ATTEMPTS TO TRANSFER ECZEMATOUS CONTACT-TYPE ALLERGY WITH WHOLE BLOOD TRANSFUSIONS*

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The problem of antibody demonstration in allergic eczematous contact-type sensitivity has intrigued dermatologists for many years and has been subjected to extensive experimental investigation. Passive transfer of allergic contact sensitivity with mononuclear blood cells and lymph node cells in guinea pigs has been accomplished with relative ease by many investigators using diverse technics following the original work of Landsteiner and Chase and Haxthausen (1, 2, 3, 4). It appeared paradoxical and puzzling that on the contrary, attempts by Haxthausen (5, 6) and by Baer, Sulzberger, Serri and Kirman (7, 8) to transfer allergic eczematous contact-type sensitivity by these same procedures in man have been unsuccessful or at best produced equivocal results.

Recently Epstein and Kligman (9) reported that they had been successful in passively transferring allergic contact sensitivity to three contact allergens in man—3-pentadecylcatechol (PDC), paranitrosodimethylaniline (NDMA), and 2,4-dinitrochlorobenzene (DNCB). This was accomplished through both intradermal and intravenous injections of leukocytes from the peripheral blood of highly sensitive donors. They attributed the failures of other investigators to the transfer of insufficient numbers of leukocytes, to inadequate sensitivity of the donors of the cells, or to "admirable conservatism" which kept previous investigators from claiming that they achieved passive transfer on the basis of a few positive results. Epstein and Kligman felt that to achieve successful passive transfer of DNCB sensitivity in man at least 170 million lymphocytes must be injected intradermally or 1 billion intravenously, from donors sensitive to dilutions as high as 1:1,000,000.

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In view of these positive findings it was decided to undertake another study on transfer of contact sensitivity to DNCB, utilizing whole blood transfusions from donors with exceedingly high degrees of sensitivity (10).

METHODS AND MATERIALS

The four volunteer donors were white males hospitalized for chronic dermatoses (atopic dermatitis, peripheral vascular disease (2 donors), and allergic contact dermatitis). They were selected from among 11 hospitalized patients all of whom had been repeatedly exposed to 2 drops (0.014 ml) of 10% DNCB in acetone extruded through a 22 gauge needle onto the subscapular area at intervals of 1–4 weeks and were found to be extremely sensitive after one to three such applications. The four selected for this study responded to challenges with concentrations of DNCB as low as .0001%.

The recipients were comprised of seven white male patients hospitalized on a ward separate from that of the cell donors to guard against inadvertent exposure to DNCB. In order to insure viability of the transferred white cell elements, phlebotomy and transfusion were so arranged as to assure completion of the transfer in a minimum period of time. Under sterile conditions, 500 cc of whole blood was collected from the donor volunteers in a Saftivac® vacuum bottle which contained 120 cc of "A-C-D Solution 'B' U.S.P."* Within minutes, the blood was administered to the compatible volunteer recipient, the elapsed time from the beginning of phlebotomy to completion of the transfusion ranging from 22 to 49 minutes. Leukocyte viability counts (11) were done on several occasions to insure that the technic was not harmful to a substantial number of the white cells (Table I).

Twenty-four hours after transfusion the recipients were skin-tested at duplicate symmetrical sites with 2 drops (.014 ml) of 0.1% and 1 drop of 1% DNCB in acetone on the subscapular area. These tests were repeated 48 hours following the transfusion.

* "A-C-D Solution 'B' U.S.P.": Each 100 cc contains Sodium Citrate U.S.P. 1. 32 Gm., Citric Acid U.S.P. (anhydrous) 0.44 Gm., and Dextrose U.S.P. 1.47 Gm.

TABLE I

Quantity and viability of leukocytes transferred

Donor	Recipient	Time Elapsed for Transfer	Total Number of Leukocytes Transferred	Total Number of Lymphocytes Transferred	Minimum % of Viable Leukocytes after Transfusion
		min			
A. N.	R. D.	38	4.5×10^9	1.5×10^9	not determined
P. McT.	J. P.	49	3.5×10^9	1.1×10^9	not determined
J. McC.	M. L.	34	8.5×10^9	2.1×10^9	91%
D. T.	F. S.	22	4.8×10^9	2.4×10^9	88%
A. N.	M. G.	24	7.5×10^9	2.1×10^9	86%
J. McC.	P. W.	32	9.5×10^9	3.8×10^9	not determined
A. N.	J. J.	30	5.5×10^9	1.8×10^9	not determined

TABLE II

Erythema response of F. S. to 1% DNCB

	Time Interval of Erythema Readings After 1% DNCB Application				
	Immediately	24 hrs.	48 hrs.	72 hrs.	1 week
DNCB applied 1 day after transfusion	+	++	+	+	0
DNCB applied 2 days after transfusion	0	+	+	0	0
DNCB applied 7 days after transfusion	0	+++*	++*	++*	0

* Edema noted; 0.1% DNCB also positive.

RESULTS AND COMMENTS

None of the 7 recipients reacted to 0.1% DNCB solution in acetone applied 24 and 48 hours following the transfusion. Two of the 7 recipients developed erythema at the sites tested with 1% DNCB solution. In one recipient, F. S., who developed a moderate erythematous response without edema (Table II) when tested with 1% DNCB 24 hours after transfusion, there was a pronounced decrease in erythema when tested 48 hours following the transfusion. In the only other patient manifesting a positive response to 1% DNCB, the intensity of erythema was the same when the test was repeated 48 hours later.

In order to permit proper evaluation of the erythematous response to 1% DNCB in 2 of the 7 cell recipients, a group of 8 volunteers who had no known previous exposures to DNCB were tested with the 1% solution in a manner identical to all recipients. Two of this group responded with unequivocal erythema without edema.

DISCUSSION

In the series of tests reported here we tried to avoid the 3 principal pitfalls which may have caused previous failures (4, 5, 6) to passively transferred eczematous contact-type sensitivity. These are 1) inadequate sensitivity of the donor; 2) inadequate number of cells transferred and 3) poor or absent viability of the cells transferred. Despite the use of what we believed to be proper technics to avoid these pitfalls, all of the 7 recipients, each of whom received at least 1 billion lymphocytes from donors hypersensitive at least to a 1:1,000,000 DNCB solution in acetone, were uniformly negative in response to challenge with 0.1% DNCB. It is somewhat difficult to harmonize the unsuccessful passive transfer in these cases with the positive findings of Epstein and Kligman in 2 out of 2 recipients of intravenously administered donor white cells. However, differences in the technic of testing may help to explain the discrepancies. Our procedure was to place 2 drops of 0.1% DNCB on each skin site and then to permit acetone to evaporate at room temperature actually leaving approximately 0.014 mg DNCB per test site. Epstein and Kligman (12) challenged their cell recipients by placing 0.25 ml of a 0.1% DNCB solution in an open end glass cylinder, 2.9 cm in diameter, which was placed against the skin test site. They then

TABLE III

Passive transfer of peripheral blood leukocytes

	Compound	Average No. of Lymphocytes	Range	No. of Recipients	Positive Transfers
		Intravenous Injection			
Epstein and Kligman...	DNCB	1.0×10^9	1.0×10^9–1.3×10^9	3	2
Harber and Baer.....	DNCB	2.1×10^9	1.1×10^9–3.8×10^9	7	0
		Intradermal Injections			
Epstein and Kligman....	DNCB	4.7×10^8	1.7×10^8–9.5×10^8	10	3
Meneghini and Levi.....	pot. dichromate	2.5×10^8	1.0×10^8–5.0×10^8	9	0
Meneghini and Levi.....	turpentine	1.6×10^8	1.5×10^8–1.6×10^8	2	0
Meneghini and Levi.....	nickel sulfate	0.7×10^8	1.6×10^8–1.6×10^8	2	0

permitted the acetone to evaporate. Thus they actually tested their site with 0.25 mg of DNCB.

Although it would be almost impossible to accurately state what size skin area the 2 drops of our solution covered as it rapidly spread on the skin surface, there can be no doubt that Epstein and Kligman deposited locally on a given area of skin surface approximately 17 times as much allergen as we did. Obviously then they applied almost twice as much DNCB to each site in their tests with 0.1% DNCB than we did in our test sites with 1% DNCB.

It is therefore likely that Epstein and Kligman's positive results with 0.1% DNCB should be considered together with our positive skin tests to 1% DNCB in two of our 7 cell recipients. Our experience and that of previous workers suggests that a concentration of 0.14 mg of DNCB per test site may well be at the borderline of primary irritancy. This is evident also from the response of mild or moderate erythema in 2 of the 8 control volunteers who were drop tested in a manner identical to the cell recipients. In one of these cases the erythema did not appear until 12 hours following application and persisted 48 hours.

In spite of these results it cannot be entirely ruled out that a transfer of specific sensitivity may have been accomplished in one of our cases (Table II). This patient had moderate erythema when challenged with 1% DNCB 24 hours following transfusion and only slight erythema when the test was repeated the following day. Six days after the original test 1% DNCB was applied again and a positive response, accompanied by edema was elicited. Furthermore a positive response to 0.1% DNCB was noted for the first time. This was interpreted as indicating that *active sensitization* had taken place due to the exposures to 1% DNCB used in the previous skin tests.

It is of interest to note that recently Meneghini and Levi (13), (Table III) failed in their attempts to passively transfer allergic hypersensitivity to common contact allergens including chromate, nickel and sulfonamide. They used quantities of leukocytes from the circulating blood and from lymph nodes ranging from 100 million to 500 million, administered to the recipients by intradermal injection. More recently Serri (14) has attempted to transfer DNCB sensitivity. His technic involved 3 daily transfusions of 30–40 cc of citrated whole blood from highly sensitized donors. The recipients were then challenged 1, 2, 4 and 6 days following the last transfusion with 0.25% DNCB. Serri too, reported no evidence of successful passive transfer.

The possibility still remains that through modification of technics it will be possible to develop a method which will consistently permit demonstration of the hypothetical antibody of allergic eczematous contact sensitization. This would seem likely to succeed in view of the success of Lawrence (15) and others with transfer of tuberculin sensitivity in man.

However it is by no means established to what degree human tuberculin-type skin sensitivity and human eczematous contact-type sensitivity to simple chemicals are related and to what degree they differ, either immunologically or otherwise. However our results suggest that it would be worthwhile to explore further the possible differences as well as the similarities.

Furthermore the fact that DNCB sensitivity in guinea-pigs can be transferred by means of white cell suspensions while our experiments did

not succeed in doing this in man suggests the possibility of significant differences in these forms of sensitivity and the mechanisms mediating them.

SUMMARY

1. Passive transfer of allergic eczematous contact sensitivity to DNCB was attempted in 7 patients. The technic used involved blood transfusions each of which contained at least one billion viable mononuclear cells from donors exquisitely sensitive to DNCB.

2. No transfer was demonstrated. None of the recipients reacted to challenge with 0.1% DNCB. Two of the 7 recipients had positive reactions to 1% DNCB. The significance of these responses is discussed. It is pointed out that DNCB in 1% concentrations approaches the borderline of primary irritancy.

3. The results suggest that there may well be significant differences between tuberculin-type and contact-type eczematous sensitivity in man and also between contact-type eczematous sensitivity in man and guinea pigs.

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DISCUSSION

DR. WILLIAM L. EPSTEIN (San Francisco, Calif.): I commiserate with my colleagues; there is nothing more discouraging than negative results and in a procedure that is as difficult to perform as passive transfer it is especially frustrating. I would like to point out that we are not the only ones who have transferred sensitivity to DNCB and DNFB in man. Robert Good at approximately the same time as we reported transfer of delayed hypersensitivity to dinitrochlorobenzene in patients with hypogammaglobulinemia. Subsequently Charles Calnan from England working at the University of Pennsylvania also was able to transfer sensitivity to dinitrochlorobenzene in man.

The technics of Dr. Harber and Dr. Baer are somewhat different from the methods we used. Besides having very sensitive donors and using the intravenous method of transmitting large numbers of lymphocytes, our subjects were healthy young males. Dr. Harber's were hospitalized; obviously they were there for a reason. My experience with hospitalized patients suggests that they do not react as briskly as active persons. Another point of difference is our testing method. We applied greater amounts of allergen to the skin. The concentrations used were borderline irritating but with experience it was possible to distinguish irritant and allergic reactions to DNCB; the prime points of distinction are delay in onset of allergic reactions and duration of the response. When dealing with very weak allergic reactions, as many of the transferred sensitivities are, it is vital to use the greatest amount of allergen possible in order to elicit a measurable

response. The answer is to have a wide experience with the chemicals in question.

Finally, I would like to suggest that Drs. Harber and Baer might have more luck transmitting sensitivity to pentadecyl catechol. There is a very important point here. Transmission of most simple chemical hypersensitivity in man is very difficult whereas the transfer of sensitivity to poison ivy and fungal and bacterial agents is relatively simple. Previous exposure to these latter substances may in some way facilitate transfer without actually sensitizing the subject. I am suggesting that an altered state of reactivity exists in the subject in whom transfer is successful. In America nonsensitive adults cannot be sensitized to Rhus allergens but children are readily sensitized by even minimal exposure (Epstein, W. L.: Rhus dermatitis, Ped. Clin. N. Amer. **6:** 843, 1959).

Dr. Albert M. Kligman (Philadelphia, Pa.): The American-British philosopher Whitehead once remarked, "A conflict is not by any means a catastrophe. It is an opportunity for enlightenment."

I feel very much in that mood as I think of what has happened here. Without taking sides in the matter, there is something very peculiar about this question of transferring delayed type sensitivity with leukocytes. The fact is that it is rather easy to do when one uses antigens which are native to the environment of the recipients. Sherwood Lawrence in New York City finds it almost impossible *not* to transfer tuberculin sensitivity no matter what he does to the donor leukocytes.

In our own work it was rather easy to transfer delayed sensitivity to the poison ivy antigen to which the recipients could have been presumed to have had some previous natural exposure. Similarly they have probably had a minor skirmish with the tubercle bacillus. The point here is that the recipients are not virgins with respect to these antigens. Passive transfer in such instances may be simply elevation of latent sensitivity to a clinical level. Now transfer of dinitrochlorobenzene sensitivity is quite another matter. This substance is novel to the recipients and passive transfer very difficult to accomplish.

Calnan in our laboratory last year went through a very considerable exercise to try to increase the efficiency of dinitrochlorobenzene transfer but most of his efforts were futile. It is a tough thing to do and one has to be extremely meticulous in all details.

Dr. Marion B. Sulzberger (New York, N. Y.): First of all I would like to second what Dr. Kligman has just said and emphasize once again that most of the regular successes in passive transfer of contact-type eczematous sensitivity in man have been with "antigens" with which the recipient individual can be presumed to have had the possibility of some previous contacts. The question then arises whether one is really performing a passive transfer of antibodies. Maybe one is just producing a "booster effect," an "anamnestic response".

It is quite conceivable that the transferred donor cells may contain traces of potent or coupled antigen sufficient to produce a powerful booster effect in recipient individuals with a pre-existing very low level of sensitivity. This explanation would fit in well with all of the time sequences observed; and also with the phenomenon that the sensitivity often generalizes all over the skin surface even when the cells are deposited only in one small site. The hypersensitivity does not remain local as it does, for instance, when one puts other types of antibodies such as Prausnitz-Kuestner reagins into a skin site. All these phenomena and still others make it appear to me that this may not actually be a passive transfer which one is accomplishing with the donor cells. Ones must have what Kligman so picturesquely described as a "virgin soil" in the recipients in order to be sure that the transfer has been a passive one through transfer of antibodies and not an active booster effect through addition of antigens. I admit, however, that there are many things about this situation that are not quite as simple as I have just expressed them.

In many discussions with Sherwood Lawrence, who works at our school, we have speculated about the possibility that these phenomena produced by cell transfers are due to booster effects. I suggested that he try sensitizing individuals with cells of donors that were sensitive to antigens to which the recipients had never been exposed. He recently used cells from coccidioidal granuloma patients in California who were skin sensitive to Coccidiomycin; and with transfer of these cells he was able to produce positive Coccidiomycin skin test sensitivity in some (but not all) recipients in New York City who pre-

sumably had had no previous exposure to the coccidioidal antigen itself.

The other thing I would like to emphasize before I sit down is that, just as stated by Harber and Baer, I am afraid that Kligman and Bill Epstein were working with borderline irritant concentrations in their skin tests with dinitrochlorobenzene. They applied so much dinitrochlorobenzene per unit of skin area that it was on the border of primary irritancy; therefore in any reactions which they saw after applications of their material it would be extremely difficult if not impossible to distinguish between possible primary irritant effects and possible passively transferred allergic eczematous sensitivity.

Dr. Leonard C. Harber (in closing): Dr. William Epstein's remarks raised several valid points which illustrate the difficulties involved in interpreting data concerning cellular transfers of allergic eczematous contact-type allergy. Dr. Good did claim to have positive results in cell transfers of dinitrofluorobenzene (DNFB) sensitivity. On the other hand, Dr. Serri attempted to transfer DNCB sensitivity with essentially the same technics as Dr. Epstein and Dr. Kligman had described in their report. His attempts as well as ours which I described today were not successful. I believe the DNCB concentration which Dr. Serri used for skin testing was 0.25%.

With respect to age and physical condition of the patients at Bellevue Hospital who were used as donors, they did come from a relatively low socio-economic status. Their ages ranged from 17 to 60. However, all of these volunteers were carefully examined before they gave whole blood in order to assure that this would not be a detriment to their health and the same is also true of the recipients of the whole blood transfusions.

Regarding the amount of allergen deposited at a given site using the technics which we described as compared with those of Dr. Kligman and Dr. Epstein, I can best express it in this manner: a truly positive response observed through our drop testing procedure consisted of an erythematous reaction about 2.5 cm. in diameter. Obviously the .014 milliliter of acetone which is initially dropped on the skin is unlikely to cover a 2.5 cm. area. It is the result of diffusion. As far as trying to prevent this by using a cup, as Dr. Epstein described, we felt that our method was preferable for the following reasons: first, it is the method commonly used in this type of experiment in man and guinea pigs; and secondly, it avoids the error of depositing primary irritant quantities of allergen on the skin; and thirdly, one does run the danger of altering the physiology of the normal skin site tested by pressure or prolonged exposure to a solvent.

Commenting on some of Dr. Kligman's pertinent remarks, we used DNCB instead of pentadecylcatechol mainly because with this compound it is always difficult to be sure that the recipient of the transfer cells was not previously sensitized by natural exposure to poison ivy. In addition, when one does a test before the transfer experiment to make sure that an individual is not already hypersensitive to the allergen which one intends to use, one can justly be criticized that the test dose itself acted as the sensitizing dose that produced the positive results in the transfer test. It was with this in mind that we definitely kept all individuals who were going to be recipients of DNCB on a different ward than that of the donors who were being sensitized to DNCB. With the use of this procedure, we felt relatively safe in assuming that the recipients had never encountered 2,4-dinitrochlorobenzene before they received the whole blood transfusion.