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MINIREVIEW

"Transfer Factor": An Update[1] (42015)

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After a short burst of activity following Lawrence's original description of the transfer factor phenomenon (1), interest in this area diminished drastically. Historically, TF was defined functionally as a biochemical entity in dialyzable leukocyte extracts (DLE) which could transfer antigen-specific reactivity as demonstrated by skin testing; it has been shown lately by more sensitive and quantitative *in vitro* assays measuring lymphokine release by T lymphocytes incubated with the appropriate antigen (2). However, only recently has it been definitively proven that TF acts in an antigen-specific manner (3). Other components present in DLE can have antigen-independent, nonspecific effects on cellular immunity (CMI) and on the inflammatory response; among these are prostaglandins, nicotinamide, ascorbic acid, serotonin, histamine, T lymphocyte or differentiation maturation factors, thymosin-like factor, monocyte chemoattractants, and neutrophil immobilizing factors. These components might well produce either generalized augmentation or even suppression (4) of immunologic activities upon clinical DLE evaluation *in vitro* and *in vivo*. In the early seventies interest in the TF phenomenon was rejuvenated when our group showed that DLE containing TF, in addition to transferring skin-test reactivity, conferred both the ability to produce lymphokines in response to specific antigens and resistance to infection in patients with genetically determined immune deficiency. Since then, several groups successfully employed DLE in immunotherapy and/or immunoprophylaxis in diseases associated with compromised cellular immunity. These diseases have included acquired or inherited "antigen-specific" or "broad spectrum" immunodeficiency diseases, neoplasia, and a variety of viral, fungal, and mycobacterial diseases. In these patients, each had failed to respond previously to any of the available antibiotics or chemotherapy and/or developed strong adverse reactions. Each patient treated with DLE served as his own control in that upon receiving DLE devoid of TF of the appropriate specificity, disease as measured by both clinical response or laboratory parameters worsened dramatically; however, DLE therapy containing TF of the *correct* specificity was substantially more clinically dramatic.

Total reliance on skin testing in TF research diminished as *in vitro* assays were developed (5). Also, TF cellular sources (helper T and target cells (T lymphocytes, natural killer cells, and possibly monocytes–macrophages)) have been identified (5–8). Animal models utilized to study TF have demonstrated the transfer of antigen-specific cellular immunity across mammalian species barriers. The enigmatic biochemical nature of TF is yielding and hypotheses have been postulated for its mode(s) of action (9, 10). For instance, two structurally distinct TF moieties have been detected in DLE for both antigen specificities (PPD and coccidioidin) extensively studied; we found one released by 37°C incubation and another released by cell rupture at 0°C. In addition, a third TF moiety exists for each TF specificity released by immune T-lymphocytes pulsed *in vitro* with specific antigen (9), different from the 37°C incubation TF only in absence of a phosphate group.

We recently applied the *in vitro* assay for TF activity to the clinical use of DLE containing TF and devised new methodologies for determining the TF potency of DLE preparations; these methods are able to predict whether a patient will benefit from TF

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immunotherapy and can monitor the recipient's response to TF therapy (10). Remarkably few side effects are elicited by DLE-TF (occasional pain or erythema at the site of injection and transient low-grade fever) and there is not overt evidence of toxicity (7).

The variable results of past DLE clinical studies may be due to inappropriate selection of recipient–donor, improper evaluation of potency and specificity of preparations, inadequate monitoring of immune responses to therapy, and inefficient conventional therapy, e.g., antibiotics, surgery to reduce the antigen load before DLE administration.

Controlled clinical trials. Four clinical trials of DLE therapy (double-blind) emphatically reinforce the potential value of DLE-TF in immunotherapy and/or immunoprophylaxis. These involved chronic aggressive hepatitis, multiple sclerosis, varicella zoster infection in leukemic children, and cutaneous leishmania infection (Ref. 11). More recent cases demonstrating the efficacy of DLE are reported below.

Behcet's syndrome. Behcet's syndrome, most frequently characterized by severe orogenital ulceration is of unknown etiology. Studies of the immune system have revealed impaired cellular immunity in Behcet's patients. Most consistently, patients had diminished peripheral "interactive" T cells (see below). Low numbers of interactive T cells frequently are found in recurrent viral infections. We postulated previously that an environmental viral agent may play a role in the etiology of Behcet's syndrome in which a deficient CMI was the predisposing factor.

Our first therapeutic trial with DLE in this disease began in 1974 in which DLE was prepared from random donors; at present we use household contact donors since the putative etiologic agent is unknown but presumably present in the patient's environment.

To date 14 patients with Behcet's syndrome have been treated with encouraging results. Of the first 6 who were administered DLE from random donors, 3 showed rapid clinical responses. Since then, 8 patients were treated with "household contact" DLE, 7 of which improved dramatically with concomitant normalization of immunological parameters. In 2 of these 7 patients, remission was short lived. One became unresponsive to DLE after 6 months, and one discontinued therapy after 2 months, after which disease exacerbation occurred. In the remaining 5 Behcet's syndrome patients, long-term remission was obtained with DLE therapy; high-dose steroid therapy was discontinued and/or reduced in others. One patient is free of symptoms 6 years after beginning DLE therapy, in spite of several relapses after temporary cessation of DLE.

Interactive T-cell enumeration has proved in our hands to be the most reliable index of immunological response in these patients.

Alopecia areata. It is generally accepted that immunological factors may participate in this disease of unknown etiology characterized by hair loss. The frequent history of autoimmune phenomena in families with this disease prompts the concept that the pathogenesis of alopecia areata is itself autoimmune. Previously, we suggested that the disease may be triggered by an environmental agent (12). Immunological studies in our laboratory of 60 patients with alopecia areata have revealed a patient subpopulation with cellular immune dysfunction. These patients have severe hair loss involving the entire scalp or body, and may have concomitant thyroid autoantibodies but without overt thyroid dysfunction. Consistent CMI defects include diminished interactive T cells, and low production of lymphokines. An open-label trial of DLE therapy in 9 patients using household contact-prepared DLE was recently conducted. Generally, an initial course of 9–12 IU was administered over a 5-day period and followed by 1 unit booster injections every 3–4 weeks. All patients responded immunologically with normalization of interactive T cells and lymphokine production. Insignificant clinical responses were noticed in 3 patients; 6 other patients demonstrated regrowth of hair from 1 to 3 months after initiation of therapy, a growth that has persisted. The first patient included in the trial continued on therapy for $1\frac{1}{2}$ years and hair growth is now cosmetically acceptable. In 3 patients, therapy was discontinued during hair regrowth (at $\frac{1}{4}$–$\frac{1}{2}$ years after onset of therapy). In each, acute hair loss after 1 month of DLE therapy discontinuance oc-

curred, and growth resumed after therapy reinitiation.

Epidermodysplasia verruciformis (EV). We recently reported the clinical and laboratory responses to DLE therapy of two EV patients; EV is a chronic cutaneous infection demonstrating a variety of human papillomavirus types (13). One patient with chronic disease (30 years) and no improvement on various therapies showed gradual yet definite resolution of extensive verrucae planae, plaque, tinea-versicolor-like, and tumor lesions scattered over the entire integument. DLE therapy cessation for 9 months resulted in recurrence of partially regressed lesions and new tumors in this patient, followed by regression upon DLE therapy reinitiation. The second patient was a grandson of the first and had minimal disease that showed no disease progression during DLE prophylaxis. A third patient was a brother of the second patient, received no DLE and served as a control. All demonstrated severely depressed T-suppressor cell levels, a defect not previously reported in EV patients. Finally, evidence was found for a possible X-linked recessive mode of inheritance for susceptibility to EV.

Mycobacterial infection. Mycobacterium fortuitum is not usually considered a human pathogen; yet, in a large series of patients with malignancy *M. fortuitum* was the second most common cause of mycobacterial disease. Recently we treated a patient in which *M. fortuitum* appeared to be causing serious pulmonary disease; *in vitro* evaluation suggested that this patient's immune system was compromised due to an antigen-selective CMI defect to the *M. fortuitum* agent. Immunotherapy with DLE TF specific for *M. fortuitum* was initiated because of chronic pulmonary fibrosis and severe pulmonary infection which proved to be refractory to the standard medications of antituberculosis therapy and amikacin; thus, the patient may have had an antigen-selective cellular immune defect to *M. fortuitum,* while his efferent CMI seemed otherwise intact. DLE has been used immunotherapeutically with clinical success in patients with similar diseases.

When DLE preparations of known *in vitro* TF potency were used and therapeutic DLE injections were administered in amounts commensurate with their TF potency, successful induction of responsiveness to PPD-F (PPD-derived from *M. fortuitum*) *in vivo* was obtained. Also, the patient gradually improved clinically during the 3-year treatment. His responsiveness to PPD-F has been maintained with prophylactic DLE injections every 6 months, while no antitubercular medications have been necessary (14).

Another elderly patient with life-threatening *M. intracellulare* pulmonary infection was found to have absent CMI response to the PPD-I antigen. After therapy for 3 months with antigen-specific DLE he improved clinically and became antigen-responsive on *in vitro* testing.

Retinitis pigmentosa. Although retinitis pigmentosa (RP) is a syndrome of uncertain etiology, progressive visual loss occurs due to degeneration of retinal photoreceptors. The majority of RP patients have no family history of disease; however, in others RP is hereditary. Although no distinctive concept of immunological mechanisms in RP has emerged, we recently evaluated 20 RP patients immunologically (15). Immune defects existed in the RP patients compared to the healthy control group. Interestingly, one patient subset showed reduced circulating T cells, deficient mitogenesis to PHA and lymphokine production, elevated circulating antibodies to IgG, interactive T cell formation (see below), and elevated serum IgG. Thus, RP is most probably a syndrome in which at least one disease entity is associated with (and perhaps due to) dysregulated immunity. DLE preparations from household contacts restored these *in vitro* abnormalities and appear to have arrested significantly overt clinical disease; whether further visual deterioration can be halted will be determined by further follow-up.

Interactive T cells. Certain human T cells from peripheral blood, termed interactive T cells, bind to established normal and malignant B cell lines to form rosettes. The percentage of such rosette formation is approximately 27.8 ± 5.3% in normals and is a stable characteristic over 1 year (16). Severely diminished interactive T-cell rosetting has been detected in certain patient groups, such as Behcet's syndrome and alopecia totalis

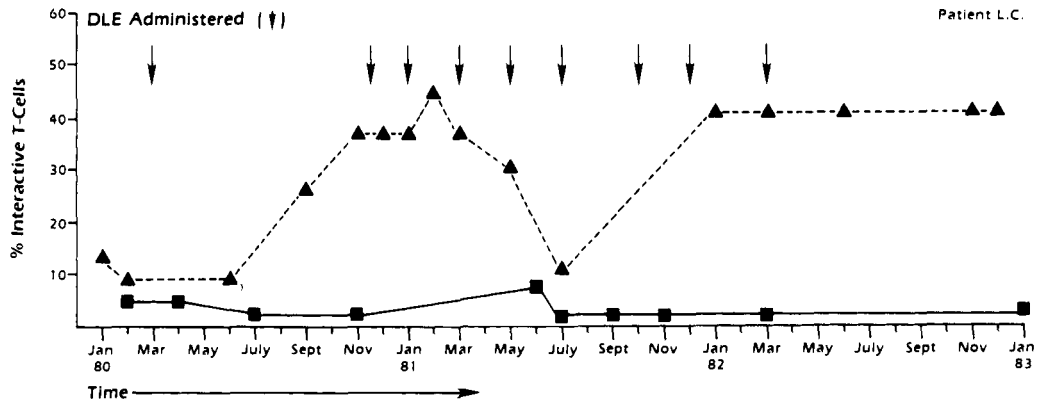

FIG. 1. Correlation of symptoms and DLE administration with interactive T-cell numbers in patient L.C. (▲) interactive T cells; (■) symptoms. Reproduced, by permission of the publisher, from Ref. (17).

universalis. In these patients, diminished lymphokine production imbalances is also present.

Interactive T cell function is still obscure, but they exist in markedly diminished numbers in certain individuals with a predisposition to severe, recurrent apparently viral infections. Family studies indicate a probable autosomal recessive inheritance.

The interactive T cell level is perhaps a mirror of DLE immunoprophylactic efficacy. Figures 1–3 show a correlation of symptoms and interactive T cells with TF administration in three patients. The first patient presented with a recurrent pharyngitis and cervical lymphadenopathy; another had severe recurrent pneumonia (presumably viral), and another (C.B.) had retinitis pigmentosa and symptoms of recurrent viral infection including aphthous ulcer. All were restored to normal immunological laboratory values by appropriate DLE administration. Each normalization was followed by cessation of infections for several months (Figs. 1–3) and diminished infection severity. Enumeration of this interactive T-cell immunocyte subset may be an important adjunct in monitoring such patients with clinical disorders that re-

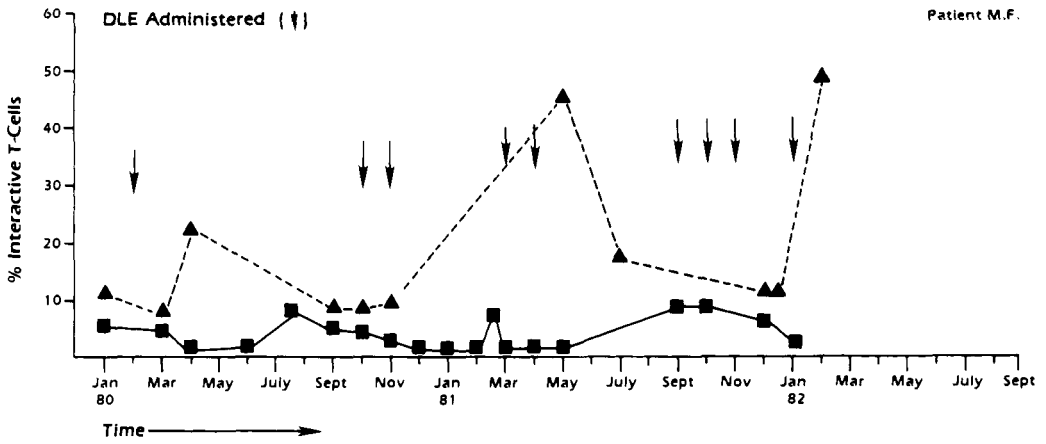

FIG. 2. Correlation of symptoms and DLE administration with interactive T-cell numbers in patient M.F. (▲) interactive T cells; (■) symptoms. Reproduced, by permission of the publisher, from Ref. (17).

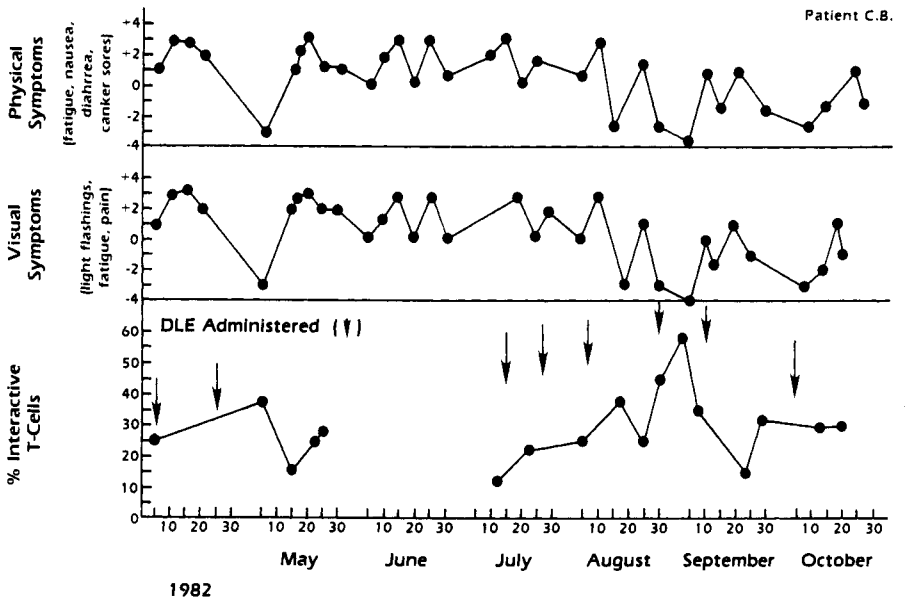

FIG. 3. Correlation of symptoms and DLE administration with interactive T-cell numbers in patient C.B. Reproduced, by permission of the publisher, from Ref. (17).

ceive DLE-TF and perhaps also other immunomodulators. For a more comprehensive recent treatise on transfer factor, the reader is referred to: "Transfer Factor" by Galbraith, and Fudenberg (17).

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