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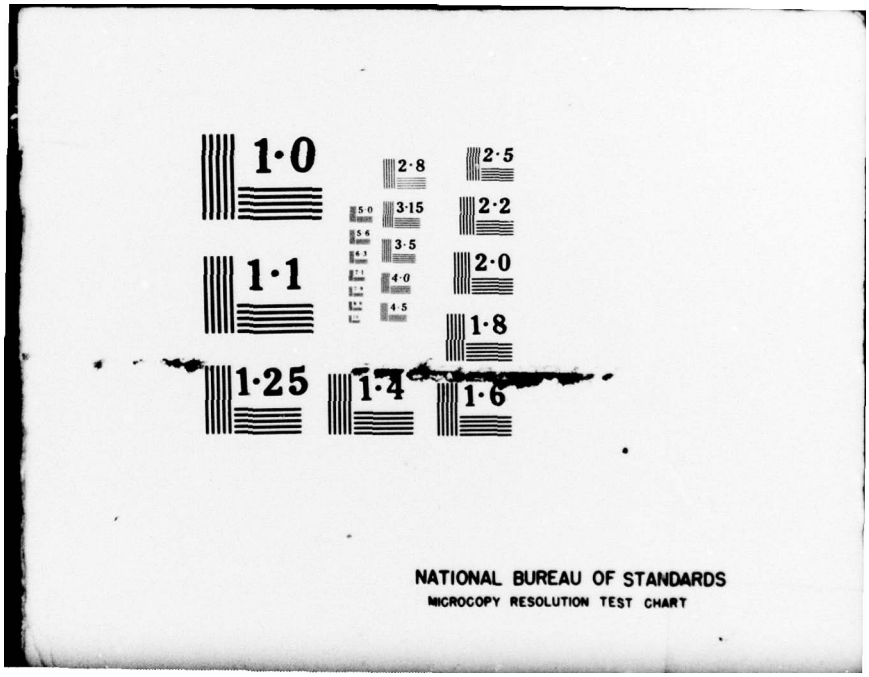
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CELLULAR TRANSFER OF CUTANEOUS SENSITIVITY TO HEMOLYTIC STREPTOCOCCI

FINAL REPORT

1963 - 1975

by

H. Sherwood Lawrence, M.D.

December 1977

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Transfer Factor (TF) has emerged as one of the more potent immunologic reagents proven effective in the initiation, augmentation or reconstitution of delayed hypersensitivity (DTH) and cell-mediated immunity (CMI) in humans. TF is a small (<10,000 M.W.) non-antigenic, non-immunoglobulin moiety that is separated from the macromolecules of human blood leukocytes by dialysis, further purified by Sephadex fractionation and concentrated by lyophilization. Dialysable TF (TF_D) and the partially purified Sephadex fractions possess the

(over)

20. Abstract

immunological activities of the viable leukocytes from which it is prepared, namely TF confers on the normal recipient for prolonged periods the DTH and CMI possessed by the immune donor.

The established effectiveness of TF_D in selectively augmenting cellular immunity has fostered its widespread application in the immunotherapy of a variety of congenital and acquired diseases characterized by deficient CMI. The congenital T-cell deficiencies that have responded to TF_D administration are: Wiskott-Aldrich Syndrome, Swiss-Type Agammaglobulinemia, Ataxia-Telangiectasia, and Severe Combined Immunodeficiency Disease. Disseminated intracellular infections caused by viruses, fungi or mycobacteria have also responded favorably to TF_D administration and include Chronic Active Hepatitis, Herpes Zoster and Simplex, Vaccinia, Cytomegalovirus, and giant cell measles pneumonia; chronic mucocutaneous candidiasis, disseminated coccidioidomycosis; histoplasmosis and torulosis, tuberculosis and lepromatous leprosy. Immunotherapy with TF_D is also being evaluated in certain types of cancer such as breast cancer, osteogenic sarcoma, malignant melanoma, nasopharyngeal carcinoma and Hodgkin's disease. The most recent applications of TF_D to immunotherapy have included certain diseases of possible infectious origin that are considered "autoimmune" such as Bechet's syndrome, Reiter's syndrome, multiple sclerosis, subacute sclerosing panencephalitis, juvenile rheumatoid arthritis and chronic active hepatitis. Although experience is still fragmentary, the general principle that appears to be emerging is that TF_D administration may be expected to benefit those patients afflicted with diseases that arise from or result in deficient CMI and its consequences. Operationally TF_D administration results in the appearance of a new population of antigen-responsive lymphocytes in the recipient's circulation which in the presence of the related antigen express the activities of the natively immune cell, namely cutaneous DTH and CMI in vivo, eradication of infectious agent, lymphocytic infiltration and rejection of tumor metastasis plus lymphocyte transformation and lymphokine production (Migration Inhibitory Factor, Lymphotoxin, Interferon) in vitro. In addition to these antigen-specific responses, TF_D administration results in a nonspecific augmentation of CMI responses, such as acquisition of lymphocytic PHA-responsiveness, E-rosette responsiveness and MLC responsiveness as well as endowment of immunodeficient children with the capacity to recognize and respond to environmental antigens (e.g., DNCB). Whether the antigen-specific activities and the nonspecific consequences observed following TF_D administration are properties of the same or different molecule(s) present in leukocyte dialysates remains to be elucidated as does the precise biochemical species and mechanism of action of Transfer Factor itself.

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Lawrence, H. Sherwood
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FINAL REPORT ON CONTRACT DA49-193-MD-2502 - "Cellular Transfer of Cutaneous Sensitivity to Hemolytic Streptococci" - H.S. Lawrence, M.D., Contractor.

This project began with our demonstration that cellular transfer of cutaneous delayed type hypersensitivity (DTH) was possible in humans using viable WBC (1,2) and that WBC extracts are as effective as living cells (3). This finding allowed us to begin the attempts to isolate and characterize the active material(s) in WBC extracts responsible for transfer of DTH and to which activity we gave the operational term Transfer Factor (TF), with the realization that more than one Factor may be involved in the results achieved. TF IN WBC EXTRACTS: Treatment of WBC extracts with DNAse, RNAse or Trypsin failed to inactivate TF and excluded known macromolecular candidates for informational transfer (3). Additionally serial transfer of antigen-specific DTH from individual A to B to C using WBC extracts tended to exclude a superantigen or unique type of antibody and suggested the recipient of TF replicates more TF of the same specificity which then appears in his circulating leukocytes (3). Seeking a relationship between TF and known immunoglobulins we studied the diphtheria-toxoid-antitoxin system and found that DTH to toxoid was readily transferred with WBC extracts but that antibody could not be detected in the skin or sera of such recipients using a sensitive biological test (4). In attempting to block transfer of DTH we incubated immune cell populations with antigen (PPD, toxoid) and found that PPD interacted with the tuberculin specific TF and liberated it into the cell-free supernatant leaving behind the toxoid specific TF in the cell pellet which had been depleted of the tuberculin specific TF (4,5). From these studies we concluded that: a) TF could only result in the transfer of DTH responses and not the capacity for antibody production nor a secondary antibody response to the same antigenic determinants; b) antigen can interact with and liberate a specific cell-bound TF while leaving behind in the cell pellet the TF's of differing antigenic specificities; and c) rapid liberation of specific activity after only 15-20 minutes exposure to antigen indicated TF to be a pre-existent moiety in immune leukocytes.

In an effort to exclude the possibility that TF merely resulted in elevation of the recipient's latent sensitivity to ubiquitous antigens, we studied the transfer of coccidioidin sensitivity to recipients who had not been exposed to this fungal infection. DTH to coccidioidin was readily transferred using WBC extracts prepared from immune donors and it was concluded that the possibility of elevation of latent sensitivity using this antigen had been greatly diminished but not entirely excluded (6).

We demonstrated the capacity of TF to transfer accelerated skin allograft rejection to nonimmune recipients. This study established that: a) individuals do not possess a pre-existent TF vs. histocompatibility antigens of other individuals; b) such allograft-specific TF does not appear after immunization with only one skin graft but requires sequential exposure to two or more grafts to reach detectable levels of activity; c) the TF raised in this fashion when injected into nonimmune people causes accelerated rejection of only that allograft used to immunize the TF donor whereas unrelated control allografts are unaffected; d) TF prepared from nonimmune donors or from inadequately immunized donors did not cause accelerated rejection of test or control skin grafts nor did hyperimmune sera (7). From this study it was concluded that a new TF

had been raised de novo; that it exhibited exquisite specificity for the HL-A antigens of the individual whose skin had been used to immunize the TF donor; and that it was unlikely that the effects were due to elevation of a latent sensitivity. Further studies undertaken to define the locus of human histocompatibility antigens in WBC extracts revealed that antigenic activity resides in the endoplasmic reticulum and to be nondialysable whereas the dialysate was unable to cause active sensitization of individuals to skin allografts obtained from the donor of the dialysate (8). This finding separated those materials (antigens) which actively sensitize, from TF which cannot actively immunize individuals to skin allografts, but can only transfer such immunity.

Our next step in the goal to identify and characterize TF in crude DNAse-treated WBC extracts was greatly facilitated by our finding that the active material(s) passed readily through a Visking Cellulose sac (9). By this one simple maneuver all of the macromolecular cell constituents, passenger viruses, AU antigens and histocompatibility antigens are left behind within the dialysis sac and the dialysate containing TF in highly purified form can be concentrated by lyophilization. We found that dialysable Transfer Factor (TFd) possessed all of the biological properties and potency for in vivo transfer of DTH as the parent viable cells or DNAse-treated WBC extracts from which it was prepared. These included the same intensity and prolonged duration (1-2 years) of transferred DTH and the immunological stability of lyophilized TFd (5 yrs.). TFd was shown to be free of papain digested gamma-globulin fragments added to the inside of the bag as markers; to be orcinol and Biuret positive; to be nonantigenic in rabbits Additionally, the activity in dialysates was found to be retarded following passage through Sephadex G-25 and eluted under a broad peak in the region where molecules of <10,000 mol. wt. emerge. By exclusion, the main candidates for the biological activity of TFd are polypeptides and/or polynucleotides (9). Thus TF was shown to be a dialysable non-IG, nonantigenic moiety of <10,000 m.w. comprised of a complex of polypeptides and/or polynucleotide residues. The dialysable nature of TF, its low molecular weight and polypeptide-polynucleotide composition have each been confirmed rapidly and in precise detail by several groups of investigators, including the same U-V absorption patterns of active peaks eluted from Sephadex G-25. We continued to explore the in vivo capacity of TFd to restore DTH and CMI responses in patients with diseases characterized by anergy, as a prelude to its possible application as an immunotherapeutic reagent. TFd was shown capable of conferring local DTH to tuberculin to some anergic sarcoid patients and both local and systemic reactivity to others which endured for a year (10). Similarly, TFd conferred local and systemic cutaneous DTH to SK-SD to a group of patients with metastatic cancers of various types (11). In the miniscule dosage of TFd employed in the above group of patients, no clinical improvement was anticipated or observed. Nevertheless, these studies demonstrated that anergic patients with disseminated disease possessed cell populations that could respond to TFd and that DTH and CMI responses could be augmented by TFd prepared from normal individuals.

The Search for an In Vitro Assay of TF - Disappointments and Consolations: It was apparent from the beginning of this project that the chief impediment

to the isolation and characterization of TF was the lack of an animal model and/or an in vitro assay system. Our earliest efforts included attempts to neutralize cells bearing TF with antigen and resulted instead in the liberation of antigen-specific TF into the cell-free supernatant solution (4,5). With David, Al-Askari and Thomas, we next turned to macrophage migration inhibition technique of George and Vaughan and in a series of papers (12-16) established its value as an in vitro correlate of DTH and CMI induced by bacteria, protein antigens and simple chemical compounds. We also adapted this in vitro system to the study of transplantation immunity (29). These preliminary studies showed that as few as 2% sensitive cells could cause normal cells to respond to antigen and suggested the possibility of detecting a guinea pig TF. No TF was detected. Nevertheless, these observations did prepare the way for the subsequent discovery of MIF by Bloom and by David and generated a whole new phase of immunological investigation at both basic and clinical levels

With Valentine we next turned to lymphocyte transformation as a possible in vitro assay of TFd and although moderately successful, the results were initially sporadic and unpredictable (17). Seeking to prepare a more potent TF for this in vitro system we incubated sensitive human blood lymphocytes with antigen and discovered instead a nondialysable, heat-labile, antigen-dependent lymphokine which alerted naive lymphocytes to transform and proliferate following exposure to the related antigen (18). We termed this activity lymphocyte transforming factor (LTF) to distinguish it from TF. With Spitler we then reported that LTF could be demonstrated in the guinea pig and it shared properties with human LTF (18).

Having detected two cell-free products that acted upon naive lymphocytes to result in transformation and proliferation when exposed to antigen, we looked for such recruitment to occur in an antigen-stimulated immune lymphocytes that were held captive for time-lapse cinematography. We failed to detect recruitment, at least when such lymphocytes were under continuous vision from 48 hours through 7 days, and instead found the large number of cells generated by the 7th day arose from far fewer than our estimated <2% antigen-reactive progenitors, entirely by clonal proliferation. Nevertheless, recruitment could be shown to occur artificially when LTF plus antigen were added to naive lymphocytes. The latter were then triggered into identical clonal proliferation, quantitatively and qualitatively, that we had observed in natively immune lymphocytes following exposure to antigen alone (20).

We next approached target-cell sickness or death as a potential in vitro assay of TF and found instead another nondialysable, heat-labile product of antigen-stimulated human lymphocytes (21). This lymphokine initially seemed to have properties of a human lymphotoxin, similar to that described for mice by Granger. However, since our material modulated HeLa cell cloning and since it interfered with tumor-cell division rather than resulting in direct cell-lysis, we named it CIF for cloning inhibitory factor (22).

Over the past few years our approach to the problem of the identity and mechanism of action of TFd has been devoted almost exclusively to the development and perfection of a suitable in vitro assay for this material(s). This strategy arose from the realization of the limitations and restrictions of our extensive in vivo experience and the obvious advantages of an in vitro system that may reflect in vivo events.

Therefore during the years 1972 through August 1975 which mark the termination of this grant we have accomplished the following: 1) developed and defined the experimental conditions whereby leukocyte dialysates containing TFd cause augmentation of antigen stimulated human lymphocyte proliferation (23); 2) having detected a new lymphokine, cloning inhibitory factor (CIF), produced by antigen or mitogen stimulated human lymphocytes and we defined and extended the preparation and properties of CIF on HeLa cell cloning (24); 3) showed that lymphokine production (lymphocyte transforming factor of blastogenic factor) occurs in antigen stimulated human lymphocyte cultures at a time when cell proliferation is prevented by culture in 95% oxygen (25); 4) evaluated the efficiency of "pooled" nonspecific TF in the immunotherapy of 5 patients with breast cancer and established the safety of administration of large doses (270 billion cell equivalents) thrice weekly for 1 year (26).

These and other recent advances in our work on Transfer Factor have been summarized and brought up to date in the author's Harvey Lecture (29).

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