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TRANSFER FACTOR FOR THE PREVENTION OF VARICELLA-ZOSTER INFECTION IN CHILDHOOD LEUKEMIA

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Abstract Sixty-one patients with leukemia and no immunity to chickenpox were given dialyzable transfer factor or placebo and followed for 12 to 30 months in a double-blind trial designed to examine the clinical efficacy of transfer factor. Sixteen patients in the transfer-factor group and 15 in the placebo group were exposed to varicella zoster, and most of them had a rise in antibody titer. Chickenpox developed in 13 of 15 exposed patients in the placebo group but in only one of 16 in the transfer-factor group ($P=1.3x10^{-5}$). In the patients treated with transfer factor and exposed to varicella without acquiring chickenpox the titer of antibody to varicella zoster was equal to that in the patients given placebo who became infected with chickenpox. Transfer factor converted negative results on skin tests for varicella zoster to positive in approximately half the recipients. Passive immunization with dialyzable transfer factor appears useful in nonimmune persons. (N Engl J Med. 1980; 303:355-9.)

WITH current modes of therapy, acute lymphocytic leukemia in childhood is curable in approximately one third of newly diagnosed patients.[1] Such progress in treatment of neoplastic disease has now made it even more critical to anticipate the infectious processes to which patients with leukemia are predisposed. Varicella-zoster disease remains one of the most common serious infectious in childhood leukemia; it is associated with a 7 per cent mortality[2] and with markedly increased morbidity when these patients are compared with normal children.[3]

Present modes of treatment for varicella in patients with leukemia include administration of zoster immune globulin or hyperimmune plasma within 72 hours after exposure,[4,5] and if disease progresses to involve the central nervous system or visceral organs, vidarabine or acyclovir, newer antiviral agents, are usually offered. Vidarabine has not yet been shown to be efficacious for disseminated varicella-zoster disease in a double-blind clinical trial .[6] A live varicella-zoster vaccine has also been developed and examined on a limited basis, and in Japan it has been given to children with acute lymphocytic leukemia.[7] However, in one recipient progressive cutaneous dissemination still occurred, so that cautious evaluation is needed before further use.

Another approach, active immunotherapy with dialyzable transfer factor, offers an alternative preventive treatment in this clinical setting. Previous studies in patients with acute lymphocytic

leukemia have demonstrated that transfer factor can transfer reactivity as measured by in vitro assays of varicella-zoster blastogenic, cytotoxic, and leukocyte-inhibitory factor and by in vivo responses to skin tests.[8,9] Perhaps more important, follow-up studies have indicated that positive responses may last for at least 17 months after a single injection.[8] We performed a randomized, double-blind, placebo-controlled clinical trial in a large group of patients with leukemia and susceptibility to varicella zoster, to evaluate the therapeutic efficacy of this approach.

METHODS

Study Population

Seventy-two children with a diagnosis of acute lymphocyte leukemia were included in the study. All had negative histories for chickenpox and negative skin tests for varicella zoster. At entry into the study, the ages of the patients ranged from five months to 14 years, with a mean of 7.1 years. Sixty-two per cent of the children were male. The basic chemotherapeutic regimen for acute lymphocyte leukemia consisted of induction of remission with prednisone and vincristine followed by cranial irradiation, maintenance therapy with 6-mercaptopurine and weekly methotrexate for three years, and periodic cyclic reinduction of remission with vincristine and prednisone. After informed consent was obtained from parents and all children over eight years of age, patients wereassigned to transfer factor or placebo by predetermined random numbers. This study population was followed from May 1977 to April 1980.

Skin-Test Antigen

Varicella-zoster virus antigen was prepared with the Scott strain of varicella-zoster and human diploid cells. The tissue-culture line employed, designated USU-500, is a fibroblast-like cell line derived from neonatal human foreskin. This tissue was minced, cultured as explants, and cryopreserved at the fourth passage. Confluent cultures representing approximately 2×10^7 cells per flask were inoculated with 1×10^6 virus-infected USU-500 cells that had been cryopreserved 24 hours after inoculation and stored in the vapor phase of liquid nitrogen to minimize loss of infectivity during long-term storage. The resulting varicella zoster infected cells diluted in Hanks' minimum essential medium were inoculated onto cell sheets and incubated at 37iC. Cells were then maintained in Eagle's minimum essential medium with 2 per cent fetal-calf serum. Identical uninoculated cultures of USU-500 cells were used to prepare control skin-test antigen and test-tube cultures for subsequent studies of viral infectivity. A cytopathic effect was first noted in virus-infected flasks approximately 60 hours after inoculation. Cultures were harvested when more than half the cell sheet demonstrated a cytopathic effect, which occurred approximately seven days after inoculation. Cells were then harvested by scraping with sterile Teflon rods and washed three times with phosphate-buffered saline; the cells were resuspended in 2 ml of sterile saline, and aliquots were obtained for infectivity, titration. Varicella-zoster skin-test antigen was prepared by sonication of the cell suspension in saline for two minutes, followed by centrifugation at 3OOOXg for 20 minutes. The supernatant was harvested and heat inactivated at 56iC for one hour; no infectivity was demonstrated and no virus was observed by electron microscopy in this preparation. Subsequent screening of the material in immune adults

demonstrated that a 1:100 dilution yielded consistently positive skin tests with 8 to 28 mm of induration and erythema.

Varicella-zoster virus was measured in the original harvested cell cultures by means of plaque titration on USU-500 cells according to previously reported techniques.[10]

Preparation of Dialyzable Transfer Factor

After screening studies for cellular and humoral immune responses to varicella zoster had been performed five donors with unusually high in vitro reactivity to varicella-zoster antigen were selected.[8] All these donors were adults convalescing from chickenpox. After informed consent had been provided, leukocytes were obtained by leukapheresis with a continuous-flow Cell-trifuge blood-cell separator (American Instrument) and separated from the cell pack with a Hypaque-Ficoll gradient. The leukocytes were freeze-thawed 10 times in the presence of DNase and were then dialyzed and concentrated by means of lyophilization according to the methods of Lawrence and Al-Askari.[11] Transfer factor from five donors was pooled into a single lot for further testing. Potency was confirmed by passive transfer of 0.1 ml of transfer factor intradermally into varicella-negative human recipients followed, in 24 hours, by skin tests with various antigens at the site where the transfer factor had beeninjected.[8] According to this method, skin test responses to varicella zoster were consistently >10 mm in previously negative recipients.

Patients with acute lymphocytic leukemia were given transfer factor by subcutaneous injection in doses of 1×10^8 lymphocyte equivalents per 7 kg of body weight. The placebo consisted of normal saline colored with trace amounts of riboflavin.

Measurement of Varicella-Zoster Antibody

Serum antibody to varicella zoster was measured with the indirect enzyme-linked immunosorbent assay (ELISA).[12] Briefly, polystyrene microtiter plates (Dynatech) were sensitized with varicella-zoster control antigens obtained by sonication of infected or uninfected human fibroblasts. Serum samples were tested at a 1:5 dilution in a previously determined optimal dilution (1:300) of horseradish peroxidase-conjugated goat antihuman immunoglobulins (Cappel). Bound peroxidase activity was assayed with o-phenylenediamine as the enzyme substrate. Serum reactivity was defined by calorimetric activity exceeding two standard deviations from the mean for serum from patients susceptible to varicella zoster.

Serum samples obtained before enrollment and selected samples obtained during convalescence were also examined for varicella zoster antibody by a technique measuring the titer of fluorescent antibody to membrane antigen (FAMA).[13] Briefly, unfixed tissue culture cells were incubated with various dilutions of test serum, washed, incubated with fluorescein-labeled antihuman IgG, and examined by fluorescence microscopy. Comparison of ELISA and the FAMA technique has demonstrated a high degree of correlation. ELISA offers the advantage of clearer end-point readings and was therefore chosen for the present investigation.

Clinical Evaluation

Any patient in the study who became infected with chickenpox was treated according to acceptable medical practice as judged by the primary physician. Zoster immune globulin, hyperimmune and antiviral chemotherapy were never withheld because of ongoing protocol.

Chickenpox was diagnosed clinically, and isolation of the virus from skin lesions of patients or their contacts was then attempted. Appropriate laboratory studies were undertaken when involvement of the visceral organs or central nervous system was suspected.

RESULTS

Clinical Features

The patients in this study have now had 12 to 30 months of evaluation since enrollment. Nine patients (four treated with transfer factor and five with placebo) either died or relapsed within six months of enrollment or were lost to follow-up and not exposed to chickenpox. In addition, two children had detectable varicella-zoster antibody on entry into the study. These 11 patients were therefore eliminated from the analysis.
The patients given transfer factor and those given placebo were comparable in age on enrollment into the study, age at diagnosis, sex, and other clinical features (Table 1).

Table 1. Demographic and Clinical Data on Patients with Acute Lymphocytic Leukemia (ALL)

FEATURE GROUP	TRANSFER FACTOR	PLACEBO
Number of patients	31	30
Sex (male/female)	20/11	21/9
Mean age at diagnosis of ALL	4 yr, 9 mo	4 yr, 5 mo
Mean interval since diagnosis	2 yr, 8 mo	2 yr, 4 mo
Mean age at enrollment in study	7 yr, 5 mo	6 yr, 9 mo
Exposed to chickenpox (no.)	16	15
Clinical chickenpox	1	13*
Disseminated disease	0	3
Mortality	0	0
Received zoster immune globulin (no.)	2	3

***P = 1.3 x 10^{-5} by Fisher's exact test.**

Exposure to Chickenpox and Disease

Thirty-one patients, 16 in the transfer-factor group and 15 in the placebo control group, were exposed to chickenpox at least once during the observation period. Only exposures considered to predispose to development of disease were recorded for evaluation. Contact with siblings who had active lesions or playmates who were with patients indoors for more than two hours were included. Of the 31 exposed children, 14 became clinically infected; 13 were in the placebo group, and only one was in the transfer-factor group ($P = 1.3 \times 10^{-5}$ by Fisher's exact test). The single affected patient in the transfer-factor group had only three skin vesicles and no systemic manifestations. Three patients in the placebo group had disseminated disease; two had pneumonia and hepatitis, and one encephalitis. There were no deaths.

Varicella-zoster Cultures

Appropriate cultures were obtained from 12 of the 14 clinically infected patients, and varicella-zoster virus was isolated from seven. Viral cultures were not available from the one child who had mild clinical chickenpox, who was in the group receiving transfer factor.

Delayed Hypersensitivity to Varicella Zoster

Skin tests for varicella-zoster antigen were negative in all 72 children at the time of enrollment. Eleven patients treated with transfer factor and not exposed to chickenpox had skin tests repeated 16 months after transfer factor was administered, and seven had converted to positive responses. Eight of 11 patients treated with transfer factor and exposed to chickenpox converted to positive responses; among the eight was the one patient who acquired clinical disease. Of eight control patients who had chickenpox after exposure, four had positive responses; none of the 12 unexposed children had skin reactivity.

Titers of Antibody to Varicella Zoster

Serum samples obtained before and after enrollment were available from 63 patients in whom adequate follow-up was completed. Final serum samples were obtained from 12 to 23 months after enrollment in the study, at least two months after the most recent exposure to chickenpox. Sixty-one patients had no demonstrable antibody by either ELISA or FAMA test at enrollment (Table 2). After exposure to chickenpox, antibody was detected by ELISA in 17 of 31 children including nine of 14 with clinical disease. One patient with no history of varicella during the observation period acquired detectable antibody. This was a placebo-treated patient whose serum sample after 16 months of follow-up was positive at a 1:5 dilution but negative at a 1:10 dilution.

Varicella-zoster antibody was measured with the FAMA assay in patients who were exposed to chickenpox but who did not seroconvert according to ELISA methodology. Three additional children had positive results in this assay. Two of these three (one treated with transfer factor and one with placebo) were exposed to chickenpox but did not become infected. The third patient had been given zoster immune globulin after exposure and had positive results in serum obtained three months later.

Another patient in the placebo group was exposed to a culture-positive sibling and subsequently had culture-positive clinical infection.

Table 2. Varicella-Zoster (VZ) Antibody in Study Patients

GROUP	TOTAL No. OF PATIENTS	No. OF PATIENTS POSITIVE FOR V-Z ANTIBODY*	RECIPROCAL GEOMETRIC MEAN ANTIBODY TITER
Transfer factor			
At enrollment	31	0**	0
At 12-30 mo follow-up VZ exposure	16	9	23
Clinical chickenpox	1	1	80
No known VZ exposure	15	0	0
Placebo			
At enrolment	30	0	0
At 12-30 mo follow-up VZ exposure	15	8	33
Clinical chickenpox	13	8	38
No known VZ exposure	15	1	0

***By the indirect enzyme-linked immunosorbent assay (ELISA), reactive at a serum dilution >1:5.**
****By ELISA and a technique using fluorescent antibody to membrane antigen (FAMA).**

Other Treatment

Zoster immune globulin was given to five children after exposure to chickenpox (Table 1). Three were in the placebo group, and two of these three acquired the disease. Neither of two similarly treated in the transfer-factor group had chickenpox as a result of this exposure, but one became infected after contact three months later. Two patients with disseminated disease were treated with vidarabine, and one with acyclovir. All have recovered without apparent residual effects. During the course of the study, 11 patients received washed packed red cells; seven were receiving placebo, and four transfer factor. This therapy did not appear to influence seroconversion.

DISCUSSION

The efficacy of transfer factor has been studied in a wide variety of infectious diseases. The results in these predominantly single cases and small clinical trials have been critically reviewed and were published after three international conferences on transfer factor. Very few studies have indicated that

transfer factor as any important role in the control of human infection. However, in some selected situations, such as the treatment of chronic mucocutaneous candidiasis in the immunodeficient host, results have been more promising.[14] Only a single double-blind controlled trial has demonstrated that transfer factor can be beneficial; in this study, transfer factor was used for the therapy of human cutaneous leishmania infection in Tehran, Iran.[15] Transfer factor continues to be used more extensively in Europe, where its production is supported by centralized blood banks.

To date, transfer factor has not been employed to prevent human infectious processes, and relatively few animal studies have evaluated its use before infectious challenge. We have reported that transfer factor protects against fatal disseminated infection from herpes simplex virus Type I in marmoset monkeys.[16] However, treatment of established disease in these animals was uniformly unsuccessful. The marmoset represents a model of cellular immunodeficiency and increased propensity to fatal dissemination of herpes viruses. The protective effect found in these animals encouraged us to evaluate the clinical efficacy of specific human dialyzable transfer factor in a similarly compromised host, the child with acute lymphocytic leukemia. Initial human trials evaluated conversion of in vitro cellular immune responses to varicella zoster after administration of transfer factor.[8] The responses studied included lymphocyte blastogenesis, direct cytotoxicity, production of leukocyte-inhibitory factor, and production of antibody as measured by FAMA and complement-fixing antibody. According to these indexes, no patients in relapse acquired immune responses, but 10 of 12 in remission acquired positive reactivity in at least one assay of cell-mediated immunity. The test of cytotoxicity was the most consistently positive after administration of transfer factor. No patient acquired antibody to varicella zoster. Most surprisingly, after 17 months of follow-up, cellular immunity was still detectable with these in vitro techniques. Subsequent studies evaluated a varicella-zoster skin-test preparation for use in determining susceptibility in the normal host and in examining immune status in the immunocompromised host.[9] Although the results of skin tests correlated well with a history of chickenpox and antibody titers in the control population, 48 of 71 patients with leukemia in remission and a history of chickenpox had negative skin tests. Therefore, skin tests alone could not be used to assess susceptibility to varicella in patients with leukemia. On the other hand, skin reactivity could frequently be converted from negative to positive with the administration of transfer factor.

These studies confirm previous results and provide proof of the efficacy of transfer factor in a double-blind and placebo-controlled trial. Two unique aspects of the clinical design probably contributed most to the successful results: selection of donors with high titers of positive varicella-zoster response, and administration of transfer factor before viral challenge. All our previous efforts to induce protection in animals had indicated that these factors were critical and would best ensure efficacy, although we have not yet tested, in human beings, the protection afforded by transfer factor prepared from nonimmune subjects.

Initial investigations of transfer factor reported by Lawrence over 30 years ago had demonstrated the importance of selecting donors in whom skin tests were strongly positive to the antigen under study, and careful selection of donors should have become a basic principle in subsequent therapeutic trials. Unfortunately, this has not always been the case. In Europe particularly, transfer factor has been prepared from numerous random blood donors and pooled for treatment of various disease states. The

potency of transfer factor in protecting against an infectious agent has usually not been determined with any meaningful index. The absence of responses in recipients of transfer factor may therefore be attributable to the use of preparations that were inadequate.

It is also apparent from previous clinical trials that transfer factor is only effective against infectious processes that are "subacute" in nature. Such diseases include chronic mucocutaneous candidiasis, subacute sclerosing panencephalitis, coccidioidomycosis, leprosy, and some tumors. Treatment of rapidly progressive disease has been largely unsuccessful. Therefore, if benefit from transfer factor for more acute and fulminant types of processes is to be evaluated, very early treatment (for example, on first exposure to the agent) should be planned. This is, of course, usually impractical. Long-term prophylaxis such as that seen in the present trial is certainly a rational and achievable alternative.

It is assumed that transfer factor will only be effective if administered to recipients who possess a population of mature lymphocytes that can elaborate positive responses to specific antigens. Our earlier studies supported this hypothesis; conversion could not be detected when patients with acute lymphocytic leukemia in relapse or very early remission were given transfer factor but was usually detected in patients who had been in remission for more than two years. These studies also demonstrated that although some degree of immunologic competence of the host is necessary, the immune system need not be completely normal.[8]

Some of the patients in this study did not convert according to tests of skin reactivity but were still protected from clinical infection. Similar results have been seen in other immunodeficient hosts, who have benefited from transfer factor without becoming totally reconstituted. The best examples are patients with Wiskott-Aldrich syndrome[18] and those with chronic mucocutaneous candidiasis.[14]Both these syndromes are associated with partial defects in cellular immunity. Benefits from transfer factor do not always correlate with conversion of in vitro or in vivo immune responses. There is one interesting difference between patients with the Wiskott-Aldrich syndrome or chronic mucocutaneous candidiasis and the patients with leukemia in our study; the former groups have required periodic injections of transfer factor to sustain positive immune responses. Treatments have been given as frequently as once a week in patients with chronic mucocutaneous candidiasis and after up to six months in those with Wiskott-Aldrich syndrome. The decision to give additional therapy has been guided by various in vitro and in vivo assays. The protocol in our study required a single injection because earlier studies had indicated that patients with acute lymphocytic leukemia would retain specific reactivity to varicella-zoster antigen for as long as 17 months. This longer duration of immunoreactivity is assumed to occur because there is a more competent existing lymphocyte population in patients with leukemia, and it may not be markedly altered by the primary disease or chemotherapy.

A possible mechanism of action of transfer factor is an adjuvant effect that follows exposure to a specific antigen. In this experimental design, the skin test could provide such an antigen. However, previous studies with the same transfer factor preparation but in which skin testing was not included have demonstrated conversion of cellular reactivity[8]. Moreover, none of our exposed patients had

chickenpox during a 17-month observation period. Thus, a direct effect of transfer factor on lymphocytes not requiring prior exposure to antigen is suggested.

Most patients treated with transfer factor had seroconversion after exposure to varicella zoster yet remained essentially asymptomatic. Antibody determinations offered the most definitive evidence that exposure was achieved and that transfer factor attenuated clinical disease. Four of 13 control patients who acquired overt chickenpox remained antibody negative by both ELISA and the FAMA assay. However, the titer of the antibody in positive children was equal to determinations in our laboratories in normal youngsters after active chickenpox disease. Other investigators have also observed variability of humeral responses to varicella zoster in similar immunocompromised patients.[19] In our subjects treated with transfer factor, antibody was not detectable until exposure to live varicella-zoster virus had occurred. It was thus assumed that this exposure induced the development of humeral immunity, which would then complement the mechanisms of cellular reactivity achieved with transfer factor. Long-term follow-up of this patient population is of course needed before protection can be considered absolute.

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