Immunologic Specificity of Transfer Factor

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ABSTRACT This study examined the immunologic specificity of transfer factor using a chromatographically purified transfer factor preparation. The specificity of transfer was examined utilizing immunity to keyhole limpet hemocyanin (KLH) and tuberculin. Transfer factor prepared from a donor immune to KLH successfully transferred KLH skin test reactivity to 10 out of 10 recipients. In contrast, comparable amounts of transfer factor from two donors not immune to KLH failed to transfer immunity to KLH in 11 recipients despite evidence for successful transfer of tuberculin reactivity. Unlike prior studies with a variety of antigens, the immunity to KLH in recipients of KLH immune transfer factor appeared comparable to that of the donor since both could be elicited with the same skin test antigen dose. These observations indicate that transfer factor can initiate a specific immune response to an antigen not previously encountered by the recipient and that in certain circumstances this immune response can be comparable to that of the donor. These observations on specificity and potency of transfer factor have important implications for the clinical use of this material.

INTRODUCTION

Transfer factor is a dialyzable, low molecular weight material derived from lysed human leukocytes, which is thought to be capable of transferring cellular immunity from an immune donor to a nonimmune recipient. Basing primarily on the transfer of immunity to histocompatibility antigens, Lawrence believes that this material transfers the specific immune reactivity of the donor and is a specific informational molecule (1). However, Bloom (2) has recently reviewed the transfer factor literature and suggested the alternate possibility that transfer factor might act nonspecifically to stimulate the reactivity of a subthreshold number of previously sensitized lymphocytes.

Most clinical studies of transfer factor have utilized the injection of leukocyte lysates or the dialysate of lysed leukocytes (1, 3–6). Such crude preparations represent a heterogeneous mixture of small molecular weight compounds. Our laboratory has recently developed a chromatographic technique for isolating the active component of transfer factor (TF_C)[1] which represents approximately 1% by weight of the dialysate and contains all the biological activity of crude transfer factor (7). The purpose of this study is to determine the activity and specificity of this purified transfer factor preparation using transfer of skin test reactivity with two antigens, tuberculin (PPD), and keyhole limpet hemocyanin (KLH).

METHODS

Antigens and skin tests. Antigens for delayed hypersensitivity skin tests included: PPD in doses of 250 tuberculin U (second strength), 5 tuberculin U (intermediate), and 1 tuberculin U (first strength)[2]; histoplasmin (1:20)[2]; coccidioidin (1:100)[3]; mumps[4]; trichophyton (1:30)[5];

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[1] *Abbreviations used in this paper:* KLH, keyhole limpet hemocyanin; PPD, purified protein derivative (tuberculin); SK-SD, streptokinase-streptodornase; TF_C, chromatographically isolated transfer factor.

[2] Parke, Davis & Company, Detroit, Mich.

[3] The Cutter Laboratories, Berkeley, Calif.

[4] Eli Lilly and Company, Indianapolis, Ind.

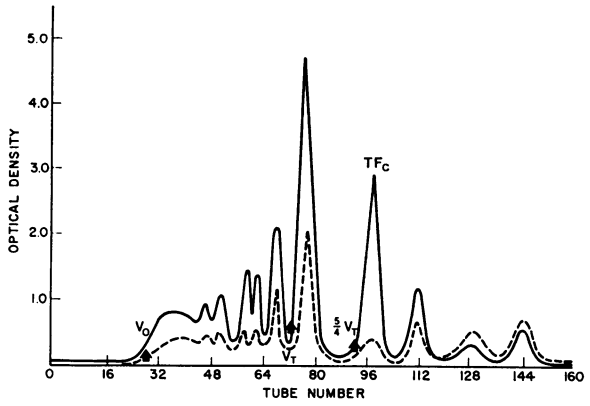

FIGURE 1 Chromatogram of dialyzable transfer factor. The dialyzate of lysed leukocytes was placed over a 2.5- × 100-cm Sephadex G-25 column and eluted with 0.01 M ammonium bicarbonate buffer at 40 ml/h. Tube volume was 10 ml. Optical density was recorded at 260 nm (——) and 280 nm (- - -). V_o, void volume; V_t, total bed volume of column. The biologically active fraction is labeled TFc.

candida (1:100)[5]; streptokinase-streptodornase (SK-SD) (50 U).[6] KLH for immunization and skin testing was kindly provided by Dr. Evan Hersh, M. D., Anderson Hospital, Houston, Tex. Immunization with KLH was accomplished using a single subcutaneous injection of 1 mg and skin testing was performed with 100 μg (8). All skin tests were applied intradermally in a volume of 0.1 cm^3. Two perpendicular diameters of induration were measured 48 h after application of the skin tests, and the average of the two diameters was recorded. Recipients of transfer factor were skin tested at a site remote from transfer factor administration.

Transfer factor preparation. Donor leukocytes were collected from donors using an Aminco blood cell separator[7] and dialyzable transfer factor was prepared by the method of Lawrence (1). The dialyzed transfer factor was lyophilized and the active component isolated by Sephadex G-25 chromatography as previously described (7) with two modifications. Column size was increased to 100 × 2.5 cm and the separation accomplished in a volatile buffer (0.01 M NH_4HCO_3, pH 7.8). Fig. 1 illustrates a typical chromatogram and the biologically active component is identified as TFc to indicate that it is chromatographically isolated transfer factor. TFc adheres to Sephadex and elutes at five-fourths the total bed volume of the column. This characteristic and the use of a volatile buffer allows quantitation of TFc in terms of weight after lyophilization. For this study, TFc was administered as a single subcutaneous injection of 250 μg, the product of approximately 4×10^8 lymphocytes.

Transfer factor donors. Donor 1 was selected because of his strong reactivity to first strength PPD (55 mm). He was immunized with 1 mg KLH and 2 wk later had a 13-mm reaction to a KLH skin test. He also had an 11-mm reaction to SK-SD and negative reactions to mumps, coccidioidin, and candida skin tests. Donor 2 was also selected because of strong reactivity to first strength PPD (42.5 mm). However, he was not immunized with KLH and had a negative KLH skin test when tested after donation of transfer factor. He also had a 9.5-mm reaction to mumps, 5.5-mm reaction to candida, and negative reactions to coccidioidin and histoplasmin. A third preparation was selected at random from a pool of normal donors collected over several years. This donor had no exposure to KLH and skin test reactivities were not available. This preparation served only as an additional control for nonspecific transfer of KLH reactivity.

[5] Hollister-Steir Laboratories Inc., Spokane, Wash.

[6] American Cyanamid Co., Lederle Laboratories Div., Pearl River, N. Y.

[7] American Instrument Co., Inc., Silver Spring, Md.

RESULTS

TFc from donor 1, who was immune to PPD and KLH, was administered to 10 healthy volunteers who were selected on the basis of having negative second strength PPD skin tests and no known contact with KLH. Six of these recipients were skin tested 2 days after TFc administration and all six had positive KLH skin tests (Table I). In addition, five out of six developed tuberculin reactivity. The other four recipients had their initial KLH skin tests performed 21 days after TFc administration and all had positive reactions. Three of these four recipients also developed reactivity to second strength PPD. None of the 10 recipients developed positive skin tests with intermediate strength PPD.

The specificity of KLH transfer was established using TFc prepared from donors 2 and 3 as controls. TFc prepared from donor 2, who was immune to tuberculin and not to KLH, was administered to five recipients who were selected on the basis of negative reaction to second strength PPD. 2 days after TFc administration, none of the five recipients had KLH reactivity while four had developed tuberculin reactivity (Table II). Transfer factor from this donor had also transferred tuberculin

TABLE I

*Skin Test Responses in 10 Recipients of TFc from Donor 1**

	KLH skin tests‡			PPD skin tests§		
Subject	Pre-TFc	2 days post-TFc	21 days post-TFc	Pre-TFc	2 days post-TFc	21 days post-TFc
1	—	9.5	—	0	13.0	16.0
2	—	8.5	—	0	6.0	6.0
3	—	11.0	—	0	0	0
4	—	13.0	—	0	12.0	—
5	—	15.0	—	0	6.0	—
6	—	9.0	—	2.0	13.5	—
7	—	—	14.0	0	12.0	16.0
8	—	—	12.0	0	11.0	13.0
9	—	—	13.0	0	0	0
10	—	—	7.0	0	13.0	10.0
Mean	—	11.0	11.5	0.2	8.7	
SD	—	2.5	3.1	0.6	5.3	

* Donor 1 had 55-mm first strength PPD and 13-mm KLH skin test reactions.

‡ Expressed as mean diameter of induration (in millimeters) to 100 μg KLH.

§ Expressed as mean diameter of induration (in millimeters) to second strength PPD.

TABLE II

*Skin Test Responses in Recipients of TFc from Donor 2**

Subject	KLH skin tests‡		PPD skin tests§	
	Pre-TFc	2 days Post-TFc	Pre-TFc	2 days Post-TFc
1	—	0	0	10.5
2	—	0	0	3.0
3	—	0	0	7.5
4	—	0	0	7.0
5	—	0	0	8.5

* Donor 2 had 42.5-mm first strength PPD and negative KLH skin test reactions.

‡ Expressed as mean diameter of induration (in millimeters) to 100 μg KLH.

§ Expressed as mean diameter of induration (in millimeters) to second strength PPD.

reactivity in three out of three recipients in a previous study (7). TFc from donor 3 who was not immune to KLH was utilized as a "negative" transfer factor preparation and administered to six healthy recipients. No skin test reactivity to KLH occurred in the recipients who were skin tested either 2 days or 21 days after TFc administration.

To further characterize the KLH reactivity in our population, we skin tested with KLH 12 healthy volunteers who did not receive TFc. Only one individual reacted (15 mm). This incidence is similar to that noted by Hersh, who found 5–10% of normal individuals in Houston, Tex., sensitive to this antigen. An additional index of specificity of transfer would be conversion of skin test reactivity to one of the other antigens used. These antigens are those which a general population may have encountered, and this conversion may represent nonspecific enhancement of a minimal cell-mediated immunity. Among the 10 subjects in group I, there were 28 initially negative skin test reactions to histoplasmin, coccidioidin, trichophyton, or candida. All remained negative after TFc administration with the exception of conversion to a positive coccidioidin skin test (8 mm) in one subject who also had a strongly positive histoplasmin skin test (24 mm) on initial testing. These two antigens are known to have cross-reactivity (9).

DISCUSSION

Many investigators have shown that transfer factor administration alters the cellular immune reactivity of the recipient (5, 7, 10). The present study once again confirms these findings with transfer of tuberculin reactivity in 15 of 18 recipients and KLH reactivity in 10 of 10 recipients. Lawrence and his coauthors have stated that this transfer is specific for those reactivities possessed by the donor (4, 6). For this reason, most investigators select donors with intense skin test reactivity to the antigenic determinant to be transferred, although pools of "normal" transfer factor have been reported to be clinically effective in treating certain immunodeficiency diseases (10). The question of specificity of transfer and donor selection becomes critical when transfer factor as an immunotherapeutic agent for specific malignancies and infectious diseases is considered. However, the concept that transfer factor transfers only those delayed hypersensitivity reactions possessed by the donor has recently been questioned (2). Nonspecific effects of transfer factor therapy have been observed in the treatment of immune deficiency diseases (11, 12). Only two studies have been directed to the immunologic specificity of transfer factor. The first involved transfer of coccidioidin reactivity. The results were inconclusive, and the authors concluded that they had "not fully demonstrated that transfer factor can confer upon the recipient a de novo sensitivity" (4). The second study reported transfer of immunity to histocompatibility antigens as determined by accelerated skin graft rejection in six recipients. However, active transfer factor preparations could only be obtained from the donors for a short period after four skin graft rejections. Thus, there is limited evidence to document specificity of transfer factor.

The use of a "neo-antigen" as a marker of transfer permits design of a study for unequivocally documenting specificity. KLH is a potent immunogen that elicits a primary immune response in man (13) that can be adoptively transferred with whole lymphocyte preparations (14). Approximately 95% of the population have no circulating antibody or skin test reactivity to this antigen but are readily immunized by the usual skin test dose (8). The present investigation shows that only TFc prepared from a donor with KLH sensitivity was capable of transferring that reactivity. 10 of 10 recipients demonstrated delayed hypersensitivity to KLH after a 250-μg dose of TFc from an immunized donor. An equivalent dose of TFc from a tuberculin-positive but KLH-negative donor did not transfer KLH reactivity in five recipients despite transfer of tuberculin reactivity. TFc from a second donor with no KLH exposure failed to transfer KLH reactivity in six recipients. This documents the specificity of transfer with TFc but does not preclude the presence of other substances with nonspecific immunologic activity in crude transfer factor preparations.

Transfer of cellular immunity with various transfer factor preparations has generally produced a modest degree of sensitivity in recipients. Donors have usually been highly sensitive to a small skin test dose of a particular antigen while detection of reactivity in recipients usually requires doses of skin test antigen that are 10–

250 times that used for donor selection (4, 5). This study demonstrates that in the KLH system, transfer is remarkably efficient with 10 of 10 recipients achieving reactivity (11 mm) to the same skin test dose of KLH as the donor (13 mm). The degree of tuberculin reactivity transferred was again small in comparison to the donors' reactions and conforms to the experience of others. The difference in the intensity of transferred immunity with different antigens may result from intrinsic differences in the antigens themselves. An alternate explanation would be that recent exposure to a particular antigen may increase the amount of specific transfer factor available in circulating leukocytes. This type of phenomenon was suggested by Lawrence, Rapaport, Converse, and Tillet's ability to systemically transfer immunity to histocompatibility antigens only when transfer factor was prepared from a donor at the peak of a fourth set skin graft rejection (6). The observation that transfer of equivalent degrees of cellular immunity can be accomplished has important implications in the use of transfer factor in immunotherapy regimens and will require studies to delineate the mechanism and factors involved.

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