Properties and activities of transfer factor

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Although there is agreement that transfer factor endows skin test–negative subjects with the ability to develop the delayed allergic responses of the transfer factor donors, there is little direct information on the mechanism of this phenomenon or on the nature of the active component(s). This report reviews some of the known effects of transfer factor or immune responses and inflammation. It is concluded that transfer factor has multiple sites of action, including effects on the thymus, on lymphocyte-monocyte and/or lymphocyte-lymphocyte interactions, as well as direct effects on cells in inflammatory sites. It is also suggested that the "specificity" of transfer factor is determined by the immunologic status of the recipient rather than by informational molecules in the dialysates. Finally, it is proposed that many effects of transfer factor may be due to changes in intracellular cyclic necleotide content, especially accumulation of cGMP, in immunologically reactive cells.

In 1969, our group at the National Institutes of Health began an investigation of the relationships of certain immunologic abnormalities to the susceptibility of patients to persistent or recurrent infections with opportunistic organisms. The model disease selected for this study was chronic mucocutaneous candidiasis.[1] It became evident that while this syndrome was clinically heterogenous, the majority of patients had abnormalities in cell-mediated immunity.[2] This finding led to two additional questions: was it possible to correct the immunologic abnormalities in these patients, and if so, would immunologic reconstitution provide the patients with the ability to clear established infections or with enhanced resistance to reinfection? The agent employed for immunologic reconstitution in these studies was dialyzable transfer factor.[3]

This article will review recent studies of the composition and biological activities of dialyzable transfer factor. It will focus on work done in this laboratory, and only pertinent reports by others will be mentioned. However, several comprehensive reviews of this subject have recently appeared.[3-5]

BACKGROUND INFORMATION

Transfer of delayed cutaneous hypersensitivity from immune donors to nonimmune recipients with lymphoid cells was first described by Landsteiner and Chase.[6] This series of experiments, conducted in guinea pigs, revealed that the duration of the passively acquired allergic state was brief unless the donors and recipients were genetically identical, presumably because the recipients rejected the transferred allogeneic cells. Furthermore, successful transfers were

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achieved only when intact, living cells were used; dead cells and cell lysates were inactive.[7, 8]

Passive transfer of delayed hypersensitivity in man by injection of peripheral blood leukocytes from skin test–positive donors was first reported by Lawrence.[9] In subsequent reports, Lawrence and his associates described several characteristics of transferred delayed allergy in man that differed from the observations in guinea pigs. For example, in humans passively acquired hypersensitivities persisted for many months even though an allogeneic relationship existed between the donors and recipients.[3, 10, 11] Moreover, in man it was possible to transfer delayed reactivity with nonviable, disrupted blood leukocytes.[10-13] Finally, perhaps the most exciting and provocative finding was the discovery that the active component in the cell lysates (transfer factor) was dialyzable[14] and therefore had a small molecular weight.

Aside from dialyzability and the apparent resistance of transfer factor to degradation with deoxyribonuclease, pancreatic ribonuclease, and trypsin,[3] little is known about the chemical nature of dialyzable transfer factor. Furthermore, the mechanism through which molecules of such small molecular weight ($<10{,}000$ daltons) could "transfer" long-lasting delayed hypersensitivity is difficult to conceive. Does transfer factor endow cells with new genetic properties that are expressed as the ability to recognize and respond to new antigens? Does it function by facilitating or derepressing cells with previously acquired potential to respond to certain antigens? Does transfer factor either directly or indirectly provide cells with antigen-specific receptor sites? Or are the essential properties of transfer factor nonspecific or antigen-independent so that it serves as an amplifier or adjuvant and thereby enhances expression of preexisting, but subclinical cellular immune responses? How many components in dialyzable transfer factor are required to elicit the phenomenon of transfer of cell-mediated immunity? Which cells serve as the "targets" for the effects of transfer factor? Finally, can passive transfer of delayed allergy with cellular dialysates be used therapeutically in patients with infectious or neoplastic diseases?

The recent resurgence of interest in transfer factor is an outgrowth of several advances in clinical immunology. Animal models of immune deficiencies that are analogous to human disorders have shown the relative independence of the humoral and cellular immunologic systems.[15] It has been recognized that deficient function of the antibody-synthesizing system predisposes patients to infections with pyogenic organisms, while defects in cell-mediated immunity are often accompanied by chronic or recurrent infections with intracellular organisms, fungi, and some viruses. Moreover, critical cellular interactions in cellular immunity have been identified and it is known that resistance to infections with certain organisms such as *Listeria monocytogenes*, *Salmonella typhimurium*, and *Mycobacterium tuberculosis* in rodents is expressed in mononuclear phagocytes with enhanced bactericidal activity.[16] This system requires contributions from two components: an antigen-specific interaction involving "sensitized" lymphocytes and microbial antigens that culminates in cell division and release of lymphokines; and mononuclear phagocytic cells that become nonspecifically "activated," probably through the actions of the lymphokines.[17] Clinical studies

have revealed abnormalities at several steps in this reaction sequence and have prompted efforts to treat patients with certain infections by restoring cellular immunity.

TRANSFER FACTOR AND IMMUNOLOGIC SPECIFICITY

Since the earliest experiments by Lawrence and co-workers, much evidence has indicated that transfer factor was specific, meaning that only the delayed hypersensitivity responses possessed by the transfer factor donors appeared in the recipients. Although the first experiments involved responses to common environmental antigens, the concept of specificity was also supported by the successful transfer of delayed hypersensitivity to coccidioidin with leukocyte lysates from skin test–positive Californians to lifelong residents of New York City,[10] by passive transfer of delayed allergy to ethylene oxide–treated human serum with lysates from sensitived donors,[11] and by transfer of allograft immunity.[18] In the last series of experiments the transfer factor donors were sensitized with skin grafts from unrelated subjects. The transfer factor in the form of leukocyte extracts was given to third subjects who were grafted with skin from both the original skin donor and the transfer factor donor. Grafts from the first skin donors were rejected in an accelerated fashion while grafts from the transfer factor donors were rejected at the normal time, suggesting that the transfer factor had specifically sensitized the recipients to react against the allogeneic antigens of the first donor. Parenthetically, it was noted that this effect of transfer factor on graft rejection was most striking when the transfer factor was injected in the vicinity of the graft, an observation compatible with local effects of transfer factor on immunologic inflammation. Recently, Zuckerman and co-workers[19] provided additional evidence for antigenic specificity of transfer factor by transferring delayed cutaneous hypersensitivity to keyhole limpet hemocyanin (KLH) to 10 successive recipients with a column-purified fraction of dialyzable transfer factor.

On the negative side, however, we have been unsuccessful in 5 attempts to transfer delayed allergy to a copolymer of glutamic acid-lysine-tyrosine (GLT) to anergic patients with mucocutaneous candidiasis with dialyzable transfer factor from sensitized donors. The same preparations were effective in transferring reactivity to extracts of candida and streptococci. The reason for these failures is unclear, but one must consider the possibility that the ability to respond to antigens such as GLT may be under genetic control and that our recipients were genetically unresponsive. Alternatively, since they were successfully sensitized to ubiquitous microbial antigens, it is possible that one must have a natural "priming" exposure to an antigen or a cross-reacting substance before one can be passively sensitized with transfer factor.

There are also a number of reports indicating that transfer factor may endow recipients with immune responses not possessed by the donors. In the study of transfer of reactivity to coccidioidin,[10] transfer factor from two coccidioidin-negative donors sensitized 6 of 8 recipients to this antigen. These donors were subsequently retested and found to have positive coccidioidin skin tests, although they were negative at the time of donation of the leukocytes for the transfer

studies. A patient with the Wiskott-Aldrich syndrome developed delayed allergy to trichophytin after receiving transfer factor from a skin test–negative donor,[20] and several patients with immunodeficiencies had positive responses to challenge doses of chlorodinitrobenzene (CDNB) after receiving transfer factor from insensitive donors.[21, 22] In other immunodeficient patients the capacity of lymphocytes to respond to allogeneic cells in mixed leukocyte reactions was restored following transfer factor therapy.[23]

Obviously these observations do not exclude specificity as a property of transfer factor, but they do suggest that transfer factor also contains activities that may activate or amplify immunologic reactions in nonspecific ways.

OTHER "ANTIGEN-INDEPENDENT" ACTIVITIES IN TRANSFER FACTOR

Identification and characterization of nonspecific or antigen-independent activities in transfer factor has been a special interest in this laboratory. Recently, we have reported that transfer factor contains a potent chemotactic activity.[24] The in vitro studies indicated that the activity was more pronounced with polymorphonuclear leukocytes than with monocytes, an observation that was somewhat unexpected in view of the fact that the inflammatory infiltrates in delayed hypersensitivity are predominantly mononuclear. However, when chemotaxis was studied in vivo by injecting small volumes of transfer factor into rhesus monkey skin and serially examining the histology of the reactions, a mononuclear cell response became apparent at 5 hours and was marked at 24 hours when the polymorphonuclear neutrophil infiltration was waning.[25] It is probable that the late influx of mononuclear cells is due in part to a second chemotactic substance that is released from the neutrophils.[26]

The relationship of this chemotactic activity to antigen-induced inflammatory responses has not been determined. Lawrence and Pappenheimer[13] reported that transfer factor was released from leukocytes in vitro by exposure of sensitive cells to antigen. If this occurs in vivo, locally produced transfer factor with its chemotactic activity could amplify weak inflammatory responses and be important in the local effects of transfer factor on skin graft rejection[18] mentioned earlier and the phenomenon of "local" transfer of delayed allergy with transfer factor in patients with sarcoidosis.[27]

There is a substantial body of evidence indicating that the intensity of several immunologic and inflammatory processes is modulated by the amounts of cyclic nucleotides in the effector cells. By increasing intracellular adenosine 3′,5′-monophosphate (cAMP), one reduces immunologic release of histamine and slow reacting substance of anaphlaxis (SRS-A) from leukocytes, mast cells, and lung,[28, 29] cytotoxic activity of sensitized T lymphocytes against allogeneic target cells,[30] release of lysosomal enzymes from neutrophils,[31, 32] and production of antibody-forming spleen cells in vitro.[33] An opposite effect is seen with agents that increase intracellular guanosine 3′,5′-monophosphate (cGMP). These agents enhance immunologic release of mediators,[34] cytotoxicity of T cells,[30] cell movement and release of lysosomal enzymes in neutrophils,[31, 32, 35] cell division of spleen cells,[36] and increase in the number of antibody plaque-forming cells in the spleens of nude mice.[33]

These observations suggested that enhancement of immunologic responses by transfer factor might involve changes in intracellular cGMP content. Indeed, when suspensions of peripheral blood leukocytes were exposed to transfer factor, 4- to 10-fold increases in intracellular cGMP content were found.[37] In other experiments, we found that the cGMP accumulation occurred predominantly, if not exclusively, in monocytes. During the short incubation intervals studied there were no effects on the cGMP content of purified lymphocytes. Moreover, transfer factor caused only slight accumulation of cAMP in leukocytes.

Not all of the components that cause cGMP accumulation have been identified. Dialyzable transfer factor contains ascorbate and serotonin, and both of these substances increase the cGMP content of leukocytes.[38, 39] By gel filtration of transfer factor we have obtained fractions that contain neither ascorbate nor serotonin, yet cause cGMP accumulation.[37] One of these fractions also contains a substance that causes conversion of delayed skin tests in anergic patients, and is probably identical to that recently reported by Zuckerman and co-workers.[19]

Alterations in monocyte or macrophage function due to changes in intracellular cyclic nucleotides could be expressed as changes in the efficiency of antigen processing or macrophage-lymphocyte interactions (the afferent limb of immunity) or as changes in phagocytic activity of cells in inflammatory sites. Although there is no supporting evidence at this time, it is possible that changes in intracellular cyclic nucleotides are involved in the DNA synthetic responses that underlie the in vitro assay for transfer factor recently described by Ascher and associates.[40]

EFFECTS OF TRANSFER FACTOR ON LYMPHOCYTE FUNCTIONS

When lymphocytes from immune normal subjects are exposed to antigens in vitro, the cells synthesize DNA and divide (lymphocyte transformation) and release lymphokines, the putative mediators of delayed allergic inflammation. These responses correlate closely with delayed skin responses; antigens to which subjects are unreactive do not stimulate DNA synthesis or lymphokine production.

There is evidence suggesting that lymphocyte transformation and lymphokine production may be functions of different populations of lymphocytes. It has been shown that when cells that are dividing in response to an antigen are killed by exposure to bromodeoxyuridine and light, the surviving, nonreplicating cells are still able to respond to the same antigen by producing MIF, a lymphokine.[41] Other evidence comes from studies of patients with immune deficiency diseases in whom there may not be concordance between the in vivo and in vitro expressions of cell-mediated immunity. For example, in most patients inability to develop a delayed skin response to an antigen is accompanied by inability to produce lymphokines to the same antigen. Yet, antigen-stimulated DNA synthesis may be either severely depressed or normal, suggesting that the immunologic lesions in some patients may be limited to the lymphokine-producing cell line, while in other patients the defect may affect both cell lines or a common precursor cell.[1]

Similar observations have been made in recipients of transfer factor. Normal

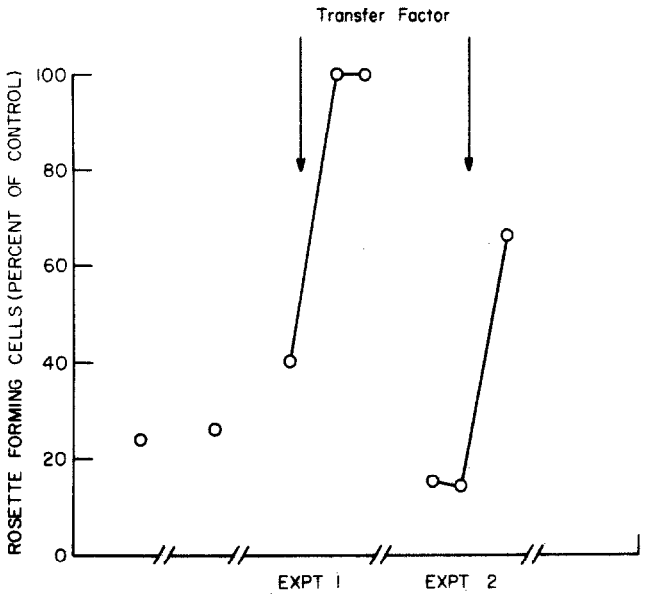

FIG. 1. Effect of transfer on number of E rosette–forming lymphocytes in the blood of a patient with chronic mucocutaneous candidiasis. The dose of transfer factor contained the extract from 6 × 10^8 lymphocytes. The rosette assays were done 1 day before the transfer factor in both experiments, and 3 and 4 days after transfer factor in experiment 1 and 3 days after transfer factor in experiment 2.

subjects respond to transfer factor by becoming reactive to specific skin tests, and their lymphocytes respond to antigens in vitro with both lymphokine production and lymphocyte transformation.[4] Immunodeficient patients with chronic mucocutaneous candidiasis or the Wiskott-Aldrich syndrome may also respond to transfer factor by conversion of delayed skin tests and development of lymphokine-producing cells.[42, 43] Correction of impaired lymphocyte transformation is usually not observed, and in the exceptional cases where it occurs the responses are usually of small magnitude and inconstant.[42, 44, 45] Thus, in these immunodeficient patients the effects of transfer factor appear to be exerted mainly on cells in the pathway that culminates in lymphokine production. It is unclear if if these effects are exerted directly on the lymphocytes or indirectly through precursor cells or macrophages.

Thymus-derived and bone marrow–derived lymphocytes may be distinguished from one another by specific properties of the cell membranes. Thymus-derived T cells spontaneously interact with sheep erythrocytes to form rosettes.[46] Bone marrow–derived B cells have immunoglobulins on the cell membranes as well as receptors for erythrocytes coated with 19S antibody and complement.[46] Wybran and associates[47] reported that patients with the Wiskott-Aldrich syndrome may have deficient numbers of rosette-forming T cells in their blood and in some cases the percentage of these cells increased following administration of transfer factor. A similar observation was made in an immunodeficient patient by Valdimarsson and associates,[48] and we have studied this phenomenon in 3 patients with chronic

mucocutaneous candidiasis and deficient numbers of T lymphocytes. In 1 of our patients, on 2 occasions administration of transfer factor was followed by normalization of the number of rosette-forming lymphocytes (Fig. 1); no effects were observed in the other 2 patients. We were unable to increase T cell rosette formation by incubating the patients' blood lymphocytes with transfer factor in vitro, perhaps indicating that the effect is exerted on noncirculating lymphoid cells.

There are also instances in which immunodeficient patients have shown improvement or normalization of blood lymphocyte responses to the T cell mitogen, phytohemagglutinin (PHA), following administration of transfer factor.[21, 22, 48-50] Although this response is only a semiquantitative measurement of T cell function, these observations and the reports of increased numbers of rosette-forming cells suggest that a component of transfer factor may promote differentiation or peripheralization of T lymphocytes.

RELATIONSHIP OF THE RECIPIENTS' IMMUNOLOGIC COMPETENCE TO THE RESPONSE TO TRANSFER FACTOR

Normal subjects are readily sensitized by adequate doses of transfer factor from strongly reactive donors, but the results with patients with immune defects are more variable. Patients with Hodgkin's disease usually fail to respond to transfer factor.[3] In sarcoidosis, patients may show the phenomenon of "local" transfer in which skin tests placed proximal to the injection of transfer factor become positive, while the same tests placed at remote sites remain negative.[27]

Recently, we studied the effects of transfer factor on delayed allergic responses in 4 patients with thymus aplasia who had no demonstrable cell-mediated immune responses, but normal immunoglobulin synthesis.[51] The diagnosis was confirmed at autopsy in 3 patients, and by the immunologic responses in the fourth. Following treatment with transfer factor none of the patients developed any positive delayed skin responses even though the potency of the transfer factor was established in anergic patients with chronic mucocutaneous candidiasis.[42]

Later, the fourth patient was given a fetal thymus transplant, following which he developed T lymphocytes as shown by normalization of lymphocyte transformation responses to mitogens and formation of rosettes with sheep erythrocytes. At this time he also showed delayed skin responses to streptococcal antigens, mumps antigen, and phytohemagglutinin and could be sensitized with CDNB. Nine months later the delayed skin responses could no longer be elicited, although the in vitro tests indicated that he still had normal numbers of circulating T cells. The patient was again given transfer factor and, in contrast to the pre-transplant experience, he now developed normal delayed cutaneous hypersensitivity responses.

The explanation for this observation is speculative. If considered with the effects of transfer factor on T cell rosette formation and PHA reactivity described above, it may indicate that transfer factor acts on cells in the thymus, or that patients must have "post-thymic" cells in order to respond to transfer factor. In this regard transfer factor may also serve as a probe for identificatioin of lesions in patients with immunodeficiency syndromes.

A MODEL FOR THE ACTION OF TRANSFER FACTOR

From the information summarized above, one can construct a tentative model for the immunologic activities of transfer factor. One must recognize that future experiments may show that the proposal is incorrect. Even so, if the model has prompted these experiments, it has been useful.

All of the currently recognized effects of transfer factor are on cellular immunity. In these responses, stem cells from the bone marrow differentiate into T lymphocytes under the influence of thymus. It is unclear if T cells are capable of responding to antigens at this stage of differentiation or require further maturation in peripheral lymphoid tissues. It is probable that the number of antigenic determinants to which a given cell can respond is quite small, perhaps only one. Contact with antigen, an event that may require macrophages or monocytes, stimulates specific antigen-responsive cells to synthesize DNA and divide, and to produce lymphokines. Presumably, cell division expands the clone of antigen-responsive cells and provides the cellular basis for anamnestic responses to subsequent encounters with the antigen. Lymphokine-producing cells may be separate from dividing cells and may be analogous to plasma cells in that they are terminally committed to producing effector molecules, but incapable of cell division. There is also evidence that bone marrow–derived B lymphocytes may produce lymphokines. The lymphokines, in turn, through their blastogenic and cytotoxic activities and their effects on cell migration and macrophage activation, produce inflammatory responses and the lesions of cellular immunity.

It seems unlikely that the multiple effects of transfer factor on immunologic inflammatory responses are due to a single activity on a single cell line. Instead, it is proposed that transfer factor acts at multiple sites and that the consequences of these activities are maturation of antigen-reactive lymphocytes and amplification of inflammatory responses.

For example, the appearance of rosette-forming and PHA-responsive lymphocytes in the blood of recipients of transfer factor implies an effect of transfer factor on T cell maturation or peripheralization, perhaps by a direct effect on the thymus. This suggestion is supported by the failure of transfer factor to cause conversion of delayed skin tests in patients with thymus aplasia and by the observation that a patient became responsive to transfer factor after receiving a thymus transplant.

Transfer factor also affects lymphocyte responses to antigens. This is illustrated by the appearance of lymphokine-producing, antigen-responsive cells in immunodeficient patients, by the development of both DNA-synthesizing and lymphokine-producing cells in normal subjects, and by the in vitro effects of transfer factor on lymphocyte transformation. Although an appealing explanation for these observations would be an antigen-specific informational effect of transfer factor, other mechanisms seem more likely. Depression of suppressed gene function could occur through the classical Jacob-Monod mechanism or could appear to occur through indirect mechanisms that improve the efficiency of antigen processing by phagocytes or lymphocytes, or enhance cell collaborations either between macrophages and lymphocytes or between lymphocyte subpopulations. This mechanism would place the "specificity" of transfer factor in the

lymphoid cells of the recipients rather than in the dialyzable material from the leukocyte lysates and in this regard is similar to the model recently proposed by Burnet.[52] It would also explain the failure of transfer factor to transfer cellular immune responses to patients with certain immune deficiencies and the failure of transfer factor to convey responsiveness to synthetic antigens.

As a corollary one would propose that treatment of a subject with transfer factor also endows his lymphoid cells with the property of responding to antigens by releasing more transfer factor. This possibility has been suggested by the reports of serial transfer of delayed allergy with transfer factor from recipients of transfer factor to second recipients,[3] an observation that we have confirmed. It also provides a mechanism for amplification of immune responses by generation of additional antigen-reactive cells to participate in lymphokine production.

Finally, transfer factor also has direct effects on inflammation through the chemotactic activity for polymorphonuclear leukocytes and monocytes, and perhaps through other effects of macrophage activation.

Thus, transfer factor has effects at different stages of immunologic inflammation. Are these effects due to different components of the dialysate or is there a single substance that causes these effects in different tissues? The answer to this question will require additional experiments. Isolation and identification of the components that cause changes in intracellular cyclic nucleotides, especially accumulation of cGMP, should be particularly informative because this response is known to increase the intensity of immunologic and inflammatory responses in other systems.

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