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TRANSFER FACTOR: PAST, PRESENT AND FUTURE

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TRANSFER FACTOR—PAST

Historical Perspective

Adoptive transfer of cell-mediated immunity to specific antigens in humans was first demonstrated by Lawrence (1) in 1949, thus opening a new avenue of research that has led both to increased understanding of basic immune mechanisms and to the development of immunomodulation therapy. Lawrence originally showed that transfer of whole viable lymphocytes from a normal tuberculin skin test-positive donor to a normal skin test-negative recipient resulted in a conversion of the recipient to skin-test positivity, but this observation created little interest in the immunologic community for several reasons. In 1955, the lymphocyte was mentioned for the first time as a possible immunologic organ. Traditionally, the lymphocyte had been studied in hematologic rather than in immunologic terms. In the third edition of his classic textbook on the anatomy of the lymphoid system, Professor A. Joffy of the University of Bristol proposed three questions to lymphocytes: "Where do you come from, where do you go and what do you do, if anything?" In 1962(2), J. Miller showed that the immune system in chickens could be divided into two components, namely the B lymphocyte derived from the bursal system (B cell) producing antibodies that protect against infection from microorganisms (pneumococcus, meningococcus, streptococcus, gonococcus, etc) and thymic-dependent (T cell) systems that protect against fungi, parasites, viruses, mycobacteria, and cancer metastases. Previously, Lawrence (3) demonstrated that delayed cutaneous hypersensitivity (DH) responsiveness could also be transferred by soluble extracts of leukocytes

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from 20 ml of blood and termed the factor responsible for this phenomenon "transfer factor" (TF). At that time, the significance of DH was unknown so TF received little attention. DH was thought due to antigen-binding at the skin injection site by antibodies of very high affinity. All immunological phenomena were thought to be an effect of antibodies; such antibodies had molecular weights of 150,000 or greater. Therefore, no attention was paid to Lawrence's finding of transfer of DH by a soluble factor of a molecular weight less than 20,000, as demonstrated by passage through dialysis membrane which retains molecules >20,000 molecular weight.

We now know that the extract obtained by the Lawrence method contains at least 200 different moieties with molecular weights from 1000 to 20,000 and that only one of them is antigen-specific TF with a molecular weight of approximately 3500 (4). Hence, we call preparations made by the Lawrence method dialyzable leukocyte extract (DLE). In 1970, we showed (5) that DLE could not only transfer skin-test positivity but also produce or intitiate other cell-mediated immunity (CMI) reactions in various immunodeficiency states (e.g. in Wiskott-Aldrich syndrome patients, the antigen-specific transfer of CMI can provide protection for a period of approximately six months, paralleled by antigen-specific production of lymphokines, such as migration inhibitory factor (MIF) for macrophages [Figures 2, 3] and leukocyte migration inhibition factor (LIF) for granulocytes [Figure 4 in section II.A.3.] [6]). (The generalized term "leukocyte migration inhibition" was adopted because the target cell was unknown when the biological activity was

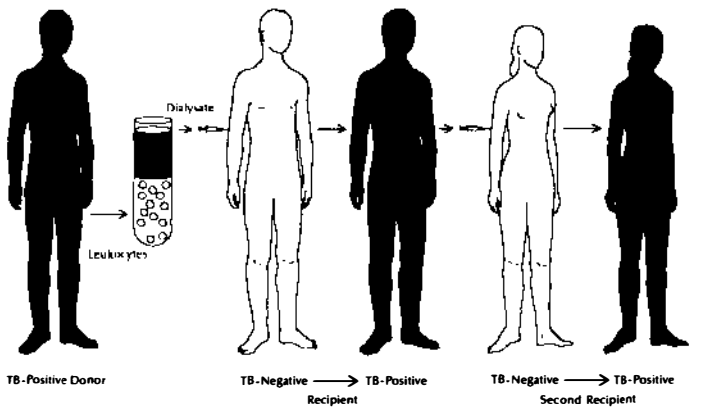

Figure 1 Transfer of skin test reactivity from a normal individual, positive by skin test to tuberculin-purified protein derivative (PPD), to a normal recipient previously negative by delayed hypersensitivity skin test to PPD, using a cell-free extract derived from donor leukocytes. Note that the cell-free extract derived from the primary recipient after transfer converted a second normal subject previously skin-test negative to PPD to skin-test positive. PPD positive status in black, PPD negative status in white (54).

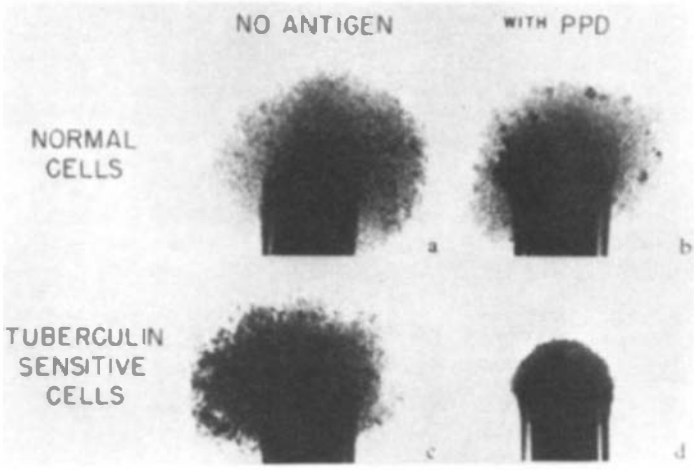

Figure 2 Macrophage migration inhibition produced when PBL from individuals either nonimmune (normal cells, a and b) or sensitive to tuberculin (tuberculin-sensitive cells, c and d) were incubated with medium alone (no antigen) or with PPD added. The extent of migration inhibition can be calculated after measuring the areas of migration either as

$$\% \text{ migration} = \frac{\text{average migration of cells in the presence of antigen}}{\text{average migration of cells without antigen}}$$

or as

$$\% \text{ inhibition} = 100 - \% \text{ migration.}$$

Generally, inhibition >20% is significant in MMI systems. (6)

first shown; when the target cell was later found to be the granulocyte, the old term was retained.) DLE can also transfer the ability to produce MIF in an antigen-specific fashion from one normal individual who has this ability to another normal individual who does not (e.g. one who has never been exposed to the specific antigen), exactly paralleling the skin test reactivity. However, both the nature and mechanisms of action of transfer factor and its potential as an immunotherapeutic agent were until recently highly controversial, partly because of semantic confusion of the terms defined below.

Definition of Terms

Disruption of buffy coat cells or lymphoid cells into aqueous solution, followed by dialysis to obtain a low molecular weight fraction, yields a crude preparation Dialyzable Leukocyte Extract (DLE). As stated above, this extract contains more than 200 distinct moieties, of which a number have

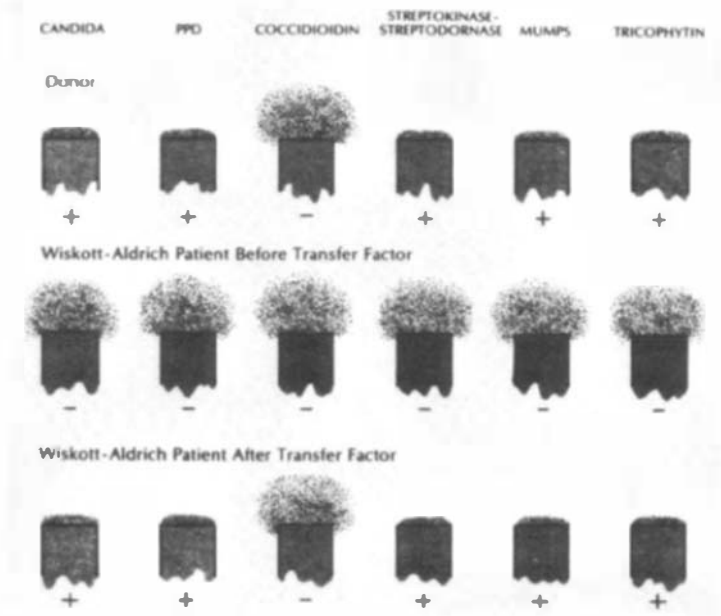

Figure 3 The specificity of Transfer Factor using the macrophage migration inhibition factor (MIF) assay. TF was admainistered to a Wiskott-Aldrich patient anergic for all six antigens listed above. Top: The donor was positive for all but coccidioidin. Middle: Before therapy, the patient was negative for all six antigens. Bottom: After TF therapy, the patient was negative for all six antigens. Bottom: After TF therapy, the patient became positive for the five antigens to which the donor was reactive, and remained negative for coccidioidin. This was the first patient ever treated with TF. (5, 54)

been identified, for example, thymosin (7) and prostaglandin (8). Certain components of DLE possess adjuvant properties (nonantigen-specific immunostimulation effects) whereas others have been shown to suppress immune responsiveness (9). We now reserve the term transfer factor (TF) for the components of DLE that mediate T-lymphocyte responses of an antigen-specific nature (10). This fraction in turn contains a multitude of factors corresponding to the sum of immune experiences of the individual subject. We use the term DLE-TF to describe our product (DLE enriched in TF) by one additional procedure that eliminates molecules greater than 12,000 molecular weight. Furthermore, we use the term DLE-TF_{as} for DLE-TF which is known to be specific for a desired antigen. Until recently, some immunologists used the term TF to describe the entire DLE. This resulted in much semantic confusion since DLE contain nonspecific immunologic adjuvant moieties.

General Characteristics of Transfer Factor

Human TFs are small molecules approximately 2000 to 3500 daltons. TF is heat labile but cold stable; indeed, the biologic activity of the factors remains unimpaired after several years of storage at −20°C to −70°C. Studies of the effect of enzymes on the antigen-specific biologic activity of TF indicate that the factors contain RNA bases attached to small peptides (11). At present it appears that these molecules are complexed in vivo, and that breaking of the RNA peptide bond destroys their biologic activity (12). Further data on TF structure is given in section IVB.

If indeed a unique TF exists of each antigen specificity, then it is likely that individual TFs differ structurally in a manner similar to the subtle variations in antigen-binding sites at the hypervariable region of immunoglobulins. In view of the molecular weight and composition, it is likely that each TF contains at least six amino acids (9). Therefore, if one considers the possible combinations of the 20 known amino acids, there are several million variations in the primary structure, and thus the number of TFs specific for different antigens exist in every normal immune inventory.

TRANSFER FACTOR: PRESENT

Immunotherapy with DLE Containing TF

INDICATIONS FOR THERAPY The criteria for selection of suitable candidates for therapy depend to some extent on the nature of the disease under consideration. Thus, treatment with DLE containing TF would be indicated in a patient with a documented selective defect in cell-mediated immunity (e.g. familial chronic mucocutaneous candidiasis) or in certain neoplastic diseases refractory to other therapy. Such a clear-cut indication is, however, relatively rare. More frequently, an antigen-specific defect may be suspected but not proven, as in certain patients with chronic or recurrent severe viral infections. A more uncertain area includes diseases of unknown etiology but presumed by many to be initiated by viral infection (e.g. Behçet's syndrome), in which evidence of cell-mediated immune defects has been obtained. Results of reported therapeutic trials with DLE must therefore be interpreted with caution, as the activity of adjuvant or inhibitory factors present in crude preparations of DLE may in certain instances be responsible for any therapeutic effect unless "control DLE", devoid of TF of desired specificity, has previously been administered to the recipient without clinical benefit (i.e. unless each patient served as his or her own control, as described below) (13).

SIDE EFFECTS OF DIALYZABLE LEUKOCYTE EXTRACT THERAPY DLE is usually administered by subcutaneous or intramuscular injection, although

oral administration is reportedly equally effective (14, 15). DLE is remarkably free from adverse side effects. Transient pyrexia may occur, but there have been no reports of hypersensitivity reactions or of long-term adverse effects. Patients with osteosarcoma or breast cancer with bone metastases treated with (and responding to) DLE containing TF of appropriate specificity have experienced severe pain at the site of primary or metastatic lesions, caused by necrosis of tumor cells; DLE devoid of TF of corresponding specificity did not cause pain (Fudenberg & Levin, unpublished observations).

CHOICE OF DONOR The selection of suitable donors of DLE is a major factor in the efficacy of therapy. Clearly, all potential donors must be screened to confirm general immunocompetence, but of equal importance is the investigation of donors for antigen-specific immune responses where appropriate, for example, when the patient is known to have an antigen-selective defect for a given microbial agent or tumor antigen. In our early studies, lack of MIF production was used as the criteria for an antigen-selective defect (see Figure 2), but in the last 10 years we have used lack of LIF production (see Figure 4).

LIF production is measured by the direct assay for leukocyte migration inhibition in agarose (LMI). Peripheral blood leukocytes, or PBL (2.0×10^8 cells/ml), are incubated with Medium 199 only (control) or with medium plus test antigen at 37°C in a humidified incubator gassed with 5% CO_2 in air (17). The optimal concentrations for all antigens are determined previously by dose response (18, 19). The PBL are then placed into wells punched in solidified agarose containing Tissue Culture 199-C in petri dishes and allowed to migrate for 18 hr at 37°C (20, 21). During this incubation period, the granulocytes (polymorphonuclear neutrophils, or PMN) randomly migrate out of the application wells under the agarose to form a circular zone of cells. Responsiveness to antigen or mitogen is expressed as a migration index (MI): Note that migration can also be expressed as percentage inhibition by the equation

$$\% \text{ inhibition} = (1 - \text{MI}) \times 100$$

For the highest concentrations of each test substance used, an MI less than 0.80 (i.e. more than 20% inhibition) indicates responsiveness [induction of LIF production (22)]. When the patient is known to have an antigen-selective defect, the potential donor is identified as having CMI for the relevant antigen by the same assay: direct leukocyte migration inhibition in agarose. When the relevant antigen is unknown, potential donors of DLE should be selected from close household contacts, not necessarily relatives of the patient, on the

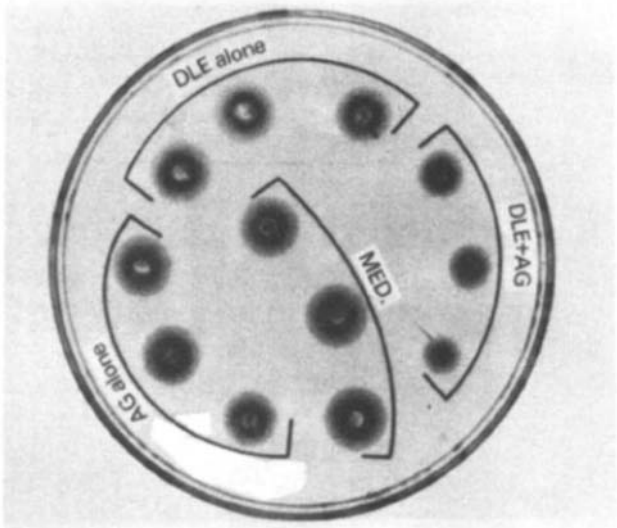

Figure 4 Standard plate pattern for testing effects of DLE in vitro by leukocyte migration inhibition in agarose (LMI). MED = PNL incubated with medium only. AG = PBL incubated with antigen. DLE = PBL incubated with DLE containing antigen-specific TF. DLE + AG = PBL incubated with DLE plus specific antigen. The migration areas shown were obtained after 18 hours. PBL were initially nonresponsive to the antigen, as shown by the MED and AG diameters of migration. At the concentration of DLE used, DLE alone had no effect ($MI_A = 1.00$). Note, however, the decreased migration when PBL were incubated with DLE + AG (20).

premise that such contacts have shared exposure to the agent and mounted a normal cell-mediated immune response to it, and therefore have not developed the disease (23).

PREPARATION OF DIALYZABLE LEUKOCYTE EXTRACT We usually obtain 10^{10} mononuclear cells (1% of total body lymphocytes; no more than 5% granulocytes) by lymphopheresis, using an IBM/Cobe cell separator, with the collection bag placed on a tray containing ICB. The cells are then disrupted by freeze-thawing and sonication. The leukocyte lysate is subsequently dialyzed to obtain a low molecular weight fraction (less than 12,000). An aliquot of the DLE is then subjected to two week culture to ensure sterility and absence of endotoxin. As mentioned above, this material, now enriched in TF, we term DLE-TF. To permit some comparison between batches, one international unit has been (somewhat arbitrarily) defined as that amount of extract derived from 5×10^8 leukocytes (24) and is usually placed in a 1 ml vial. Thus, since the ratio of polymorphonuclear cells to mononuclear cells in pooled buffy coat is approximately 2:1, our preparations (95% mononuclear cells) contained 40 international units per 1 ml vial. It should be emphasized

Table 1 Migration indices and their significance

Term	Equation	Definition
1. Migration index (MI)	Area of random migration of PMN when subject's PBL are incubated in culture medium plus antigen. Area of random migration of PMN when subject's PBL are incubated in culture medium only.	The MI value indicates the extent of antigen responsiveness of the subject's MNL prior to incubation with TF. MI values <0.90 indicate some response (some LIF-induced inhibition of migration).
2. MI_A	Area of random migration of PMN when subject's PBL are incubated with a test substance. (TF or some other test substance) and culture medium only. Area of random migration of PMN when subject's PBL are incubated in culture medium only.	The MI_A value quantitates changes in random PMN migration produced independent of antigen stimulation and not promoted by LIF. An MI_A value <0.80 indicates such an antigen-independent effect.
3. MI_B	Area of migration of PMN when subject's PBL are incubated with a test substance plus antigen and culture medium. Area of migration of PMN when subject's PBL are incubated with antigen and culture medium.	The MI_B value quantitates changes in random migration that are dependent on LIF release by MNL made responsive to antigen by incubation with TF. The term differs from the MI_A since the MI_B indicates that a two-step process has occurred: (a) induction of responsiveness by TF and then (b) induction of LIF release from newly sensitized MNL induced by the binding of the appropriate antigen.
4. D_B	$1-(MI_B/MI_A)$	The D_B is the antigen-dependent, LIF-dependent percentage decrease in random PMN migration induced by pulsing previously nonresponsive MNL with TF and antigen. This term indicates the magnitude of MNL activation induced by TF. By dividing the MI_B value by the MI_A value one can eliminate any contribution to a reduction in migration promoted by antigen or other constituents alone, thereby obtaining the reduction in random migration attributable only to LIF secreted by cells pulsed with TF and antigen.
5. D_A	$1-(MI_A/0.90)$	The D_A is the antigen-independent LIF-independent percentage decrease in random PMN migration. It quantitates the activity of components in DLE which cause direct effects on PMN.

[a] DLE, dialyzable leukocyte extract; LIF, leukocyte migration inhibitory factor; MNL, mononuclear leukocytes (monocytes and lymphocytes); PBL, peripheral blood leukocyte; PMN, polymorphonuclear neutrophils (granulocytes). (Excerpted from ref. 130 with permission).

that unit standardization provides no information on potency of either the TF moiety or of antigen-nonspecific fractions of the extract. Two batches of extract containing TFs of the same specificity and prepared from the same number of lymphocytes may differ twenty-fold in potency. We determine potency units using production of LIF (25). That dilution of the DLE preparation which produces 20% inhibition of LIF production in an antigen-specific manner determines the potency index. Although for scientific purposes purification and structure for the TFs of differing specificities is mandatory, for clinical purposes a mixture of at least several moieties is probably preferable since nature has designed, at least in the immune system, molecules that work synergistically when combined. An example is provided by experience with interferon. The first product termed gamma-interferon was provided by the Helsinki Blood Bank, which shipped leukocyte extracts to Sweden for clinical trials. In clinical trials, this material was reported to benefit 50% of patients with a wide variety of cancers. Great emphasis (and much research funding) was placed on purifying gamma-interferon free of contaminants. However, when this was finally achieved, and recombinant interferon became available, it produced clinical benefit in only 1% of cancer patients. Presumably, the "contaminants" were responsible, at least in part, for the favorable results of the original mixtures.

IN VITRO DETERMINATION OF POTENCY Several years ago, we showed by use of LMI assay that batches of dialyzable leukocyte extract, prepared from different donors and standardized in identical manner, varied markedly in their ability to induce lymphokine production by immunologically normal cells exposed to specific antigen (17). This work made possible the standardization of TF by potency units. The LMI assay for potency of DLE is based on a direct investigation of leukocyte migration inhibitory factor (LIF) production in response to specific antigen (26). If the lymphocytes respond normally to the antigenic challenge, LIF is liberated and prevents or reduces the normal PMN leukocyte random migration. We use this LMI assay extensively in the diagnosis of antigen-specific cell-mediated immune defects and in donor selection. The addition of DLE to this system has two potential effects: first, an antigen-independent inhibition of migration at high concentrations of extract, and second, antigen-specific induction or enhancement of LIF production at lower concentrations of extract (22).

The method for determining DLE-TF potency is briefly summarized as follows (17): (*a*) Aliquots of target cells (PBL) from three normal donors previously shown to be nonresponsive to the test antigen (in this case PPD) by LMI (MI ≥ 0.90) are incubated in either (1) medium alone, (2) medium plus antigen (100 g/nl), (3) DLE (in at least 10 different concentrations) in medium, or (4) DLE (in the same ten concentrations) plus antigen in medium

for 30 min at 37°C in a humidified incubator gassed with 5% CO_2 in air, before neutrophil migration (22). (*b*) After neutrophil migration (18 hr), the effects of antigen alone, DLE alone, and DLE plus antigen were quantitated by determining three migration indices termed, respectively, the MI, MI_A, and MI_B. The MI_A value quantitates antigen-independent (LIF-independent) LMI produced by non-TF components. MI_A values ≤ 0.80 indicate significant antigen-independent effects. The MI_B value quantitates antigen-dependent LMI induced by LIF released from T lymphocytes newly sensitized by TF in the presence of specific antigen (22). An MI_B value quantitates antigen-dependent LMI induced by LIF released from T lymphocytes newly sensitized by TF in the presence of specific antigen (22). An MI_B value < 0.90 indicates meaningful antigen-dependent LMI. All concentrations of DLE are tested in six replicate cultures. The MI, MI_A, and MI_B values given are the mean ± SEM for all determinations.

DLE preparations are initially tested over a concentration range of I to 50 μl DLE per 100-μl cell suspension to determine a "working range" of concentrations for evaluating TF potency. When the proper working range of a DLE preparation is determined, the highest concentrations of DLE produce mild antigen-dependent LMI (MI_A between 0.80 and 0.70), which should be accompanied by antigen-dependent specific LMI when an appropriate antigen is employed (i.e. PPD in this patient). At intermediate concentrations of DLE, only antigen-dependent specific LMI is produced, whereas at low or suboptimal concentration of DLE only antigen-independent enhancement of mi-

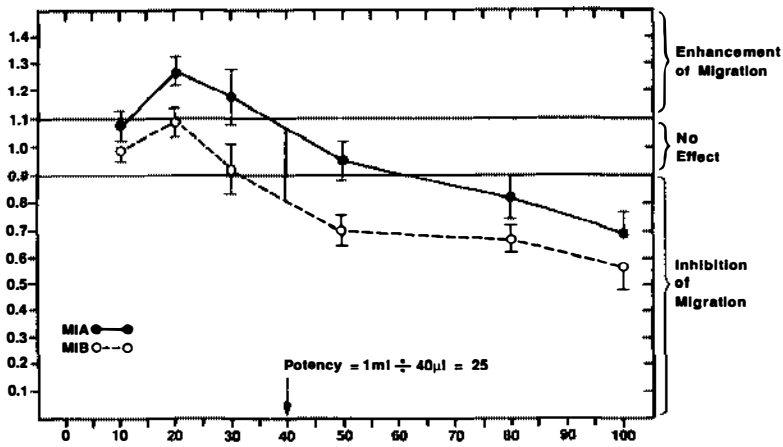

Figure 5 Potency assay. Direct leukocyte migration in agarose. In each panel, the top line represents random migration; the bottom line represents inhibition of migration by one or another activity of the Sephadex G25 fractions (17).

gration ($MI_A > 1.10$) or no effect at all is seen (20). The potency is determined by using the intermediate concentration range, where only antigen-dependent LMI is produced.

We have defined one potency unit of extract as the amount that produces a 20% antigen-dependent decrease in LMI when added to 4×10^6 target lymphocytes derived from the potential recipient of the extract (26). The value of the LIF assay in determinations of antigen-specific activity has been confirmed by others (e.g. 27). For example, if 40 μl of a given DLE-TF preparation are necessary to produce 20% inhibition then 1000 μl (we store DLE frozen in 1 ml vials) is divided by 40 and this DLE preparation therefore contains 25 potency units. A similar system in which the T cell mitogen phytohemagglutinin is substituted for antigen can be employed to standardize preparations of extract intended for therapy of patients with immune defects of unknown specificities. This LMI assay also identifies batches of DLE that for unknown reasons contain significant amounts of immunoinhibitory factors (about 10–15% of DLE preparations). In many of these the RNA peptide bond has been cleaved, presumably by enzymes of disrupted contaminating granulocytes.

LABORATORY MONITORING OF CLINICAL RESPONSE Striking differences in clinical response to DLE therapy occur between individual recipients (28, 29). Responses vary according to (*a*) the disease process, (*b*) the severity of disease, (*c*) the potency of the extract preparation (as discussed above), and (*d*) the amount of antigen burden (H. H. Fudenberg & K. Tsang, unpublished observations). Thus, two patients with the same disease may well display quite disparate responses to the same batch of extract (29). It is therefore imperative that appropriate in vitro laboratory evaluation of patients is performed in any trial of DLE so that the scheduled amount and frequency of therapy may be titrated against both the clinical and immunologic responses (13). Several in vitro immunologic assays are currently available for monitoring recipients, and in general their use has superseded that of skin testing for monitoring immunologic response, since skin testing is time-consuming and difficult to evaluate in a quantitative manner; furthermore, skin testing introduces antigen (possibly for the first time) into the recipient (13).

ANTIGEN-SPECIFIC RESPONSES The previously mentioned direct LMI test is, in our opinion, the best way available at present to investigate antigen-specific immune response. Certain patients may show a clinical improvement in response to the adjuvant moieties of DLE in the absence of an antigen-specific response; the LMI test clearly identifies such patients. Although antigen-specific TF confers on the previously unresponsive individual the ability to produce lymphokines in response to specific stimulation, it does not

produce de novo the ability to respond to antigen by lymphocyte DNA synthesis. However, DLE-TF will enhance antigen-dependent DNA synthesis in already committed lymphocytes (M. P. Arala-Chaves & H. H. Fudenberg, unpublished observations; 32). This activity is found in the adjuvant moiety of DLE that is quite distinct from the moiety responsible for the de novo induction of mediator production (33). Thus antigen-dependent lymphocyte DNA synthesis is not an appropriate test either for in vitro screening of preparation of extract or for monitoring of recipient response. Indeed, no improvement in deficient DNA synthesis was found in certain patients who responded well to extract therapy in terms of skin-test conversion, lymphokine production, and clinical improvement. In addition, DLE contains immunologically active peptides that boost the recipient's own CMI in an antigen-specific manner (28, 34). These moieties, molecular weights approximately 1500–2000, are now termed Imregs (35).

Another valuable immunologic test is antigen-specific T cell cytotoxicity—a test frequently employed in the investigation of patients with a variety of neoplastic diseases. Purified T-lymphocytes are cultured with viable tumor cells (labeled with ^{51}Cr) derived either from the patient's own tumor or from a cell line of the same tumor type. Tumor cells of other types plus matching fibroblasts are used as a control. The specific cytotoxic activity of the lymphocytes results in lysis of the tumor cells and release of the radioisotope into the medium in amounts far greater than release of radioisotope from other tumor types. Addition of DLE derived from a donor proven by this test to be responsive to the tumor antigens enhances the specific cytotoxicity of the patient's lymphocytes in a dose-dependent manner (36).

ROSETTE FORMATION BY ACTIVE T CELLS WITH SHEEP RED BLOOD CELLS (SRBC) This test enumerates a subset of T-lymphocytes that form immediate rosettes with sheep erythrocytes; this percentage of "active" T cells usually correlates more closely with cell-mediated immune status than does the percentage of total T cells as measured by rosette formation with SRBC at 18 hr. The two tests are performed at different temperatures and different T cell/SRBC ratios (37, 38). We (38) and others (39) have demonstrated that in immunodeficient patients suffering from recurrent viral, fungal, or mycobacterial infections, or from certain malignancies, response to DLE-TF therapy is associated with a normalization of the active T cell population. In vitro investigations have shown that the fraction of DLE-TF that mediates this effect is also responsible for the transfer of antigen-specific reactivity (40).

INTERACTIVE T CELLS We have increasing evidence that the enumeration of so-called "interactive" T cells (IAT) is of particular value in monitoring response to DLE therapy in certain diseases (41). This subpopulation of T

cells is identified by its ability to form rosettes with human transformed B lymphocytes (such as cells from the RAJI cell line). The rosette reaction appears to involve a membrane receptor on the lymphoblastoid cell that is distinct from, for example, Fc receptors. Interactive T cells are frequently reduced in patients with recurrent viral and/or fungal infections. The membrane markers of interactive T cells do not correspond to any known T cell subpopulation (e.g. CD4+, CD8+, etc.).[1] A reduction in circulating IAT correlates in several diseases with deficient mitogen-dependent lymphokine production (e.g. 42) and low percentages in this subset are frequently observed in many cell-mediated immunodeficiency states, as described below. When decreased, normalization of the IAT population is one of the first events to occur following administration of DLE containing TF of the relevant specificity. An example of the value of such tests is illustrated by the course of one representative patient followed for 7 years. The DLE derived from the lymphoid cells in her spouse was far more effective than that derived from cells of a normal random donor (Figure 6a). Note also that clinical improvement was reflected by a rise in IAT. Furthermore, of at least a dozen batches of DLE obtained from her husband, one (obtained shortly before a onset of a mild viral infection) was only one third as potent as the usual DLE; Figure 6b depicts clinical and laboratory response to the usual DLE and to that obtained shortly before onset of viral infection in husband.

OTHER IN VITRO EFFECTS OF DLE Although little is known of the mechanisms of action of TF, much has been learned of the results of activity of both TF and DLE both in vivo and in vitro. In vitro, DLE increases macrophage activation and IL-1 production (43). It also augments resynthesis of the receptor (the CD11 site) for Trypsinized sheep RBC (Trypsin destroys these receptors) (44). (The CD11-antigen site of T cells is one site involved in T cell activation). DLE inhibits spontaneous loss of such receptors; it also inhibits antibody-dependent cell-mediated cytotoxicity (ADCC) activity of normal peripheral blood lymphocytes (45); it augments DNA synthesis by normal lymphocytes (46); it stimulates mixed lymphocyte culture reactivity (47); and enhances defective leukocyte chemotaxis (48) and deficient natural killer (NK) cell function (49). In vivo, the extract enhances graft rejection (50) and in deficient patients, augments both ADCC (51) and NK activity (49). This wide variety of effects reflects the activities of many moieties of crude DLE, including nonspecific adjuvant or inhibitory functions. Antigen-specific properties of extract ascribed to the TF moiety include the ability to

[1]This subset may be the in vitro equivalent of a T cell–B cell interaction that takes place in vivo. Indeed, certain authors have suggested that B cells are more important than monocytes in antigen-presentation to T cells in vivo (148).

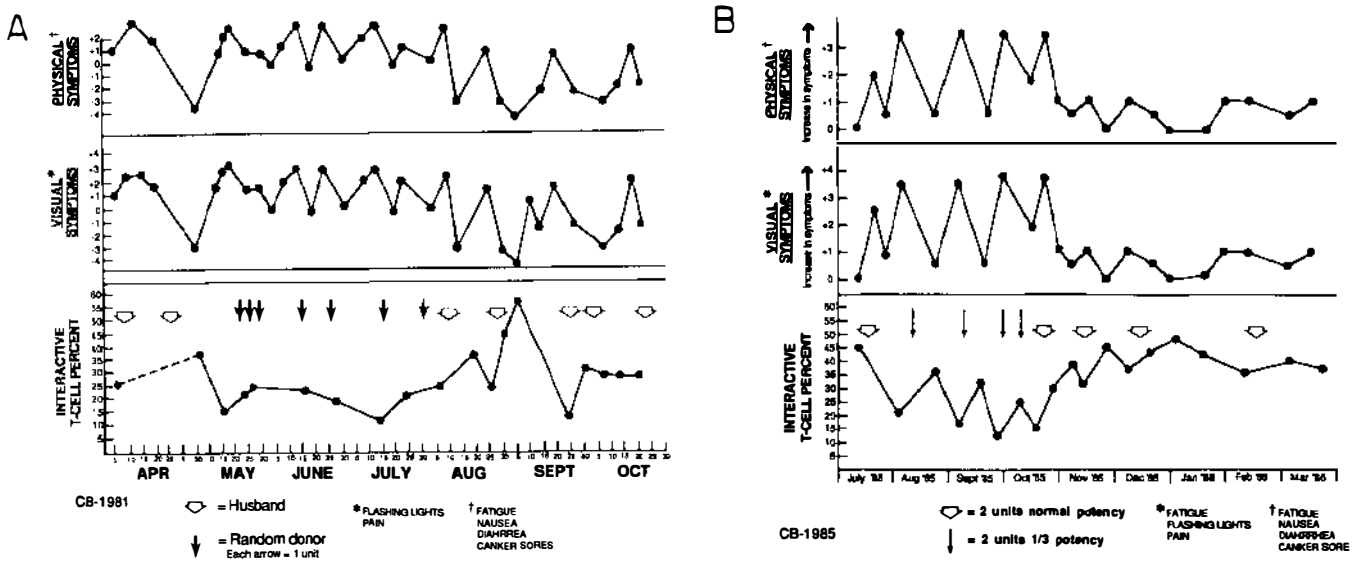

Figure 6 Dialyzable leukocyte extract, interactive T cells, and clinical symptoms in patient C.B., a 40 year-old white female, first seen 1981, with retinitis pigmentosa and recurrent viral infections, many of which triggered visual symptoms.

6a. Interactive T cells and visual and physical symptoms in response to DLE from cells of husband and DLE from cells of random donors. Note that her husband's DLE was much more effective in raising IAT and preventing viral and visual symptoms than was DLE from random donors. Note that following administration of DLE from husband, visual and physical symptoms were completely eradicated and there was a marked increase in IAT to supranormal levels.

6b Interactive T cells and visual and physical symptoms in response to DLE from husband, normally, and DLE from husband, obtained 5 days prior to viral infection. Note that one batch of DLE from husband during the 5 years had approximately ⅓ the usual potency, and administration of this material raised IAT and reduced visual and physical symptoms far less than did DLE obtained from husband on other occasions. For example, note the difference between Sept. 1, 1985,–mid-October 1985 and Dec. 1985 to March 1986. Symptoms are lower and IAT are higher.

confer on nonresponsive lymphocytes the potential to react with the relevant antigen in vivo and to produce lymphokines in vitro (5) (and presumably in vivo as well) and to enhance antigen-specific T cell cytotoxicity against tumor antigens, for example, by previously nonresponsive cells (52). These effects must be considered in both in vitro testing of preparations of DLE and monitoring of patient response to therapy with extract, as discussed below.

Results of Therapy with DLE Containing TF

Since DLE contains many moieties in addition to TF and since these other moieties may account for 10–15% of immunologic activity in DLE, we used each of the first 100 patients as his or her own control. This was accomplished by making TF from the cells of a donor lacking immunity to the given antigen, e.g. herpes 1, Mycobacterium fortuitum, cytomegalovirus. This DLE lacking CMI for the relevant antigen was administered over 6 weeks. No improvement was noted either clinically or in immunologic parameters during this period or over a subsequent 6 weeks without DLE. Each patient was subsequently given DLE from a donor with high CMI to the antigen over a 6 week period, with 80% of patients improving; improvement was always accompanied by or preceded by improvement in formerly deficient laboratory parameters. The above experiments where the patient served as his or her own control provide compelling evidence that TF is directly responsible for the clinical and laboratory improvements.

We have previously presented a detailed review of DLE therapy in a wide variety of diseases. Thymus-derived T cells, acting by production of soluble mediators of cell-mediated immunity, in some undefined manner protect us against protozoal infection (e.g. Pneumocystis carinii), fungal infections (e.g. candidiasis), mycobacterial infections, parasitic infection (e.g. leishmaniasis), viral infections, and protect, at least partially, against cancer metastases (53). The next section describes the results of DLE-TF therapy in selected disorders.

Fungal Diseases

CHRONIC FAMILIAL MUCOCUTANEOUS CANDIDIASIS (CFMC) CFMC, a fungal disease, has been frequently cited as a classic example of an antigen-specific cell-mediated immunodeficiency that may respond to DLE-TF_{as} therapy (54). We first described the therapeutic use of DLE in CFMC in 1972 (6); since then, several reports from other centers have indicated that DLE prepared from candida-sensitive donors frequently improves the immunologic response of the CFMC patient, but has a variable effect on the patient's ability to eliminate the pathogen (55, 56). In our first report of 11 cases of CFMC treated with DLE, about half of the patients responded clinically (57). Figure 7 shows an example of good clinical response to DLE-TF, i.e. patient's hands

before and after therapy; patient's feet were the same. It is now recognized that CFMC is not a single disease but a syndrome consisting of several entities, each with a distinct immunologic profile (58). In retrospective analysis of our own data, it is clear that only patients with one of these entities respond to DLE therapy—furthermore, large doses of DLE containing TF in conjunction with antifungal therapy were required. More recently the influence of other immunologic factors in CFMC has been recognized, such as the presence of circulating inhibitory factors; in one patient evidence of candida-specific immunodeficiency was found both by skin testing and by LIF (59). (The authors suggest that the inhibitory factors are antigen [candida]-antibody complexes.) This patient thus apparently fulfilled the criteria for potential DLE-TF_{as} therapy. However, further investigation showed a circulating suppressor factor, and a trial of therapeutic plasma exchange was performed. After the second exchange, the patient's immune response became normal, and after three weeks of continued antifungal therapy, the patient was disease free and remained so for at least a year. These examples illustrate both the disease-specificity and recipient-specificity of potential response to DLE-TF_{as} therapy, and probably explain in part the variable results of such therapy, at least in CFMC. Antigen-specific TF has also proved useful in other fungal diseases [e.g. coccidiomycosis (60)].

Mycobacterial Diseases

LEPROMATOUS LEPROSY This manifestation of the disease caused by *Mycobacterium leprae* is characterized by absent cell-mediated immune responses to the lepromin-antigen derived from the mycobacterium or to the organism itself (61). Other cell-mediated immune defects complicating this disease include reduction in peripheral and lymph node T cells, impaired response to mitogens, and the anergy to common recall antigens. These latter defects are reversed by successful antimycobacterial therapy, although apparently cured patients often remain anergic to lepromin. An early report of the effect of lepromin-specific DLE in this disease described induction of lepromin-responsiveness in six of nine patients following one injection (62). In a later report, four patients treated with a 12-week course of antigen-specific DLE in conjunction with antimycobacterial therapy had enhanced elimination of mycobacteria from lesions during DLE therapy and also became lepromin-positive as determined by skin testing (63).

Subsequently, Leser et al (64) performed a double-blind trial of DLE therapy in 15 patients with lepromatous-leprosy, using nonantigen-specific DLE prepared from spleen and cadaveric kidney donors. Five patients received sulfone therapy in conjunction with saline placebo injections, five received sulfones and DLE, and five received placebo tablets and DLE.

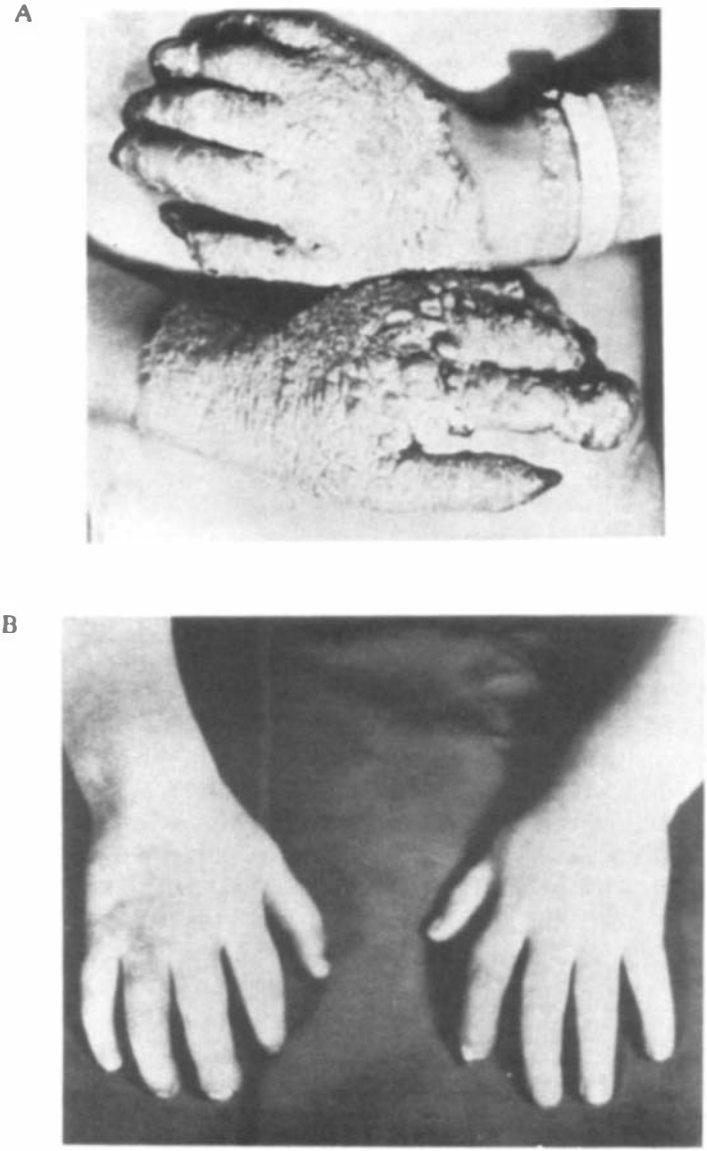

Figure 7 Efficacy of DLE containing candida-specific TF prepared from lymphocytes of a donor with high CMI to candida on the first patient with chronic mucocutaneous candidiasis so treated. Photographs show the hands of child before (Figure 7a) and 3 months after (Figure 7b) TF. Feet show similar change in appearance. Candidiasis also involved lower half of face, and this too cleared with therapy (54).

Clinical and bacteriologic improvement occurred in patients in all three groups. Immunologic studies revealed no changes in T-or B lymphocyte numbers following therapy in any group. Therapy with DLE containing TF corrected previously defective mitogen-dependent lymphocyte DNA synthesis; however, there was no conversion of negative lepromin skin tests in any of the patients. This is not surprising, since antigen-specific DLE was not used. However, these results and those reported previously suggest that extract may be therapeutically beneficial in lepromatous leprosy, and that larger trials of therapy with DLE prepared from lepromin-sensitive donors are indicated.

Other mycobacterial diseases that have responded dramatically to DLE containing antigen-specific TF include tuberculosis vulgaris (8) and Mycobacterium fortuitum (MF) refractory to antibiotic therapy (17). It is noteworthy that DLE-TF obtained from cells of a donor with high CMI for Mycobacterium tuberculosis (PPD Standard), but no detectable CMI for (PPD-MF) antigen, produced no improvement, but DLE-TF positive for PPD-MF and negative for PPD-S produced dramatic improvement in both immunologic test and clinical symptoms.

Parasitic Infections

CUTANEOUS LEISHMANIASIS Cutaneous leishmaniasis, a parasitic infection which is usually self-limiting in humans, may in certain patients persist for years. It is generally presumed that an antigen-specific defect underlies the persistent disease. Sharma (65) reported the effects of antigen-specific DLE in cutaneous leishmaniasis; intensive therapy with DLE-TF for several months produced dramatic healing of lesions in three patients whose disease had existed for 8–30 years. Sharma then conducted a therapeutic trial of extract in 23 patients (66). Her study is one of the few well-controlled trials of DLE therapy reported. Patients received either placebo (saline injections, nonantigen-specific DLE) or DLE prepared from patients who had recovered from leishmaniasis. Eight patients with acute infection showed no response, although healing was observed in three patients with acute disease. The nonresponders in this group were all negative to the pathogen by skin testing, whereas all three responders were skin-test positive, suggesting a nonantigen-specific adjuvant effect of DLE in these patients. Finally, twelve patients were treated with DLE containing TF specific for leishmania antigen. In this group, all eight patients with acute disease rapidly responded to therapy as did two of four with persistent infection. Similar results were obtained in a subsequent study by Delgado et al (67); they administered DLE prepared from leishmania-responsive healthy donors to seven patients without antigen-specific CMI response. Four of the seven with acute leishmaniasis had complete resolution of their disease; this was accompanied by conversion to

antigen responsiveness. None of the other three patients, all of whom had the disease for at least 10 years, responded (67).

Viral Infections

VARICELLA ZOSTER This example illustrates the potential prophylactic use of antigen-specific DLE. Varicella zoster, a viral infection, is a significant cause of mortality and morbidity in children with acute lymphocytic leukemia who are therapeutically immunosuppressed. Many children with leukemia in remission die of cerebral varicella. In a well-designed prophylactic trial, Steele et al (68) administered placebo or antigen-specific DLE in double-blind manner to 61 children with leukemia and no immunity to chickenpox as assessed by skin testing and serum antibody levels. DLE was prepared from adults convalescing from chickenpox who demonstrated skin-test positivity. Of the 15 children in the placebo group, 13 became infected, and of these, 3 had disseminated disease. Only 1 of the 16 exposed patients in the group receiving DLE contracted disease, which was clinically not severe. It is noteworthy that 7 of 11 patients treated with DLE containing TF specific for varicella zoster but not exposed to chickenpox became skin-test positive to varicella zoster antigen. Furthermore, of the 15 patients receiving DLE and exposed to chickenpox but who did not become clinically infected, 9 seroconverted and 7 acquired skin-test positivity. These dramatic results indicate that DLE containing antigen-specific TF induced antigen-specific CMI to varicella zoster that prevented overt clinical infection in these children.

HERPES SIMPLEX We have had excellent results with DLE-TF_{as} in treatment and prevention of recurrent herpes simplex infections; such therapy promptly eradicated lesions and subsequent prophylactic DLE-TF markedly decreased the frequency of recurrence. The most dramatic example was eradication of ophthalmologic herpes within 6 hr of administration (69).

Other viral infections (e.g. cytomegalovirus) have also been shown to respond to DLE containing TF of appropriate specificity (e.g. 19, 70).

Putative Viral Diseases

BEHÇET'S SYNDROME (B.S.) This syndrome, which we (71) and others (72, 73) believe to be of viral origin, is characterized by severe orogenital ulceration and inflamation of the large joints (e.g. knee joints); because of blood vessel involvement, many organs may be involved, but the ophthalmologic and neurologic consequences are the most serious. Although the syndrome is rare in the United States, it is relatively common in the Mideast (e.g. Turkey and Lebanon) and Japan; several of our patients first developed B.S. after a prolonged (3–6 months) febril illness while traveling in Japan or the Mideast (71). In two B.S. patients, one in California, one in New Orleans,

DLE-TF from cells of random normal donors was ineffective, whereas DLE-TF prepared from lymphocytes of a household contact of a B.S. patient in New Hampshire induced clinical and immunologic normalization in a B.S. patient in California, implying that a similar, if not identical, environmental agent (presumably viral) is involved in the etiology or pathogenesis of most or all cases of B.S. We have found that patients with Behçet's syndrome have impaired cell-mediated immunity. Lehner reported that all patients with Behçet's syndrome and also all with recurrent aphthous stomatitis (RAS) had autoreactivity to mucosal antigens (74). Our most consistent finding as diminution in function of interactive T cells in peripheral blood (see ref. 41). The CMI defect may be a predisposing factor; alternatively, the diminution of function of interactive T cells or a defect resulting from the disease permits its persistence. Another explanation is that B.S. results from an aberrant immune response to the putative virus.

We first began therapeutic trials of DLE in patients with B.S. in 1974, initially employing DLE prepared from random donors, but have subsequently used household-contact donors, since the putative etiologic agent is unknown but presumed environmental. These trials were uncontrolled, and the therapeutic regimen was tailored for each patient according to clinical and immunologic responses. Despite these deviations from the ideal trial protocol, the clinical results were most encouraging (75). We have since treated 14 patients with B.S. with DLE-TF, in some patients derived from household contacts, in others from random donors. Disease presentation and severity varied widely in these patients. Since that early study, eight patients have been treated with household contact DLE-TF; seven had both normalization of immunologic parameters and dramatic clinical improvement. In two of the seven, remission was short-lived, one becoming unresponsive to therapy after 6 months, and one discontinuing therapy after 2 months, when exacerbation of disease occurred. In the remaining 5 patients with Behçet's, long-term remission was obtained with continued DLE-TF therapy; high-dose Prednisone therapy (80 mg per day) was discontinued in one and much reduced in two.

Two patients with severe RAS of several years duration who were treated with household-contact DLE-TF showed rapid and dramatic clinical responses within 2 weeks of onset of therapy and enhanced cell-mediated immunity and were able to gradually reduce Prednisone from 80 mg/day to 0 mg/day over a 4 week period. One patient remained in remission with monthly injections of extract for 6 months, when therapy was discontinued because the patient became pregnant and exacerbation of severe disease occurred. The other patient has remained in partial remission for over one year, with occasional episodes of ulceration only. As mentioned above, enumeration of interactive T cells has proved to be the most reliable index of

immunologic response in these patients (71); their function became normal shortly before patients noted clinical improvement. Others have reported variable effect of DLE in double-blind trials (73); the variability probably was due to nonselection of donors. Different pools of cells of normal individuals were used.

ALOPECIA TOTALIS This disease, presumably caused by a virus, is characterized by hair loss that involves the entire body. The frequent personal or family history of autoimmune phenomena suggests that the pathogenesis of alopecia totalis is itself autoimmune, although the evidence is far from conclusive (76) and autoreactivity to hair follicles has not been convincingly demonstrated; mononuclear cell infiltration has been found at the scalp biopsy site. We believe that in certain patients with a genetic predisposition, the disease process is triggered by an environmental agent (probably viral) (77), as in Behçet's syndrome. This subpopulation with alopecia totalis has concomitant thyroid autoreactivity in the absence of thyroid dysfunction; consistent CMI defects were found in this population, including diminished function of interactive T cell populations and deficient mitogen-dependent lymphokine production. We have recently performed an open-labeled trial of DLE therapy in nine patients with severe alopecia (totalis or universalis) and documented CMI defects, using DLE prepared from household contacts. The therapeutic regimen was determincd according to the individual patient responsiveness; in general, an initial course of 9–12 international units administered over a 5 day period was followed by booster injections of one unit every 3-4 weeks. All patients responded immunologically to therapy, with normalization of interactive T cell percentages and conversion to positivity of mitogen-dependent LIF production, with one exception to the latter. No significant clinical response was observed in three patients. However, in the others regrowth of hair began between 1 and 3 months following onset of therapy, and growth persisted. The first patient included in the trial has rcccived therapy for 18 months, and has no noticeable hair loss. In three patients, therapy was discontinued during regrowth (at 3–6 months following the onset of therapy). In each patient acute hair loss was noted within 1 month of withholding DLE, and growth returned with continued therapy (42, 77).

OTHER PUTATIVE VIRAL DISEASES Other putative viral diseases in which DLE has produced dramatic clinical improvement include pemphigus vegetans (78), chronic discoid lupus erythematosis (79), and epidermal dysplasia (80), a widespread dermatologic malignancy (caused by one form of papovavirus). Several investigators found some patients with psoriasis had impaired CMI and good clinical response to transfer factor (e.g. 81, 82). DLE has also produced marked clinical benefit in an animal model of immunologic (type I)

diabetes (83), namely the obese rat. In this model there are no antibodies to pancreatic islet cells, but histology of the pancreas indicates that these cells are replaced by a characteristic feature of CMI, namely infiltration by monocytes and lymphocytes.

Malignant diseases

MELANOMA The immunologic aspect of malignant melanoma has been widely studied, and information available to date suggests that patients with melanoma respond immunologically to the tumor and that immune mechanisms may influence the disease course (84). Melanoma would thus appear to be a disease suitable for therapy with specific DLE. Several therapeutic trials with DLE have been performed; however, the results obtained were highly variable, with overall poor clinical response to therapy (85–87). (These investigators believe that negative results were due to use of DLE derived from random normal donors.) These early trials, including our own, were performed before the development of many of the assays used for the selection of donors and monitoring of response, and the significance of the results obtained is questionable. Perhaps because of this, the investigation of antigen-specific DLE therapy in melanoma has not been pursued.

Human Osteosarcoma (OS)

We previously reported clinical and immunologic parameters in osteosarcoma patients who received OS-specific DLE (36, 88). Among seven patients bearing primary tumors (surgically removed prior to the beginning of OS-specific DLE therapy), 80% were dead by 24 months with single agent chemotherapy, radiation therapy, or no therapy. These had no improvement in survival time as compared with historical controls. However, five of six patients apparently free of overt metastases after tumor resection treated with OS-specific DLE for 24 months, were alive and disease-free at the end of that period and also at last follow-up, 62–82 months after therapy (A. H. Levin & H. H. Fudenberg, unpublished observations). Compared with 5 year survival computed from historical controls, this data is statistically significant (> 0.008). This increase in survival time is due to prevention of pulmonary metastases in patients treated with OS-specific DLE. In seven patients with surgical removal of the primary, there was no clinical benefit prior to DLE administration, using DLE devoid of TF-specific for OS antigen—all died with lung metastases within 24 months (36).

These results have been reproduced in an animal model of human osteosarcoma (90). Fetal hamsters at day 14 of fetal life were tolerized to OS antigen in utero with purified human-osteosarcoma antigen; they were inoculated with 2 million live human osteosarcoma cells by day 3 after birth. By day 14, palpable tumors were present. Untreated hamsters or hamsters without sur-

gical removal of the primary were all dead of pulmonary metastases by day 60. In all instances, as is true in humans with osteosarcoma, death was due to metastases to the lungs; the metastatic cells were of human origin as shown by cytogenetic studies. Osteoid formation was also present on microscopic examination. DLE-OS extended life span to normal in 60% of the animals. DLE specific for fibrosarcoma, breast melanoma, etc, had no effect on extension of life span. As in humans, the DLE-OS was effective only if the primary was removed prior to therapy (90).

DISTANT METASTASES FOLLOWING AMPUTATION OF PRIMARY TUMOR

We have treated two patients with breast carcinoma who developed 2 or 3 bone metastases 3–4 years after removal of primary tumor. Breast cancer-specific DLE-TF caused eradication of metastases within 7 days (H. H. Fudenberg, unpublished observations). We have also treated two patients with hypernephroma (HN) who developed cerebral metastases, as shown by pineal shift, on pneumoencephalogram 3–4 years after resection of the primary. In both cases, DLE-TF (HN) caused disappearance of cerebral symptoms, and disappearance of pineal to normal within 24 hr (54). One other patient who developed HN and bone metastases after surgical removal of the primary (HN) is still alive 10 years later, receiving DLE-TF (HN) every 2 weeks. (When this was stopped for 6 weeks, additional metastases occurred and DLE-TF (HN) was reinstituted; examination six months later showed no new metastases nor increase in size of old metastases, and stable clinical status.) (93).

Bukowski et al have noted objective regression of tumor in one of 25 patients with metastatic hypernephroma using DLE-TF, 16 with 9 without other immunostimulants (87).

Neurologic Diseases

DLE-TF has also been effective in subsets of certain neurologic disorders which are probably syndromes rather than distinct entities. Among these disorders are Alzheimer's disease (94), autism (95), and retinitis pigmentosa (96). Approximately 20% of patients with Alzheimer's disease (94), 70% of patients with autism (95), and 50% of patients with retinitis pigmentosa (97) have antibodies to neuron axon filament proteins (NAFP), presumably the hallmark of unconventional virus infection of the central nervous system (98). Six of nine AD patients with such antibodies were given DLE derived from household contacts and showed considerable improvement, e.g. regain of speech, regain of capacity to recognize spouse, improvement in cognitive function, and in one instance regain of ability to walk unaided (previously in a wheel chair). Twenty-one "Charleston units" (roughly equivalent to 1000 international units) were given over a 3 day period. Beneficial effects lasted

about one month; after a subsequent month without therapy, the patient fell back to baseline levels. Three patients without antibodies had no clinical improvement. This series of events was repeated three times with exactly the same results. Use of DLE from nonhousehold contacts had no beneficial effect. Again, patients with antibodies in NAFP (predominantly 70 kD) improved while those lacking such antibodies did not.

Fifteen of 19 patients with autism, onset usually at age 18 months, (sudden cessation of verbalization and sudden loss of attention span, marked hyperkinesis, etc) treated with DLE-TF from cells of parental donor, showed considerable improvement, including normalization of sleep (8–10 hr sleep per night rather than 2), marked decrease in hyperkinesis, regain of verbalization capacity, marked increase in attention span, etc (95).

In retinitis pigmentosa, nine of ten patients treated with DLE from a household contact have had no further visual loss during the 5 year period. The patients usually do not notice visual difficulties until 65–70% of visual fields are lost and usually lose 15% of their remaining visual fields thereafter (J. Fleischman, personal communication). (The retina is the end of the second cranial nerve so that, though located in the eye, it is really part of the central nervous system and probably its most rapidly metabolizing portion.)

We have also had considerable success with DLE-TF administration with 21 "Charleston units" over 4 days, every 6–8 weeks in one of three patients with amytrophic lateral sclerosis; only he had antibodies to NAFP in serum and spinal fluid (100). This patient is of particular interest since he improved dramatically clinically (e.g. became able to ambulate after previously being confined to a wheel chair) with DLE-TF obtained from a close contact, but subsequent administration of the same amount of DLE-TF from the spouse of a patient with multiple sclerosis yielded absolutely no improvement, either in immunologic function tests or in clinical status (100). We have treated one patient with a familial neuro-degenerative disease, probably Creutzfeld-Jacob's disease (both mother and sister died of CJD at age 32) and with antibodies to NAFP with good results (100); after three days, the patients symptoms disappeared for 4–6 weeks. She has received injections approximately every 6 weeks for the last 3½ years, is now 35 years old, and her clinical status is improved compared to when first seen here (e.g. can drive both car and boat, play the piano, etc).

We have used DLE-TF from household contacts in the so-called post-viral chronic hyperfatigability-cognitive dysfunction syndrome (formerly and erroneously termed chronic Epstein-Barr virus disease) (101). Nine patients, seven previously unable to work (all patients with this disorder have severe chronic hyperfatigability and marked cognitive impairment, e.g. temporary amnesia, inability to think clearly, etc.) received household-contact DLE-TF for one year or more. These patients had experienced severe symptoms for

time spans of 1–5 yr. Eight of the nine improved considerably on DLE-TF and seven of the eight have returned to work. We have followed 20 additional patients for less than one year, and although some have already shown considerable clinical improvement, we hesitate to draw conclusions before one year of therapy.

Yang and co-workers reported good results from semipurified DLE derived from both human and porcine lymphocytes in myasthenia gravis (102).

Therapeutic Failures

Failure of transfer factor in multiple sclerosis was reported by Fog et al (103). However, these investigators used pooled cells from 100 random blood donors to make the DLE in Switzerland, after which it was administered by a neurologist in Copenhagen. No immunologic tests were performed on either donors or recipients. In contrast, Basten et al (104) reported 2 years later in the same journal that in a double-blind study, DLE from carefully selected household contacts (HSV positive) administered for 18 months slowed the rate of progression of the disease in patients in stage I and II, although it was not effective at the dose and frequency used in more seriously affected patients (stages III and IV). The above data on multiple sclerosis indicate that TF made in one laboratory and that made in another differ as much in efficacy as a Rolls-Royce does from a Model T Ford, though both are called automobiles. Nonetheless, the "failures" of DLE-TF in one or another disease rarely mention whether or not the recipient was monitored before, during, and after therapy by tests of immunologic functions, and fail both to select appropriate recipients and to provide data necessary for optimal quantity and frequency of administration. Hence, most physicians are skeptical of the clinical efficacy of DLE-TF. The reasons for these failures are multiple: some are listed below (H. H. Fudenberg, E. M. Paulling & J. A. Emerick, unpublished observations):

Donor Factors

1. Use of pooled donors (50–100 obtained from blood bank red-cell donors during a given day). If the recipient has an infection or a tumor that occurs only rarely in the general population, the pooled DLE will have little, if any, activity.
2. Use of a donor who (*a*) has little or no cell-mediated immunity (CMI) to the infectious agent or to the relevant tumor-cell antigen (see Figure 6a) or (*b*) has a defect across a broad spectrum in CMI.
3. Viral infection of donor within 4 weeks prior to donation due in most instances to antigen-antibody complexes that inhibit the effector arm of CMI [Again, see Figure 6b].

Collection and/or Storage

1. Too many granulocytes in collected material plus no cooling of collection bag during collection. (Granulocyte enzymes destroy TF at temperatures as low as 80°F. As noted earlier, our pheresis unit provides us with leukocyte preparations containing 95% mononuclear cells.)
2. Storage at 4°C rather than −20°C (the peptide-nucleotide bond is split by proteases active at 4°C).

DLE Administration

1. Inadequate amounts or low potency of the DLE-TF administered.
2. Recipient not monitored by immunologic tests to ascertain whether immunologic deficiency is corrected.

Recipient Factors

1. Defective monocyte number or function in recipient: a small amount of monocytes (or monocyte supernatants) is necessary for optimal effect of DLE-TF (M. P. Arala-Chaves & H. H. Fudenberg, unpublished observations).
2. Allergies to ragweed, pollens, etc: If patient has allergies and TF is given during the high pollen season, TF may not be effective. If given prior to pollen season, TF seems to diminish the severity of allergic symptoms (e.g. hay fever, allergic rhinitis) (95).
3. Impaired endocrine function;
   (*a*) Clinical hypothyroidism as gauged by hypersensitivity to cold, hypohydrosis and abnormally low first a.m. pulse and temperature (in some patients with one or another immunologic defect, thyroid function tests, e.g. free T3, free T4 and TSH may be normal, but the patient is clinically hypothyroid) (101).
   (*b*) Adrenal cortical hypofunction,
   (*c*) Impaired estrogen production (in pre-menopausal females).
   (*d*) Deficiencies in purine enzyme pathway (95).

Factors in Either Donor or Recipient

1. Marijuana use in donor or in recipient (H. H. Fudenberg, unpublished observations).
2. Patient encounters a virus not previously encountered by his donor (107). One of our patients with recurrent viral infection that disappeared after maintenance of prophylaxis with DLE-TF from a household contact came in contact in London with a British family with an upper respiratory infection; he developed severe viral infections, presumably due to a virus that the DLE-TF donor had never encountered. Another individual with

similar symptoms, also doing very well on DLE-TF maintenance prophylaxis, developed severe URI while in Russia, presumably due to an organism that the donor had not previously encountered).

It should be noted that if the patient receiving DLE-TF has a viral infection at the time of, or shortly before, DLE-TF administration, the action of DLE-TF is blocked by an unknown mechanism, not only during the symptomatic phase, but also for 5–7 days prior to the onset of symptoms and 3–4 weeks after the symptoms disappear. If the patient has a bacterial infection, the action of DLE-TF is blocked for the duration of symptoms only.

Current "Hot Spots" in TF Research

POTENCY The use of potency assays for TF when the antigen from the etiologic organism is known and available has greatly increased the incidence of complete responses (e.g. Mycobacterium fortuitum, herpes 1, systemic candidiasis, etc). This is important since one donor with positive CMI for a given antigen may donate DLE that is 20-fold more potent than another with positive cell-mediated immunity to the same antigen. In instances where the etiologic agent is not known, we still rely on "Charleston units" (amount of DLE derived from 5×10^8 lymphocytes). However, DLE from household contacts almost invariably has far greater potency than DLE from random donors (105). (Again, see Figure 6a.)

ANIMAL TRANSFER FACTORS DLE has been demonstrated in cows (108), burros (109), dogs (110), rabbits (111), guinea pigs (112), hamsters (90), rats (113), and mice (114), and it also appears present in chickens (115). Antigen-specific TF from one species can transfer antigen-specific CMI to another species without significant loss of potency. For example, bovine DLE made against the parasite coccidioides protects not only cows but also mice from an LD90 dose of this parasite, whereas bovine DLE devoid of TF of this specificity has no protective effect (116). Bovine DLE containing TF specific for nematodes is effective in sheep (117), and bovine colostral DLE has been used for both prophylaxia and treatment of various viral and parasitic diseases of dogs (e.g. canine parvovirus) (118), pigs (e.g. swine transmissible gastroenteritis) (118), and chickens (e.g. Marek's disease, Newcastle disease, laryngotracheitis, infectious bursal disease) (118, 119). Porcine spleen TF of varied specificities has also been used in treating viral and parasitic diseases of animals, including cows (bovine epizootic fever) and chickens (Marek's disease) (120). Furthermore, we have shown that rabbit TF protects against lung metastases in hamsters bearing human osteosarcoma (90) and that human TF is effective in rats, guinea pigs, etc (121). It seems that the specific activity of TF in intraspecies transfer is equivalent to that obtained in transfer to the same species.

TF derived from infrahuman species has recently been used in therapeutic attempts in a variety of diseases. Bovine TF has been given repeatedly to humans with no adverse reactions (14, 122–125). Louie et al administered it for 4 weeks to eight patients with AIDS and cryptosporidia (an organism that causes diarrhea and is found only in humans with immune deficiency); four of the eight responded in that diarrhea disappeared, and ova and parasites were not present in the stool during therapy and for 4 weeks thereafter (126). Bovine DLE has also been repeatedly given to patients with malignancy; it produced antigen-specific transfer of reactivity (e.g. to KLH) in individuals previously lacking such reactivity. More importantly, it has no adverse effects; indeed, Chng et al have given both bovine lymph node and colostrol TF to one patient repeatedly over four months without adverse reaction (148). Presumably, the molecule is too small to be immunogenic despite repeated administration. Porcine spleen DLE was given repeatedly to humans without adverse effects (126), and has been beneficial in 14 of 16 patients with myasthenia gravis (102). Murine DLE given repeatedly to humans with AIDS caused no adverse reactions (128). Because of the large volumes that can be obtained, it seems likely that bovine colostral transfer factor will be increasingly used in human diseases associated with T cell dysfunction when no other therapy is available.

TRANSFER FACTOR—FUTURE

Structure

Although no one has yet purified TF of any specificity, in the DLE we obtain from 10^{10} mononuclear cells the concentration of antigen-specific TF is 1 part per million. After a series of fractionation processes (e.g. Sephadex G25 permeation chromatography [Figure 8], Biogel P2, High Pressure Reverse Phase Liquid Chromatography [HPLC] [Figure 9], boronate affinity chromatography, and isoelectric focusing [129, 130]), we estimate the final concentration at approximately 12 parts per 100. Acrylamide gel electrophoresis showed 6 bands when stained with the usual stains for RNA and protein, but the biologically active material elutes from a position between the bands (131). Nonetheless, we can arrive at a simplest case model based on enzyme inactivation experiments on DLE obtained from PBL of humans (130), burros (109), and cows (131); DLE liberated by antigen (132); bovine dialyzable lymph node extract (B-DLN) (133); DLE obtained by incubation of bovine lymph nodes at 37°C (131); and bovine colostrum DLE (134).

Results of enzyme inactivation experiments by ourselves and others, (e.g. Burger et al [135]) reviewed and recently extended by Paddock (134)) strongly suggest that 3 different moieties exist with TF activity in vitro, as compared with two years ago when we knew of only 2 (see Figure 10) (130, 131). Two are present in HPLC peak 5: the first is probably the precursor of TF, hereafter

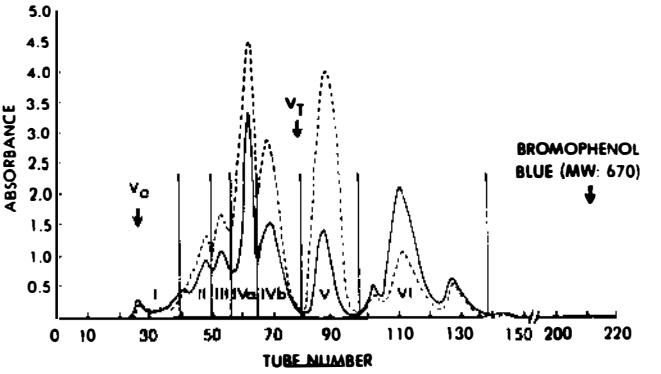

Figure 8 Sephadex G25 gel permeation chromatography containing PPD-positive TF and negative for coccidiomycose antigen (cocci). Biological activity as measured by production of Leukocyte Migration Inhibition fractor was confined to fraction IVB (127).

termed TF_{pre}, and the second the secretory TF that is secreted, transported across the cell membrane, and exported, hereafter termed TF_{sec}. (This is the probable intercellular immune messenger [131].) Another moiety with TF activity is confined to HPLC peak 7; this appears to be membrane-bound TF, hereafter termed TF_{mem}. All three of these moieties are oligoribonucleotide-peptides, since biologic activity, as measured by LIF production, is destroyed by P1 nuclease and pronase, but not by DNase (see Table 2) (130, 131).

TF_{sec} differs from TF_{pre} in that the latter has a phosphate on the 3' end. (This external phosphate appears to be the only difference between TF_{pre} and TF_{sec}.) TF_{mem} has no phosphate at its 2',3' end, but it does have a phosphate sensitive to removal by bacterial alkaline phosphatase. This phosphate is required for activity. With no other site available, presumably the phosphate is on the peptide moiety, where it could possibly be attached to any of several amino acids.

The RNA data strongly suggest that TF_{pre} and TF_{sec} have internal purine residues, since activity was destroyed by RNase T1 and RNase U2, but not by RNase A (136). In contrast, TF_{mem} has an internal pyrimidine residue, since activity was destroyed by RNase A. (In a sense, this is reminiscent of the situation with IL-1, which exists in three forms, namely IL-1a, IL-1b, and IL-1 precursor; current evidence suggests that most of the membrane-associated IL-1 is the alpha form, and that only the beta form is secreted in extracellular fluid [137, 138].) In all three TF's (pre, sec, and mem), the N terminal end of the peptide is joined to the oligonucleotide, through the 5' phosphate.

The peptide moieties in both TF_{pre} and TF_{mem} are free, since carboxypeptidase A destroyed activity. (Carboxypeptidase is an exonucleotide that hy-

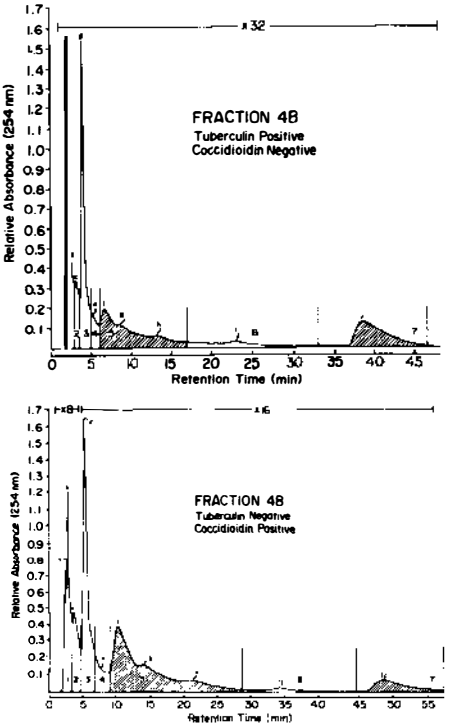

Figure 9 High pressure reverse phase liquid chromatography of Sephadex fraction IVB from two DLE preparations, one containing TF for tuberculin (PPD) and devoid of cocciiodin TF activity (top), and another devoid of TF for tuberculin but with biological activity for coccidioidies (bottom). Note the strong similarity of patterns. In both preparations, biological activity was confined to peaks 5 and 7 (129).

drolyzes a peptide bond adjacent to the C-terminal end of a polypeptide). The amino terminal end of both TF_{pre} and TF_{mem} are blocked since leucine aminopeptidase (that hydrolyzes any peptide bond adjacent to a free amino group) did not destroy biologic activity.

TF_{sec} and TF_{mem} activity were destroyed by snake-venom phosphodiesterase (SVP), indicating that the nucleotide's 3' end is free (i.e. not linked to peptide) and also not blocked at either the 2' or 3' position of the sugar residue. In contrast, TF_{pre} activity was not destroyed by SVP. However, TF_{pre} and TF_{sec} oligoribonucleotides lack free 5' ends since spleen phosphodiesterase (SP) also failed to destroy activity. Bacterial alkaline phosphatase (BAP) alone did not destroy the activity of TF_{pre} or TF_{sec}; however, BAP followed by SVP destroyed TF_{pre} activity and also, of course, TF_{sec} activity. This indicates that the 3' end of TF (pre, sec, or mem) is not blocked by a peptide but the 3' end of TF_{pre} is blocked by a phosphate. With regard to TF_{mem}, BAP

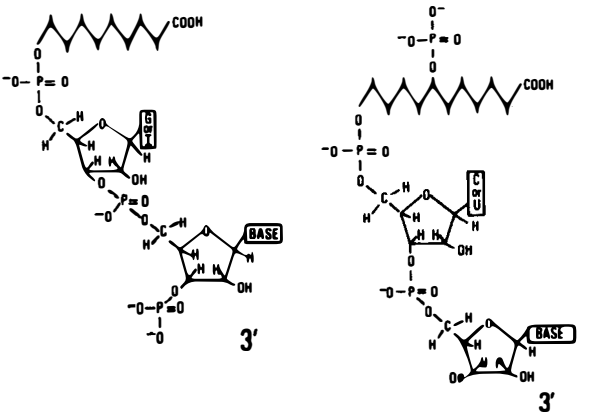

Figure 10 Simplest case models of TF-5 and TF-7 based on enzyme inactivation studies. (130)

alone destroyed activity, but SP did not. By analogy with the other two moieties, we suggest a phosphate somewhere on an amino acid residue sensitive to BAP. Thus TF_{mem} has an external phosphate that is required for its activity, whereas the TF_{pre} external phosphate is not required for activity (134). The simplest case models are shown in Figure 11. Results identical to TF_{pre} and TF_{mem} from human DLE were obtained with TFs of burro DLE.

The pyrophosphate linkage proposed by Burger (135) is probably erroneous since no degradation of TF activity resulted on exposure to tobacco-acid pyrophosphatase (139). The external phosphate is on the 3' end of TF_{pre} because its biologic activity is degraded by snake-venom phosphodiesterase I only if the phosphate is first removed by phosphatase. This external phosphate appears to be the only difference between TF_{pre} and TF_{mem}. Some confirmation of the proposed structures is provided by their affinity for 2',3' cis-diols. If our models are correct, then TF_{sec} and TF_{mem} should bind to boronate and TF_{pre} should not unless the 3' phosphate is removed, and indeed those were the results that we obtained. Antigen-liberated TF, TF obtained by incubation of lymphocytes at 37°C, and bovine colostrum TF gave identical results in enzyme inactivation experiments.

Mechanisms of Action

The mechanism(s) whereby TF participates in the cell-mediated immune response are still unknown. (Indeed, the processes by which T-lymphocytes recognize and respond to antigenic stimulation are as yet poorly understood.) In simplistic terms, T cell responses require specific antigen presentation by monocyte/macrophage cells or cells derived from them in which the Ia-like antigens (products of the so-called immune response genes) play an important role (140). However, Ia antigens are not present on the TF polypeptide (135).

Table 2 Enzymatic degradation studies of human TF transfer factor activity in vitro (leukocyte migration inhibition assay)[c]

Enzyme incubation	Type of cleavage	HPLC Fraction 5	HPLC Fraction 7	Bovine TF[b]
RNase T1, 5 units, 1 hr, 37°C	Endoribonuclease specific for Gp or Ip pApGp/CpUpGp	+	0	+
RNase A, 5 units, 1 hr, 37°C	Endoribonuclease specific for pyrimidines, Cp and Up pApApGpCp/Up/Gp	0	+	0
RNase U2	Endoribonuclease specific for purines, Gp and Ap pAp/Ap/Gp/Cp/Up/Gp	+	0	+
P1 nuclease, 10 units, 5 hr, 37°C	Endonuclease→ 5' NMP pA/pA/pG/pC/pU/pG/p	+	+	+
Bacterial alkaline phosphatase, 25 μg,	Removes external 5' and 3' phosphate p/ApApGpCpUpG/p	0	+	N.T.
Spleen phosphodiesterase (phosphodiesterase II), 0.05 units, 12 hr, 37°C	5' exonuclease→ 3' NMP 5'HO Ap/Ap/Gp/Cp/Cp/Up/Gp3'	0	0	0
Bacterial alkaline phosphatase, then spleen phosphodiesterase	Nucleotide products + Pi	+	0	0
Snake venom phosphodiesterase (phosphodiesterase I), 10 μg, 1 hr 37°C	3' exnouclease→ 5'NMP 5' pA/pA/pG/pC/pU/pG OH 3'	+	0	+
DNase I, 200 units, 1 hr, 37°C	Digests DNA to small oligonucleotides	0	0	0
Pronase, 1 P.U.K., 18 hr, 37°C	General protease	+	+	+
Phospholipase A2, 10 units, 4 hr, 37°C	Hydrolyzes fatty acyl ester at 2-positions	+	0	N.T.
Carboxypeptidase A_a, 5 units, 1 hr, 25°C	Exopeptidase which hydrolyzes the peptide bone adjacent to the C-terminal end of a polypeptide	+	+	+
Leucine aminopeptidase, 5 units, 6 hr, 25°C	Exopeptidase which hydrolyzes the peptide bond adjacent to a free amino group	0	0	0

[a] Pronase and carboxypeptidase A were independently confirmed to be devoid of ribonuclease activity.
[b] Bovine colostrum TF and TF liberated from antigen gave results very similar to those with Bovine TF.
[c] LIF activity was considered destroyed when the decrease in migration was <10%. 0 = no destruction of LIF activity; + = destruction of LIF activity. (Excerpted from ref. 130 with permission)

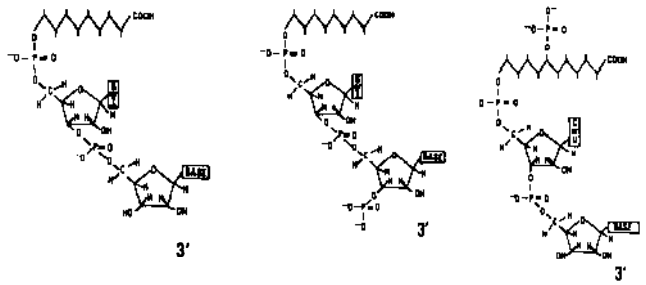

Figure 11 Simplest case models of 3 TF's, from left to right, TF secretory, TF_{sec}, TF precursor, TF_{pre}, and TF membrane, TF_{mem}, based on enzyme inactivation studies of DLE obtained from peripheral blood cells, liberated by incubation of antigen, and obtained by incubation of bovine lymph nodes at 37°C (131).

T cell recognition of antigen involves membrane receptor sites. One hypothesis for the mechanism of action of TF is that TF forms a part of the T-lymphocyte receptor (T-AR) for antigen. If so, since T-cell activation is triggered by the binding of the Ia antigen determinants by monocytes and monocyte-derived cells, then obviously TF would be necessary for T-cell activation. This is supported by the fact that TF specific for PPD binds PPD but no other antigens such as candida and coccidiomycoses immitis (141). However, additional data must be furnished so that the hypothesis is compatible with, first, the activity of TF in the normal T cell-mediated immune response, and second, the ability of TF to transfer such immunity to a previously nonresponsive recipient in an antigen-specific fashion.

In the antigen-responsive subject, a small number of T cells bearing receptors for a given antigen are continually present. These membrane receptor sites probably include the TF moiety. Specific antigen binding to the appropriate receptor probably initiates production and release of more TF, which then binds to immunologically uncommitted T-lymphocytes, thus rendering them antigen-sensitive and responsive. Similarly, in the transfer of immunity to the nonresponsive host, the TF introduced to the subject presumably binds to the "immunologically virgin" cells. The binding of TF to membranes presumably results in the expression of the T cell-antigen receptor, perhaps by modification of existing membrane structure (since TF binding would require the presence of a receptor for TF, it is possible that this TF-binding site is capable of binding all TFs and that the resulting complex of antigen-specific TF and its membrane binding site forms the specific antigen receptor). However, de novo synthesis of the receptor or exposure of the relevant receptor by allosteric effects of the membrane proteins have not been excluded. Several additional hypotheses have been put forward, but the mechanism is probably so unusual that it has not yet been proposed.

Recent findings that DNA is present on the surface of 50% of T-lymphocytes in circulating peripheral blood (142, 143) raise another intriguing possibility, namely that the oligoribonucleotide portion of TF binds to complementary DNA on the cell surface and that both the DNA and the oligoribonucleotide bound to it are then internalized via the DNA receptor. Furthermore, the TF might be cleaved, so that the peptide portion of TF remains at the cell surface and the TF peptide becomes part of the antigen receptor. DNA, acting as a ligase, could facilitate incorporation of the peptide into the antigen receptor.

Cech et al (144) have shown that RNA enzymes ("ribozymes") catalyze RNA splicing reactions like enzymes that act on other substances. The folded RNA, like a true enzyme, speeds up an otherwise slow reaction (in fact the rate of cell splicing is increased 10 billion times). Since RNA is an informational macromolecule, these findings may have relevance to the oligoribonucleotide portion of transfer factor. Ribozymes do not merely accelerate the rate of the biochemical reaction, but show extraordinary specificity with respect to the substrate they act upon and the products they generate. McFarlane Burnett suggested decades ago (personal communication) that the chief role of transfer factor was to rapidly increase synthesis of more transfer factor. Perhaps this ribozyme mechanism is involved.

Most investigators in the TF area neglect the fact that induction of LIF secretion requires specific antigen and collaboration between T-lymphocytes and monocytes. Whether the two cell types are simultaneously involved or whether TF acts on one cell directly or acts indirectly on one cell population after the first cell population results in liberation of lymphokines that activate the second is unknown. DLE-TF had no effect in one recipient in whom no monocytes were detected in peripheral blood; when monocytes spontaneously reappeared about six months later, subsequent administration of DLE-TF produced improvement (29).

Purification and Characterization

Future progress in this area depends on complete isolation and purification of transfer factors of different specificities. We believe that the RNA bases are responsible for activity and the peptides for antigen-specificity (if any of the 20 known amino acids can be at any of the 6–8 positions in the peptides, the number of different specificities can be in the billions). Such delineation would make possible production of huge quantities of TFs of different specificities by solid phase synthesis of both peptides and nucleotides. This would also provide data helpful in understanding the mechanism of action of TF; many have been proposed (e.g. depressor), but the authors believe none thus far proposed are correct. It is conceivable that TF binds to the surface of

virgin T cells and by allosteric or other effect either uncovers the relevant receptor for the antigen in question, or fits into a "plastic" receptor to activate the receptor for antigen. Possibilities as far-fetched as transport to microsomes or nucleoli by transport proteins, and subsequent synthesis of the TF constituents at these sites also warrant exploration.

CONCLUSION

Fundamental information regarding the nature of transfer factor has accumulated slowly, because DLE contains 200 moieties rather than 1, as originally thought. Progress has also been hampered by those who refuse to believe in its clinical efficiency until the mechanism is known. However, penicillin was used in pneumococcal pneumonia for 10 yr with dramatic clinical results before the uncovering of its mechanism of action, namely inhibition of replication of bacterial cell walls. Hopefully, in the next few years our understanding of the structure and mechanisms of TF will be greatly extended, and TFs of various specificities will be produced by recombinant DNA methodology.

Although knowledge of its mechanism and/or structure is unknown, DLE containing TF has become widely used since it is relatively inexpensive and since there are no satisfactory alternatives in some diseases (various viral infections, etc). In Czechoslovakia, one state institute has prepared a massive pool of DLE enriched in TF by Sephadex G25 gel filtration and has distributed it to every hospital in the country (V. Mayer, personal communication). In Japan, the forty Red Cross Centers are providing DLE from pooled leukocytes of normal healthy donors to 400 hospitals for use in a wide variety of conditions (145). In China, porcine DLE has been prepared in several centers and has been widely used in human diseases (146). Some of the disorders are listed in Table 3.

Table 3 DLE-Transfer factor - beneficial results

Reported by us, confirmed by others

1. Familial T-lymphocyte dysfunction with severe recurrent infection
2. Herpes infection
3. Cytomegalovirus infection
4. Candidiasis
5. Parasitic infection (e.g. pneumocystis carinii, cryptosporidiosis, etc.)
6. Mycobacterium tuberculosis infection refractory to antibiotics
7. Behçet's syndrome
8. One set of lupus erythematosus
9. Pemphigus vegetans (skin disease)
10. Wiskott-Aldrich syndrome

Table 3 *(continued)*

Reported by us

1. Mycobacterium fortuitum infection
2. Mycobacterium avian infection
3. Alopecia totalis
4. Alzheimer's disease (one subset)
5. Chronic Hyperfatigability Syndrome
6. Autism (one subset, 70%)
7. Retinitis pigmentosa (one subset, 50%; DLE-TF does not reverse the disease but prevents additional visual loss)
8. Amyotrophic lateral sclerosis (one subset)
9. Osteosarcoma (DLE-TF prevents metastases to lungs)
10. Bone metastases after surgical removal of breast cancer
11. Bone metastases after surgical removal of kidney cancer
12. Epidermal dysplasia (multiple cutaneous malignancies)
13. Certain food and chemical hypersensitivities
14. Coccidioidosis

Reported by others

1. Lepromatous leprosy
2. Lieshmaniasis
3. Rat diabetes (Type I–immunologic) (trials in humans not yet reported)
4. Myasthenia gravis
5. Subacute sclerosing panencephalitis
6. Atopic dermatitis
7. Bronchial asthma
8. Recurrent otitis media
9. Varicella
10. Hepatitis B

In view of the lack of adverse side effects in humans receiving DLE-TF from infra-human species, it is likely that use of such TFs (especially bovine colostrum) will become standard therapy in any disease for which no current therapy exists, or when such therapy has toxic side effects. Bovine colostral DLE can be made in huge quantities at much lower cost and in much less time than is required for the production of human DLE-TF.

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Literature Cited

1. Lawrence, H. S. 1949. The cellular transfer of cutaneous hypersensitivity to tuberculin in man. *Proc. Soc. Exp. Biol. Med.* 71:516–22
2. Miller, J. H. F. 1962. Immunologic significance of the Thymus in the adult mouse. *Nature* 195:1318–19
3. Lawrence, H. S. 1955. The transfer in humans of delayed skin sensitivity to streptococcal M substance and to tuberculin with disrupted leukocytes. *J. Clin. Invest.* 34:219–30
4. Fudenberg, H. H., Smith, C. L. 1981. Immunomodulation and immunotherapy: An overview of biologic and synthetic agents and their effects on the human immune system. *EOS Riv. Immunol. Immunopharmacol.* 1:3–11
5. Levin, A. S., Spitler, L. E., Site, D. P., Fudenberg, H. H. 1970. Wiskott-Aldrich Syndrome, a genetically determined cellular immunologic deficiency: Clinical and laboratory responses to therapy with transfer factor. *Proc. Natl. Acad. Sci. USA* 67:821–28
6. Fudenberg, H. H., Spitler, L. E., Levin, A. S. 1972. Treatment of immune deficiency. *Am. J. Pathol.* 69:529–36
7. Sargent, I. L., Myer, R. S., Valdimarsson, H. 1979. Effects of Transfer Factor (TF) and Thymosin on the Recovery of E-Rosetting Capacity in Trypsinised Lymphocytes. See Ref. 24, pp. 129–36
8. Wilson, G. B., Jonsson, H. T. Jr., Halushka, P. V., Garner, B. P., Berkaw, M. N., et al. 1979. Contribution of prostaglandins to the biological activity of Dialyzable Leukocyte Extracts containing Transfer Factor Activity. See Ref. 24, pp. 137–50
9. Fudenberg, H. H., Wilson, G. B., Goust, J. M., Nekam, K., Smith, C. L. 1980. Dialyzable leukocyte extracts (transfer factor): A review of clinical results and immunological methods for donor selection, evaluation of activities, and patient monitoring. In *Thymus, Thymic Hormones and T Lymphocytes,* ed. F. Aiuti, H. Wigzell. *Proc. Sereno Symp.* 38:391–421. London: Academic
10. Wilson, G. B., Fudenberg, H. H. 1983. Is controversy about "transfer factor therapy" nearing an end? *Immunol. Today* 4:157–61
11. Wilson, G. B., Welch, T. M., Fudenberg, H. H. 1976. Human transfer factor in guinea pigs: Partial purification of the active component. In *Transfer Factor: Basic Properties and Clinical Applications,* ed. M. S. Ascher, A. A. Gottlieb, C. H. Kirkpatrick, pp. 409–23. New York: Academic
12. Fudenberg, H. H., Keller, R., Wilson, G. W. 1988. Interactive T cells: measurement and significance. In prep.
13. Fudenberg, H. H., Wilson, G. B., Tsang, K. Y. 1984. Evaluation of "transfer factor" potency and prediction of clinical response. In *Immunomodulation: New Frontiers and Advances,* ed. H. H. Fudenberg, H. Whitten, F. Ambrogi, pp. 115–30. New York: Plenum
14. Jeter, W. S., Kibler, R., Soli, T. C., Stephens, C. A. L. 1979. Oral administration of bovine and human dialyzable transfer factor to human volunteers. See Ref. 24, pp. 451–60
15. Schwartz, R. S., Jeter, W. S. 1981. Oral-administration of human dialyzable transfer factor in a patient with psoriasis. *Arch. Dermatol.* 117:3–4
16. Deleted in proof
17. Wilson, G. B., Metcalf, J. F. Jr., Fudenberg, H. H. 1982. Treatment of *Mycobacterium-fortuitum* pulmonary infection with "Transfer Factor" (TF): New methodology for evaluating TF potency and predicting clinical-response. *Clin. Immunol. Immunopathol.* 23:478–91
18. Metcalf, J. F., John, J. F. Jr., Wilson, G. B., Fudenberg, H. H., Harley, R. A. 1981. Mycobacterium fortuitum pulmonary infection associated with an antigen-selective defect in cellular-immunity. *Am. J. Med.* 71:485–92
19. Kyong, C. U., Wilson, G. B., Fudenberg, H. H., Goust, J. M., Richardson, P., Eckerd, J. 1980. Chorioretinitis with a combined defect in T and B lymphocytes and granulocytes: A new syndrome successfully treated with dialyzable leukocyte extracts (Transfer Factor). *Am. J. Med.* 68:955–61
20. Wilson, G. B., Fudenberg, H. H. 1981. Leukocyte migration inhibition as a method for assaying transfer factor activities. In *Lymphokines,* ed. E. Pick, M. Landy, 4:107–73. New York: Academic
21. Hoffman, P. M., Sputler, L. E., Hsu, M., Fudenberg, H. H. 1975. Leukocyte migration-inhibition in agarose. *Cell. Immunol.* 18:21–30
22. Wilson, G. B., Fudenberg, H. H., Horsmanheimo, M., 1979. Effects of Dialyzable Leukocyte Extracts (DLE) with Transfer Factor (TF) activity on leukocyte migration *in vitro*. I. Antigen-dependent inhibition and antigen-inde-

pendent inhibition and enhancement of migration. *J. Lab. Clin. Med.* 93:800–18

23. Vasily, D. B., Miller, O. F., Fudenberg, H. H., Goust, J. M., Wilson, G. B., 1984. Epidermodysplasia verruciformis: Response to therapy with dialyzable leukocyte extract (transfer factor) derived from household contacts. *J. Clin. Lab. Immunol.* 14:49–57
24. Khan, A., Kirkpatrick, C. H., Hill, N. O., ed. 1979. *Immune Regulators in Transfer Factor*. New York: Academic
25. Fudenberg, H. H., Wilson, G. B., Tsang, K.Y. 1983. Evaluation of potency and predictability of clinical response to DLE containing transfer factor. In *Immunomodulation and Thermotherapy,* ed. H. H. Fudenberg, P. Pontiggia, C. Ogier, pp. 141–52. Rome: Acta Medica
26. Fudenberg, H. H., Wilson, G. B., Keller, R. H., Metcalf, J. F., Paulling, E. E., et al. 1983. Clinical applications of the leucocyte migration inhibition assay—new methods for determining transfer factor potency and for predicting clinical response. In *Immunobiology of Transfer Factor*, ed. C. H. Kirkpatrick, H. S. Lawrence, D. R. Burger, pp. 293–310. New York: Academic
27. Borkowsky, W., Lawrence, H. S. 1979. Effects of human leukocyte dialysates containing transfer factor in the direct Leukocyte Migration Inhibition (LMI) assay. *J. Immunol.* 123:1741–48
28. Arala-Chaves, M. P., Fudenberg, H. H. 1976. Specificity of transfer factor. *Nature* 262:155–56
29. Arala-Chaves, M. P., Silva, A., Porto, M. T., Picoto, A., Ramos, M. T. F., Fudenberg, H. H. 1977. *In vitro* and *in vivo* studies of the target cell for dialyzable leukocyte extracts: Evidence for recipient specificity. *Clin. Immunol. Immunopathol.* 8:430–47
30. Deleted in proof
31. Deleted in proof
32. Cohen, L., Holzman, R. S., Valentine, F. T., Lawrence, H. S. 1976. Requirement of precommitted cells as targets for the augmentation of lymphocyte proliferation by leukocyte dialysates. *J. Exp. Med.* 143:791–804
33. Wilson, G. B., Welch, T. M., Knapp, D. R., Horsmanheimo, A., Fudenberg, H. H. 1977. Characterization of Tx, an active subfraction of human dialyzable transfer factor. I. Identification of the major component of TFg, a precursor of Tx, as hypoxanthine. *Clin. Immunol. Immunopathol.* 8:551–69
34. Gottlieb, A. A., Sutcliffe, S. B. 1982. *In vivo* modification of delayed-type hypersensitivity by small molecular-weight components derived from human-leucocytes: partial-purification of components causing amplification of response. *Clin. Exp. Immunol.* 50:434–41
35. Gottlieb, A. A., Farmer, J. L., Matzura, C. T., Hester, R. B., Rosenberg, J. S. 1984. Modulation of human T-cell production of migration-inhibitory lymphokines by cytokines derived from human-leukocyte Dialysates. *J. Immunol.* 132(1):256–60
36. Levin, A. S., Byers, V. S., Fudenberg, H. H., Wybran, J., Hackett, A. J., et al. 1975. Osteogenic sarcoma: Immunologic parameters before and during immunotherapy with tumor-specific transfer factor. *J. Clin. Invest.* 55:487–99
37. Wybran, J., Carr, M. C., Fudenberg, H. H. 1972. The human rosette-forming cell as a marker of the population of thymus-derived cells. *J. Clin. Invest.* 51:2537–43
38. Wybran, J., Levin, A. S., Spitler, L. E., Fudenberg, H. H. 1973. Rosette-forming cells, immunologic deficiency diseases and transfer factor. *New Engl. J. Med.* 288:710–13
39. Horowitz, S., Groshong, T., Albrecht, R., Hong, R. 1975. The "active" rosette test in immunodeficiency diseases. *Clin. Immunol. Immunopathol.* 4:405–14
40. Nekam, K., Kalmar, L., Gergely, P., Kelemen, G., Fekete, B., Lang, I. 1977. *In vitro* effect of transfer factor on active rosettes and leukocyte migration of patients with cancer. *Clin. Exp. Immunol.* 27:416–20
41. Goust, J. M., Fudenberg, H. H. 1983. T-cell binding to B-lymphoid cell-lines in humans: A marker for T—B cell-interaction? *J. Immunol. Methods* 59: 29–38
42. Galbraith, G. M. P., Fudenberg, H. H. 1985. Transfer factor. In *Dermatologic Immunology and Allergy,* ed. J. Stone, pp. 889–98. St. Louis, Mo: Mosby
43. Dorfling, P., Schroder, I. 1987. Effect of the dialyzable leukocyte extract and its different fractions on the production of H2O2 and IL-1 by macrophages. In *Leukocyte Dialysates and Transfer Factor,* ed. V. Mayer, J. Borvak, pp. 141–45. Inst. Virol., Slovak Acad. Sci.
44. Nekam, K., Fudenberg, H. H., Mandi, B., Lang, I., Gergely, P., Petranyi, G. 1981. Resynthesis of trypsinized sheep red blood cell receptors on human lymphocytes: Comparison of the effects of immunopotentiators of biological and synthetic origin *in vitro*. *Immunopharmacology* 3:31–39
45. Nekam, K., Lang, I., Torok, K., Kal-

mar, L., Gergely, P., Petranyi, G. 1979. Effects of therapy with dialyzable leukocyte extracts containing transfer factor activity on antibody-dependent cytotoxic activity in humans. *Clin. Immunol. Immunopathol.* 13:407–12

46. Uotila, A., Krohn, K., Marnela, K.-M., Antonen, J. 1983. Mechanism of the *in vitro* augmentation of lymphocyte transformation by transfer factor and by other cellular Dialysates. See Ref. 26, pp. 227–36
47. Dupont, B., Ballow, M., Hansen, J. A., Quick, C., Yunis, E. J., Good, R. A. 1974. Effect of transfer factor therapy on mixed lymphocyte culture reactivity. *Proc. Natl. Acad. Sci. USA* 71:867–71
48. Gallin, J. I., Kirkpatrick, C. H. 1974. Chemotactic activity in dialyzable transfer factor. *Proc. Natl. Acad. Sci. USA* 71:498–502
49. Lang, I., Nekam, K., Gergely, P., Petranyi, G. 1982. Effect of *in vivo* and *in vitro* treatment with dialyzable leukocyte extracts containing transfer factor activity on human natural killer cell activity. *Clin. Immunol. Immunopathol.* 25:139–44
50. Lawrence, H. S. 1969. Transfer factor. *Adv. Immunol.* 11:195–266
51. Nekam, K., Perl, A., Gergely, P., Lang, I., Gonzales-Cabello, R., Feher, J. 1987. Effects of dialyzable leukocyte extracts on lectin-dependent cell-mediated cytotoxicity *in vitro*. See Ref. 43, pp. 171–75
52. Byers, V. S., Levin, A. S., Hackett, A. J., Fudenberg, H. H. 1975. Tumor-specific cell mediated immunity in household contacts of cancer patients. *J. Clin. Invest.* 55:500–13
53. Fudenberg, H. H. 1986. Transfer factor: Past, present and future. *Plenary Lecture 5th Int. Symp.*, Bratislava, Czech.
54. Fudenberg, H. H., Levin, A. S., Spitler, L. E., Wybran, J., Byers, V. 1974. The therapeutic uses of transfer factor (Invited review). *Hosp. Pract.* 9:95–104
55. Kirkpatrick, C. H., Greenberg, L. E. 1979. Treatment of chronic mucocutaneous candidiasis with transfer factor. See Ref. 24, pp. 547–52
56. Littman, B. H., Rocklin, R. E., Parkman, R., David, J. R. 1978. Transfer factor treatment of chronic mucocutaneous candidiasis: Requirement for donor reactivity to candida antigen. *Clin. Immunol. Immunopathol.* 9: 97–110
57. Spitler, L. E., Levin, A. S., Fudenberg, H. H. 1975. Transfer Factor II: Results of therapy. In *Primary Immunodeficiency Diseases in Man*, ed. D. Bergsma, R. A. Good, J. Finstad, N. W. Paul. *Birth Defects: Original Articles Ser.* 11(1):449–56. White Plains, New York: Natl. Found.
58. Valdimarsson, H., Higgs, J. M., Wells, T. S., Yamamara, M., Hobbs, J. R. Holt, P. J. L. 1973. Immune abnormalities associated with chronic mucocutaneous candidiasis. *Cell. Immunol.* 6:348–61
59. Lee, W. M., Holley, H. P., Stewart, J., Galbraith, G. M. P. 1986. Refractory esophageal candidiasis associated with a plasma inhibitor of T-lymphocyte function: Response to plasma exchange. *Am. J. Med. Sci.* 292:47–52
60. Catanzero, A., Spitler, L. E. 1976. Clinical and immunologic results of TF therapy in coccidiomycosis. See Ref. 11, pp. 477–94
61. Myrvang, B., Godal, T., Ridley, D. S., Froland, S. S., Song, Y. K. 1973. Immune responsiveness to *Mycobacterium leprae* and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy. *Clin. Exp. Immunol.* 14:541–53
62. Bullock, W. E., Fields, J. P., Bandvias, M. W. 1972. An evaluation of transfer factor as immunotherapy for patients with lepromatous leprosy. *New Engl. J. Med.* 287:1053–1059
63. Hastings, R. C., Morales, M. J., Shannon, E. J., Jacobson, R. R. 1976. Preliminary results in the safety and efficacy of transfer factor in leprosy. See Ref. 11, pp. 465–76
64. Lesser, P. G., Byers, V. S., Fudenberg, H. H., Wybran, J., Hackett, A. J., et al. 1975. Cell mediated immunity in patients with Virchowian hanseniasis before immunotherapy with tumor-specific transfer factor. *J. Clin. Invest.* 55:487–99
65. Sharma, M. K., Anaraki, F., Ala, F. 1979. Preliminary results of transfer factor therapy of persistent cutaneous leishmania infection. *Clin. Immunol. Immunopathol.* 12:183–90
66. Sharma, M., Firouz, R., Ala, F. 1979. Transfer factor therapy in human cutaneous leishmania infection (CLI): A double-blind clinical trial. See Ref. 24, 563–70
67. Delgado, O., Romano, E. L., Belfort, E., Pifano, F., Scorza, J. V., Rojas, Z. 1981. Dialyzable leukocyte extract therapy in immunodepressed patients with cutaneous leishmaniasis. *Clin. Immunol. Immunopathol.* 19:351–59
68. Steele, R. W., Myers, M. G., Vincent, M. M. 1980. Transfer factor for the prevention of varicella zoster infection in

childhood leukcmia. *New Engl. J. Med.* 303:355–59
69. Fudenberg, H. H. 1985. Ophthalmologic *Herpes Zoster"* Complete rcmission in six hours with dialyzable transfer factor. *J. Clin. Lab. Immunol.* 18:49–51
70. Prochazkova, J., Parizkova, E., Horacek, J., Pozler, O., Chylkova, V., et al. 1987. Clinical and immunological improvement in infants with HCMV infection treated with dialyzable leukocyte extract. See Ref. 43, 403–11
71. Freiburger, K., Fudenberg, H. H. 1980. Behcet's Disease: Pitfalls in therapy and diagnosis. *Hosp. Pract.* 15(11):40g–40j
72. Hooks, J. J. 1978. Possibility of a viral etiology in recurrent aphthous ulcers and Behcet's syndrome. *J. Oral Pathol.* 7:353–64
73. Heim, L. R. 1979. Atopic dermatitis, specific virus infections, and Behcet's syndrome, transfer factor therapy. See Ref. 24, pp. 531–36
74. Lehner, T. 1978. Immunological aspects of recurrent oral ulceration and Behcet's syndrome. *J. Oral Pathol.* 7:424–30
75. Wolf, R. E., Fudenberg, H. H., Welch, T. M., Spitler, L. E., Ziff, M. 1977. Treatment of Behcet's syndrome with transfer factor. *J. Am. Med. Assoc.* 238:869–71
76. Rook, A. 1977. Common baldness and alopecia areata. In *Recent Advances in Dermatology,* ed. A. Rook, pp. 223–47. New York: Churchill Livingstone
77. Galbraith, G. M. P., Thiers, B. H., Vasily, D. B., Fudenberg, H. H. 1984. Immunological profiles in alopecia areata. *Br. J. Dermatol.* 110:163–70
78. Wolf, R. E., Fudenberg, H. H., Gilliam, J. N. 1978. Transfer factor therapy in a case of pemphigus vegetans associated with chronic mucocutaneous candidiasis. *Clin. Immunol. Immunopathol.* 10:292–97
79. Fudenberg, H. H., Strelkauskas, A. J., Goust, J. M., Osborne, D., Fort, D., Vasily, D. 1981. "Discoid" lupus erythematosus: Dramatic clinical and immunological response to dialyzable leukocyte extract (Transfer Factor). *Trans. Assoc. Am. Physicians* 94:279–91
80. Vasily, D. B., Miller, F., Fudenberg, H. H., Goust, J. M., Wilson, G. B. 1984. Epidermodysplasia verriciformis: response to therapy with dialyzable leukocyte extract (transfer factor) derived from household contacts. *J. Clin. Lab. Immunol.* 14:49–57
81. Rovensky, J., Schroder, I., Pekarek, J., Svejcar, J., Vlcek, F. 1979. Transfer factor treatment in patients with psoriasis. See Ref. 24, 605–32
82. Vlcek, F., Rovensky, J., Urbanek, T., Svec, V., Lukac, J., et al. 1987. Immunomodulatory thcrapy with dialyzable leukocyte extract (DLE) in psoriasis. See Ref. 43, pp. 565–70
83. Dwyer, J. M., Topper, E., Sherwin, R. 1987. The prevention of diabetes in BB rats by the administration of transfer factor. In 5th International Symposium on Transfer Factor, ed. V. Mayer, J. Borvak, pp. 275–84. Bratislava, Czechoslovakia: Inst. Virol., Slovak Acad. Sci.
84. Clark, W. H., Mastrangelo, M. J., Ainsworth, A. M., Berd, D., Bellet, R. E., Bernardino, E. A. 1977. Current concepts of the biology of human cutaneous malignant melanoma. *Adv. Cancer Res.* 24:267–338
85. Bukowski, R. M., Deodhar, S., Hewlett, J. S., Greenstreet, R. 1983. Randomized controlled trial of transfer factor in stage II malignant melanoma. *Cancer* 51:269–72
86. Vetto, R. M., Burger, D. R., Nolte, J. E., Vandenbark, A. A. 1976. Transfer factor immunotherapy in cancer. See Ref. 11, pp. 523–36
87. Bukowski, R. M., Hewlett, J. S., Deodhar, S. H. 1979. Immunotherapy of stage II malignant melanoma and renal cell carcinoma with transfer factor: Clinical results. See Ref. 24, pp. 581–90
88. Byers, V. S., Levin, A. S., Hackett, A. J., Fudenberg, H. H. 1975. Tumor-specific cell mediated immunity in household contacts of cancer patients. *J. Clin. Invest.* 55:500–13
89. Deleted in proof
90. Fudenberg, H. H., Tsang, K. Y. 1985. In utero osteosarcoma tolerized hamsters; a model for human cancer and immunocyte differentiation. In *Theories and Models in Cellular Transformation,* ed. L. Saanti, L. Zardi, pp. 23–43. London: Academic
91. Deleted in proof
92. Deleted in proof
93. Morrison, S. K., Fudenberg, H. H., Vasily, D. B. 1988. Ten year survival of a patient with osteosarcoma and pulmonary metastases. In prep.
94. Fudenberg, H. H., Singh, V. K. 1988. Immunodiagnosis and immunotherapy of paticnts with Alzheimer's discase. In *Immunology and Alzheimer's Disease,* ed. A. Pouplard-Barthelaux, J. Emile, Y. Christen. Berlin: Springer-Verlag
95. Fudenberg, H. H., Coleman, M., Rosberger, D., Singh, V. J., 1988. Immunotherapy of Autistic children. *Clin. Res.* In press
96. Galbraith, G. M. P., Fudenberg, H. H. 1984. One subset of patients with retini-

tis pigmentosa has immunologic defects. *Clin. Immunol. Immunopathol.* 31:254–60

97. Galbraith, G. M. P., Emerson, D., Fudenberg, H. H., Gibbs, C. J., Gajdusek, D. C. 1986. Antibodies to neurofilament protein in retinitis pigmentosa. *J. Clin. Invest.* 78:865–69
98. Bahmanyar, S., Moreau-Dubois, M. C., Brown, P., Cathala, F., Gajdusek, D. C. 1983. Serum antibodies to neurofilament antigens in patients with neurological and other diseases and in healthy controls. *J. Neuroimmunol.* 5(2):191–96
99. Deleted in proof
100. Deleted in proof
101. Fudenberg, H. H., Fudenberg, Hl., Emerick, J. A., Paulling, E. M. 1989. Immunodiagnosis and immunotherapy in CHF, *Clin. Res.* (Abstr.) In press
102. Yang, B., Li. Z., Cui, Z., Luo, Y. 1989. Mechanisms of development of myasthenia gravis and the treatment effects of transfer factor. *Int. J. Immunopharmacol.* In press
103. Fog, T., Pedersen, L., Raun, N. E., Kam-Hansen, S., Mellerup, E., et al. Long-term transfer factor treatment for multiple sclerosis. *Lancet* 1(8069):851–53
104. Basten, A., Pollard, J. D., Stewart, G. J., Frith, J. A., McLeod, J. G. et al. 1980. Transfer factor in treatment of multiple sclerosis. *Lancet.* 2:931–34
105. Deleted in proof
106. Deleted in proof
107. Deleted in proof
108. Klesius, P. H., Fudenberg, H. H. 1977. Bovine transfer factor: *in vivo* transfer of cell-mediated immunity to cattle with alcohol precipitates. *Clin. Immunol. Immunopathol.* 8:238–46
109. Wilson, G. B., Morin, M. L., Stuart, L. D., Williams, A. M., et al. 1983. Transfer of cell-mediated immunity in vitro to human lymphocytes using dialyzable leukocyte extracts from immune burros. See Ref. 26, pp. 213–32
110. Lewis, D. E., Cramer, J. D., and Reed, R. E. 1979. Detection of a new protein component in canine plasma after transfer factor administration: correlation with delayed-type skin reactivity. See Ref. 24, pp. 93–116
111. Tsang, K. Y., Fudenberg, H. H., Pan, J. F. 1985. Transfer of osteosarcoma specific cell-mediated immunity in hamsters by rabbit dialyzable leukocyte extracts. *Cell Immunol.* 90:295–302
112. Welch, T. M., Triglia, R., Spitler, L. E., Fudenberg, H. H. 1976. Preliminary studies on human "transfer factor" activity in guinea pigs: systemic transfer of cutaneous delayed-type hypersensitivity to PPD and SKSD. *Clin. Immunol. Immunopathol.* 5:407–15
113. Liburd, E. M., Pabst, H. R., Armstrong, W. D. 1972. Transfer factor in rat coccidiosis. *Cell. Immunol.* 5:487–89
114. Arala-Chaves, M. P., Klesius, P. H., Fudenberg, H. H. 1979. Evidence for specific and non-specific effects of dialyzable leukocyte extracts (containing transfer activity) in mice. See Ref. 24, pp. 15–26
115. Giambrone, J. J., Klesius, P. H., Yu, M. 1983. Adoptive transfer of delayed wattle reactivity in chickens with a dialyzable leukocyte extract containing transfer factor. *Poultry Sci.* 62(5):767–71
116. Klesius, P. H., Qualls, D. F., Elston, A. L., Fudenberg, H. H. 1978. Effects of bovine transfer factor (TFd) in mouse coccidiosis (Eimeria ferrisi). *Clin. Immunol. Immunopathol.* 10:214–21
117. Klesius, P. H., Kirkpatrick, C. H. 1983. Dialyzable leukocyte extract containing transfer factor—its future in veterinary medicine. See Ref. 26, pp. 129–42
118. Wilson, G. B., Fort, J. D. 1987. Interspecies transfers of cell-mediated immunity using specific immunity inducers with potency-prevention in selected diseases. See Ref. 43, pp. 333–58
119. Wilson, G. B., Poindexter, C., Fort, J. D., Ludden, K. D. 1988. *De novo* initiation of specific cell-mediated immune responsiveness in chickens by transfer factor (specific immunity inducer) obtained from bovine colostrum and milk. *Acta virol.* 32:6–18
120. Li, Z. 1987. Studies on porcine spleen cell dialysate: experimental applications of TF-p in veterinary and human medicine. See Ref. 43, pp. 478–90
121. Arrenbrecht, S., Aker, O., Dubs, R., Grob, P. J. 1979. Human transfer factor in rats. See Ref. 24, pp. 643–48
122. Burger, D. R., Klesius, P. H., Vandenberk, A. A., Vetto, R. M., Swann, A. I. 1979. Transfer of keyhole limpet hemocyanin dermal reactivity to man with bovine TF. *Cell. Immunol.* 43:192–96
123. Huo, B.-L. 1987. Clinical application of transfer factor in China. See Ref. 43, pp. 451–59.
124. Viza, D., Rosenfeld, F., Phillips, J., Vich, J. M., Denis, J., et al. 1983. Specific bovine transfer factor for the treatment of herpes infections. See Ref. 26, pp. 245–60
125. Jones, J. F., Schumacher, M. J., Jeter, W. S., Hicks, M. J. 1983. Oral bovine transfer factor (OTF) use in the hyper-

IgE syndrome. See Ref. 26, pp. 261–72

126. Louie, E., Borkowsky, W. S., Klesius, P. H., Haynes, T. B., Gordon S., et al. 1987. Treatment of Cryptosporidiosis with oral bovine transfer factor. *Clin. Immunol. Immunopathol.* 44(3):329–34
127. Wilson, G. B., Fudenberg, H. H. 1979. Effects of dialyzable leukocyte extracts with transfer factor activity on leukocyte migration in vitro. II. Separation and partial characterization of the components in DLE producing antigen-dependent and antigen-independent effects. *J. Lab. Clin. Med.* 93:819–37
128. Moulias, R. 1988. Use of murine transfer factor in AIDS. *Instit. Virol.* In press
129. Wilson, G. B., Paddock, G. V., Fudenberg, H. H. 1979. The chemical nature of the antigen-specific moiety of transfer factor. *Trans. Assoc. Am. Physicians* 92:239–56
130. Wilson, G. B., Paddock, G. V., Fudenberg, H. H. 1981. Effects of dialyzable leukocyte extracts with transfer factor activity on leukocyte migration *in vitro*. V. Antigen-specific lymphocyte responsiveness can be initiated by two structurally distinct polyribonucleotides. *Thymus* 2:257–76
131. Wilson, G. B., Paddock, G. V., Fudenberg, H. H. 1982. Bovine "transfer factor": an oligoribonucleopeptide which initiates antigen-specific lymphocyte responsiveness. *Thymus* 4:335–50
132. Wilson, G. B., Fudenberg, H. H., Paddock, G. V., Tsang, K. Y., Williams, A. M., Floyd, E. 1983. Mechanism(s) of action of human transfer factor: Insights obtained from studying "antigen liberated transfer factor" specific for tuberculin. See Ref. 26, pp. 331–46
133. Wilson, G. B., Newell, R. T., Burdash, N. M. 1979. Bovine dialyzable lymph node extracts have antigen-dependent and antigen-independent effects on human cell-mediated immunity in vitro. *Cell. Immunol.* 47:1–18
134. Paddock, G. V. 1987. Comparative structural analysis of transfer factor from different species and sources: relationships with other immunopotentiators. See Ref. 43, pp. 2–30
135. Burger, D. R., Vandenbark, A. A., Vetto, R. M., Klesius, P. 1983. Human Transfer Factor: Specificity and structural models. See Ref. 26, pp. 33–49
136. Paddock, G. V., Wilson, G. B., Fudenberg, H. H., Wang, A. C., Lovins, R. E. 1979. Purification and structural analysis of the Transfer Factor-like activity detected *in vitro* by leukocyte migration inhibition. See Ref. 24, pp. 419–32
137. Dinarello, C. A. 1988. Biology of interleukin-1. *FASEB J.* 2(2):108–15
138. Singer, I. I., Scott, S., Hall, G. L., Limjuco, G., Chin, J., Schmidt, J. A. 1988. Interleukin 1-β is localized in the cytoplasmic ground substance but is largely absent from the golig-apparatus and plasma-membranes of stimulated human-monocytes. *J. Exp. Med.* 167: 389–407
139. Paddock, G. V., Wilson, G. B., Williams, A. M., Fudenberg, H. H. 1983. Human Transfer Factor: Exogenous labelling, purification, and role of ribonucleic acid segment. See Ref. 26, pp. 51–63
140. Fudenberg, H. H., Pink, J. R. L., Wang, A. C., Ferrara, G. B. 1984. *Basic Immunogenetics.* New York: Oxford Univ. Press. 3rd ed.
141. Paddock, G. V., Wilson, G. B., Lin, F.-K., O'Leary, Fudenberg, H. H. 1981. In *Electrophoresis '81,* ed. R. Allen, P. Arnaud, pp. 479–86. New York: de Gruyter
142. Bennett, R. M., Gabor, G. T., Merritt, M. M. 1985. DNA binding to human leukocytes; evidence for a receptor-mediated association, internalization, and degradation of DNA. *J. Clin. Invest.* 76:1–9
143. Bennett, R. M., Hefeneider, S. H., Bakke, A., Merritt, M., Smith, C. A., et al. 1988. The production and characterization of murine monoclonal antibodies to a DNA receptor on human leukocytes. *J. Immunol.* 140:2937–42
144. Cech, T. R., 1987. The chemistry of cell splicing of RNA and RNA enzymes. *Science* 235:1531–39
145. Sasakawa, S., Takenouchi, K., Matsumoto, C., Mura, T., Saito, S., et al. 1987. Clinical trials of Dialyzable leukocyte extract (RCTF-1) in Japan. See Ref. 43, pp. 419–35
146. Huo, B. 1987. Clinical application of transfer factor in China. See Ref. 43, pp. 451–59
147. Ashwell, J. D. 1988. Are lymphocytes-B the principal antigen-presenting cells *in vivo? J. Immunol.* 140(11):3697–3700
148. Chng, H. H., Shaw, D., Klesius, P. H., Saxon, A. 1989. Inability of oral bovine TF to eradicate cryptosporidial infection in a patient with congenital dysgammaglobulinemia. *Clin Immunol.* In press