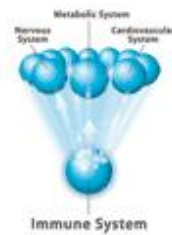


## About Transfer Factor



Transfer factors are natural, microscopic molecules that reside in the bodies of all animals. They are messengers, passing immunity information about the presence of an immune threat—whether external or internal—and how to properly respond, from immune cell to immune cell.

## FEATURED ARTICLE



### Dr. H. Sherwood Lawrence: Transfer Factors and Immunology Pioneer

The knowledge of transfer factors owes its existence to immunology pioneer Dr. H. Sherwood Lawrence. In 1949, Lawrence discovered that by injecting an extract from the leukocytes of someone previously infected with tuberculosis into someone as of yet uninfected with it, immunity was conferred to the recipient, sparing him/her from developing the infection. Dr. Lawrence named the extract *transfer factor* and the possibility of sharing natural immunity between people and even animals and people became real.

Dr. Lawrence graduated from the NYU School of Medicine in 1943. From 1943 to 1949, he served as a medical intern and then as a medical officer with the U.S. Navy, seeing activity in southern France and Japan and receiving two Bronze Stars. He served on the faculty of the NYU School of Medicine from 1947 to 1959 and from 1959 until his retirement in 2000, was head of infectious diseases and immunology at NYU. He also served as co-director of medical services at Bellevue and New York University hospitals from 1964 to 2000, director of the NYU cancer center from 1974 to 1979 and director of the NYU AIDS research center from 1989 to 1994. He was a member of the National Academy of Sciences and honorary chairman of the International Transfer Factor Society (ITFS), a scholarly organization committed to the worldwide exchange of information pertaining to the immunologic properties of leukocyte dialysates.

## FEATURED ARTICLE



### Transferable Active Immunological Memory: The Case for Antigen Specific Transfer Factor

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#### Abstract

Immunological memory that provides for rapid recognition and response to infection is a unique and critical function of the immune system. Functional memory represented by specific antibody provides an example of immune memory and in the form of colostrum the immune experience of the maternal system is passively transferred to the offspring. Evidence now clearly suggests that cell mediated immune memory is highly antigen specific, resides in a small polypeptide and is transferred as active immunity in colostrum secretions. This review discusses the scientific evidence implicating transfer factor as the cell mediated immune memory molecule.

#### Introduction

The ability of the immune system of higher animals to accurately recognize and respond to the unique

antigenic structure of foreign microbes is fundamental to the health and survival of the species. Yet the understanding of the precise mechanisms of immunological recognition remains unclear. Similarly critical is the ability of the immune system to store this immunological recognition information in a manner that provides for efficient recollection and response. This phenomenon of active immunological memory is the practical basis for all successful vaccine strategies. However the mechanism by which the specific stereo-chemical identity of microbial antigens is created, stored and communicated within the immune physiology remains mostly unknown.

Scientific evidence and practical experiences over the last 50 years provide a basis for advancing the theory for immunological memory encoded in simple protein structures. Corollaries for this proposal can be found in the specificities of antibody antigen interaction. The ability of an antibody to bind to a unique antigen lies in the specific epitope region of the antibody. The epitope portion of the antibody is comprised of relatively few amino acids compared to the balance of the large molecular structure of an antibody. These amino acid sequences are a product of cellular gene reorganization and ribosomal RNA controlled protein synthesis. The successful gene reorganization results in an antibody that binds with the offending antigen. Subsequent binding to antigen by B cell surface antibody there after stimulates cloned selection of that antibody producing cell and large quantities of highly specific antibody can be produced. Similar mechanisms likely operate in the T cell lymphocyte line for the selection and development of antigen specific T cells.

This review traces scientific investigations of immune responses and the biology of immunological communication within individuals and with their offspring. The accumulating evidence suggests that immunological memory is encoded in a small polypeptide; a small portion of which is uniquely structured to contain important antigenic structural information. This polypeptide called transfer factor (TF) is the basis for immune memory.

### **Biological and Clinical Evidence**

As early as 1922, Smith and Little, (1922) implicated the importance of immune memory and its transfer in the observation that new born calves that did not receive colostrum typically perished. They speculated that a factor in colostrum protected calves in some manner. Many years later, McGuire et al (1976) clearly documented that the quantitative failure of passive transfer of antibodies resulted in degrees of susceptibility to infectious disease. Yet others, including Kerr (1956) showed that some calves seemed to have more effective immune responses to certain disease agents including Tuberculosis (TB). Given that not all disease agents, like TB, are effectively controlled by antibody activities alone, the observation may have been interpreted to include that colostrum contained factors other than antibodies that contributed to the full immune competence of the newborn.

Throughout the late 1900's most researchers attributed the benefit of colostrum in animals and human disease resistance solely to the passive protection of absorbed and circulating maternal antibodies. At this time there was little if any scientific recognition for a role of cell mediated immunity conveyed via colostrum.

In another avenue of investigation, the 1949 experiments of Lawrence suggested another means by which specific active immunological memory could be transferred. Lawrence produced a cell free dialysate of lysed human lymphocytes (DLE) from a subject that was skin test positive to tuberculin extract. When the dialysate was injected into another human subject that heretofore was TB skin test negative, the subject rapidly become TB skin test positive. This unknown substance that transferred immune reactivity was called transfer factor. Given the protracted nature of TB infection and the time needed to convert a patient to a TB reactor it is highly unlikely that the dialysate was a source of infection.

The bovine and human observations with TB reactors suggest a chemical or cellular signal is transferring information. However the results may be explained by some non-specific adjuvant like activities contained in mycobacterium and not necessarily a transfer of microbial antigen information.

Subsequent investigations into the immune biology of the mammary gland clearly show that its immune functions are much more than the concentration of antibody for colostrum synthesis. In 1978, Bennett and Jasper were evaluating white blood cell numbers in non-lactating mammary gland secretions. In the

weeks prior to parturition the healthy non diseased gland produced myriads of mononuclear cells in the secretions. Latent disease was speculated to be the case; however examination of young non-infected cows revealed a similar pattern. The presence of the mononuclear cells during colostrum formation suggested a role of cell mediated immunity for colostrum immune protection of the neonate.

A decade later Benoco et. al. (1987) demonstrated large populations of T and B cell lymphocytes in the non lactating mammary secretions. Perhaps for the first time, the researchers suggested this may be an essential event in the transfer of cellular immunity via colostrum. The belief that colostrum antibody was only a source of immune protection was now in question.

The transfer of active immune information and function in human colostrum and milk was suggested by the work of Schlessinger and Covelli (1977). Infants from TB-PPD test positive mothers had circulating lymphocytes reactive to tuberculin by in vitro assays, whereas infant from non reactive mothers statistically did not. Similar to the work of Lawrence these researchers suggested a transfer factor like substance or intact cells transmitted Tuberculin responsiveness.

Another important piece of the puzzle is provided by Yang et. al. (1997) Tracing markers for the CD4+ and CD8+ lymphocyte sub populations, they reported dynamic lymphocyte population fluxes in mammary secretions as the animal approaches parturition. These regulatory and antigen specific types of T cells are present in large numbers in colostrum. The authors speculate that these cells survive and mature in the gastro-intestinal tract of the newborn. However they did not evaluate this potential. Given the monogastric digestive processes of the neonate and the potential for host versus graft and graft versus host histo-incompatibility the functional viability of these cells is highly questionable.

The evidence for colostrum derived factors that specifically educate and activate the immune system of the neonate was advanced significantly by Wilson and et al. (1988). Their work demonstrated several important attributes of the cellular immune enhancing properties of colostrum extracts. Infectious agents unique to poultry were used to vaccinate cattle prior to parturition and colostrum production. Colostrum lymphocyte dialysates were injected into pathogen free poultry. Birds injected with the dialysate demonstrated *in vivo* and *in vitro* reactivity to the specific pathogens used to vaccinate the cattle. Birds that did not receive the dialysate did not react in either assay. Cross reactivity was not observed indicating specificity of the vaccine to test antigen system. The work also shows that this "transfer factor" works across widely divergent species lines. More over the transfer factor in colostrum can be influenced by exogenous vaccination.

Additional work by Wilson and Shuler (1988) provided evidence for the successful licensing of a transfer factor based vaccine for Transmissible Gastroenteritis Virus in swine. TGE is a highly lethal viral infection of newborn pigs. The researchers immunized cattle with a TGE vaccine and others received no vaccine. Piglets receiving the colostrum transfer factor from vaccinated cows had significantly less mortality than those receiving colostrum transfer factor from non vaccinated cows. This observation clearly speaks to the ability of transfer factor to produce effective and specific immunity and to do so in a period of time much shorter than the process of active immunization.

The recognition of the specificity of transfer factor was further advanced by the research of Amaidov and Tziporkov (1996). Rabbits were immunized with *Salmonella ch. Suis* to produce a lymphoid dialysate (DLE). The dialysate was injected intra-peritoneally into mice. When challenged with *Salmonella ch suis* the dialysate injected mice had 70% effective protection. The DLE injection provided no protective effect for *Salmonelladublin* challenge.

In a human clinical trial, Borkowsky et. al ( 1987) treated eight patients with clinical AIDS and co-infected with *Cryptosporidium parvum* with an oral DLE bovine transfer factor. Four of eight clinically improved and one remained *C. parvum* free for 2 years after discontinuation of transfer factor therapy. The immune system of the AIDS patient is highly disrupted by the viral infection of the CD4+ and the CD8+ lymphocytes and yet transfer factor from calves immune to *C. parvum* was specific and able to improve the immune function in a portion of the patients

These lines of research are highly suggestive that transfer factors are quite specific. However the researchers used whole microbe preparations that contain a wide array of antigens. Since many

microbes share similar antigenic structures, the question of molecular specificity for transfer factor remained as a matter of speculation until recently.

### **Molecular Evidence**

The molecular basis and specificity of transfer factor became very clear after the works of Charles Kirkpatrick and coworkers. As early as 1985 Kirkpatrick et. al., demonstrated specific binding of transfer factor and the antigens used to generate them. These specific transfer factors could be selectively bound and eluted and specific immunological reactivity transferred to recipient mice. Subsequent work confirmed the molecular basis for transfer factor specificity.

Kirkpatrick (1996) reports on the work of his laboratory and that of others. The researchers in separate investigations reveal affinity purification methods for transfer factors. In particular Rozzo and Kirkpatrick (1992) demonstrate affinity purification of specific transfer factor that binds to specific antigen and purity confirmed by HPLC. Kirkpatrick (1996) demonstrates transfer factor specificity by using unique antigenic molecules such as ferritin and cytochrome C to vaccinate mice for the production of transfer factor. Oral administration of the purified transfer factor and subsequent *in vivo* CMI foot pad assay demonstrated clear and distinct specificity to these small and unique antigens.

In a second study Thull and Kirkpatrick (1996) evaluated cytokine production in mice sensitized to Herpes simplex virus by vaccination or by the administration of HSV specific transfer factor. Spleen cell cultures derived from the animals were tested for cytokine production in response to killed virus exposure. Spleen cells from mice sensitized with HSV-TF produced a different pattern of cytokine production. The pattern of cytokine production was typical of the T Helper 1 phenotype where interferon gamma is the predominate cytokine.

These data not only provide additional molecular evidence for the specificity of the transfer factor message but also reveal a selective signal enabling cell mediated immune functions of the TH 1 cell system.

In an attempt to identify the molecular identity of transfer factor Kirkpatrick (2000) investigated the amino acid sequence of the transfer factor molecule; the results reveal a common sequence of amino acids for all TF regardless of the origin. It appears this portion of the molecule is conserved across species lines. Another smaller region of the molecule is hyper variable in its amino acid composition. This variable region most likely contains the inverse stereo chemical image that allows for highly selective binding to antigen. It is also that portion of the molecule that encodes for the active immune recognition of the antigen. These observations are consistent with our current understanding of the specificity of antibodies, wherein the epitope of the light chain is determined by as few as 10 amino acids. Such appears to be the case for the specificity of transfer factor. However in this case the transfer factor molecule provides key antigenic information to uncommitted T cells and T Helper 1 cells and enables them to sustain active immune functions for some time.

### **Conclusion**

In the course of discovery of immune memory a similar pattern of antibody and cellular specificity has emerged. From a teleological and biological perspective, the communication of immune memory within a species and between mother and offspring is central to its survival.

Transfer factor is the small and information rich polypeptide that stores the unique cell mediated immune experience of the animal. The picture of how transfer factor performs this function in the whole animal and at the molecular level is becoming increasingly clear.

Given the critical and central role of cell mediated immunity in disease resistance, the potential to harvest this molecule for disease prevention and clinical intervention in virtually all vertebrate species is staggering.

Additionally the adverse effects of modified living and inactive pathogen vaccine preparations may be

avoided by the application of specific transfer factor immunoprophylaxis. The recognition of antigen specific transfer factor and active research toward these goals holds great potential for the near future.

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