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Transfer Factor Revisited: Treatment of Congenital Cytomegalovirus Infection

J. Kelly Smith[1,2*]

[1]Department of Academic Affairs, James H. Quillen College of Medicine, East Tennessee State University, USA
[2]Department of Biomedical Sciences, James H. Quillen College of Medicine, East Tennessee State University, USA

ABSTRACT

A 7-month-old girl with congenital cytomegalovirus (CMV) infection underwent an immune assessment in anticipation of Transfer Factor therapy. She had been symptomatic since birth, with jaundice, rhinorrhea, diarrhea, pneumonia, hepatosplenomegaly, chorioretinitis, hydrocephalus (for which she was shunted), motor retardation, and failure to thrive. Her sputum and urine cultures were positive for CMV and her IgM anti-CMV antibody titer was positive at 1:16-1:32 dilutions. Her baseline immune assessment was normal except for a failure of her peripheral blood mononuclear cells to produce migratory inhibitory factor in response to CMV antigen. Treatment with transfer factor prepared from CMV seropositive donors resulted in clinical improvement, clearance of the virus, normal migratory inhibition factor responses to CMV antigen, and subsequent development of normal growth and development parameters.

Keywords: Transfer factor, dialyzable leukocyte extract, cytomegalovirus, congenital cytomegalovirus, migration inhibition factor, immunotherapy, CMV, MIF, ganciclovir, valganciclovir.

***Correspondence to Author:**
J. Kelly Smith
Department of Academic Affairs, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee.

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Introduction

In 1942 Landsteiner and Chase performed an experiment that was to provide a new paradigm for the field of immunology. They removed peritoneal exudates from guinea pigs previously sensitized intraperitoneally with picryl chloride and conferred delayed cutaneous hypersensitivity (DCH) to the compound by injecting the washed exudates into the abdominal cavities of skin test negative guinea pigs[1]. They had demonstrated the transfer of DCH by leukocytes, and, in so doing, provided the first convincing evidence of the existence of separate cellular and humoral immune compartments.

In 1949 Dr. Sherwood Lawrence and his associates extended the observations of Landsteiner and Chase by transferring DCH to tuberculin using leukocytes from tuberculin skin-test positive human donors and non-reactive human recipients. They found that the transfer activity resided largely within lymphocytes, and that its activity was rapid in onset and of long duration. They found that leukocyte lysates and dialysates imparted DCH in a manner similar to the intact cell, and that the activity was not abrogated by trypsin, deoxyribonuclease and ribonuclease. They concluded that the active material was a low molecular weight “informational” or “activator” molecule which conferred specific antigen-reactivity to naïve lymphocytes, possibly by effecting gene de-repression. The substance was named transfer factor (TF)[2,3].

We now know that TF contains more than 200 highly polarized, hydrophilic, low molecular weight peptides, and that it is capable of transferring both specific and non-specific immunity to healthy recipients. TF can be extracted from human and animal white blood cells, cloned lymphocytes grown *in vitro*, and colostrum, and is capable of functioning across species[4].

Case Report

A 7-month-old girl was diagnosed as having disseminated cytomegalovirus (CMV) infection at birth. Her post-natal physical examination revealed low grade fever, jaundice, rhinorrhea, diarrhea, pneumonia, hepatosplenomegaly, chorioretinitis, hydrocephalus (for which she was shunted), motor retardation, and substandard height and weight gain (Fig. 1). Her sputum and urine cultures were positive for CMV and her IgM anti-CMV antibody titer was positive at 1:16-1:32 dilutions. Her baseline immune assessment was normal except for a failure of her peripheral blood mononuclear cells to produce migratory inhibitory factor (MIF) in response to CMV antigen.

Within a week of receiving 0.3 billion leukocyte equivalent units (LEU) of TF prepared from a CMV seropositive donor, her MIF response to CMV antigen was positive, and by ten days she demonstrated clinical improvement. One week after receiving a total dose of 3.3 billion LEU her urine cultures became negative for CMV and remained so over the 34 months of post-treatment monitoring. Empirically, she received an additional 4.0 billion LEU before treatment was discontinued (Fig. 2). After discharge, her growth and development parameters return to normal (Fig 3), and she was well enough to take ballet lessons at the age of two. She completed her high school and college education, free of any clinically significant neurologic residual.

Discussion

Cytomegalovirus is the commonest cause of congenital infection with an estimated incidence of 0.6-0.7% of live births worldwide[5]. In utero infection with CMV can result in death of the fetus or permanent neurodevelopment disabilities including microcephaly, hydrocephalus, and mental retardation[6-8]. Congenital CMV is the commonest cause of congenital sensineuronal hearing loss worldwide[9,10].

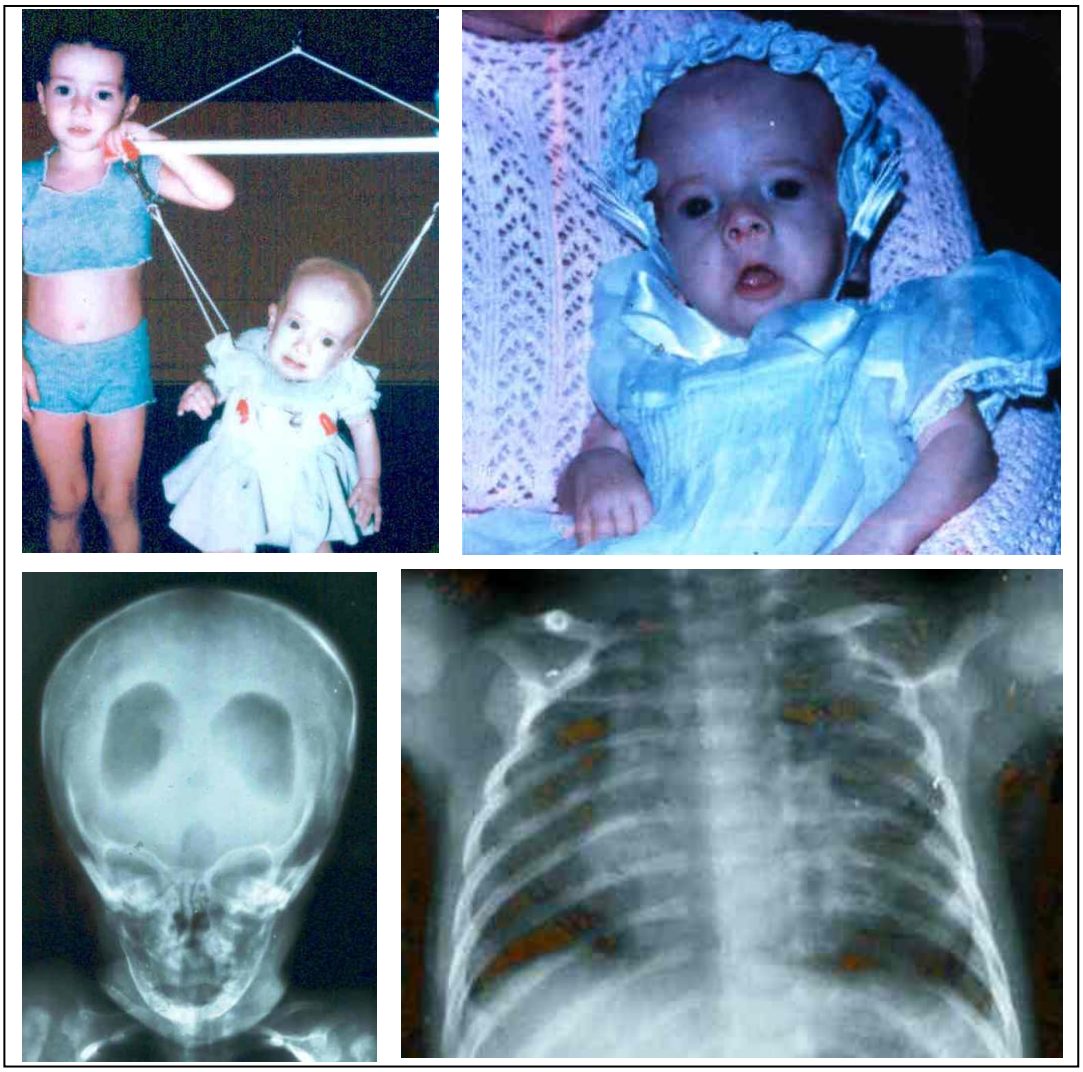

Figure 1. Pretreatment images. The upper left image shows the child suspended in a sling at 4 months of age next to her older sister. Note her hydrocephalus, esotropia, wasting, and lack of muscle tone. At six months of age, hydrocephalus and chronic rhinorrhea are evident in the upper right image. Radiographic evidence of hydrocephalus and bilateral CMV pneumonia are shown in the lower left and right images, respectively.

Side Bar

Preparation of dialyzable leukocyte extract (transfer factor)

TF was prepared by the method of Lawrence[4]. For each batch of TF, 500 mL of whole blood taken from a candida skin test positive donor was collected in a heparinized polyethylene bag and transferred into a sterile glass container. Six percent dextran in saline (25% of final volume) was added, and the red blood cells allowed to sediment at room temperature for 90 minutes. The top layer (white blood cells and plasma) was removed and centrifuged at 4°C for 20 minutes at 367g. The supernatant was decanted, and the cell layer suspended in ~ 6 mL lots of sterile distilled water. The suspended cells were then lysed by alternate freezing and thawing in dry ice and 37°C water. Lysates were pooled and dialyzed against 800 mL of sterile distilled water at 4°C for ~ 12 hours, using membranes that permitted egress of molecules of <10,000 molecular weight*. The dialysate was lyophilized in ~150 mL lots, and the lyophilized extracts concentrated by adding 5 mL of sterile distilled water to each lot, pooling the solutions, and re-lyophilizing overnight. The concentrated dialysate was stored at -70°C until use. Dialysate reconstitution was done by adding 3 mL of sterile distilled water and passing the solution through a Millipore filter. The solutions were checked for endotoxin by the limulus amoebocyte lysate test. Sterile precautions were taken during the entire procedure.

Use of transfer factor as a therapeutic agent in this case was approved by the Institutional Review Board.

*Cytokines are too large to pass through this filter

TF Dose (10^9 LEU)	0.3	1.0	2.0			1.5	1.0	1.5		
CMV Titer	16	32	16	8		8	16	8	8	8
MIF Response	0	+	0	+	+					
CMV culture	+	+	+	0	0	0	0	0	0	0
	0	**1**	**2**	**3**	*3*	*5*	*7*	*11*	*17*	*34*

Time post treatment

Figure 2. Microbiological and immunological response to TF therapy initiated when the child was 6 months of age. Urine CMV cultures became and remained negative after a total of 3.3 billion LEU of TF given over a two week period. She developed a consistently normal MIF response to candida antigen 3 weeks after initiation of therapy, and her IgG anti-CMV antibodies fell from a titer of 16-32 to a titer of 8. Bold letters on x-axis = weeks; italic letters on x-axis = months. LEU, leukocyte equivalent units; TF, transfer factor; CMV, cytomegalovirus; MIF, migratory inhibitory factor.

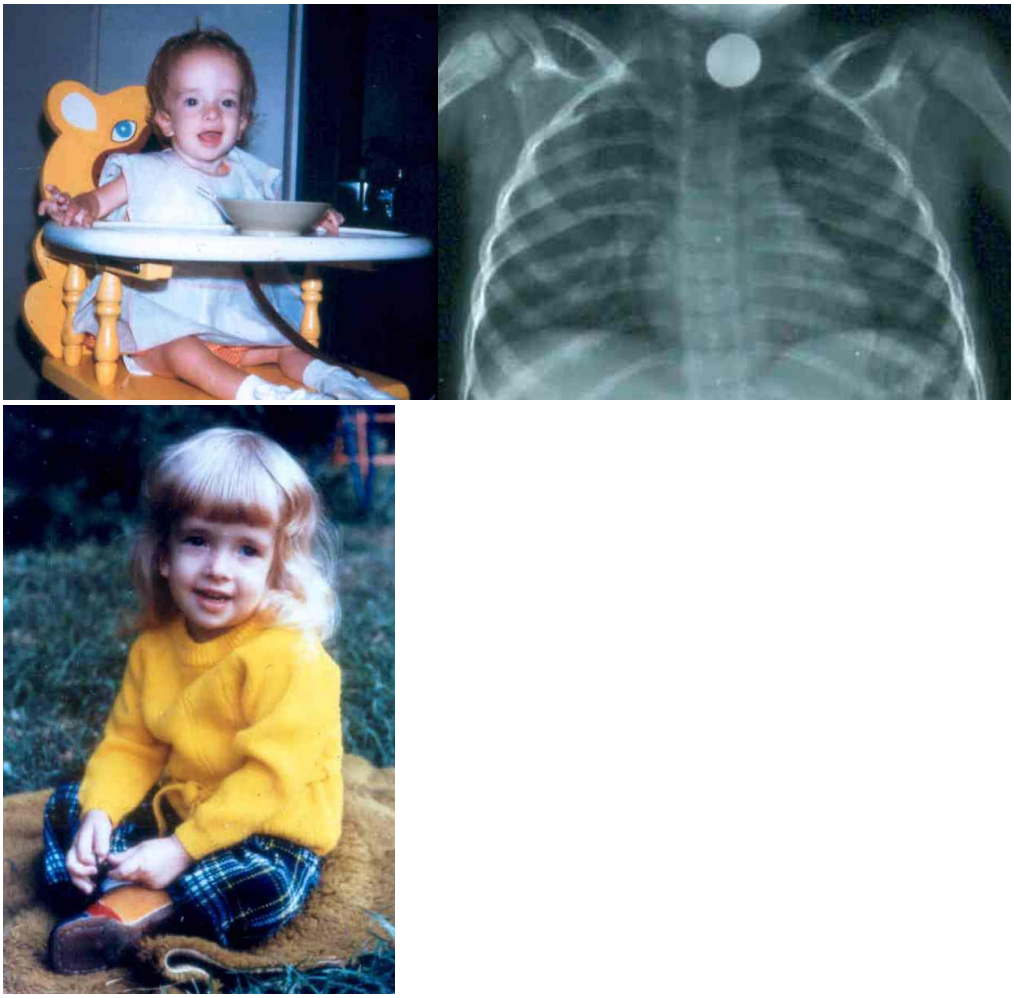

Figure 3. Post-treatment images. The upper left image shows the child at 16 months of age, 10 months after the initiation of transfer factor therapy. Her CMV pneumonia has cleared (upper right image) and she is aviruric with nearly normal developmental parameters. The lower left image shows the child at 27 months of age. Her developmental parameters are normal and she is participating in a number of activities, including ballet.

Antimicrobial treatment for symptomatic congenital CMV infection has been available for thirty years, beginning with ganciclovir (GCV) and more recently with valganciclovir (VGCV). The Collaborative Antiviral Study Group of the National Institute of Allergy and Infectious Diseases initiated studies to determine the efficacy of these drugs in preventing hearing loss or improving the hearing in children born with congenital CMV infection and CNS symptoms. Based on these studies, 6 months of oral VGCV (16 mg/kg twice daily) improved hearing outcomes by 77%; the language component of neurodevelopmental scores also improved on treatment. However, 27% of treated subjects developed neutropenia, and there is evidence of carcinogenicity and gonadotoxicity of GCV in some animal models, making the decision to start antiviral therapy problematic in some cases[11-16].

In our patient, administration of TF prepared from CMV seropositive donors was successful in eradicating her infection and establishing normal growth and development parameters. This case predated the availability of anti-viral agents with activity against CMV and thus provided the rationale for attempting immune reconstitution with TF. Others have also reported success in treating CMV infection with TF[17-20]. The reader is referred to a recent review article on transfer factor as an immune modulator[21].

We subsequently treated three other children with disseminated CMV infection with similar doses of TF. Case 1 was a 14-month-old boy with microcephaly and severe psychomotor retardation; case 2 was a 2-month-old boy with hepatosplenomegaly; case 3 had postnatally acquired CMV pneumonia. All three children had positive CMV urine cultures, positive serology, and deficient MIF responses to CMV antigen. They became culture negative with normal MIF responses to CMV antigen following treatment with TF prepared from CMV seronegative donors. There were no adverse reactions to treatment in any of these children.

In many ways our results parallel those described with thymosin-1α (T1α), a pleiotrophic prothymosin polypeptide derivative found in high concentrations in the thymus and in lesser concentrations in other tissues, including peripheral blood mononuclear cells (PBMC)[22] . Synthetic T1α has been used to treat some viral infections[23], although seemingly with less efficacy than we found with TF. In humans, T1α has been shown to activate immature and mature mDC and pDC subsets, to promote T cell and NK cell maturation, and to stimulate cytokine production and CTL-mediated cytotoxic responses[24-26]. T1α has been shown to modulate the expression of a variety of gene transcripts in peripheral blood mononuclear cells *in vitro*, possible accounting for its pleiotropic effects. Prothymosin α, the precursor of T1α, is a histone H1-binding protein which synergizes with CREB-binding protein to stimulate AP1- and NF-κB-dependent transcription by chromatin remodeling[27]. Whether T1α (or TF) have similar modus operandi in transcription regulation is not known. However, the conclusion of Lawrence and associates that TF may work by “gene de-repression”[2,3] may well prove to be correct.

Conclusion

TF is a safe and effective treatment option for symptomatic congenital and postnatally-acquired CMV infection.

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