Transfer Factor and Other Mediators of Cellular Immunity

H. S. Lawrence, MD and F. T. Valentine, MD*

DELAYED-TYPE HYPERSENSITIVITY, OR CELLULAR IMMUNITY, comprises a class of immune responses that result in tissue damage and that are distinctive in their induction and expression from responses mediated by humoral antibody. Moreover, until recently an accepted characteristic of cell-mediated immunity was the absolute requirement of living lymphoid cells, rather than any constituent or product of such cells, to initiate this type of inflammatory response. This conviction arose from Landsteiner and Chase's pioneering discovery of the cellular transfer of delayed hypersensitivity in the guinea pig. Thus, by this one simple observation, they clearly separated cellular from serum antibody responses and provided an immunologic reagent for analysis of mechanisms of cellular immunity. The need for living cells was fostered by the short-lived duration of sensitivity in outbred experimental animals and by the failure to achieve transfer *in vivo* with extracts of lymphoid cells.[1] Thus matters stood, until the adaptation of cellular transfer to human species.[2–5] Cellular transfer in humans was accomplished at first with viable circulating leukocytes [6–8] and the results paralleled those in the guinea pig; namely, the requirement for a specifically sensitive donor and an adequate dosage of cells to achieve transfer. Early departures from the experience in animals centered around the lower dosages of cells effective in man and the long duration of transferred sensitivity (months to 1–2 years).[5] These clues led to the demonstration that leukocyte extracts are as effective in the transfer of delayed sensitivity in man as viable cells.[9] Since this critical finding marked a major departure from observations in experimental animals, it was understandably accorded a certain skepticism. Nevertheless, with subsequent repeated and extensive confirmation of the

From the Infectious Disease and Immunology Division, Department of Medicine, New York University School of Medicine and Bellevue Hospital, New York, NY.

Supported by Grant AI-01254-15 from the US Public Health Service and in part by the Streptococcal and Staphylococcal Commission, Armed Forces Epidemiological Board.

Address for reprint requests: Dr. H. S. Lawrence, Infectious Disease and Immunology Division, Department of Medicine, New York University School of Medicine, 550 First Avenue, New York, NY 10016.

* Recipient of Career Scientist Award I-637 from the Health Research Council of the City of New York and supported in part by a grant from the John Polachek Foundation for Medical Research.

efficacy of leukocyte extracts in the transfer of delayed hypersensitivity in man, this finding is no longer in question.[10–15]

The value of transfer with leukocyte extracts lay in the opportunity it afforded to begin the search for the identity and character of the active moiety, which we have termed transfer factor. This approach led to the findings that the activity in leukocyte extracts is not abolished by either endogenously liberated nucleases or lysosomal hydrolases or by exogenous addition of DNAse, RNAse or DNAse plus trypsin.[9] Immunologic studies revealed transfer factor does not function as antigen or superantigen in that it was unable either to transfer or to actively induce antibody formation in the recipient despite the appearance of delayed sensitivity to diphtheria toxoid.[16, 17] We were also unable to detect antitoxin either in the sensitive leukocyte extracts used to transfer delayed sensitivity or in the recipient of transfer. This interesting observation was later confirmed by Good and his colleagues [18] in agammaglobulinemic recipients of transfer as well as in our subsequent studies demonstrating the transfer of coccidioidin sensitivity.[19, 20] Thus, when circulating leukocytes or their extracts are used as vehicles of transfer factor, the recipient expresses delayed cutaneous sensitivity in the absence of specific circulating immunoglobulins. Whenever multiple sensitivities have been transferred in humans, the recipient expresses the exact pattern exhibited by the donor.[5, 12] In effect, the recipient of transfer factor is endowed with the precise cellular immunologic memories of the donor, for prolonged periods of time.

This highly selective immunologic specificity of transfer factor is also evident in results achieved in transfer of accelerated skin homograft rejection.[21] Here we found that a new transfer factor was synthesized by each individual actively sensitized to any other individual's skin homograft. Injection of an unrelated recipient with a transfer factor versus A's skin resulted in accelerated rejection only of target skin grafts from A and not control skin grafts from B, C or D. As a result of this specificity of the host's transfer factor for the histocompatibility antigens of the transplant donor, we have suggested elsewhere that the immunosuppressive effects of antilymphocyte serum result from its engagement, among the total population, of the very few cells bearing transfer factor directed against that particular organ transplant.[22]

Progress in the search to identify and characterize transfer factor was greatly facilitated by our subsequent finding that it is a dialyzable moiety of $< 10{,}000$ mol wt that is neither an immunoglobulin nor a light chain.[23] Dialyzable transfer factor has all the biologic activities

possessed by the parent leukocyte extract or the viable cells from which it is prepared.

Thus in one simple step transfer factor is separable from all of the macromolecular cell constituents including transplantation antigens.[24] Our earlier studies using diphtheria toxoid demonstrated that transfer factor could not induce antibody synthesis (antitoxin) in recipients;[16] more recent attempts to make an antibody to dialyzable transfer factor have also been equally unsuccessful since it is not antigenic when injected repeatedly in rabbits or man.[5] Of interest in this connection is our additional finding that human histocompatibility antigens in leukocyte extracts that function to actively sensitize the host to related skin grafts are nondialyzable, whereas the dialysate containing transfer factor cannot sensitize to the related skin graft. Thus, in the homograft system, the material (transfer factor) that serves to transfer accelerated rejection is separable in form and function from histocompatibility antigens that actively induce such sensitization.[24]

Moreover, addition of dialyzable transfer factor alone either to nonsensitive human lymphocytes or to specifically sensitive lymphocytes in culture has no discernible effect on such cells, as measured by radioautography or thymidine uptake.[5,15] Were transfer factor an antigen or superantigen in the form of a polynucleotide antigenic fragment, one would anticipate lymphocyte transformation and thymidine uptake to occur since this *in vitro* system will detect as little as 0.001 μg of PPD. This cumulative evidence would seem to exclude the possibility that transfer factor is an antigenic fragment—or, as postulated to fulfill its potent biologic activities, a new species of superantigen. The biologic, biochemical and immunologic properties of dialyzable transfer factor are summarized below.[5]

Biologic	*Biochemical*	*Immunologic*
Properties of whole extract:	Soluble, dialyzable, lyophilizable	Not immunoglobulin
Prompt onset (hours)	<10,000 mol wt	Not immunogenic
Long duration (>1 yr)	No protein, albumin, α or γ globulin	Immunologically specific
Equal intensity	Orcinol-positive	Converts normal lymphocytes *in vitro* and *in vivo* to antigen-responsive state
Dissociable from transplantation antigens	Polypeptide/polynucleotide composition	Transformation and clonal proliferation of converted lymphocytes exposed to antigen
Small quantities → magnified effects	Inactivated at 56 C for 30 min	Informational molecule/ derepressor/receptor site?
	Resists pancreatic RNAse	
	Retains potency (5 yr)	

Thus, transfer factor has emerged as a dialyzable moiety of < 10,000 mol wt that is neither antigen nor antibody. By exclusion, the

only candidates in the dialysate to qualify for the potent biologic activities of transfer factor are polynucleotide and polypeptide chains. The demonstration that transfer factor is inactivated by heating at 56 C for 30 min [25] and its consistent resistance to the action of pancreatic RNAse [23, 25] suggest that if indeed it turns out to be a polynucleotide, it shares certain properties with double-stranded RNA polynucleotides. The dialyzable nature of transfer factor, its low molecular weight and polynucleotide-polypeptide composition, as well as its Sephadex patterns and nonantigenic properties have been repeatedly confirmed by several workers in explicit detail.[26–30]

Activities of Transfer Factor In Vitro

The earliest studies *in vitro* of transfer factor were done with AM Pappenheimer and showed that incubation of sensitive blood leukocytes with specific antigen (PPD) desensitized the cells and liberated preformed transfer factor into the cell-free supernatant solution within 30 min of incubation.[16, 17] In experiments using leukocyte populations sensitive to both diphtheria toxoid and tuberculin, incubation with PPD resulted in release of the tuberculin transfer factor into the supernatant while the toxoid transfer factor remained in the cells.[17] These experiments suggested that antigen interacts with transfer factor but does not block its activity; the interaction is immunologically specific; and the release occurs promptly within 1 hr of incubation and results in the liberation of preformed transfer factor. This stands in contrast to the synthesis *de novo* of the extracellular proteins LTF, MIF, and LT (discussed below), which require a 12–18 hr latent period after triggering with antigen, before detectable levels are reached.

Transfer Factor and Lymphocyte Transformation

The findings that dialyzable transfer factor is not antigenic in animals or man [31] and that it is freed of histocompatibility antigens have allowed it to be added to nonsensitive lymphocytes in culture without discernible effects, as measured by thymidine uptake or autoradiography. Taking advantage of this property, Fireman *et al* [29] and Valentine and Lawrence [32] found that dialyzable transfer factor can convert nonsensitive lymphocytes in culture to an antigen-responsive state, which is revealed by transformation of such lymphocytes to lymphoblasts after but not before addition of the appropriate antigen. It was also observed that administration of dialyzable transfer factor to negative recipients *in vivo,* in addition to initiating specific delayed cutaneous reactivity, also conferred on the recipient's lymphocytes the

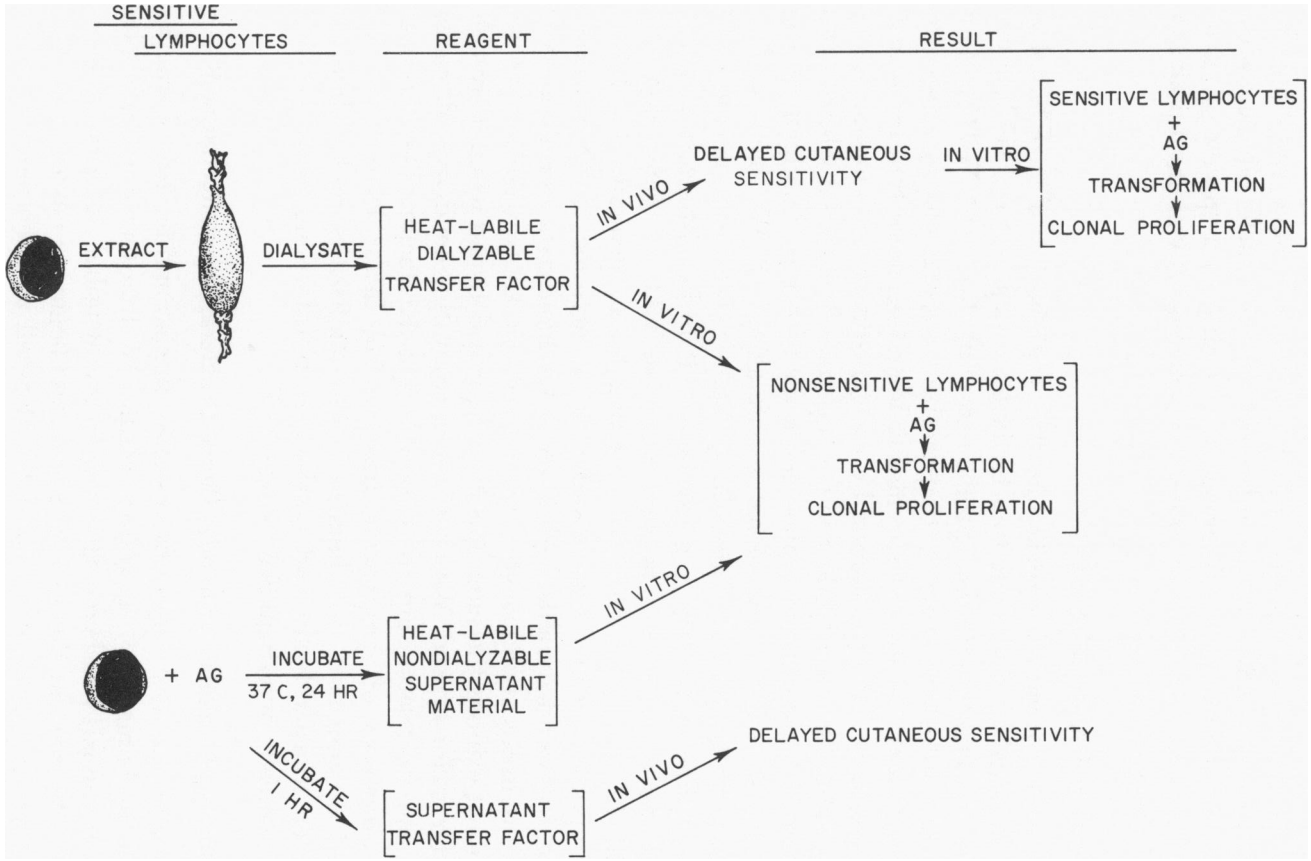
capacity to respond to appropriate antigen with transformation and clonal proliferation *in vitro*. Adler and Smith [33] have subsequently shown that dialyzable human tuberculin transfer factor also converts nonsensitive mouse spleen lymphocytes *in vitro* to a specific antigen-responsive state detected by transformation and thymidine uptake in the presence of tuberculin.

Lymphocyte Transforming Factor

We were not entirely satisfied by the very small number of lymphocytes engaged by dialyzable transfer factor (—*eg*, about 5% at 7 days) despite the highly selective, specific nature of this event. We therefore adapted the technic of incubating sensitive lymphocytes *in vitro* with antigen [17] with the idea of specifically liberating more transfer factor. This resulted in the production by antigen-stimulated lymphocytes of a nondialyzable, heat labile (56 C) supernatant material which, upon addition to nonsensitive lymphocytes in the presence of antigen, caused their transformation and clonal proliferation.[34, 35] We have termed this activity *lymphocyte transforming factor* (LTF) and since it is not sedimentable at 100,000 $\times$ *g*, we believe it to be distinct from histocompatibility antigens. The production of LTF is immunologically specific in its requirements and its activity is antigen-dose dependent. LTF appears distinct from transfer factor in some of its properties (—*eg*, it is nondialyzable). Although it functions as a recruiting agent for nonsensitive lymphocytes, we do not yet know whether this recruitment exhibits the same specificity that is detected when dialyzable transfer factor is used. We continue to study this nondialyzable molecule and its relationship to dialyzable transfer factor. The existence and properties of lymphocyte transforming factor have been confirmed in animals [36] and humans.[37] The similarities and differences between transfer factor and lymphocyte transforming factor are diagrammatically oversimplified in Text-fig 1.

Escalation of Cellular Immunity: Clonal Proliferation vs. Recruitment

A major question arising from studies *in vivo* and *in vitro* of transfer factor in humans [5, 15] and from studies of transferred radio-labeled cells in animals had been: How can so few lymphocytes accomplish so much? Answers to this question have just begun to emerge from several lines of investigation. Recently Marshall and the authors of this paper [38, 39] secured time-lapse cinematographic evidence that as few as $< 2\%$ of circulating human lymphocytes are antigen-responsive *in vitro*. It is these few cells that give rise to the 20% transformed



TEXT-FIG 1. Diagrammatic comparison of activities *in vivo* and *in vitro* of dialyzable transfer factor, antigen-liberated transfer factor and lymphocyte transforming factor. (From Lawrence, HS [5])

lymphocytes (blast cells) detected after 7 days' incubation with specific antigen. This increment occurs by a process of repeated cell division and the proliferation of a clone of lymphocytes. Although clonal proliferation could adequately entirely account for the increase in lymphoblasts to 20%, the design of these experiments cannot exclude recruitment of uncommitted lymphocytes to the pool of responsive cells in the first 48–72 hr of test tube culture. We would suggest that recruitment of uncommitted cells may actually occur in this early period after the few responsive lymphocytes are engaged by antigen. This view arises from the demonstration, by this laboratory, of two agents that actually do recruit nonresponsive cells; namely, transfer factor and lymphocyte transforming factor [15, 35] discussed above. The magnitude of the reality of antigen-stimulated clonal proliferation of lymphocytes is illustrated in Fig 1.

Migration Inhibitory Factor

Other attempts at detecting transfer factor activity *in vitro* were undertaken with David, Al-Askari and Thomas [40] using the capillary migration technic of George and Vaughan.[57] These studies led to extensive application of the technic and to the discovery of migration inhibitory factor (MIF) [41, 42] as Dr. Bloom discusses in detail below. However, Dr. David and I were unable to detect transfer factor in the guinea pig system and our efforts to adapt human cells were inconclusive. Subsequently, Thor and Dray[55] successfully applied the capillary migration technic to human cells with results essentially similar to those reported in the guinea pig. Thor[43] has also reported, using human cells, the transfer *in vitro* to normal lymphocytes of the capacity to make MIF when stimulated with the appropriate antigen by means of an RNA species prepared from specifically sensitive lymphocytes.[43] This RNA material differs from transfer factor in its larger molecular size and in its inactivation after treatment with pancreatic RNAse.

A recent clue to the relationship of transfer factor to the other mediators of cellular immunity was secured by Rocklin and David and colleagues.[44] These investigators were treating a patient suffering from disseminated candidiasis with dialyzable transfer factor prepared from candida-positive donors. Before the injection of transfer factor *in vivo,* the patient had a negative skin test to candida antigen, and her lymphocytes failed to make MIF when incubated with antigen. After transfer, delayed cutaneous reactivity to candida appeared and her lymphocytes acquired the capacity to produce MIF upon antigenic stimulation.

Thus, from this and other data discussed above, transfer factor functions to convert nonsensitive lymphocytes to a specific antigen-responsive state. Once this conversion has occurred, such lymphocytes, upon exposure to the right antigen, express all the properties detected in natively sensitive cells. This latter includes cutaneous reactivity *in vivo*, lymphocyte transformation, clonal proliferation and production of effector molecules such as MIF.

Lymphotoxin

Dr. Granger discusses in detail the nature and properties of lymphotoxin below. Lebowitz and Lawrence[45] have also reported on lymphotoxin (LT) produced by tuberculin-sensitive human blood lymphocytes incubated with tuberculin for 36 hr. Rather than assaying incorporation or release of a radiolabel from target cells as the endpoint, we chose to assay cytotoxic activity by the effect of LT on the cloning efficiency of individual HeLa cells in culture. The human LT we have studied reduces HeLa cell clones by 98% of the control value.

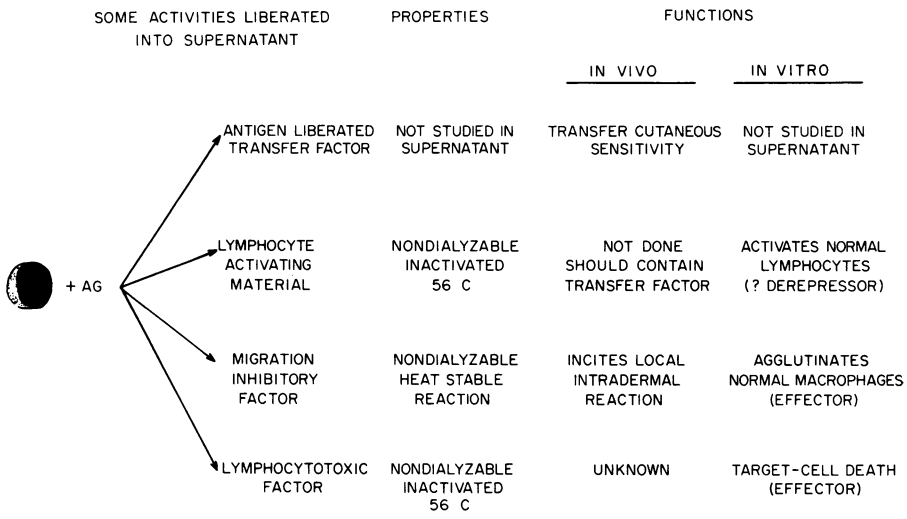
Our preparations of human LT were found to be nondialyzable and inactivated by heating to 56 C for 30 min. We then undertook time-lapse cinematography studies of the effects of human LT on HeLa cells in culture and found the main effects to be rounding-up of the cells, the appearance of abnormal forms, fusion of cells and finally the cessation of cell division after a variable number of cycles. Cytolysis or detachment of target cells from the plastic surface of Falcon flasks was not observed.[2]

Although we have demonstrated that transfer factor can convert normal cells to a specific antigen-responsive state, and such converted cells have been shown by David[44] to produce MIF in the presence of antigen, we have not yet demonstrated that antigen-stimulated cells recruited by transfer factor will produce lymphotoxin as well.

Mediators of Cellular Immunity

Thus the recent application of correlates *in vitro* of delayed hypersensitivity or cellular immunity have revealed a wide variety of latent responses residing in specifically sensitive circulating lymphocyte populations that are triggered into activity by interaction with specific antigen and result in the production of a family of effector molecules. The latter, their properties and functions currently delineated are diagrammatically oversimplified in Text-fig 2.

Two points to be made here are (1) each of these reagents is present



TEXT-FIG 2. Activities produced by or liberated from antigen-responsive human blood lymphocytes after interaction with specific antigen. *Transfer factor:* liberated after 1 hr incubation with antigen.[16,17] *Lymphocyte activating material:* produced after 24 hr incubation with antigen.[34] *Migration inhibitory factor:* produced after 24 hr incubation with antigen—heat-stable, MIF is produced by human lymphocytes,[55] some properties listed in this figure were obtained in the guinea pig.[41,42] *Lymphocytoxic factor:* produced after 36 hr incubation with antigen.[45,56] (From Lawrence HS[5])

in the same supernatant after interaction of sensitive lymphocytes with specific antigen; and (2) the activities listed are in the nature of a progress report to which recent additions—*eg*, interferon and leukotactic factor [46] should be made.

The workers in this area are faced with at least two problems that are currently being actively pursued. One problem posed is whether lymphocyte transforming factor, migration inhibitory factor and lymphotoxin represent a family of distinct molecules or whether there is but one molecule with variable functions, depending on its concentration. The other problem relates to the demonstration of functions *in vivo* for molecules produced *in vitro*. So far only MIF has been shown to cause an "accelerated" cutaneous reaction of the delayed type when injected intradermally in guinea pigs. Similar activities *in vivo* are actively being sought to correlate with lymphocyte transforming factor and lymphotoxin.

It has been apparent that transfer factor is a distinctive molecule separable from LTF, MIF and LT by virtue of its physicochemical properties alone—*eg*, dialyzable, < 10,000 mol wt, nonantigenic, *etc*). The properties of transfer factor *in vivo* are also distinctive in the transfer of delayed cutaneous reactivity,[3-8] homograft rejection [21] and

recovery from disseminated intracellular viral [47,48] and fungal infections.[49,50] Moreover, in addition to transferring the above states of reactivity *in vivo*, transfer factor confers on the recipient's blood lymphocytes the capacity to respond to antigen *in vitro* in the ways that natively sensitive lymphocytes can, such as lymphocyte transformation, clonal proliferation and MIF production.

Thus we have come to view transfer factor as an initiator of cellular immunity and distinct from the effector molecules. Moreover, transfer factor is immunologically specific, whereas although the production of the effector molecules is specific, their activities are largely nonspecific. The effector molecules are not immunoglobulins.[2]

Transfer Factor and Immunologic Homeostasis

We have emphasized the tissue damaging effects of transfer factor and the uncommitted cells it recruits into similar activity, with resultant sickness or death of target cells bearing the correct antigenic configuration. It is appropriate here to consider the normal function of transfer factor and its contribution to cellular homeostasis in multicellular organisms. It should be stressed that the functions of transfer factor uncovered in the recipient take place in the donor whenever he is exposed to antigen, whether in the form of living microbes and viruses or their products or in the form of an organ transplant.

Thomas [51] was the first to suggest that the mechanism of cellular immunity evolved as a defense against neoplasia, where mutant cells are recognized by the host as "foreign" and rejected as a homograft. This concept has since been termed "immunological surveillance" by Burnet.[52]

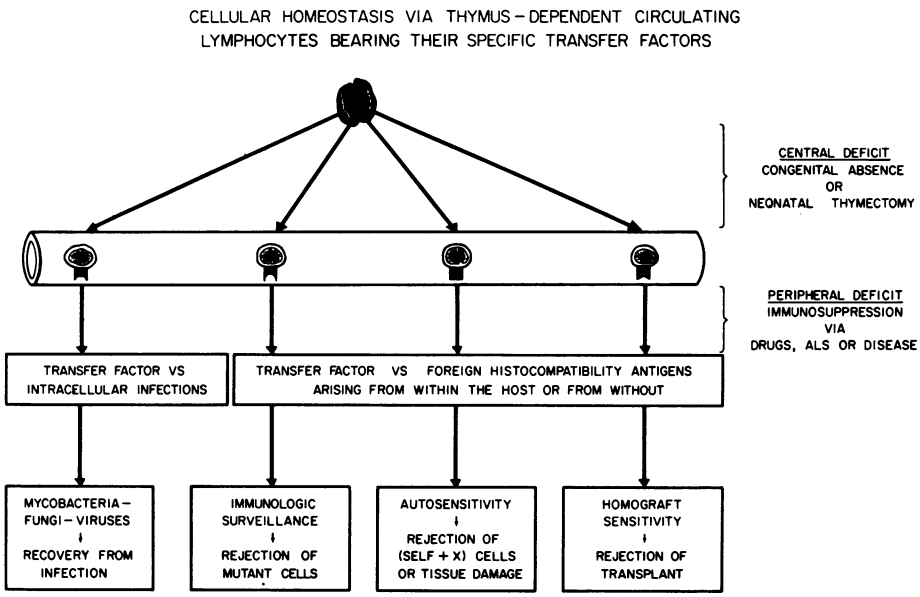
We have suggested elsewhere in the self + x hypothesis [53] that the prolonged, relatively amicable, intracellular residence of certain bacteria, fungi and viruses results in alterations of host histocompatibility antigens which induce a specific transfer factor versus the self + x complex. This postulate virtually identified all delayed hypersensitivity or cellular immune responses as homograft reactions, where the host's histocompatibility antigens complexed with tuberculin come to be regarded as foreign and lymphocytes bearing the appropriate transfer factor initiate the attempt to reject such tissues. In addition to such activities lymphocytes equipped with an appropriate transfer factor are concerned with normal resistance to or recovery from disseminated disease caused by bacteria, fungi or viruses that result in intracellular infections. Treatment with transfer factor has been shown

to eradicate disseminated vaccinia [47, 48] and disseminated candidiasis [49, 50] in such patients exhibiting depressed cellular immunity.

Thus one of the main activities of circulating, thymus-dependent lymphocytes is, by virtue of the specific transfer factor they possess, the policing of the host of unwanted cells that are foreign to it and the attempt to reject them. Such cells may arise within the host and acquire a foreign caste in consequence of mutation, neoplastic change, or alteration of histocompatibility antigens by intracellular microbial or viral residence; overtly foreign cells may be presented to the host from without in the form of an organ transplant.

It appears to be no accident, therefore, that the same cell lineage, when equipped with the appropriate transfer factor, will reject those cells containing tubercle or leprosy bacilli, fungi or viruses and bring disseminated infections caused by such microbes to a halt. These cellular homeostatic functions of thymus-dependent circulating lymphocytes bearing specific transfer factors are diagrammatically oversimplified in Text-fig 3.

It is understood that, once interaction of such specifically sensitized cells and the correct antigenic configuration occurs, there is initiated lymphocyte transformation and clonal proliferation with concomitant production of lymphocyte transforming factor, macrophage in-



TEXT-FIG 3. Predilection and specificity of transfer factor for foreign histocompatibility antigens arising from within or without the host. (From Lawrence HS[58])

hibitory factor and lymphotoxin each to perform tasks *in vivo* that should turn out to at least resemble, if not mimic, their performance *in vitro*.

References

1. Bloom BR, Chase MW: Transfer of delayed-type hypersensitivity. A critical review and experimental study in the guinea pig. Progr Allerg 10: 151–245, 1967
2. Mediators of Cellular Immunity. Edited by HS Lawrence, M Landy. New York, Academic Press, 1969, pp 1–469
3. Lawrence HS: Transfer of hypersensitivity of the delayed type in man, Cellular and Humoral Aspects of the Hypersensitive States. Edited by HS Lawrence. New York, Paul B. Hoeber, Inc, 1959, pp 279–318
4. Lawrence HS: Some biological and immunological properties of transfer factor. Cellular Aspects of Immunity, Ciba Foundation Symposium. Edited by GEW Wolstenholme, M O'Connor. Boston, Little, Brown and Co, 1960, pp 243–271
5. Lawrence HS: Transfer factor. Advances Immun 11:195–266, 1969
6. Lawrence HS: The cellular transfer of cutaneous hypersensitivity to tuberculin in man. Proc Soc Exp Biol Med 71:516–522, 1949
7. Lawrence HS: The cellular transfer in humans of delayed cutaneous reactivity to hemolytic streptococci. J Immun 68:159–178, 1952
8. Slavin RG, Garvin JE: Delayed hypersensitivity in man: Transfer by lymphocyte preparations of peripheral blood. Science 145:52–53, 1964
9. Lawrence HS: The transfer in humans of delayed skin sensitivity to streptococcal M substance and to tuberculin with disrupted leukocytes. J Clin Invest 34:219–230, 1955
10. Freedman SO, Fisher JP, Cooke RA: A study of leukocyte antibodies in allergic patients. J Allerg 28:501–513, 1957
11. Maurer PH: Immunologic studies with ethylene oxide-treated human serum. J Exp Med 113:1029–1040, 1961
12. Jensen K, Patnode RA, Townsley HC, Cummings MM: Multiple passive transfer of the delayed type hypersensitivity in humans. Amer Rev Resp Dis 85:373–377, 1962
13. Baram P, Mosko MM: Chromatography of the human tuberculin delayed type hypersensitivity transfer factor. J Allerg 33:498–506, 1962
14. Brown RE, Katz M: Passive transfer of delayed hypersensitivity reaction to tuberculin in children with protein calorie malnutrition. J Pediat 70:126–238, 1967
15. Lawrence HS: Specific recruitment of immunocompetent cells by transfer factor, Mediators of Cellular Immunity. Edited by HS Lawrence, M Landy. New York, Academic Press, 1969, pp 143–175
16. Lawrence HS, Pappenheimer AM Jr: Transfer of delayed hypersensitivity to diphtheria toxin in man. J Exp Med 104:321–336, 1956
17. *Idem*: Effect of specific antigen on release from human leukocytes of the factor concerned in the transfer of delayed hypersensitivity. J Clin Invest 36:908, 1957
18. Good RA, Varco RL, Aust JB, Zak SJ: Transplantation studies in patients with agammaglobulinemia. Ann NY Acad Sci 64:882–924, 1957

19. Rapaport FT, Lawrence HS, Millar JW, Pappagianis D, Smith CE: Transfer of delayed hypersensitivity to coccidioidin in man. J Immun 84:358–367, 1960
20. *Idem*: The immunologic properties of coccidioidin as a skin test in man. J Immun 84:368–373, 1960
21. Lawrence HS, Rapaport FT, Converse JM, Tillett WS: Transfer of delayed hypersensitivity to skin homografts with leukocyte extracts in man. J Clin Invest 39:185–198, 1960
22. Lawrence HS: Transfer factor, homograft rejection and the effects of antilymphocyte serum in man. Fed Proc 29:183–185, 1970
23. Lawrence HS, Al-Askari S, David J, Franklin EC, Zweiman B: Transfer of immunological information in humans with dialysates of leukocyte extracts. Trans Assoc Amer Phys 76:84–89, 1963
24. Rapaport FT, Dausset J, Converse JM, Lawrence HS: Biological and ultrastructural studies of leukocyte fractions as transplantation antigens in man. Transplantation 3:490–500, 1965
25. Lawrence HS, Zweiman B: Transfer factor deficiency response—a mechanism of anergy in Boeck's sarcoid. Trans Assoc Amer Phys 81:240–247, 1968
26 Baram P, Mosko MM: A dialyzable fraction from tuberculin-sensitive human white blood cells capable of inducing tuberculin-delayed hypersensitivity in negative recipients. Immunology 8:461–474, 1965
27. Baram P, Yuan L, Mosko MM: Studies on the transfer of human delayed-type hypersensitivity. 1. Partial purification and characterization of two active components. J Immun 97:407–420, 1966
28. Arala-Chaves MP, Lebacq EG, Heremans JF: Fractionation of human leukocyte extracts transferring delayed hypersensitivity to tuberculin. Inter Arch Allerg 31:353–365, 1967
29. Fireman P, Boesman M, Haddad ZH, Gitlin D: Passive transfer of tuberculin reactivity in vitro. Science 155:337–338, 1967
30. Brandress MW: Attempt to transfer contact hypersensitivity in man with dialysate of peripheral leukocytes. J Clin Invest 41:2152–2157, 1968
31. Valentine FT, Franklin EC, Lawrence HS: Unpublished observations
32. Lawrence HS, Valentine FT: Transfer factor in delayed hypersensitivity. Ann NY Acad Sci 169:269–280, 1970
33. Adler WH, Smith RT: In vitro stimulation of mouse spleen cell suspensions. Fed Proc 28:813, 1969
34. Valentine FT, Lawrence HS: Transfer of cellular hypersensitivity in vitro. J Clin Invest 47:98a, 1968
35. Valentine FT, Lawrence HS: Lymphocyte stimulation: Transfer of cellular hypersensitivity to antigen in vitro. Science 165:1014–1016, 1969
36. Spitler LE, Lawrence HS: Studies on lymphocyte culture: Products of sensitive lymphocyte-antigen interaction. J Immun 103:1072–1077, 1969
37. Maini RN, Bryceson ADM, Wolstencroft RA, Dumonde, DC: Lymphocyte mitogenic factor in man. Nature 222:43–44, 1969
38. Marshall WH, Valentine FT, Lawrence HS: Cellular immunity in vitro. Clonal proliferation of antigen-stimulated lymphocytes. J Exp Med 130:327–343, 1969
39. Marshall WH, Valentine FT, Lawrence HS: Lymphocyte proliferation in

tuberculin-stimulated and mixed leukocyte cultures investigated by continuous time-lapse cinematography. Third Annual Leukocyte Culture Conference. Edited by WO Rieke. New York, Appleton-Century-Crofts, 1969, pp 475–481

40. David JR, Al-Askari S, Lawrence HS, Thomas L: Delayed hypersensitivity in vitro. I. The specificity of inhibition of cell migration by antigen. J Immun 93:264–273, 1964
41. Bloom BR, Bennett B: Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science 153:80–82, 1966
42. David JR: Delayed hypersensitivity *in vitro:* Its mediation by cell-free substances formed by Lymphoid cell-antigen interaction. Proc Nat Acad Sci 56:72–77, 1966
43. Thor, DE: Delayed hypersensitivity in man: A correlate *in vitro* and transfer by an RNA extract. Science 157:1567–1569, 1967
44. David JR: Discussion, Mediators of Cellular Immunity. Edited by HS Lawrence, M Landy. New York, Academic Press, 1969, pp 133–134, 237
45. Lebowitz A, Lawrence HS: Target-cell destruction by antigen-stimulated human lymphocytes. Fed Proc 28:630, 1969
46. Ward PA, Remold HG, David JR: Leukotactic factor produced by sensitized lymphocytes. Science 163:1079–1081, 1969
47. Kempe CH: Studies on smallpox and complications of smallpox vaccine. Pediatrics 26:176–189, 1960
48. O'Connell CJ, Karzon DT, Barron AL, Plaut ME, Ali VM: Progressive vaccinia with normal antibodies. Ann Int Med 60:282–289, 1964
49. Buckley RH, Lucas ZJ, Hattler BG, Zmijewski CM, Amos DB: Defective cellular immunity associated with chronic mucocutaneous moniliasis and recurrent staphylococcal botryomycosis: immunological reconstitution by allogenic bone marrow. Clin Exp Immun 3:153–169, 1968
50. Levin AS, Stites DP, Spitler LE, Sahud MA, Fudenberg HH: Induction of "delayed hypersensitivity" in a Wiscott Aldrich patient by transfer factor. Clin Res 18:428, 1970
51. Thomas L: Discussion, Cellular and humoral aspects of the hypersensitive states. Edited by HS Lawrence, New York, Paul B. Hoeber, Inc, 1959, pp 529–532
52. Burnet FM: Immunological aspects of malignant disease. Lancet 1:1171–1174, 1967
53. Lawrence HS: Homograft sensitivity—An expression of the immunologic origins and consequences of individuality. Physiol Rev 39:811–859, 1959
54. Editorial, Transfer factor and leprosy. N Eng J Med 278:333–334, 1968
55. Thor DE, Dray S: A correlate of human delayed hypersensitivity: Specific inhibition of capillary tube migration of sensitized human lymph node cells by tuberculin and histoplasmin. J Immun 101:51–98, 1968
56. Granger GA, Kolb WP: Lymphocyte *in vitro* cytotoxicity: mechanisms of immune and non-immune small lymphocyte mediated target L cell destruction. J Immun 101:111–175, 1968
57. George M, Vaughan JH: *In vitro* cell migration as a model for delayed hypersensitivity. Proc Soc Exp Biol Med 111:514–521, 1962
58. Lawrence HS: Transfer factor and cellular immune deficiency disease. New Eng J Med 283: 1970 (in press)

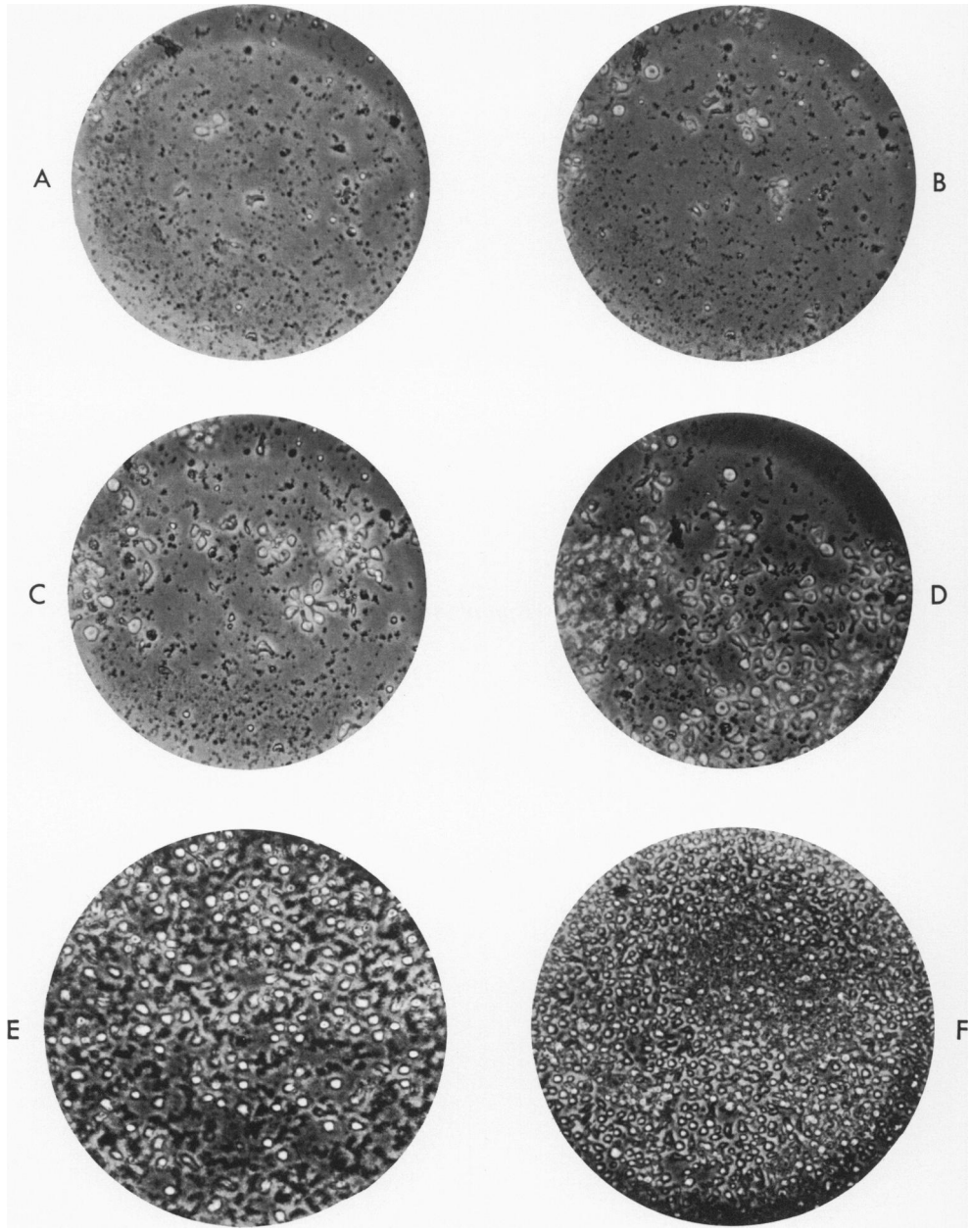


Fig 1. Frames from time-lapse cinematography show lymphoblastic proliferation in an individual microchamber after culture of lymphocytes stimulated with tuberculin. **Sequence A through D** begins on Day 3 of culture and continues through Day 6; **sequence E and F,** not followed with time-lapse, illustrate magnitude clones may reach by Day 7 or 8. Experiment suggests that clonal formation (at least after Day 3) results from repeated cell division of a few antigen-sensitive cells rather than from recruitment. (From Marshall WH *et al.*[38])

[*End of Article*]