Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 126 OCTOBER 1967 No. 1

Contact Hypersensitivity to Simple Chemicals. Incubation of Sensitized Guinea Pig Peritoneal Exudate Cells.* (32351)

R. K. GUTHRIE, W. L. ELLIS, AND C. H. BROCK
Department of Biology, North Texas State University, Denton

Previously we have reported(1) that peritoneal cell extracts from guinea pigs sensitized to 1-fluoro-2,4-dinitrobenzene would transfer sensitivity if collected 17 days after beginning sensitization but were not successful if collected 13 days after initial sensitizing contact. The transfer of delayed type hypersensitivity by crude cell extracts has been previously reported by many investigators(2,3,4,5,6,7,8), although reports of unsuccessful or nonspecific results have been made when animals other than the human being have been used (9,10,13). The differing capabilities of cell extracts at the time periods above have been advanced as one possible explanation for discrepancy of results reported. To investigate further the differences in cell transfer capabilities at these time periods, the following study was made.

Materials and methods. Albino guinea pigs, weighing 350-500 g, of the random bred Hartley strain, were sensitized by painting with 5 drops of a 2% solution of 1-fluoro-2,4-dintrobenzene (DNFB) in absolute ethyl alcohol on the shoulders and neck, after removing the hair with a fine clipper. This application of DNFB was repeated daily for 6 days in the same location.

Actively sensitized guinea pigs were skin tested on either the ninth day or the thirteenth after initial contact sensitization by applying one drop of a solution of DNFB in olive oil to an area of the flank which had been cleared of hair by clipping. The DNFB solutions of 0.5 and 0.75% were rubbed gently into the skin with a polished glass rod. Skin tests were read at 4 and 24 hours after application of the olive oil solution, and were graded ++++, marked homogenous erythema; +++, homogenous erythema; ++, patchy erythema; +, slight erythema; 0, no reaction. An additional designation of ± to indicate minimal reaction has been added.

After reading of skin tests, hair was clipped from the abdominal surface and 15 ml of sterile light mineral oil was injected intraperitoneally to evoke exudative cells. Seventy-two hours after oil injection, animals were exsanguinated by intracardial puncture, and peritoneal exudate cells were collected. For this collection, the abdominal wall was opened and the cavity washed 3 times with sterile Hanks balanced salt solution(11), modified by the addition of 0.1% gelatin.

* This work was supported by the Faculty Research Fund, North Texas State University.

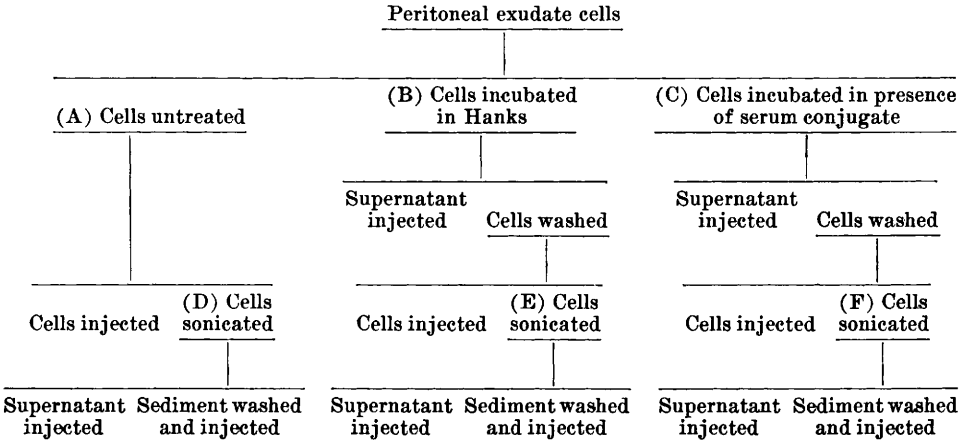

FIG. 1. Procedures for handling peritoneal exudate cells.

Collected cells were sedimented by centrifugation at 2,000 rpm for 20 minutes. Packed cells were washed once in Hanks solution, resedimented at 2,000 rpm for 10 minutes and the volume recorded. Washed cells were resuspended in 10 ml Hanks solution and divided into portions to contain 0.75 ml packed cells, which is equivalent to approximately 9-10 $\times$ 10^7 cells. These cell aliquots were then handled according to one of the following procedures (Fig. 1), and injection into recipient animals was completed within 5 hours of exsanguination.

A). Cells were injected intraperitoneally, without further treatment into normal recipient guinea pig. B). Hanks solution was added to a total volume of 5 ml and the cells were then incubated at 37 C for 30 minutes. After incubation the peritoneal exudate cells were sedimented at 2,000 rpm for 10 minutes, the supernatant removed and injected intraperitoneally into recipient. Packed cells were washed 3$\times$ with Hanks solution and injected into recipient animal. C). Hanks solution was added to a volume of 5 ml and an equal volume of guinea pig serum conjugate of DNFB was added. The cells were then incubated and treated as in section B above. D). Hanks solution was added to a volume of 5 ml and the cells were then subjected to treatment by the Branson Sonifier until microscopic examination of the suspension showed no cells intact. To avoid excessive heating, all sonifier treatment was interrupted at intervals, and the cell container was immersed in tap water. Suspensions thus treated were then centrifuged at 2,000 rpm for 20 minutes. The supernatant fluid was removed and injected intraperitoneally into a normal recipient animal. Sediment was washed once in Hanks, resuspended and injected into a recipient. E). Cells were incubated as in B, the supernatant removed and injected into a recipient. The cells were then treated as in D. F). Cells were incubated as in C, and then treated as in D.

In incubation experiments normal control recipient guinea pigs were injected with Hanks solution and with guinea pig serum conjugate in volumes equal to that used in incubation of cell aliquots.

Axillary lymph nodes were removed following harvest of peritoneal exudates. After the fat was trimmed away, the nodes were finely minced with scissors and were strained through a fine aluminum screen to separate cells. Cells so collected were then washed once in Hanks solution and sedimented for 10 minutes at 2,000 rpm. Cells were suspended in 5 ml of Hanks for injection into recipients.

For use in testing and incubation experiments, serum was taken from normal guinea pigs, pooled and conjugated with DNFB. The conjugate was prepared according to the method of Kabat and Mayer(12).

Results. All actively sensitized donor animals showed reactivity of at least ++ level and the majority of the animals showed +++ and ++++ reactions.

After transfer of the appropriate material, *i.e.*, incubation supernates, whole cells or

TABLE I. Skin Test Reactions to 0.75% DNFB Recipients Receiving Lymph Node Cells and Unincubated Peritoneal Exudate Cells.

Group test numbers	Lymph node cells		Peritoneal exudate cells*		
	Volt transferred	Reaction	Volt transferred	Reaction Whole cell	Reaction Sonic extract
13-day cells					
Test 1	.30	++++	.75	+++	not done
2	.30	++++	.75	++	"
3	.20	++	.75	++	"
4	.25	+	.75	++	"
5	.10	+	.75	+	—
6	.25	+++	.75	+	—
7	.45	+	.40	+	—
17-day cells					
Test 1	.30	++++	.75	++	not done
2	.10	+++	.60	+	"
3	.10	+	.75	+	"
4	.35	+	.75	++	+
5	.35	+	.75	++	+
6	.90	+++	1.0	++	++

* These results refer to procedures in column A, Fig. 1.
† Volume here refers to total packed cells used for whole cell transfer. A volume equal to that used for whole cell peritoneal exudate was used to prepare sonic extracts.

TABLE II. Skin Test Reactions to 0.75% DNFB in Recipients Receiving Peritoneal Exudate Cells and Supernatant Hanks Solution After Incubation at 37 C.*

Group test numbers	Peritoneal exudate cells following incubation			Supernatant Hanks solution following incubation	
	Volt transferred	Incubated whole cell reaction	Sonic extract reaction	Volt transferred	Reaction
13-day cells					
Test 1	.75	+++	not done	not done	not done
2	.75	++	"	"	"
3	.75	++	"	.75	+
4	.75	++	"	.75	+++
5	.75	+	—	.75	++
6	.75	+	—	.75	+
7	.3	+	—	.3	+
17-day cells					
Test 1	.75	±	not done	not done	not done
2	.60	±	"	"	"
3	.75	—	"	.75	++
4	.75	±	—	.75	++
5	.75	±	—	.75	+
6	1.00	±	±	1.0	++

* Results in this Table refer to procedures in column B, Fig. 1.
† Volume here refers to volume of packed cells incubated in 5 ml Hanks solution.

crude cell extracts, to normal guinea pigs by the intraperitoneal route, recipients were skin tested with 0.75 and 0.50 per cen DNFB in olive oil. For each test at least 10 donor animals were used for the transfer preparations. Results of skin tests in Tables 1-3 represent the reaction of the recipient animal following transfer of one preparation. Tests No. 1 in the 13-day and the 17-day cells represent concurrent transfers on the same date from donor animals sensitized for the 13 and 17 day periods. For example, cells were harvested from 13 day donors, and divided into three aliquots. One aliquot was treated according to procedures in column A, Fig. 1, and results are shown in Table I. A second was treated according to column B, with results shown in Table II, and the third was subjected to treatment shown in column C, with results shown in Table III. Each subsequent test number represents an experiment carried out at a different time, with each test involving separate

TABLE III. Skin Test Reactions to 0.75% DNFB in Recipients Receiving Peritoneal Exudate Cells and Supernatant Hanks with *DNP*-Guinea Pig Serum Conjugate After Incubation at 37 C.*

	Peritoneal exudate cells following incubation			Supernatant Hanks with DNP-guinea pig serum following incubation	
Group test numbers	Vol transferred	Incubated whole cell reaction	Sonic extract reaction	Vol† transferred	Reaction
13-day cells					
Test 1	.75	—	not done	.75	+
2	.75	—	"	.75	+
3	.75	—	"	.75	+
4	.75	—	"	.75	±
5	.75	—	—	.75	+
6	.75	—	—	.75	+
7	.40	—	—	.40	+
17-day cells					
Test 1	.75	++	not done	.75	±
2	.60	++	"	.75	±
3	.75	++	"	.75	—
4	.75	+	±	.75	±
5	.75	+	±	.75	—
6	1.00	++	++	1.0	—

* Results in this Table refer to procedures in column C, Fig. 1.

† Volume here refers to volume of packed cells incubated in 5 ml Hanks solution containing DNP-guinea pig serum conjugate.

lots of donor animals.

Control animals tested for each of the transfers included (1) normal, uninjected guinea pigs, (2) normal guinea pigs injected intraperitoneally with Hanks solution and (3) normal guinea pigs injected intraperitoneally with DNF-guinea pig serum conjugate. No control animal showed a positive skin test to either concentration of DNFB. Donors and recipients from each test were also subjected to contact skin test with picryl chloride, o-chlorobenzoyl chloride and citraconic anhydride in olive oil at one per cent concentration. No non-specific cross reactions were observed.

Discussion. Specific sensitivity to DNFB was transferred to normal recipients at both 13 and 17 day periods by lymph node cells and by peritoneal exudate cells. Crude sonic extracts of peritoneal exudate cells transferred specific sensitivity at the 17 day period only. Washed sediments did not transfer sensitivity in any experiment.

Following collection, washing, and incubation at 37 C, of peritoneal exudate cells in Hanks solution, the supernatant incubation fluid successfully transferred specific sensitivity from cells of both the 13 and 17 day groups (Table II). After incubation however, only those cells from the 13 day groups were capable of transfer. Cells from 17 day sensitized animals had lost transfer capability. These results appear to indicate that the "transfer factor" is more securely retained by the 13 day cells. When either type of cell was sonicated following incubation, sensitivity was not transferred by either sonic extract or sediment.

When cells of the 13 and 17 day groups were incubated at 37 C in Hanks containing DNP-guinea pig serum conjugate, a somewhat different picture was presented. The supernatant fluid from this incubation did transfer sensitivity from 13-day cells, but not from 17-day cells. Following this incubation, cells from 13-day animals had lost all ability to transfer, while those from 17-day animals retained transfer ability practically unchanged. These results indicate a different effect on the cells produced by the presence of the chemical allergen in the form of a protein conjugate. When the 17-day cells were used to prepare the sonic extracts following incubation, the extract was capable of transfer in one experiment where a larger volume of cells was used and where the whole cell reaction was relatively strong. It is apparent that the presence of DNP-conjugate caused the rapid

release of transfer factor from 13-day cells, but had no effect on 17-day cells, again indicating that the factor is attached to the cell differently at the 13-day period. The effect of an inhibitor as suggested by Tsuji *et al*(7), is not apparent in the reactions of these two cell types. Rather, in this experiment there appear to be two different mechanisms of release of the transfer factor from the cell.

Attempts at analysis of the transfer factor released in incubation experiments to the present have indicated that this material is not an antibody in terms of the reactivity usually expressed by antibody molecules. The transfer factor is neither neutralized nor bound in the presence of the specific hapten in a conjugate. In repeated tests following concentration of the incubation supernatant fluid which is capable of transfer, the transfer factor fails to produce positive reactions in precipitation, hemagglutination, passive cutaneous anaphylaxis and passive systemic anaphylaxis. The transfer factor from 17-day cells in incubation Hanks solution is stable at room temperature for at least 8 hours, and at 37 C for at least 2-4 hours.

The apparent complete release of the transfer factor upon incubation in balanced salt solution could further explain some failures in attempts at passive transfer with either whole cell or extract preparations. The results reported here, indicate the necessity for careful control of procedures and handling of peritoneal exudate cells to prevent the loss of transfer capacity in supernatant fluids discarded after collecting or washing cells.

Summary. The incubation of peritoneal exudate cells in Hanks balanced salt solution at 37 C, causes the release of a factor into the solution capable of specific transfer of contact sensitivity to DNFB. The release of this factor from 17-day cells appears to be complete since neither whole cells nor sonic extracts will transfer following incubation. After incubation, 13-day whole cells will transfer, yet sonic extracts of 13-day cells fail to transfer before or after incubation. The transfer factor is also released from 13-day cells in the presence of the specific DNP-protein conjugate, and in this case again, the release appears complete. DNP-protein conjugate does not cause the release of transfer factor from 17-day cells, nor does incubation in the presence of the conjugate interfere with transfer capability of the whole cell or, possibly, of the sonic extract as seen in one experiment. Transfer factor, when released, is specific and does not possess reactive capacities associated with classical antibody.

1. Guthrie, R. K., Lowke, G. E., Ferguson, J. K., Ellis, W. L., J. Invest. Dermat., 1966, v46, 224.

2. Jeter, W. S., Tremaine, M. M., Seebohm, P. M., Proc. Soc. Exp. Biol. and Med., 1954, v86, 251.

3. Jeter, W. S., Laurence, K. A., Seebohm, P. M., J. Bact., 1957, v76, 680.

4. Cummings, M. M., Patnode, R. A., Hudgins, P. C., Am. Rev. Tuberc. & Pul. Dis., 1956, v73, 246.

5. Lawrence, H. S., J. Clin. Invest., 1955, v34, 219.

6. Baram, R., Mosko, M. M., Immunology, 1965, v9, 461.

7. Tsuji, S., Oshima, S., Oshiro, M., Izumi, T., J. Immunol., 1964, v93, 838.

8. Kochan, I., Bendel, W. L., J. Allergy, 1966, v37, 284.

9. Bloom, B. R., Chase, M. W., Goldstein, G., Bact. Proc., 1961, v61, 108.

10. Turk, J. L., Int. Arch. Allergy & Appl. Immunol., 1962, v21, 321.

11. Hanks, J. H., J. Cell. & Comp. Physiol., 1948, v31, 235.

12. Kabat, E. A., Mayer, M. M., In Experimental Immunochemistry, Charles C Thomas, Springfield, Ill., 1961, 601.

13. Bloom, B. R., Chase, M. W., Progr. Allergy, 1967, v10, 151.

Received December 12, 1966. P.S.E.B.M. 1967, v126.