Stimulation of Monocyte cGMP

by Leukocyte Dialysates

AN ANTIGEN-INDEPENDENT PROPERTY OF DIALYZABLE TRANSFER FACTOR

JEFFREY A. SANDLER, TERRILL K. SMITH, VINCENT C. MANGANIELLO, and
CHARLES H. KIRKPATRICK

*From the Laboratory of Cellular Metabolism, National Heart and Lung Institute,
and the Laboratory of Clinical Investigation, National Institute of Allergy and
Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014*

ABSTRACT We studied the effects of dialysates from leukocyte lysates containing dialyzable transfer factor activity and other leukocyte dialysates devoid of transfer factor activity on accumulation of cyclic nucleotides in human leukocytes. Dialysates from normal leukocytes produced 4- to 11-fold increases in leukocyte cGMP, and experiments with purified cell populations revealed that the increases were predominantly, if not entirely, in blood monocytes. Substances that increased monocyte cGMP could be obtained from several cell populations including mononuclear cells from Hypaque-Ficoll gradients, plastic-adherent monocytes, nonadherent lymphocytes, and neutrophils, but were not present in dialysates of leukemic lymphocytes from patients with the Sézary syndrome. Moreover, dialysates that increased leukocyte cGMP had essentially no effect on intracellular cAMP.

Dialysates of lysed mononuclear cells contained serotonin, ascorbate, and an unidentified cholinergic activity, agents known to increase leukocyte cGMP. After passage of dialyzable transfer factor from mononuclear cells through a gel-filtration column, four fractions were obtained that increased leukocyte cGMP. Two of these fractions contained ascorbate; two other active fractions, including one that also caused conversion of delayed skin tests, did not contain detectable ascorbate or serotonin. The dialysate of lysed neutrophils also increased cGMP, but this activity was limited to the column fractions which contained ascorbic acid.

These observations raise the possibility that alterations in monocyte cGMP content could modulate either the

specific antigen-dependent, or, more likely, the antigen-independent activities in preparations of transfer factor.

INTRODUCTION

Although the chemical nature and mechanisms of action of dialyzable transfer factor are still unknown, there is now substantial evidence that it markedly affects expression of cell-mediated immune responses. After receiving transfer factor, anergic patients with chronic mucocutaneous candidiasis (1), coccidioidomycosis (2), or the Wiskott-Aldrich syndrome (3) developed positive delayed cutaneous hypersensitivity responses, and in some cases their lymphocytes responded to antigenic stimulation in vitro with lymphokine production (1-3). Others have reported that after administration of transfer factor from specifically reactive donors, patients with osteogenic sarcoma developed cell-mediated cytotoxicity (4), and that lymphocytes from a patient with alveolar soft-part sarcoma responded in vitro to an extract of the tumor with production of migration inhibitory factor (5).

It has also become evident that changes in the intracellular concentration of cyclic nucleotides modulate a number of immune responses. Agents that increase intracellular cAMP have suppressive effects on immunologic release of mediators such as histamine and slow-reacting substance of anaphylaxis (SRS-A)¹ (6-8), lymphocyte-mediated cytotoxicity (9, 10), and antibody responses in vivo and in vitro (9, 11, 12). A reciprocal

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¹ *Abbreviations used in this paper:* SRS-A, slow-reacting substance of anaphylaxis; HBSS, Hank's balanced salt solution; CLL, chronic lymphocytic leukemia.

effect is seen with agents that increase intracellular cGMP. Addition of cholinergic agonists or analogues of cGMP enhances immunologic release of mediators (13), cellular cytotoxicity (10), and in vitro production of antibody-forming plaques by spleen cells from nude mice (12).

In the experiments described below, we examined the effects of dialysates of leukocyte lysates containing transfer factor activity and other leukocyte dialysates that were devoid of transfer factor on accumulation of cyclic nucleotides in human leukocytes. Exposure of leukocytes to dialysates had little effect on cAMP content. In contrast, the dialysates markedly increased leukocyte cGMP and this response occurred predominantly, if not exclusively, in blood monocytes. Some of the components of the dialysates that produced this effect were identified.

METHODS

Collection and separation of leukocytes. Peripheral blood leukocytes were collected from normal donors by use of either a continuous flow blood cell separator (American Instrument Co., Silver Spring, Md.) or a Latham blood processor (500 Incorporated, Waltham, Mass.). The cells were washed three times with cold Hank's balanced salt solution (HBSS) and counted with a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.); differential counts were made on polychrome-methylene blue-stained smears (Ames Co., Elkhart, Ind.). The washed preparations contained 60–80% lymphocytes, 15–25% monocytes, 0–5% neutrophils, and 0–3% basophils. For some experiments leukocyte-rich plasma (Universal Therapeutics, Laurel, Md.) was used instead of cells obtained by leukopheresis.

Suspensions rich in mononuclear cells were obtained by centrifuging cells through Hypaque (Winthrop Laboratories, New York)-Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) gradients according to the method of Böyum (14). After three washes with HBSS the cells were counted. Differential counts were made on both stained smears and unfixed cells in 0.05% neutral red which facilitated identification of monocytes. These preparations contained 60–75% lymphocytes, 25–35% monocytes, and 0–3% basophils.

Neutrophils were isolated by suspending the pellets from the Hypaque-Ficoll preparations in plasma and adding 3.6% dextran (Sigma Chemical Co., St. Louis, Mo.), (mol wt 254,000 daltons) in saline to a final concentration of 1%. The erythrocytes were allowed to sediment in glass cylinders and the neutrophil-rich suspensions were removed by aspiration, washed, and counted. These preparations contained 95–100% neutrophils.

Cells from Hypaque-Ficoll gradients were separated into adherent and nonadherent populations by incubating on plastic surfaces. Cells were suspended in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 10% heat-inactivated fetal calf serum (Grand Island Biological Co.) to a final concentration of 5.0×10^6 cells per ml. 5 ml of the cell suspension was placed in 100-mm plastic Petri dishes (Falcon Plastics, Oxnard, Calif.) and incubated in 5% carbon dioxide-95% air for 16–20 h. Nonadherent cells were removed from the dishes by decantation and vigorous washing with cold HBSS. The nonadherent

cells were washed and counted; the viability and composition of the cell suspension was determined by staining cells with trypan blue, neutral red, and Wright's stain. These preparations contained 10–20% neutral red-positive cells and over 95% of the cells were viable.

To remove adherent cells, the dishes were chilled and scraped with a rubber policeman. These preparations contained 80–90% neutral red-positive cells and at least 90% viable cells.

Adherent and nonadherent cells were also separated by passing Hypaque-Ficoll cells through glass bead (3M Co., St. Paul, Minn.) columns (15). The nonadherent cell suspension contained approximately 95% lymphocytes, 2% monocytes, and 3% neutrophils; 90% of the cells were viable by trypan blue exclusion.

Sterile reagents and glassware were used in all of the above procedures.

Preparation of leukocyte dialysates. The method was essentially the same as that described previously for preparation of dialyzable transfer factor (1) except that the DNase digestion step was not always used. Cells were disrupted by multiple cycles of freezing and thawing and then dialyzed against distilled water. The dialysate was lyophilized and reconstituted with either distilled water or phosphate-buffered saline so that the extract from 3×10^8 cells (lymphocytes unless other indicated) was contained in 1 ml. Dialysates were sterilized by filtration (Millipore Corp., Bedford, Mass.).

In this report dialysates of lymphocytes and monocytes prepared in this manner are referred to as dialyzable transfer factor although the molecular identity of the components responsible for conversion of delayed skin tests and increases in leukocyte cGMP has not been shown. However, transfer factor activity in the whole dialysates was demonstrated by conversion of delayed cutaneous hypersensitivity responses in patients with cellular immune deficiencies as described previously (1).

Assay of cyclic nucleotides. Leukocyte cGMP was measured by a previously reported method (16). Briefly, leukocyte suspensions containing 10^7 – 10^8 cells in 2.0 ml Krebs-Ringer-Tris buffer were incubated for 10 min before additions of 0.4 ml of dialysate or buffer. After 5 min of incubation at 37°C, 2.2 ml of the reaction mixture was added to 1.0 ml of cold 10% perchloric acid and homogenized for 45 s at 4,000 rpm with a Brinkmann Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.). A trace amount of [3 H]cGMP (< 1 pmol, sp. act 4.47 Ci per mmol) (New England Nuclear, Boston, Mass.) was added to each homogenate for calculation of recoveries, and the samples were centrifuged at 1,000 *g* for 30 min. The supernates were neutralized with KOH, again centrifuged, and applied to columns of AG 1-X8-formate (100–200 mesh) (Bio-Rad Laboratories, Richmond, Calif.). The columns were washed first with water, then with 0.5 N formic acid before the cGMP was eluted with 4 N formic acid. The samples were then lyophilized, reconstituted in 50 mM sodium acetate buffer, pH 6.2, and assayed as previously described (16) using a commercial radioimmunoassay (Collaborative Research, Waltham, Mass.).

In each experiment cells were also exposed to 100 μ M 5-hydroxytryptamine (serotonin, Sigma Chemical Co.) which causes accumulation of cGMP in monocytes (16) to confirm responsiveness of the cells.

For determination of cAMP, incubations were terminated by adding 0.5 ml of 30% trichloroacetic acid to 2.2 ml of cells in medium. After homogenization with the Polytron, 0.2 pmol of [3 H]cAMP (sp act 20 Ci per mmol, New Eng-

land Nuclear) was added for calculation of recoveries, and cAMP was isolated and assayed by the method of Gilman (17) as previously described (18).

Gel filtration of leukocyte lysates. The lyophilized dialysate of the extract from 3×10^8 cells was reconstituted in 1 ml of distilled water and passed through a 2.6×85 -cm column of Sephadex G-25 (Pharmacia Fine Chemicals) with 0.02 M ammonium bicarbonate, pH 7.4, as the eluting buffer. 3-ml fractions were collected and the absorbance was monitored at 255 nm, the absorbance peak for lysates containing dialyzable transfer factor (19). Appropriate tubes were pooled, lyophilized, and assayed for transfer factor activity, ascorbate, serotonin, and effects on leukocyte cGMP. For certain experiments, the column was calibrated by filtration of lysates containing either 2×10^5 cpm of [^{14}C]ascorbic acid or [^{14}C]serotonin creatinine sulfate (New England Nuclear).

Transfer factor activity in fractions from the gel filtration column. After passage of crude dialyzable transfer factor through the gel filtration column, the tubes corresponding to Fraction IV of Fig. 3 were combined into pool A. All other fractions were also combined and designated pool B. Both pools were lyophilized and then reconstituted so that the extract from 3×10^8 cells was contained in each milliliter. Because of the previous report by Zuckerman et al. (20), it was assumed that all of the transfer factor activity in the crude material would be contained in only one of the two pools. After Millipore filtration and sterility testing, 2 ml of a pool was injected subcutaneously into immunologically deficient patients for assessment of transfer factor activity. Intradermal skin tests were applied 5–7 days later and the delayed cutaneous responses were measured as described previously (1). Two patients were first given the material from pool A, then skin tested. The third subject received material from pool B, was skin tested, and was subsequently given material from pool A and then retested.

Assays for substances known to increase cellular cGMP. The content of serotonin in the dialysates was determined by an isotope dilution derivative assay (21), by Dr. Michael Beavan. Ascorbate was measured by the method described by Caraway (22). The possibility that leukocyte lysates might contain cholinergic activity was examined by measuring contraction of an isolated guinea pig ileum with an ADAPS Bioassayer (ADAPS, Inc., Dedham, Mass.), first with the ileum suspended in Tyrode's buffer containing the antihistamine mepyramine and then in Tyrode's buffer containing both mepyramine and 5×10^{-7} M atropine.

RESULTS

Effect of leukocyte dialysates on mononuclear cell cGMP. The effects of five preparations of dialyzable transfer factor from five normal donors on cGMP content of mononuclear cells are shown in Fig. 1. In experiments in which the duration of incubation was varied, a near-maximal rise in cGMP was seen at 1 min and persistent elevation at 120 min. Untreated mononuclear cells contained 1.2–2.7 pmol of cGMP per 10^7 cells, while in cells incubated for 5 min with transfer factor-containing dialysates, the cGMP content was increased 4- to 11-fold.

The relationship of the amount of transfer factor-containing dialysates to the change in mononuclear cell

FIGURE 1 Effect of transfer factor-containing leukocyte dialysates on mononuclear cell cGMP. Cells were incubated for 5 min with dialysate (1.2×10^8 lymphocyte equivalents) or Krebs-Ringer-Tris buffer. Values represent mean of duplicate samples plus one-half the range. Buffered saline processed in a manner similar to the leukocyte dialysates was used as the reagent blank.

cGMP content is shown in Fig. 2. Because the assay method required a one-sixth dilution of dialysate (0.4 ml added to 2.0 ml of leukocyte suspension), experiments with extracts from more than 1.2×10^8 lymphocytes were not done. The dialyzable material from leukocyte lysates themselves, when purified and assayed in a

FIGURE 2 Effect of different amounts of transfer factor-containing leukocyte dialysate on mononuclear cell cGMP. The amount of transfer factor is expressed both as the number of lymphocytes from which the dialysate was prepared and, in parentheses, as the final dilution of crude material that contained the extract from 3×10^8 lymphocytes per ml. Experiments performed and data reported as in Fig. 1.

TABLE I
Effect of Dialyzable Material from Leukocyte Lysates on cGMP Content of Human Leukocytes

Exp	Leukocyte preparation		Monocytes	cGMP content		
				Buffer	Dialysate	Δ due to dialysate
			%	<i>pmol/10⁷ cells</i>		
(1)	Mononuclear	Adherent	86*	1.5 $\pm$ 0.4	16.1 $\pm$ 1.0	+14.6
		Nonadherent	11	0.4 $\pm$ 0.1	2.1 $\pm$ 0.2	+1.7
(2)	Mononuclear	Adherent	86*	3.1 $\pm$ 1.7	16.0 $\pm$ 1.2	+12.9
		Nonadherent	20	0.7 $\pm$ 0.1	1.4 $\pm$ 0.5	+0.7
(3)	Mononuclear	Adherent	86*	3.5 $\pm$ 1.5	20.0 $\pm$ 0.9	+16.5
		Nonadherent	7	2.8 $\pm$ 1.1	6.0 $\pm$ 1.4	+3.2
(4)	Mononuclear	Precolumn	15†	2.1 $\pm$ 0.2	11.2 $\pm$ 0.4	+9.1
		Postcolumn	2	2.8 $\pm$ 0.3	3.2 $\pm$ 0	+0.4
(5)	Neutrophils			0.9 $\pm$ 0.4	0.8 $\pm$ 0.2	-0.1
(6)	Neutrophils			1.5	2.5 $\pm$ 0.2	+1.0

Cells were incubated with buffer or leukocyte dialysate (1.2×10^8 lymphocyte equivalents) for 5 min at 37°C. Values are reported as mean of duplicates $\pm$ one-half the range. In expts 1-3 the increment in cGMP content of adherent cells was $+14.7 \pm 1.0$ pmol/10⁷ cells (mean $\pm$ SE, $P < 0.005$), and in nonadherent cells the increment was 1.8 ± 0.7 pmol/10⁷ cells ($P > 0.1$).

* Scored as monocytes by neutral red uptake.

† Scored as monocytes on stained smears rather than neutral red.

manner identical to the experimental samples, did not contain detectable cGMP.

Identification of the "responding" cells. The effects of the dialyzable material on cGMP accumulation in purified preparations of neutrophils, monocytes, and lymphocytes are summarized in Table I. The most striking increases in cGMP occurred in preparations of plastic-adherent cells that were rich in monocytes (exp 1, 2, and 3); nonadherent, lymphocyte-rich preparations did not show significant increases in cGMP. Moreover, passage of mononuclear cells through a glass bead column removed the responding cells (exp 4). Neutrophils failed to respond with increases in cGMP (exps 5 and 6).

Cellular source of the material that increases leukocyte cGMP. As seen in Table II, dialysates from normal mononuclear cells (which contain transfer factor activity) produced an increase in cGMP content of mononuclear cells, but preparations from the lymphocyte-rich peripheral blood of two patients with the Sézary syndrome, a variant of lymphocytic leukemia in which the lymphocytes have properties of T cells (23), produced little, if any, change in cGMP content. A single preparation from a patient with chronic lymphocytic leukemia (CLL) in which the cells have membrane

TABLE II
Effects of Dialyzable Material from Different Leukocyte Populations on cGMP in Mononuclear Cells

Source of dialysate	cGMP		
	Buffer	Dialysate	Δ *
	<i>pmol/10⁷ cells</i>		
Leukemic cells			
Sézary cells	1.4 $\pm$ 0.5	1.8 $\pm$ 0.1	0.4
Sézary cells	2.8 $\pm$ 1.1	4.8 $\pm$ 0.8	2.0
CLL cells	2.8 $\pm$ 1.1	6.8 $\pm$ 0.2	4.0
Normal mononuclear cells			
Adherent/nonadherent cells			
Adherent	3.1 $\pm$ 1.7	9.7 $\pm$ 1.3	6.6
Nonadherent	3.1 $\pm$ 1.7	10.0 $\pm$ 1.0	6.9
Normal leukocytes			
(1) Mononuclear cells	2.8 $\pm$ 1.1	10.3 $\pm$ 1.9	7.5
Neutrophils	2.8 $\pm$ 1.1	12.0 $\pm$ 0.4	9.2
(2) Mononuclear cells	1.2 $\pm$ 0.5	6.7 $\pm$ 0.8	5.5
Neutrophils	1.2 $\pm$ 0.5	20.9 $\pm$ 3.8	19.7
(3) Neutrophils	1.2 $\pm$ 0.5	18.6 $\pm$ 2.1	17.4

* Δ = increment over value with buffer alone. Data reported as in Table I.

TABLE III
Effect of Transfer Factor-Containing Dialysate
on Leukocyte cAMP

Exp		cAMP <i>pmol/10⁷ cells</i>	Δ*
(1)	Control	67.6±18.5	—
	Dialysate	107.5±1.9	40
	Polystyrene beads	496.7±4.0	429
(2)	Control	56.0±13.0	—
	Dialysate	80.0±28.0	24
	Polystyrene beads	552.0±55.0	496

* Δ = increment over value with buffer alone. Data reported as in Table I.

properties of B cells, produced a smaller change in mononuclear cell cGMP content than preparations from normal mononuclear cells (Table II and Fig. 1).

Dialyzable material from lysates of equivalent numbers of adherent and nonadherent mononuclear cells were equally active in increasing mononuclear cell cGMP content (Table II).

Leukocyte preparations from individual donors were separated into mononuclear cells by centrifugation through Hypaque-Ficoll gradients and neutrophils by dextran sedimentation. Dialysates of the lysed cells were reconstituted so that the extract from 3×10^8 cells was contained in 1 ml. As shown in Table II, preparations from both mononuclear cells and neutrophils increased intracellular cGMP.

In summary, the results of these experiments indicate that the cells that respond with increased cGMP are probably monocytes, whereas material capable of producing this effect can be obtained from several populations of normal leukocytes.

Effect of leukocyte lysates on cAMP. As shown in Table III, dialyzable transfer factor produced small increases in the cAMP content of mononuclear cells from Hypaque-Ficoll gradients. Addition of polystyrene beads (Dow Chemical Co., Midland, Mich.) to the Hypaque-Ficoll cells produced marked increases in cAMP (16).

Concentrations in dialysates of substances known to increase cGMP. In each of the three preparations examined (Table IV), the concentrations of serotonin ranged from 42 to 88 μ M and were in the range previously shown to increase monocyte cGMP (16). Six preparations of dialyzable transfer factor and three preparations from leukemic cells contained ascorbic acid (Table IV) in concentrations that have been shown to increase cGMP.²

² Sandler, J. Unpublished observation.

The dialyzable material from both buffy-coat leukocytes and Hypaque-Ficoll-purified mononuclear cells from normal donors produced brisk contraction of the mepyramine-treated guinea pig ileum. The activity in seven preparations was equivalent to 0.33-2.33 μ M acetylcholine and in each case the contractile effect was blocked by atropine. Dialysates of Sézary and CLL cells were essentially devoid of contractile activity.

Gel filtration of leukocyte dialysates. Preparations containing 1 ml of dialyzable transfer factor were passed through a Sephadex G-25 column that had been calibrated with [¹⁴C]serotonin and [¹⁴C]ascorbic acid (Fig. 3). By both the isotopic markers and direct assay of fractions, ascorbate appeared early (Fraction II) while serotonin eluted well beyond the column volume (V_t). Dialysates containing dialyzable transfer factor yielded several fractions that increased mononuclear cell cGMP. In each of four experiments activity was found in Fraction II, the fraction that eluted in the region of the ascorbate marker and was found to contain ascorbate. Fractions I, III, and IV also increased mononuclear cell cGMP. Fraction I contained variable amounts of ascorbic acid, but Fractions III and IV did not contain detectable levels of ascorbate or serotonin. The material that eluted beyond Fraction IV did not increase mononuclear cell cGMP.

When the dialysate of lysed neutrophils was passed through the same column, only Fractions I and II

TABLE IV
Concentrations of Serotonin and Ascorbate in Dialyzable
Transfer Factor and Other Leukocyte Dialysates

Preparation	Serotonin μ M	Ascorbate mM
Normal cells		
H-F*	88.54	0.333
Buffy coat†	42.31	0.150
H-F	54.11	ND‡
H-F	ND	0.424
H-F	ND	0.333
H-F	ND	0.410
Buffy coat	ND	0.260
Leukemic cells		
Sézary syndrome	ND	0.167
Sézary syndrome	ND	0.111
CLL	ND	0.308

Assays were performed on dialysates which contained 3×10^8 lymphocyte equivalents per ml.

* H-F, mononuclear cells from Hypaque-Ficoll gradients.

† Buffy coat, cells from leukophereses that were washed but not separated into mononuclear cells and neutrophils.

‡ ND, assay not done.

FIGURE 3 Sephadex G-25 gel filtration of 1 ml of transfer factor-containing leukocyte dialysate. The column was calibrated with [^{14}C]ascorbic acid and [^{14}C]serotonin creatinine sulfate ($\bullet$) and the absorbance of each fraction at 255 nm was determined ($\circ$). Over 90% of the radioactivity was recovered. Pooled fractions were assayed for cGMP-increasing activity as described. V_0 , void volume of column; V_t , column volume; n , number of experiments from which the mean cGMP stimulation was calculated. Column dimensions, 2.6×86 cm; eluting buffer, 0.02 M ammonium bicarbonate; pH, 7.4; temperature, 4°C .

caused accumulation of cGMP in monocytes and these fractions contained ascorbic acid (Fig. 4).

Transfer factor activity in column fractions. As shown in Table V, only the material in pool A (Fraction IV) transferred delayed hypersensitivity responses to the patients. Successful transfers were obtained in three of three instances with protein purified derivative, two of two instances with candida and one of two opportunities with streptokinase-streptodornase. Tricho-

phytin reactivity was not present in the donor and did not appear in any recipient.

DISCUSSION

The experiments summarized above show that dialysates from lysed human blood leukocytes, including preparations that contained dialyzable transfer factor, markedly stimulated accumulation of intracellular cGMP in blood monocytes. Preparations from neutrophils, plastic-adherent monocytes, and nonadherent lymphocytes from normal donors produced this effect, but those from leukemic lymphocytes did not. The role of platelets as a source of cGMP-stimulating activity was not examined directly. However, the preparations from the Sézary cells were rich in platelets, yet were not stimulatory, whereas the dialysates from purified neutrophils which were devoid of platelets were active.

Serotonin (16), ascorbate,⁹ and cholinergic stimuli (24-26) can increase the cGMP content of leukocytes. When preparations of dialyzable transfer factor were fractionated on a G-25 column, cGMP-stimulating activity was found in the ascorbate-containing fractions and in two additional fractions that did not contain ascorbate or serotonin. In contrast, while the dialysates of lysed neutrophils also increased mononuclear cell cGMP, this activity was limited to the ascorbate-containing fractions. Material from dialysates of either

FIGURE 4 Sephadex G-25 gel filtration of the dialysate of lysed blood neutrophils. Column dimensions and experimental conditions were the same as Fig. 3. Only the ascorbate-containing fractions increased mononuclear cell cGMP content. Absorbance at 255 nm and elution profile of [^{14}C]ascorbic acid shown as in Fig. 3.

TABLE V
Transfer of Delayed Cutaneous Hypersensitivity with Fractions from Whole Transfer Factor

Antigen	Transfer factor donor	Delayed cutaneous hypersensitivity responses*							
		Pool A recipients‡						Pool B recipient‡	
		1		2		3		3	
		Before	After	Before	After	Before	After	Before	After
Protein purified derivative	4.0	0	1.2	0	0.5	0	0.7	0	0
Candida	1.5	0.4	1.1	0	1.2	0	1.0	0	0
Streptokinase-Streptodornase	1.0	0	1.7	0	0	1.0	1.5	1.4	1.4
Trichophyton	0	0	0	0	0	0	0	0	0

* Centimeters induration at 24 h.

‡ Recipients 1 and 2 were given material from pool A, then skin tested. Recipient 3 was first given material from pool B and skin tested, then given material from pool A and retested.

mononuclear cells or neutrophils that eluted in the region of the serotonin marker did not increase mononuclear cell cGMP. The nature of the "cholinergic" activity in the dialysates has not been defined. Serotonin produces contractions of the guinea pig ileum similar to those produced by acetylcholine and this activity, too, is blocked by atropine (27). Carbamylcholine in concentrations equivalent to those suspected to be present in the dialysates caused only small and variable effects on cGMP in mononuclear cells.³

A number of studies have demonstrated the important role of intracellular cyclic nucleotides in regulation of immunologic and inflammatory reactions. Generally, agents that increase intracellular cAMP suppress the intensity of responses, while increasing intracellular cGMP enhances or augments responses. This phenomenon has been shown for immunologic release of histamine and SRS-A from basophils and mast cells (6, 7, 13), T-lymphocyte-mediated cytotoxicity against allogeneic cells (9, 10), and release of lysosomal enzymes from neutrophils (25, 26). Of importance in understanding the pathogenesis of inflammatory responses are the report by Estensen et al. (28) that cholinergic agonists and 8-bromo-cGMP enhance movement of neutrophils; the observations by Goetzl et al. (29) that ascorbic acid increases random motility and chemotactic responses of leukocytes; and the findings that ascorbic acid and serotonin, agents that increase intracellular cGMP, enhance monocyte chemotaxis in the presence of endotoxin-treated serum.³

The mechanism through which dialyzable transfer factor transfers cell-mediated immune responses, and the role that cGMP may play in this process are unknown. A recent review has discussed whether transfer factor might contain specific immunologic information or

function as an adjuvant or amplifier of subthreshold immunologic responses (30). The concept that transfer factor contains antigen-specific activities is supported by reports of transfer of delayed skin responses to nonenvironmental antigens such as denatured human serum albumin (31) and keyhole limpet hemocyanin (20), specific cytotoxicity against tumor cells (4, 5), and accelerated rejection of skin allografts (32). On the other hand, reports of appearance of immunologic responses in recipients of transfer factor or leukocyte lysates that were not present in the donor (3, 33-37) and the recent demonstration of chemotactic activity in transfer factor preparations (38) suggest that transfer factor contains adjuvant-like, antigen-independent activities.

The role that cGMP plays, if any, in the transfer of delayed hypersensitivity is also uncertain at this juncture. Our findings suggest that increases in cGMP alone would not be sufficient to bring about conversion of skin test reactivity. Neutrophil dialysates, which contain ascorbic acid, promote an increase in cGMP content of mononuclear cells. Yet dialysates of neutrophils from skin test-positive donors do not cause conversion of delayed cutaneous hypersensitivity in skin test-negative recipients.⁴ On the other hand, Fractions III-IV of mononuclear cell dialysates which did not contain ascorbic acid or serotonin, did promote an increase in cGMP content of the plastic-adherent mononuclear cells. Fraction IV was also found to contain the component(s) responsible for conversion of delayed skin responses, confirming the observations of Zuckerman and co-workers (20). While it is conceivable that the same component could both cause accumulation of cGMP and convey the information needed for the conversion of the immunologic response, it seems more likely to us that cGMP may be involved in regulation of the antigen-independent activities of transfer factor, especially

³ Sandler, J. A., J. I. Gallin, and M. Vaughan. Unpublished observations.

⁴ Kirkpatrick, C. H. Unpublished observations.

in view of the presumed regulatory role of cyclic nucleotides in immunologic and inflammatory reactions.

We propose that the phenomenon of passive transfer of cellular immunity with transfer factor requires contributions from both antigen-specific and antigen-independent activities, perhaps requiring several components in Fraction IV of the dialysates. Lymphocytes from transfer factor recipients respond to specific antigens by producing lymphokines (1-3, 5), and Lawrence and Pappenheimer (39) have reported that in vitro stimulation of lymphocytes with antigens causes specific transfer factor to be released into the culture fluid in a manner analogous to the production of lymphokines. It has also been shown that recipients of transfer factor may in turn serve as sources of transfer factor for other skin test-negative subjects (40).⁴ Thus, the recipient of transfer factor responds to antigens by local production of more transfer factor and lymphokines which are essential for the immunologic-inflammatory responses seen in delayed cutaneous hypersensitivity and skin graft rejection. We feel that transfer factor through its immunologically specific properties (which may be modulated by cGMP) recruits naive cells into the antigen-responsive population, and through its antigen-independent properties (i.e. chemotactic activity) which are modulated by cGMP, provides additional intensification of antigen-induced inflammation. Elucidation of a putative role for cGMP in the transfer of delayed hypersensitivity will require further purification of the active components in dialyzable transfer factor.

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