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Transfer Factor - Current status and future prospects

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Abstract

We have detected new clues to the composition and function of "Transfer Factor" using the direct Leucocyte Migration Inhibition (LMI) test as an in vitro assay of Dialysates of Leucocyte Extracts (DLE). This approach has revealed two opposing antigen-specific activities to be present in the same >3500 <12,000 DA dialysis fraction - one activity is possessed of Inducer/Helper function (Inducer Factor). The opposing activity is possessed of Suppressor function (Suppressor Factor).

When non-immune leucocyte populations are cultured with Inducer Factor they acquire the capacity to respond to specific antigen and inhibition of migration occurs. This conversion to reactivity is antigen- specific and dose-dependent. When immune leucocyte populations are cultured with Suppressor Factor their response to specific antigen is blocked and Inhibition of Migration is prevented.

Abbreviations: DLE: dialysable leucocyte extracts; LMI: leucocyte migration inhibition test; TF: transfer factor.

The roadblocks to a clear resolution of the nature and functions of Transfer Factor have arisen from impediments encountered during attempts at its isolation, purification and chemical characterization. The studies reviewed here may begin to provide approaches to clarify these critical questions and to characterize further the Inducer Factor and Suppressor Factor detected in Transfer Factor (TF) preparations.

After a long and arduous journey Transfer Factorhas finally reached the stage of the "art of the soluble". A brief backward glance may help to focus our outlook for the design of new approaches to the ultimate solution of this challenging problem. It seemed like progress when we discovered that transfer of cutaneous delayed type hypersensitivity (DTH) could be accomplished with extracts of leucocytes in humans and then found that the active material was dialysable. We had stumbled thus into a biochemists nightmare - trying to isolate and purify one small molecule swimming around among myriads of other small biologically active molecules in the dialysate (e.g. serotonin, histamine, bradykinin, ascorbate, cyclic nucleotides, prostaglandins, thymosin). This predicament was complicated further when it was found that the active material in the dialysate bound to sephadex columns — a property which lead to conflicting reports from different laboratories detecting activity in different void volumes and that smearing of activity occurred rather than elution under a single peak [1].

An approach to this dilemma was reported by Burger et al. [2] using HPLC to separate the antigen-specific activity from contaminants and deduced from the results of sequential enzymatic treatments that Transfer Factor was a small ribonucleotide. Wilson and Fudenberg [3] independently derived essentially similar results and reached similar conclusions.

We had been studying the Leucocyte Migration Inhibition as an assay for TF activity [4] and about this time we had detected antigen specific TF activity in human leucocyte dialysates (DLE) using the Leucocyte Migration Inhibition (LMI) assay [5]. To analyse this activity further we then used polystyrene wells coated with antibody or with antigen as solid phase immunoadsorbents. Preparations of dialysate (DLE) were exposed to the respective immunoadsorbent before pulsing non-immune cells in the LMI assay. For example Diphtheria Toxoid Immune DLE was depleted of all antigen-specific activity after absorption

with Diphtheria Toxoid but not affected by absorption with Diphtheria antitoxin IgG. Additionally, depletion of the toxoid specific inducer activity by exposure to Diphtheria Toxoid was prevented when the polystyrene bound toxoid was coated with the Diphtheria antitoxin IgG prior to absorption of DLE. We also found that antigen bound specific activity could be recovered and function following treatment of the immunoadsorbent with 8m urea [5]. Upon further purification the antigen specific activity was found to reside in the >3500 MW and <12,000 MW dialysis fraction and not in the <3500 MW fraction which contains most of the pharmacologically active molecules capable of exerting non-specific effects (Figure 1) [4–6].

Exploiting this approach we detected two opposingantigen-specific activities to be present in the same dialysate - one activity is possessed of Inducer/Helper function of Transfer Factor and is termed Inducer Factor [6, 7, 10]. The opposing activity is possessed of suppressor function and is termed Suppressor Factor [8–10]. Non-immune leucocyte populations cultured with Inducer Factor (TiF) acquire the capacity to respond to specific antigen with resultant inhibition of migration. This effect is dose dependent and antigen-specific.

In contrast, incubation of immune leucocyte populations with Suppressor Factor (TsF) blocks the response to specific antigen and inhibition of migration is prevented [6, 8–10]. Additionally, we observed that Diphtheria toxoid immunized BALB/c mice following intraperitoneal injection with human toxoid-specific crude dialysate or with dialysate depleted of Inducer Factor resulted in in vivo suppression of antigen- specific footpad reactivity by about 40% that of control mice [9].

Both Inducer and Suppressor Factor appear to function in an immunoregulatory network. They appear to evade MHC restriction and species restriction by virtue of binding to Ia antigen on recipient macrophages. Properties of interest of Inducer Factor (TiF) and Suppressor Factor (TsF) are compared in Figure 2 below.

In Vivo Studies of Inducer Factor: Independently and at the same time Peterson et al. [11] also reported that prior treatment of murine dialysates with the related antigen prevented the transfer of DTH responses between BALB/c mice. Additionally Burger et al. [12] showed that KLH specific Transfer Factor preparations following exposure to KLH immunoadsorbent columns greatly decreased the capacity to transfer KLH reactivity in humans.

Kirkpatric and his colleagues have done much to establish the inbred mouse as a reliable animal model to study Transfer Factor. Their transfers of DTH to synthetic antigens in BALB/C mice has added strong support to the claims for the antigenic specificity of Transfer Factor [13]. The findings of specificity were reinforced in their studies using antigen-bound ferritin or cytochrome-C as immunoadsorbents to bind and remove the related Transfer Factor from the dialysate [14]. The most revealing findings however emerged from their studies on the genetic control of Transfer Factor using synthetic antigens in high-responder and low-responder inbred mice [15]. Here it was found that high-responder donors were potent sources of antigen-specific TF and low-responder mice were either poor donors or totally unable to transfer DTH. Moreover, it was determined that administration of TF from high-responder donors to low-responder recipients resulted in the conversion of the recipients to the high-responder phenotype. From these observations the authors conclude that TF production is controlled by Ir genes but its function is not. In addition to the theoretical and practical import of these findings, they also broaden the range of approaches to the study of TF at the molecular level using special strains of mice (e.g. Transgenic, Knockout, SCID, Nude).

The detection and delineation of some of the properties of Inducer Factor and Suppressor Factor have given new clues to the composition, structure and function of Transfer Factor. The finding that Inducer Factor binds to specific antigen has had both a theoretical and practical impact on our approaches to the problem. First it weakens the possibility that Transfer Factor is a fragment of the Th cell receptor for antigen since T-cell receptors do not interact with native antigens. At a purely descriptive level Transfer Factors could provide or trigger an alternate pathway for T-cell recognition of antigen as Kirkpatrick [15] suggests or be a unique Ir gene product of T cells which assists in antigen presentation to other T cells as we have suggested [6, 7]. At the practical level, the binding to antigen allowed our laboratory to initiate studies with Dr. Blas Frangione on such antigen bound and recovered purified Transfer Factor to determine its amino acid sequence. The goal was to derive the nucleic acid sequence, construct a plasmid and produce recombinant Transfer Factor of desired specificity in E. Coli with the help of biochemist Dr. Nigel Godson. As might be expected, this was not to come about easily, since we found ultimately that there was a blocked amino terminus. Two other investigators have since encountered the same

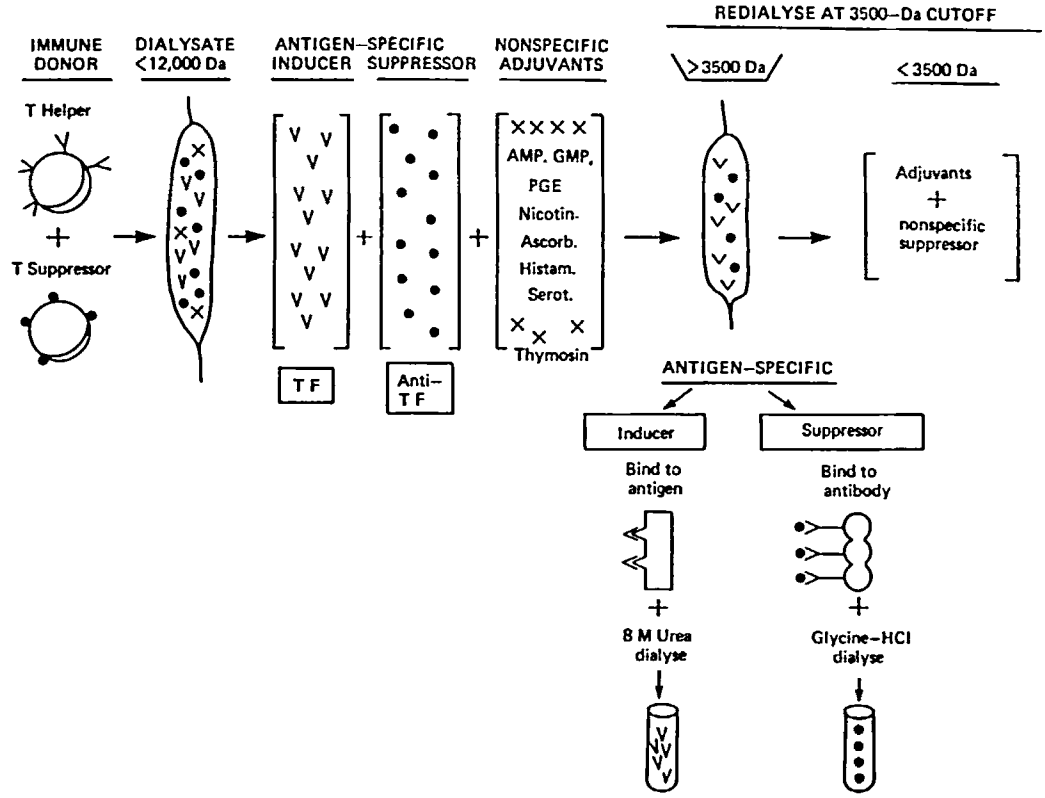

Figure 1. Qualitative analysis of leukocyte dialysates containing Inducer Factors and Suppressor Factors of varying specificities - isolation and purification by differential dialysis and affinity immunoadsorption. Reprinted, with permission, from Reference 6.

LEUKOCYTE DIALYSATE <12,000 m.w.

TRANSFER FACTOR (INDUCER-HELPER ACTIVITY)	ANTI-TRANSFER FACTOR (SUPPRESSOR ACTIVITY)
1. BINDS TO RELATED ANTIGEN	1. BINDS TO RELATED ANTIBODY IgG
2. BINDS TO ANTI-V_H ANTIBODY - NOT V_K	2. BINDS TO ANTI-V_K ANTIBODY - NOT V_H
3. BINDS TO ANTI-Ia ANTIBODY - NOT $BETA_2M$	3. BINDS TO ANTI-Ia ANTIBODY - NOT $BETA_2M$
4. >3500 <12,000m.w. FRACTION	4. >3500 <12,000m.w. FRACTION
5. ORIGIN T_H CELLS	5. ORIGIN T_S CELLS
6. ABSORBED BY T_S CELLS AND MACS	6. ABSORBED BY T_H CELLS AND MACS
7. EQUIPS NON-IMMUNE CELLS WITH ANTIGEN-BINDING MOIETY	7. BLOCKS INDUCER-HELPER ACTIVITY ON NON-IMMUNE CELLS; ABROGATES RESPONSE OF IMMUNE CELLS
8. ? DIALYZABLE FRAGMENT OF T-CELL AG-RECEPTOR	8. ? DIALYZABLE FRAGMENT OF ANTI-IDIOTYPIC T-CELL RECEPTOR

Figure 2. Properties of antigen-specific Inducer/Helper activity and antigen- specific Suppressor activity present in leukocyte dialysates. Reprinted, with permission, from reference 6.

problem, I have learned. I consider this a temporary deterrent that will be solved in the end.

The clinical trials of Transfer Factor therapy to date have generally used leucocyte dialysates (<12,000 MW) which could be expected to contain both Inducer Factor and Suppressor Factor perhaps in differing concentrations and potency which we now realize may neutralize each other on occasion in vivo and result

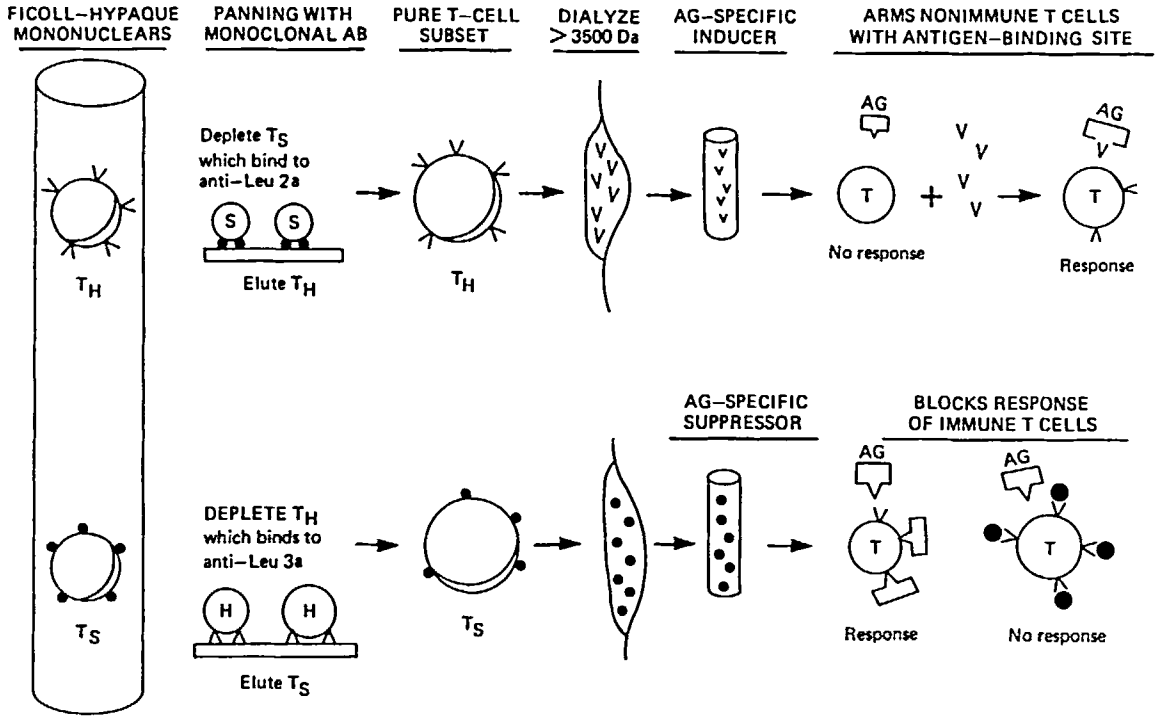

Figure 3. Preparation of Inducer Factor from enriched Th cell populations and Suppressor Factor from enriched TS cell populations. Reprinted, with permission, from Reference 6.

in unexpected failures or less than optimal clinical improvement.

Hence it might be advantageous for future clinical applications to remove the suppressor activity by binding it to specific antibody if the goal is endowment and/or enhancement of cell-mediated immunity. The application of purified Suppressor Factor could be even more effective particularly in crippling autoimmune diseases such as lupus erythematosus and rheumatoid arthritis, juvenile onset insulin-dependent diabetes, as well as various endocrinopathies, ophthalmological and neurological syndromes of autoimmune origin. (Figure 3)

As we have also proposed on prior occasions [6, 16], there is perhaps a special place for such antigen-specific Suppressor Factor therapy in organ and/or bone marrow transplantation. The in vitro production of a Suppressor Factor specific for the HLA- antigens of the prospective tissue or organ donor could be readily accomplished and purified. Pre-treatment or concomitant treatment of the transplant recipient with the dialysable Suppressor Factor could be expected to block the response of a selective subpopulation of Th cells which become sensitized to the target cells of the donor graft.

Of course in the meanwhile there is a pressing need to produce a recombinant antigenic-specific Suppressor Factor such as found in the dialysate.

Therein lies the hope for the future for both-Transfer Factor and Suppressor Factor: clinical trials with known amounts and dosages of pure standardized materials, of known composition and predictable effects available in adequate quantities for complete courses of therapy, and tailored to meet not only the needs of the specific patient, but also to selectively cope with the demands of the specific disease.

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