred locally and systemically with peripheral leukocytes, and the punch biopsies of the recipients' skin reaction showed histologic changes compatible with those found in other accepted delayed skin reactions.² The immediate type of skin reactivity could be transferred by the donor's serum² and the serum factor possessed characteristics compatible with reagin.³ It is understandable that those who were naturally sensitive to ragweed pollen had such high titers of sensitizing antibody that any moderate increase of this antibody from the injection of the emulsified antigen could not be expected to be discernible. However, in a large series of observations, we failed to find a delayed skin reactivity to ragweed following injection of such an emulsified antigen in those who were naturally sensitive to ragweed.

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This disparity between the atopic and nonatopic individuals has posed both clinical and basic questions. The investigations to be described were undertaken with the hope of shedding some light on the nature of this difference.

PROCEDURE AND RESULTS

Allergic and nonallergic male inmates at a state penal institution comprised the subjects for this study. Antigens used were extracts of pollen of either ragweed, timothy, or English plantain, 10,000 pollen units of one or of each of two, emulsified in Arlacel A-Drakeol in the proportions of 1 part Arlacel A, 9 parts Drakeol, and 10 parts aqueous extract. In certain experiments, histamine was substituted for a pollen extract. The volume injected in each instance was 1 ml. Intradermal tests were made weekly for a number of weeks and then less often, beginning one week and, in several instances, one day after the emulsion injection. The tests were made with 0.05 ml. aqueous extracts of pollen containing 1,000 pollen units per milliliter, and also with weaker solutions where indicated. Almost always, disposable syringes and needles were employed in such testing. Immediate reactions were noted and the sites examined 24 hours and at intervals thereafter. Delayed reactions were recorded positive if they were 10 mm. in diameter or larger, lasted 48 hours or longer, and showed induration. A number of reactions occurred which began within 24 hours, lasted less than 48 hours, had no induration, and were not reproducible over a long period. Such reactions were not recorded in the figures, but will be discussed later in this paper. Skin biopsies were made of the immediate, delayed, and questionable reactions at different intervals after the intradermal injections. The histologic findings of these biopsies will be described at a later time.

Most of the investigations and results can best be described under each particular question for which an answer was sought.

1. *Is the atopic person constitutionally incapable of having the delayed reactivity induced by this method?*

Nineteen atopic individuals were given an emulsified pollen antigen other than the one to which they were naturally sensitive. All 19 developed immediate skin reactivity and 8 of these developed delayed sensitivity. Of the 12 nonatopic subjects who were given emulsions of timothy pollen extract, 3 showed immediate reactivity, 7 delayed, and 2 both.

2. *Does the immediate antigen-antibody reaction from the emulsified antigen hinder the induction of delayed sensitivity? If it does, is the effect a specific one or the result of vascular effects from liberated histamine or, perhaps, other nonspecific influence?*

This could conceivably occur by diffusing the antigen so as to prevent its attachment to skin cells, by its destruction by mediator substances, or by its consumption in the antigen-antibody reaction. Nine subjects were injected with an emulsion containing two pollen antigens, one to which the subject was allergic and the other to which he was not. As can be seen in Table I, 8 de-

veloped immediate reactivity, 3 of whom also had delayed sensitivity. Of the 4 nonatopic persons receiving an emulsion combining both timothy and ragweed pollen extracts, 2 developed an immediate reactivity, 2 delayed, and 1 combined.

TABLE I. INDUCED SKIN SENSITIVITY IN 56 ATOPIC AND NONATOPIC SUBJECTS INJECTED WITH EMULSIFIED ANTIGENS

	EMULSION INJECTIONS	NUMBER	IMMEDIATE REACTIVITY	DELAYED REACTIVITY	COMBINED REACTIVITY
<i>Atopic Subjects</i>					
Allergic to ragweed		15			
	Timothy	13	13	5	5
	English plantain	2	2	2	2
Allergic to Alternaria	Ragweed	4	4	1	1
Allergic to ragweed		9			
	Timothy + Ragweed	4	4	1	1
	Plantain + Ragweed	5	4	2	2
	Histamine + Timothy	3	2	1	1
	Histamine + Plantain	3	3	2	2
Totals		34	32	14	14
<i>Nonatopic Subjects</i>					
	Timothy	12	3	7	2
	Timothy + Ragweed	4	3	2	1
	Timothy + Histamine	6	2	3	0
Totals		22	8	12	3

Six atopic subjects were given an emulsion containing 0.2 mg. of histamine (in terms of base) combined with a pollen extract to which they were not sensitive. Five developed immediate sensitivity and 3 of these also had delayed reactions. Of 6 nonatopic subjects receiving a similar emulsion, 2 developed immediate reactivity, 3 delayed, and none combined.

3. *Is the antigen to which the individual is spontaneously sensitive consumed in the antigen-antibody reaction from the emulsified antigen so that it is not available to induce delayed reactivity?*

This possibility cannot be answered at present, but investigations are under way in an attempt to answer this point.

4. *Does the allergic person who has been given an emulsified antigen in fact possess the delayed sensitivity state but fails to elicit a delayed skin reaction because of interference by the immediate reaction?*

It is conceivable that, in the reaction from the skin test, the antigen may be diffused, destroyed, or used up, thus preventing a delayed skin reaction in spite of the presence of a state of delayed hypersensitivity. If this assumption were correct it would be expected that the peripheral leukocytes of such allergic persons who received the emulsified antigen to which they were sensitive would

I = Immediate reaction.
D = Delayed reaction.
± In delayed reaction column indicates erythema less than 10 mm. lasting less than 48 hours.
O = No reaction.
Numbers in reaction column indicate degree of response.
Signifies test not made because of isolation or parole, or test regarded as unreliable for some special reasons.

be capable of transferring such a delayed hypersensitive state. Preliminary investigations along this line thus far have been negative. In other experiments, preliminary evidence indicates that a naturally acquired delayed skin reactivity is quantitatively modified when the antigen responsible for it is injected intradermally together with an immediate reacting antigen.

5. *Have the cells of the individual who has developed spontaneous immediate type of allergy become specialized for the production of skin sensitizing antibodies and not available for stimulation by the same antigen to produce delayed sensitivity?*

The possibility also exists that those who have developed spontaneously the immediate type of allergy have passed through a stage of delayed hypersensitivity early in their allergic experience. An approach to answer this question is obviously difficult.

OTHER OBSERVATIONS

Table II gives a detailed picture of some other pertinent findings. The high incidence of induced immediate reactivity and the relatively low incidence of delayed reactivity in the atopic group are striking. It is to be noted in this group also that none developed delayed reactivity solely. (Those marked $\pm$ are not included in the statistics, since their status is questionable at present.) In the nonatopic group, the tendency is for greater development of delayed reactivity and for a lesser trend toward the immediate type. In those atopic persons who had both induced types of reactivity, some developed the immediate first, some the delayed, while in others both types appeared simultaneously.

Immediate reactivity was obtained in a few atopic subjects within a week after the emulsion injection, in a large percentage by the end of 2 weeks, and in most by the end of 8 weeks. In 3 instances, immediate reactivity was noted one day after the emulsion injection and was reproducible for several weeks thereafter. In occasional instances, 5 to 8 months were required to develop such sensitivity. Delayed reactivity also became evident in most of those of this group who developed it 2 to 4 weeks after emulsion injection; none required longer than 6 weeks to acquire it. In the nonatopic subjects, immediate reactivity appeared to take longer to develop, whereas delayed sensitivity was induced at about the same time as in the nonatopic group.

There was very little evidence of conversion of a state of delayed reactivity into one of immediate, or vice versa. In most instances of either immediate or delayed reactivity, the sensitive state was present at least 12 weeks after the sensitizing dose, and persisted for at least 20 to 37 and 75 weeks in those who were followed that long. This persistence did not apply to the special type of delayed reaction previously described and designated as $\pm$ in Table II, but not included in Table I.

DISCUSSION

It is apparent that the atopic person can develop delayed sensitivity from the injection of an emulsified pollen antigen providing he has no spontaneous

immediate type sensitivity to that antigen. Since the development of such delayed sensitivity is accompanied by delayed inflammatory reactions at the site of injection of the emulsion, sometimes followed by abscess formation, it is obviously important that care be used to assure one's self that the patient is allergic to such an antigen before it is given in an emulsion. From the data at hand it is also apparent that neither the antigen-antibody reaction nor the inflammation produced by histamine prevents the second antigen in the emulsion to which the subject had no spontaneous allergy from inducing either delayed or immediate sensitivity. The immediate skin reaction from an antigen does appear to reduce the delayed reaction from another antigen. However, such inhibition is not complete. It could be argued, of course, that, while an antigen-antibody reaction does not interfere with an unrelated antigen, it might interfere with its own antigen, perhaps by tying it up with antibody so that it is not available for the production of delayed sensitivity. The occurrence at times of both immediate and delayed sensitivity to the same antigen in some spontaneous allergies and, of course, the induced combined reactions in some of our own experimental subjects argue against this explanation for the failure of the atopic individual to give a delayed skin test with the same antigen to which he is spontaneously sensitive and which he has received in emulsion. It is true that these discrepancies might possibly be explained by the fact that, in some instances of spontaneous allergy, two separate antigens are active in the testing extract, one which gives an immediate reaction and the other a delayed. It should also be kept in mind that in most instances the first test was made a week after the sensitizing dose and an early delayed type of reactivity might have been missed. On the other hand, this argument is weakened by the fact that the observed delayed sensitivities persisted for a long time, and, if the delayed reactivity had occurred in the first week, it should have been present later also.

The possibility of the presence of a cell transfer factor after an injection of emulsified pollen to which the subject is sensitive, in spite of absence of delayed skin reactivity, still remains to be settled. It is possible that the person with a spontaneous immediate type allergy, such as to ragweed pollen, may not be able to revert to a delayed reactivity because he may have already gone through this stage in the early stages of his allergy development. If this is true, the course is certainly different from that of induced sensitivity in our series of atopic persons when a new antigen is used. Here almost all develop immediate sensitivity, and, in the few who develop delayed reactivity, the immediate state may precede the delayed.

Thus the question of the difference in behavior of the allergic and non-allergic individual in the development of induced delayed hypersensitivity to pollen antigens remains unanswered, except for the fact that certain possibilities have been ruled out. Further approaches are in progress with regard to other possibilities.

We have previously referred to a group showing modified delayed reactions not included in our figures in Table I. These consisted of erythematous

areas, usually under 10 mm. in diameter, without induration, appearing in less than 24 hours and disappearing by 48 hours. In many of these the state of reactivity was not lasting, thus differing from the other delayed reactions induced by us. Raffel,⁴ in describing somewhat similar reactions induced by antigen-antibody complexes in antibody excess or by small doses of antigen alone, refers to them as the Jones-Mote type. While Raffel regards these as a type of delayed reaction, and we are inclined to agree with him, we are not as yet certain of their significance. It is even possible that they may be a type of Arthus reaction. At any rate, further studies with particular attention to serum transfer, cell transfer, and histologic examinations are being conducted on this group.

Our studies indicate that the pattern of induced sensitivity in the atopic and nonatopic groups differ. In the atopic group, pollen extract in emulsion produces almost uniformly immediate type of sensitivity and does so early. (It is very possible that those 3 subjects who showed immediate reactivity one day after injection of the emulsion represent some kind of technical error, since such a finding is difficult to believe.) Delayed sensitivity is less prominent in the atopic group. In nonatopic persons, the ratio of induced immediate to delayed reactivity is reversed. The consistent production of a sensitizing antibody in atopic individuals with a new antigen by this method suggests, but does not prove, that an emulsified antigen to which the person is already sensitive may increase his titer of sensitizing antibody. It should be stressed that in our investigations thus far reported a standard dose of antigen (10,000 pollen units) was used in the emulsion in the sensitizing injections. It is possible that smaller or larger doses, in process of investigation, may produce different results. In animal experiments, Salvin⁵ has shown that reducing the dose of antigen may diminish the chance for antibody formation and may shorten the duration of delayed reactivity. He has also demonstrated that the addition of *Mycobacterium* to the emulsion increases the intensity of delayed reactivity and defers the onset of immediate reactivity.

In our investigations, either immediate or delayed reactivity appeared usually in 2 to 4 weeks. In studies of induced skin reactivity in man from repeated intradermal injections of rabbit serum, Jones and Mote⁶ recorded the earliest delayed reactions 7 weeks after the first inoculation. Immediate reactivity was added subsequently to the delayed. Finally the delayed reactivity disappeared leaving only a state of immediate responsiveness. In induced sensitivity in man from weekly intradermal injections of guinea pig serum, Simon and Rackemann⁷ obtained delayed reactivity in one week, and in several cases examined more frequently it was present in as short a time as 3 days after the first dose. Those who had delayed reactivity converted to the immediate type sometimes after the second week, more often after the third week, and sometimes later. Six of their subjects had immediate reactivity one week after the first injection; these did not develop delayed reactivity later. By injecting antigen-antibody complexes in antibody excess in guinea pigs, Uhr and associates⁸ obtained delayed reactivity beginning on the third to fourth day, but

antibody was not detectable until at least 2 to 3 weeks thereafter. On injecting diphtheria toxoid in incomplete adjuvant in guinea pigs, Salvin⁵ found delayed sensitivity on the fourth day and combined (delayed and Arthus) beginning on the twelfth or thirteenth day. By reducing the dose of antigen, the latent period before the occurrence of delayed sensitivity was lengthened.

An impression prevails that delayed reactivity is a necessary preliminary stage in the development of the immediate type of sensitivity. This concept is supported strongly by the findings of Uhr and associates and of Salvin with antigen-antibody complexes and with some antigens alone in guinea pigs. Salvin also finds that a small dose of antigen may produce a nonreactive period between the disappearance of delayed and appearance of immediate reactivity. Jones and Mote, and Simon and Rackemann, found an order of progression in man from the injection of foreign sera intradermally. However, the tendency in their cases was to change from one type of reactivity to the other. Furthermore, as noted earlier in this paper, some of the subjects of Simon and Rackemann developed immediate sensitivity only. The experience in our series does not support the concept that delayed reactivity is a necessary step in the development of the immediate type. It must be remembered, however, that, in the investigations conducted by us and others, substantial differences in technique exist with regard to nature of antigen, dose, medium in which administered, route of administration, and animal species used.

As noted earlier with our subjects, there is a decided tendency for both immediate and delayed induced sensitivity to persist for months and perhaps years. We realize that the general clinical impression is that many induced clinical sensitivities (penicillin and others) tend to disappear. Simon and Rackemann reported that in their series of subjects with induced sensitivity to guinea pig serum the immediate reactivity was still present one year in most cases tested. In sensitivity induced by Uhr's group in guinea pigs with antigen in complete adjuvant, delayed reactivity persisted for at least 10 weeks.

SUMMARY

1. Atopic individuals develop immediate and delayed skin reactivity to emulsified pollen antigens to which they have had no previous sensitivity.

2. The development of such new sensitivity is not hampered by the production of a nonspecific inflammatory reaction (from histamine) or a specific antigen-antibody reaction at the site of injection of the emulsified new antigen.

3. With the type of antigen, dose, and method used, the atopic person almost regularly develops immediate skin reactivity and less often delayed reactivity. Nonatopic individuals, on the other hand, have a greater tendency to develop delayed sensitivity.

4. Our findings do not support the contention that delayed hypersensitivity is a necessary step in the process of development of the immediate type in all instances and with all techniques.

5. The reason for the failure of the atopic person to develop induced delayed skin hypersensitivity to the antigen to which he is spontaneously sensitive

still remains unsolved. Some possible explanations are offered, but these must await further study.

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