Immunologic Specificity of Transfer Factor

KENNETH S. ZUCKERMAN, JAMES A. NEIDHART, STANLEY P. BALCERZAK, and ALBERT F. LOBUGLIO

From the Division of Hematology and Oncology, Department of Medicine, Ohio State University, Columbus, Ohio 43210

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 lyzed human leukocytes, which is thought to be capable of transferring cellular immunity from an immune donor to a nonimmune recipient. Based 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 (TFc) 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); histoplasmin (1:20)†; coccidioidin (1:100)‡; mumps §; trichophyton (1:30)★★★.

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

§Parke, Davis & Company, Detroit, Mich.
★★★The Cutter Laboratories, Berkeley, Calif.
★Eli Lilly and Company, Indianapolis, Ind.
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.

RESULTS

TFo 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 TFo administration and all six had positive KLH
skin tests (Table 1). In addition, five out of six developed tuberculin reactivity. The other four recipients had
their initial KLH skin tests performed 21 days after TFo 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 TFo prepared from donors 2 and 3 as controls. TFo
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 TFo 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

![Graphic Image]

**Figure 1** Chromatogram of dialyzable transfer factor. The dialyze of lysed leukocytes was placed over a 2.5- x
100-cm Sephadex G-25 column and eluted with 0.01 M ammonium bicarbonate buffer at 40 ml/h. Tube volume was
10 ml. Optimization by void volume $V_c$ observed after injection of TFo at 260 nm (- -). $V_t$, total bed volume of
column. The biologically active fraction is labeled TFo.

candida (1:100); streptokinase-streptodornase (SK-SD)
(50 U). KLH for immunization and skin testing was
kindly provided by Dr. Evan Herah, M. D., Anderson Hospi
tal, Houston, Tex. Immunization with KLH was accom
plished using a single subcutaneous injection of 1 mg
and skin testing was performed with 100 $\mu$g (6). 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 Amino blood cell separator
and dialyzable transfer factor was prepared by the method
of Lawrence (1). The dialyzed transfer factor was lyophi
lized and the active component isolated by Sephadex G-25
chromatography as previously described (7) with two
modifications. Column size was increased to 100 x 2.5 cm
and the separation accomplished in a volatile buffer (0.01 M
NH$_4$HCO$_3$, pH 7.8). Fig. 1 illustrates a typical chromato
gram and the biologically active component is isolated as
TFo to indicate that it is chromatographically isolated
transfer factor. TFo adheres to Sephadex and elutes at five
fourths the total bed volume of the column. This character
istic and the use of a volatile buffer allows quantitation of
TFo in terms of weight after lyophilization. For this study,
TFo was administered as a single subcutaneous in
jection of 250 $\mu$g, the product of approximately 4 x 10$^9$
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, coc
cidioidin, and candida skin tests. Donor 2 was also selected

---

* American Cyanamid Co., Lederle Laboratories Div.,
  Pearl River, N. Y.
* American Instrument Co., Inc., Silver Spring, Md.

---

<table>
<thead>
<tr>
<th>Subject</th>
<th>KLH skin test$^2$</th>
<th>PPD skin test$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-1TFo 2 days</td>
<td>Post-1TFo 21 days</td>
</tr>
<tr>
<td>1</td>
<td>9.5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>11.0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>13.0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>9.0</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>14.0</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>12.0</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>13.0</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>7.0</td>
</tr>
<tr>
<td>Mean</td>
<td>11.0</td>
<td>11.5</td>
</tr>
</tbody>
</table>

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

$^1$ Expressed as mean diameter of induration (in millimeters) to 100 $\mu$g KLH.

% Expressed as mean diameter of induration (in millimeters) to second
  strength PPD.
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 document 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-

### Table II

<table>
<thead>
<tr>
<th>Subject</th>
<th>KLH skin test</th>
<th>PPD skin test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-TFc</td>
<td>2 days</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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...
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.

ACKNOWLEDGMENTS

The authors thank Dr. Henry Wilson for the use of the blood cell separator, which is supported by the Joseph Jeffrey and Trudy Bell Memorial Funds and the Pace Fund.

This work was supported by grants from the National Institutes of Health (CA 12786 and CA 14327) and Clinical Research Center Grant (44-34).

REFERENCES