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Structural Nature and Functions of Transfer Factors

CHARLES H. KIRKPATRICK

Conrad D. Stephenson Laboratory for Research in Immunology
National Jewish Center for Immunology and Respiratory Medicine
Denver, Colorado 80206

INTRODUCTION

Successful transfer of cell-mediated immune responses from immune donors to nonimmune recipients was first described by Landsteiner and Chase in the early 1940s.[1,2] Guinea pigs were sensitized to express contact allergy to picryl chloride or delayed hypersensitivity to tuberculin. After washed peritoneal exudate cells from the sensitized donors were given to unsensitized recipients, the recipients acquired the ability to express the cell-mediated immune responses of the donors. Further studies indicated that long-lasting and regularly successful transfers were observed when there were syngeneic relationships between the donors and recipients, and only when intact, living donor cells were used. Attempts to transfer these reactivities with serum or dead cells were unsuccessful.

Later experiments by Crepea and Cooke[3] and Jeter and associates[4] disclosed that under certain conditions it was possible to transfer contact hypersensitivity to simple chemicals such as poison ivy antigens and chlorodinitrobenzene (CDNB) from sensitized guinea pigs to unsensitized recipients with unliving lymphoid cells, allogeneic cells, and even cell extracts. The disparate results between these reports and the work of Chase have never been completely explained.

The active component of the cell extracts was called "transfer factor," and transfer of delayed-type hypersensitivity in humans with transfer factors was studied extensively by Lawrence and associates[5] in the 1950s. Their experiments involved lysates of blood leukocytes from donors who had positive delayed-hypersensitivity to antigens such as tuberculin (PPD), diphtheria toxoid, streptococcal M protein, or coccidioidin. The recipients were skin test-negative to the test antigens. Within hours after receiving the transfer factor, the recipients were able to express delayed hypersensitivity to antigens to which the donors were reactive. The effects appeared to be antigen-specific.[6]

The mechanisms were unknown and the phenomenon itself was not understood. Things became even more confusing in 1963, when Lawrence and associates[7] reported that the transfer factor would pass through a dialysis membrane with a nominal cutoff of 12,000 Da.

Lawrence's findings were viewed with skepticism by some immunologists. It was difficult to understand how substances with such small molecular weights could transfer cell-mediated immune responses in an antigen-specific manner. Alternative explanations were offered. One was based on the point that Lawrence's experiments always employed antigens from common infectious agents or components of common vaccines. It was suggested that the recipients of the transfer factors already had "priming" exposures to them, and the transfer factor merely served as a booster or amplifier of these pre-existing sensitivities. Subsequent experiments with synthetic antigens[8] and controlled studies in mice[9] have ruled out this possibility. Others postulated that the

dialysates contained fragments of antigen that were highly immunogenic and that transfer factors actually produced their effects by active sensitization of the recipients. In this regard, it should be emphasized that the immunologic effects of a transfer factor on recipients could be demonstrated within 24-48 hours after administration. This model was proposed long before the mechanisms of antigen processing and presentation were known.[10] This possibility has not been entirely excluded, and it will be important to compare the structures of the immunizing antigens with the structures of the resulting transfer factors once they are determined.

In the 1970s there was a resurgence of interest in transfer factors when it was learned that they could provide a means for reconstituting certain immune deficiency syndromes.[11,12] However, most of the diseases that were studied were rare and the clinical reports often described only a few patients. Appropriate controls were not often done. It was several years before formal clinical trials with transfer factors in certain infections and malignant diseases were reported.

This report summarizes some of the recent work that defines certain properties of transfer factors.

TRANSFER FACTORS ARE ANTIGEN-SPECIFIC

It is now clear that the immunologic effects of transfer factors are specific for the antigens that were used to immunize the donor. This was suggested by some of the earlier experiments with both microbiological and synthetic antigens. Rapaport *et al.*[6] prepared dialysates of leukocytes of coccidioidin skin test-positive donors from California and tested them for transfer factor activity in life-long residents of New York City. Successful transfers of delayed-type hypersensitivity were observed in 28 of 35 recipients. However, one of 9 recipients of a preparation from a coccidioidin skin test-negative donor also acquired delayed hypersensitivity to coccidioidin. Maurer[13] in 1961 reported that disrupted leukocytes from persons who had been given ethylene oxide-treated human serum would transfer delayed hypersensitivity to this synthetic antigen to unsensitized recipients.

Specificity was confirmed by experiments in which mice were sensitized with various antigens and transfer factor-containing dialysates were prepared from the donor's spleen cells.[8,9] The transfer factors were administered to unimmunized recipients that were tested for delayed hypersensitivity to the immunizing antigen and to other antigens in the test panel 24 hours later. The recipients reacted only to the antigens to which the donors were sensitive (TABLE 1). These experiments also contained controls in which spleen cell dialysates from unimmunized donors were administered to recipients prior to footpad testing. These recipients did not develop delayed hypersensitivity responses. Thus, no adjuvant-like activity was present.

TRANSFER FACTORS BIND TO ANTIGENS

Subsequent experiments[14-16] described a unique and specific interaction between transfer factors and the antigens that were used to induce them. This was first noted in experiments in which a transfer factor was mixed with antigen to study possible adjuvant activity.[14] Instead the results showed that the transfer factor activity was neutralized or lost when antigen was added and this effect was antigen-specific. When these experiments were repeated with antigen on a plastic surface, it was possible to selectively remove a single transfer factor from a preparation that contained several

TABLE 1. Specificity of Transfer Factors

Experiment	Donor Sensitivity	Recipient Response		
		Antigen	Footpad (Δ mm $\times$ 10^{-2})	p
1	HRPO	HBSS	7.17 ± 3.87	—
		HRPO	35.00 ± 6.09	<0.01
		Ferritin	5.50 ± 2.46	NS
2	Cyto C	HBSS	0.33 ± 2.20	—
		Cyto C	22.00 ± 4.20	<0.001
		Ferritin	2.00 ± 3.08	NS
3	Ferritin	HBSS	3.50 ± 1.12	—
		Ferritin	18.33 ± 1.84	<0.001
		Cyto C	1.67 ± 1.99	NS
4	GAT[10]	HBSS	0.2 ± 2.27	—
		GAT[10]	34.40 ± 4.01	<0.001
		Cyto C	1.50 ± 1.31	NS
		GLA[5]	4.20 ± 0.58	NS
5	GLA[5]	HBSS	1.17 ± 2.36	—
		GLA[5]	67.33 ± 2.29	<0.001
		Cyto C	−0.67 ± 2.54	NS
		GAT[10]	4.67 ± 0.95	NS

NOTE: HBSS = Hanks' balanced salt solution; HRPO = horseradish peroxidase; Cyto C = pigeon breast muscle cytochrome C; GAT[10] = random terpolymer of glutamic acid, alanine and tyrosine; GLA[5] = random terpolymer of glutamic acid, lysine and alanine; NS = not significant (p > 0.05). These data are from Petersen et al.[9] and Kirkpatrick et al.[8]

transfer factors.[16] The transfer factor could then be eluted from the antigen and recovered in an immunologically active and antigen-specific form.

These experiments provided formal proof of the existence of multiple molecules with transfer factor activity and provided additional evidence for antigen specificity of transfer factor molecules.

IMMUNOLOGIC EFFECTS OF TRANSFER FACTORS

The effects of administration of transfer factors on immune responses of recipients has been evaluated in some detail. However, most of these studies have been done in patients with immunodeficiency diseases and infections with opportunistic microorganisms,[11,12] and it is possible that the observations made in these subjects may not reflect the full spectrum of effects of transfer factors on immune functions.

Two effects are consistently observed in recipients: expression of delayed-type hypersensitivity and *in vitro* secretion of certain lymphokines by antigen-activated T lymphocytes.[11,12,17] In each study there was good concordance between "transfer" of antigen reactivity as measured by these assays and clinical benefits in the patients. On the other hand, acquisition of antigen reactivity in the lymphocyte proliferation assay occurs less commonly in recipients of transfer factors, a finding that suggests that transfer factors act on "effector" T lymphocytes rather than "memory" T lymphocytes. However, Dwyer[18] in his study of transfer factor therapy of recurrent infections with

Herpes simplex noted that the treatment was accompanied by increases in *in vitro* T lymphocyte proliferation responses to *H. hominis* antigen.

Less work has been done with the cytotoxicity assay. Levin and associates[19] reported that patients with osteogenic sarcoma developed T lymphocytes that were cytotoxic for osteogenic sarcoma cells *in vitro* after the patients had been treated with a dialysate from the blood leukocytes of household relatives.

It is noteworthy that transfer factors do not prime recipients to express antibody responses.

Interesting studies of genetic factors on production of and responsiveness to transfer factors have been reported.[20] Mice do not produce transfer factors after immunization with antigens to which they are genetically determined low responders, but they produce transfer factors to antigens against which they can express delayed-type hypersensitivity. High-responder mice produce specific transfers after immunization. When a transfer factor from a high-responder donor is given to a low-responder recipient, the recipient acquires the ability to express delayed hypersensitivity to the test antigen. This change in the phenotype of the low-responder recipient may explain the effects of transfer factors in patients with certain immune deficiency syndromes.

PROPERTIES OF TRANSFER FACTORS

Many models have been proposed for the structure of transfer factors. Most propose that they are nucleopeptides in which the specificity is encoded within the structure of the peptide moiety. There is general agreement that the molecular weights are greater than 3,500 Da and less than 6,000 Da.

Recently, we have succeeded in purifying two specific transfer factors to apparent homogeneity.[21] The entire mass of the purified product could be attributed to the amino acid composition. Spectral analyses did not provide evidence for any nucleic acids in the final product. The molecular weights of our purified materials are 5,000–5,500 Da.

TRANSFER FACTORS IN HUMAN DISEASES

There are many reports in which small numbers of patients with recurrent or chronic infections have responded to treatment with leukocyte dialysates that were presumed to contain a transfer factor. In many cases the treatments were "last ditch" efforts with failing patients, and long-term follow-up results are not given.

However, there are several studies in which adequate numbers of patients were studied and appropriate controls were used and conclusions concerning efficacy could be reached. For example, several investigators have reported beneficial effects of "transfer factor" (these studies were done with leukocyte dialysates) therapy of chronic or recurrent infections with *Herpes simplex*.[18,22,23] In each case the treatment produced either complete remissions or significant reductions in the frequency and severity of exacerbations. One study[18] was especially informative because an identical dialysate from donors who lacked cell-mediated immunity to *Herpes simplex* was also studied. When the patients were blindly reassigned from *Herpes*-specific material to the inactive material, they relapsed. However, they responded again when treatment was changed to the *Herpes*-specific preparation.

A recent report[24] describes beneficial effects of transfer factor therapy of intestinal cryptosporidiosis, a parasitic infection that causes severe or even fatal diarrhea in

patients with AIDS. Six of the 7 treated patients had improvement, but there was only 1 remission in the seven patients who received a placebo preparation.

Chickenpox has always been a serious infection in children with acute leukemia. At a time when chickenpox immune globulin was in short supply, there was a comparison of zoster (chickenpox)-specific transfer factor with placebo in a group of leukemic children.[25] In the placebo group 15 children had significant exposures to chickenpox; 13 of them developed chickenpox and 3 of these had disseminated infections. In the transfer factor group, 16 children were exposed, but only one developed chickenpox and this infection was very mild. This important study suggests that specific transfer factors may provide a mechanism for induction of protective immunity for infections in which the cell-mediated immune system is essential.

Our studies[26] in patients with chronic mucocutaneous candidiasis, a disease in which immunodeficient children have disfiguring skin infections due to infections with *C. albicans*, showed that treatment with *Candida*-specific transfer factor restored *Candida*-specific cell-mediated immunity. However, this did not allow the patients to be completely clear of extensive infections. Therefore we adopted a protocol in which the infections were first cleared with an antifungal drug and the transfer factor was used to restore immune competence. This combination protocol was tested in a clinical trial in which transfer factors from either *Candida*-immune donors or *Candida*-nonimmune donors were compared. The results clearly showed a relationship between successful restoration of *Candida*-specific cellular immunity and prolonged remissions from candidiasis with *Candida*-specific transfer factor. None of the recipients of the preparation that lacked *Candida*-specific transfer factor activity acquired immunity to *Candida*, and these patients suffered relapse.

SUMMARY AND CONCLUSIONS

Transfer factors are molecules that "educate" recipients to express cell-mediated immunity. This effect is antigen-specific. The most consistent effects of transfer factors on the immune system are expression of delayed-type hypersensitivity and production of lymphokines such as macrophage migration inhibitory factor (MIF), which is probably identical to γ-interferon in response to exposure to antigen.

Transfer factors bind to antigens in an immunologically specific manner. This discovery has enabled us to isolate individual transfer factors from mixtures that contain several transfer factors. This reactivity probably explains the specificity of individual transfer factors, and it has provided a method for purification of individual transfer factors to apparent homogeneity. The purified materials are immunologically active and antigen-specific. They have molecular weights of approximately 5,000 Da and appear to be composed entirely of amino acids.

Transfer factors appear to offer a novel means of molecular immunotherapy for certain patients with defective cell-mediated immunity.

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