Transfer factor and the immune system

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During the past 20 years, understanding of the mechanisms involved in immune responses has greatly increased. With this better understanding has come the ability to intervene therapeutically in the functioning of the immune system. This intervention may be suppressive (e.g., corticosteroids, cytotoxic drugs, and antilymphocyte serum) or reconstitutive (e.g., gamma globulin, bone marrow transplantation, and fetal thymus transplantation). One of the oldest known but most recently clinically applied modalities in the latter category is transfer factor (TF).[1] Totally rational use of TF at this time is impossible, since its exact role in the immune system is still controversial. The lack of animal systems in which to study TF has greatly hindered advances in this area.

We review the functional relationships of the basic parts of the immune system and consider the possible role of TF in the immune system. It is important to determine which patients would most likely benefit by a trial of TF therapy and to devise new means of interpreting the effects of such therapy.

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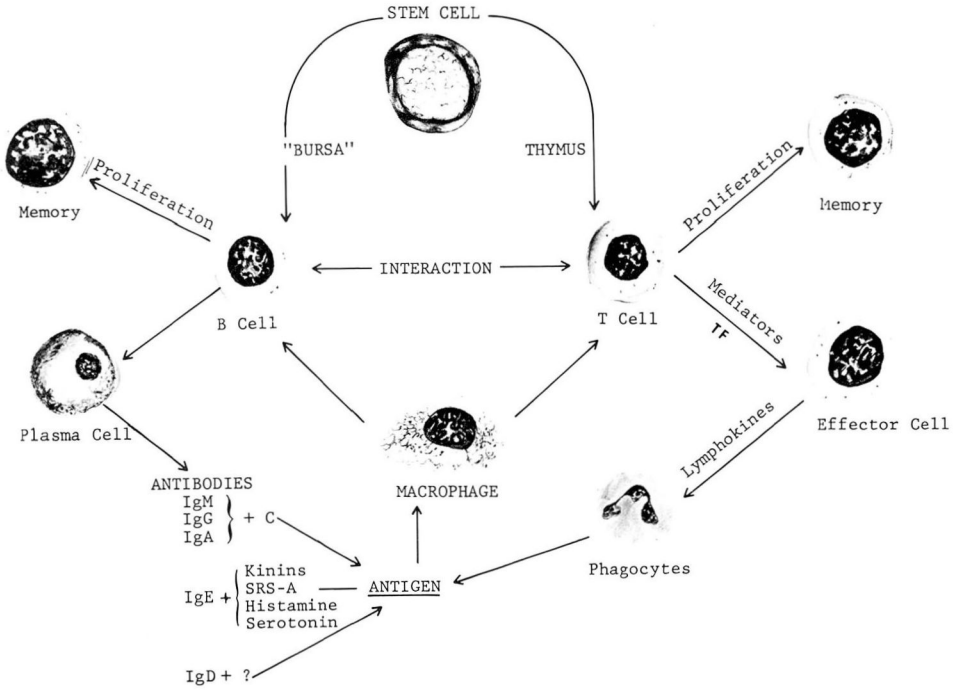
Organization of the immune system

The cellular basis of the immune system is the



IMMUNE SYSTEM

Figure. The immune system. This scheme demonstrates the basic functional interrelationship and the origin of the cellular and humoral immune mechanism. The proposed site of transfer factor (TF) intervention is indicated.

lymphocyte.[2] Lymphocytes constitute a heterogeneous population of immunologically competent cells which can be divided into two categories designated by the letters T and B. Both groups of cells arise in the bone marrow from pluripotential stem cells (*Figure*). From the bone marrow some of these cells are transported to the thymus gland where they undergo unknown influences resulting in the functioning maturation to T-lymphocytes. These cells, which are long-lived, leave the thymus gland and are distributed throughout the peripheral lymphoid tissues in characteristic locations: paracortical areas of lymph nodes, white pulp of spleen surrounding the penicilliary arteries, and bloodstream where they constitute the majority of the circulating small lymphocytes (60% to 80%). These cells provide the basis of "cell-mediated immunity" which is important in resisting intracellular infections, homograft rejection, graft versus host reactions, tumor surveillance, and probably in autoimmunity. The classic evaluation for competence of the T-cell system is delayed hypersensitivity skin testing.

The other major group of small lymphocytes which arises in the bone marrow matures in an unknown location other than the thymus gland; in chickens this occurs in the bursa of Fabricius, a hind-gut organ. In mam-

mals suggested locations for this maturation include gut-associated lymphoid tissue (GALT), appendix, sacculus rotundus, and bone marrow. There is no strong evidence to support any of these hypotheses. B-lymphocytes are found not only in the circulation (20% to 40% of small lymphocytes) but also in the germinal centers and far cortical areas of lymph nodes, spleen, and GALT. They are important in the production of humoral antibodies, which are the mainstay of the defense against pyogenic bacterial infections.

Deficiencies may occur in either part of the lymphoid system. B-cell deficiency is exemplified by X-linked infantile agammaglobulinemia (Bruton type) in which B-cell numbers may be greatly reduced, and immunoglobulin and antibody production are virtually absent. T-cell deficiency occurs in thymic hypoplasia (Di George syndrome) in which T-cells are reduced in number and cell-mediated immune functions are absent. A number of very severe combined immunodeficiency disorders also exist, as do milder variable immunodeficiency states affecting either system.

The immune response

An antigen entering this system normally first encounters the macrophage which phagocytizes it, processes it, and presents it to the appropriately programmed T- and B-cells.[3] These lymphocytes then proliferate and form a memory bank for future reactions (secondary on anamnestic) with the same antigen. The ability of lymphocytes to proliferate may be tested by various plant derivatives called mitogens. T-cell proliferation may be evoked by phytohemagglutinin and concanavalin-A. No specific B-cell mitogens are known, although pokeweed mitogen apparently stimulates B-cells and some subpopulations of T-cells.

In addition to proliferation, stimulation of T-cells induces recruitment of effector lymphocytes to produce lymphokines. The mechanism by which this recruitment occurs is not known, but it is postulated that chemical mediators such as TF may play a role. Lymphokines produced by effector lymphocytes include migration inhibition factor (MIF); chemotactic factors for neutrophils, eosinophils, and macrophages; macrophage agglutinating factor; lymphocytotoxin, interferon, and others. These agents act on the stimulating antigen either directly or through a phagocytic intermediary, completing the chain of the cell-mediated immune response. If the antigen resides intradermally, and if the subject has been presensitized (has a memory bank) the result is a positive delayed skin test.

B-cell stimulation initiates a chain of events leading to the production of mature plasma cells, which produce specific antibody in the five immunoglobulin classes (IgG, IgM, IgA, IgD, and IgE). These proteins have specific affinity for the antigen which triggered their formation; they bind with this antigen and affect it by activating a number of different effector mechanisms. IgG- and IgM-containing immune complexes activate the classic complement pathway.[4] IgA-containing immune complexes probably activate the alternate complement pathway. IgE, fixed to the surface of mast cells, binds antigen, then releases kinin activators, histamine, and slow-reacting

substance of anaphylaxis (SRS-A). The action of IgD is not known.

Transfer factor

Detailed understanding of the immune system has recently assumed more practical importance clinically, with increased awareness of opportunities for intervening to correct defective immunologic processes.[5-7] Several methods have been used, the oldest of which is gamma globulin replacement in patients with agammaglobulinemia. Other methods of reconstitution have involved transplanting living cells, either from bone marrow, thymus, or fetal liver; although these methods have the ability to reconstitute cellular immune processes, they have as a major drawback the risk of graft versus host disease in the recipient. The work of Lawrence [1, 5] has pointed out the intriguing possibility of reconstituting cell-mediated immune processes using a cell-free extract of lymphocytes which he calls TF, eliminating the risk of graft versus host disease. Lawrence[8] first transferred cellular immunity to a nonsensitized individual with TF in 1954. This extract was made from lymphocytes of a tuberculin positive donor. By breaking up the lymphocytes through freezing, thawing, and subsequent treatment with ribonuclease and deoxyribonuclease, dialysis, and lyophilization of the end-product, Lawrence[8] produced the active material. An alternate method of obtaining TF is available. To a presensitized lymphocyte culture the specific antigen is added. TF is released from the lymphocytes into the supernatant and subsequently harvested. After subcutaneous administration of reconstituted TF into a tuberculin negative recipient, a positive skin reaction to tuberculin developed in the recipient within 24 hours. The skin test remained positive for 6 months. TF made from this positive recipient's lymphocytes could successfully transfer tuberculin reactivity to a second tuberculin negative recipient. TFs are the active uncharacterized products of lymphocytes which pass through a dialysis membrane; their molecular weight is less than 10,000 daltons, probably about 4,000 daltons. TF activity persists after treatment with trypsin, deoxyribonuclease, and ribonuclease. TF is nonantigenic, nontoxic, and nonpyrogenic. With TF one can confer the property of immunological reactivity upon T-lymphocytes; this can be demonstrated by antigenic challenge (e.g., tuberculin). A single effective dose of TF requires 8.5×10^7 lymphocytes as starting material. The characteristics of TF are being further defined by fractionation studies.

The mode of action of TF remains mysterious, because experimental animal models are lacking. Lawrence[5] proposed that TF derepresses lymphocytes which then demonstrate specific antigenic activity. Some investigators believe that TF induces specific receptor sites on T-cells or possibly is the actual receptor site. Others believe that the action of TF is nonspecific, that it produces a generalized adjuvant effect;[9] thus, the question of specificity or nonspecificity of TF remains controversial.

Clinically, TF has been employed successfully in infectious diseases and in certain deficiencies of cell-mediated immunity (*Table*).[10-16] An example of the clinical application of TF was reported be Whitcomb and Rocklin[11]

in the successful treatment of a patient with resistent tuberculosis. The patient was initially tuberculin skin-test negative and a nonresponder to all commonly used chemotherapeutic agents. TF obtained from a tuberculin-positive donor was injected subcutaneously. Subsequently the patient's skin test to tuberculin became positive. Only then did he respond to antituberculous drugs.

One might speculate that the action of TF produced during a normal immune response is recruitment of T effector cells resulting in amplification of the immune response (*Figure*). Evidence for this has been reported by Kirkpatrick et al[17] in studies of patients with chronic mucocutaneous candidiasis treated with TF, and has been confirmed in our laboratory by treating a patient who had ataxia telangiectasia with TF.[15] TF appears to restore the ability to produce lymphokines, such as migration inhibition factor, without affecting the ability of T-cells to proliferate in response to nonspecific mitogens such as phytohemagglutinin and concanavalin-A. There is also some evidence for effects of TF on macrophages, including the restoration of IgG receptor sites on these cells as well as restoration of the ability of these cells to respond to chemotactic stimuli.[18, 19] Whether these are direct effects or are mediated through lymphokine production is not clear.

The most promising application of TF would seem to be in patients in whom cell-mediated immunity is deficient while humoral immunity is relatively normal. Because the administration of TF is benign, a trial of reconstitution with this agent seems warranted before other more drastic procedures involving live tissue transplantation are undertaken. TF may also be useful in individuals whose immune systems are intact but need bolstering, such as patients with tuberculosis or certain fungal infections not responsive to chemotherapy and patients with certain types of tumors. The use of TF should always depend on rational donor selection; the donor should be demonstrated to possess specific immunological responsiveness to the tumor or infectious agent under attack by appropriate skin testing or *in vitro* cellular reactivity. The recipient's response to TF should be studied by following conversion of skin tests from negative to positive, and by noting the ability to make MIF against

Table. Clinical applications of transfer factor[10]

- I. Therapy of infectious disease
  - A. Tuberculosis[11]
  - B. Candidiasis
  - C. Lepromatous leprosy[12]
  - D. Vaccinia
  - E. Herpes zoster
  - F. Multiple sclerosis[13]
- II. Therapy of cell-mediated deficiency states[14]
  - A. Congenital
    1. Ataxia-telangiectasia[15]
    2. Dysgammaglobulinemia
    3. Combined immunodeficiency
    4. Wiskott-Aldrich syndrome
  - B. Acquired
    1. Malignancy
       a. Leukemia
       b. Lymphoma
       c. Lymphosarcoma
       d. Osteogenic sarcoma[16]
    2. Nutritional
       a. Kwashiorkor
       b. Marasmus
    3. Sarcoid

specific antigen. Other immunologic parameters should be followed as well; if lymphocyte transformation to nonspecific mitogens was reduced initially, it is likely to remain low. Variable data have been obtained on the response of circulating numbers of T-cells to TF administration; if low initially it may either remain low or may rise during a period of several weeks. Since there is no *in vitro* assay for the potency of TF and since the kinetics of its handling within the human body are not known, optimal timing of repeated administration of TF is guesswork to a certain extent. One can use skin tests and MIF production as a rough guide; however, there is evidence to suggest that clinical effectiveness of TF may wane before positive skin tests disappear.

TF is likely to remain controversial until more information is gained concerning its nature and its mechanism of action. This will require purification, chemical characterization, and the development of nonhuman models in which TF may be manipulated more freely. At present there are limited indications for the use of TF in patients such as those described above, in whom conventional modalities of treatment have failed. As a last resort, before using potentially life-threatening transplantation procedures TF may be employed as a method of immunologic intervention. Still to be studied are the effects of TF in autoimmunity, which has been associated with depressed cell-mediated immunity.

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