ABSTRACTS OF THE COMMUNICATIONS PRESENTED AT THE 10TH INTERNATIONAL SYMPOSIUM ON TRANSFER FACTOR

IN THE ERA OF AIDS

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TRANSFER FACTOR - CURRENT STATUS AND FUTURE PROSPECTS

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We have detected new clues to the composition and function of "Transfer Factor" using the direct Leucocyte Migration Inhibition (LMI) test as an in vitro assay of Dialysates of Leucocyte Extracts (DLE). This approach has revealed two opposing antigen-specific activities to be present in the same >3500 <12,000 DA dialysis fraction - one activity is possessed of Inducer/Helper function (Inducer Factor). The opposing activity is possessed of Suppressor function (Suppressor Factor). When non-immune leucocyte populations are cultured with Inducer Factor they acquire the capacity to respond to specific antigen and inhibition of migration occurs. This conversion to reactivity is antigen-specific and dose-dependent. When immune leucocyte populations are cultured with Suppressor Factor, their response to specific antigen is blocked and Inhibition of Migration is prevented.

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TRANSFER FACTOR IN THE AGE OF MOLECULAR BIOLOGY - A REVIEW

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Current data suggests that the transferring of immunologically specific information by transfer factor molecules requires interaction with a cell that has been genetically programmed to be antigen reactive but at the time of interaction is unprimed. Contact with transfer factor molecules would allow a naive recipient, on a first encounter with antigen, to make a secondary rather than a primary immunological response. Transfer factor molecules for each and every antigenic determinant are thus necessary. Transfer factors made from animals or humans are capable of transferring antigen specificity across a species barrier. Even primitive species have cells from which one can make transfer factors. The molecules are, therefore, well conserved and it is reasonable to suggest that they are important for normal immunological functioning. Proposed mechanisms of action must explain the fact that transfer factors obtained from the cells of high responder animals are capable of transferring delayed hypersensitivity to low responder animals while the reverse is not true. Transfer factor molecules are likely to interact with the variable regions of the alpha and/or beta chain of T cell receptors to change their avidity and affinity for antigen in a way that otherwise would only occur after an encounter with antigen.

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ACTIVITIES AND CHARACTERISTICS OF TRANSFER FACTORS

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This report summarizes three components of our transfer factor research program. Several clinical studies have used oral administration of transfer factor containing materials. Sceptics have rejected these findings by assuming that the acidic and enzymatic environment of the gastrointestinal tract would destroy the factors. To further examine this issue, we have conducted dose-response studies of the delayed-type hypersensitivity reaction in mice that were given transfer factor either by gavage or subcutaneously. There was no difference in the responses that were related to the route of administration. We conclude that oral route of administration is efficacious and should be used when possible. We have also studied the effects of transfer factors on immune responses by recipients. The details of this research are presented in the paper by Dr. Alvarez-Thull. Briefly, the study showed that recipients of a specific transfer factor responded to the antigen for which the factor was specific by secreting gamma-IFN, but no other cytokines. The structures of transfer factor molecules are unknown. We have developed a process for isolating transfer factors in pure form and we have obtained preliminary data concerning amino acid sequences. Our goal is to obtain the complete primary structure of several transfer factor molecules.

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AIDS AND TRANSFER FACTOR: MYTHS, CERTAINTIES AND REALITIES

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At the end of the 20th century, the triumph of biology is as indisputable as that of physics was at the end of the 19th century, and so is the might of the inductive thought. Virtually all diseases have been seemingly conquered and HIV, the cause of AIDS, has been fully described ten years after the onset of the epidemic. However, the triumph of biological science is far from being complete. The toll of several diseases, such as cancer, continues to rise and the pathogenesis of AIDS remains elusive.

In the realm of inductive science, the dominant paradigm can seldom be challenged in a frontal attack, especially when it is apparently successful, and only what Kuhn calls "scientific revolutions" can overthrow it. Thus, it is hardly surprising that the concept of transfer factor is considered with contempt and the existence of the moiety improbable: over forty years after the introduction of the concept, not only its molecular structure remains unknown, but also its putative mode of action contravenes dogmas of both immunology and molecular biology. And when facts challenge established dogmas, be in religion, philosophy or science, they must be suppressed. Thus, results of heterodox research become henceforth nisi - i.e., valid unless cause is shown for rescinding them, because they challenge the prevalent paradigm. However, when observations pertain to lethal disorders, their suppression in the name of dogmas may become criminal. Because of the failure of medical science to manage the AIDS pandemic, transfer factor, which has been successfully used for treating or preventing viral infections, may today overcome a priori prejudice and rejection more swiftly. In science, as in life, certainties always end up by dying and Copernicus' vision by replacing that of Ptolemy.

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RATIONALE AND CLINICAL RESULTS OF USING LEUCOCYTE-DERIVED IMMUNOSUPPORTIVE THERAPIES IN HIV DISEASE

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Leucocyte dialysates contain a number of substances which exert important effects on human cell-mediated immunity. In this report, we describe several properties of a designated subfraction, IMREG(R)-1, which is obtained by a second dialysis against a membrane having a 3500 m.w. cutoff. These include the ability to augment and accelerate reactions of delayed hypersensitivity against antigens to which the text subject has been previously sensitized, and the ability to enhance the expression in vitro on CD4 lymphocytes of the p55 subunit of the receptor for Interleukin-2. We also report our observation that in a patient with advanced HIV disease whose lymphocytes had lost there ability to properly express the IL-2 receptor, treatment with IMREG(R)-1 over a period of months restored the expression of the IL-2 receptor on the patient's CD4+ lymphocytes towards normal.

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PRELIMINARY OBSERVATIONS USING HIV-SPECIFIC TRANSFER FACTOR IN AIDS

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Twenty five HIV-1-infected patients, at various stages (CDC II, III and IV) were treated orally with HIV-1-specific transfer factor (TF) for periods varying from 60 to 1870 days. All patients were receiving antiviral treatments in association with TF. The number of lymphocytes, CD4 and CD8 subsets were followed and showed no statistically significant variations. In 11/25 patients the number of lymphocytes increased, whilst in 11/25 decreased; similarly an increase of the CD4 lymphocytes was observed in 11/25 patients and of the CD8 lymphocytes in 15/25. Clinical improvement or a stabilized clinical condition was noticed in 20/25 patients, whilst a deterioration was seen in 5/25. In 12/14 anergic patients, daily TF administration restored delayed type hypersensitivity to recall antigens within 60 days. These preliminary observations suggest that oral HIV-specific TF administration, in association with antiviral drugs, is well tolerated and seems beneficial to AIDS patients, thus warranting further investigation.

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PRELIMINARY RESULTS IN HIV-1-INFECTED PATIENTS TREATED WITH TRANSFER FACTOR (TF) AND ZIDOVUDINE (ZDV)

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The efficiency of HIV-1 specific transfer factor (TF) administration, combined with Zidovudine (ZDV), in asymptomatic persistent generalised lymphadenopathy, or AIDS related complex (ARC) patients was evaluated.

Twenty patients were randomly assigned to receive only ZDV (1st group) or ZDV together with HIV-1-specific TF (2nd group). HIV-1-specific TF was administered orally at 2 x 107 cell equivalent daily for 15 days, and thereafter once a week for up to 6 months. There were no significant differences between the two groups in clinical evolution, red blood cells, haemoglobin, lymphocytes, CD20 subset, transaminases, a-2-microglobulin, p24 antigen. White blood cells, CD8 lymphocytes as well as IL-2 levels increased in the second group, while the CD4 subset increased in the first group. The combination treatment with ZDV and TF appeared to be safe and well tolerated. Furthermore, levels of serum cytokines were investigated in 10 patients (8 asymptomatic and 2 ARC) treated with ZDV, and compared with 5 patients of the 2nd group (3 asymptomatic and 2 ARC) treated with ZDV plus HIV-1-specific TF. Peripheral lymphocytes, CD4, CD8 subsets, IL-2, TNFa, IL-6, p24 antigen, IL-2 soluble lymphocyte receptors (sR), CD4sR, CD8sR and a-2-microglobulin were evaluated at the baseline and at the 3rd month. The CD4 subset was not significantly different in the two groups, whilst IL-2 increased in the 2nd group, receiving ZDV plus TF, suggesting an activation of the Th1 secretion pattern.

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INHIBITION OF IN VITRO HIV INFECTION BY DIALYSABLE LEUCOCYTE EXTRACTS

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Dialysable Leucocyte Extract (DLE) is a low molecular weight dialysable material of disrupted peripheral human leucocytes with widespread effects on the immune system. We described the in vitro anti-HIV activity of DLE as well as its three chromatographic fractions (Fa, Fb and Fc). To determine the levels of inhibition on HIV replication by DLE we infected MT-4 cell cultures, using the Bru viral isolate at 0.05, 0.1, 0.5 and 1 m.o.i. Previously, MT-4 cells cultures were treated with DLE or fractions at non-toxic concentrations. Reverse transcriptase (RT) activity and p24 antigen were evaluated in culture supernatants at seven days postinfection. No effect was observed when MT-4 cells were incubated with DLE for 3 h. Whereas inhibition of HIV production was observed when MT-4 cells were pre-treated for a longer periods of time. DLE inhibited p24 production and RT activity more than 50% at 0.1 m.o.i. More than 80% of inhibition was observed for all doses of DLE tested at 0.05 m.o.i. Higher viral doses (m.o.i. 0.5 and 1) were used to assess the antiviral activity of DLE fractions. Fraction Fb inhibits viral production more than 80%. Otherwise, fractions Fa and Fc did not show inhibitory effect for any viral dose used. These results indicate that DLE is able to modulate cell susceptibility to viral infection in vitro.

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TRANSFER FACTOR WITH ANTI-EBV ACTIVITY AS AN ADJUVANT THERAPY FOR NASOPHARYNGEAL CARCINOMA: A PILOT STUDY

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Overall survival of nasopharyngeal carcinoma (NPC) at UICC stage IV still remains unsatisfactory even with combination chemotherapy (CT) and radio-therapy (RT). In view of the association of reactivation of Epstein-Barr virus (EBV) with the development and recurrence of NPC, immunotherapy in the form of transfer factor (TF) with specific activity against EBV (TF-B1) was suggested as an adjuvant to a combination of CT and RT in order to improve the survival. In the present study, 6 UICC Stage IV patients received TF-B1 and another 6 patients matched for disease stage were given TF prepared from peripheral blood leucocytes (TF-PBL). Results were compared with another 18 patients matched by age, sex, and stage of disease who received standard therapy without TF during the same period (C group). After a median follow up of 47.5 months, the survival for the TF-B1 group was found to be significantly better (P=3D<0.05) than the PBL and C group. While the 8 patients with distant metastasis (DM) not treated with TF-B1 (6 in the control and 2 in the PBL group) died due to progressive disease (average survival being 14.3 months), both patients with DM in the TF-B1 group had complete remission: one died of tuberculosis after surviving for 3.5 years and another is still alive, disease free, after 4.2 years. Although the series involved a small number of cases, the apparent effect of adjuvant immunotherapy in the form of TF with anti-EBV activity is of considerable interest.

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A PRELIMINARY REPORT ON THE USE OF TRANSFER FACTOR FOR TREATING STAGE D3 HORMONE-UNRESPONSIVE METASTATIC PROSTATE CANCER

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As conventional treatments are unsuccessful, the survival rate of stage D3 prostate cancer patients is poor. Reports have suggested the existence of humoral and cell-mediated immunity (CMI) against prostate cancer tumour-associated antigens (TAA). These observations prompted us to treat stage D3 prostate cancer patients with an in vitro produced transfer factor (TF) able to transfer, in vitro and in vivo, CMI against bladder and prostate TAA. Forty four patients entered this study and received one intramuscular injection of 2-5 units of specific TF monthly. Follow-up, ranging from 1 to 9 years, showed that complete remission was achieved in 2 patients, partial remission in 6, and no progression of metastatic disease in 14. The median survival was 126 weeks, higher than the survival rates reported in the literature for patients of the same stage.

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TRANSFER FACTOR AS AN ADJUVANT TO NON-SMALL CELL LUNG CANCER (NSCLC) THERAPY

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The rationale for using transfer factor (TF) in lung cancer patients is that the possibility of improving their cell-mediated immunity to tumour associated antigens (TAA) may improve their survival. From Jan 1984 to Jan 1995, 99 non- small cell lung cancer (NSCLC) resected patients were monthly treated with TF, extracted from the lymphocytes of blood bank donors. In the same period, 257 NSCLC resected patients were considered as non-treated controls. The survival rates of the TF treated group appear significantly improved both for patients in stages 3a and 3b, and patients with histological subtype "large cell carcinoma" ($P<0.02$). Survival of TF treated patients is also significantly higher ($P<0.02$) for patients with lymphnode involvement (N2 disease). The results of this study suggest that the administration of TF to NSCLC resected patients may improve survival.

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USE OF ANTI HHV-6 TRANSFER FACTOR FOR THE TREATMENT OF TWO PATIENTS WITH CHRONIC FATIGUE SYNDROME: TWO CASE REPORTS

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A specific Human Herpes virus-6 (HHV-6) transfer factor (TF) preparation, administered to two chronic fatigue syndrome patients, inhibited the HHV-6 infection. Prior to treatment, both patients exhibited an activated HHV-6 infection. TF treatment significantly improved the clinical manifestations of CFS in one patient who resumed normal duties within weeks, whereas no clinical improvement was observed in the second patient. It is concluded that HHV-6 specific TF may be of significant value in controlling HHV-6 infection and related illnesses.

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LESSONS FROM A PILOT STUDY WITH TRANSFER FACTOR IN CHRONIC FATIGUE SYNDROME

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Transfer factor (TF) was used in a placebo controlled pilot study of 20 patients with chronic fatigue syndrome (CFS). Efficacy of the treatment was evaluated by clinical monitoring and testing for antibodies to Epstein Barr virus (EBV) and Human Herpes virus-6 (HHV-6). Of the 20 patients in the placebo-controlled trial, improvement was observed in 12 patients, generally within 3-6 weeks of beginning treatment. Herpes virus serology seldom correlated with clinical response. This study provided experience with oral TF, useful in designing a larger placebo-controlled clinical trial.

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THE USE OF TRANSFER FACTORS IN CHRONIC FATIGUE SYNDROME: PROSPECTS AND PROBLEMS

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Chronic fatigue syndrome (CFS) is a heterogeneous disorder characterized by severe prolonged unexplained fatigue and a variety of associated symptoms such as arthralgias, myalgias, cognitive dysfunction, and severe sleep disturbances. Many patients initially present with an acute onset of apparent infectious origin with either an upper respiratory or gastrointestinal illness, fever, chills, tender lymphadenopathy, and malaise suggestive of a flu-like illness. In some cases, specific viral infections can be identified at the outset, particularly herpes viruses such as Epstein-Barr virus (EBV), human herpes virus-6 (HHV-6), and cytomegalovirus (CMV). Transfer factor (TF) with specific activity against these herpes viruses has been documented. With some studies suggesting that persistent viral activity may play a role in perpetuation of CFS symptoms, there appears to be a rationale for the use of TF in patients with CFS and recent reports have suggested that transfer factor may play a beneficial role in this disorder. This report focuses on the heterogeneity of CFS, the necessity for randomized coded studies, the importance of patient selection and sub-classification in clinical trials, and the need to utilize specific end-points for determining efficacy of treatment.

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THE INFLUENCE OF AGE ON TRANSFER FACTOR TREATMENT OF CELLULAR IMMUNODEFICIENCY, CHRONIC FATIGUE SYNDROME AND/OR CHRONIC VIRAL INFECTIONS.

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A group of 222 patients suffering from cellular immunodeficiency (CID), frequently combined with chronic fatigue syndrome (CFS) and/or chronic viral infections by Epstein-Barr virus (EBV) and/or cytomegalovirus (CMV), were immunologically investigated and treated with transfer factor (TF). The age range was 17-77 years. In order to elucidate the influence of aging on the course of the disease and on treatment, 3 subgroups were formed: 17-43 years, 44-53 years, and 54-77 years. Six injections of Immodin (commercial preparation of TF by SEVAC, Prague) were given in the course of 8 weeks. When active viral infection was present, IgG injections and vitamins were added. Immunological investigation was performed before the start of therapy, and subsequently according to need, but not later than after 3 months.

The percentages of failures to improve clinical status of patients were in the individual subgroups, respectively: 10.6%, 11.5% and 28.9%. The influence of age in increasing the low numbers of T cells was evident: 10.6%, 21.2% and 59.6%. In individuals unaffected by therapy, persistent absolute lymphocyte numbers below 1,200 cells were found in 23.1%, 54.5% and 89.3% in the oldest group. Statistical analysis by Pearson's Chi-square test, and the test for linear trend proved that the differences among the individual age groups were significant. Neither sex, not other factors seemed to influence the results.

The results of this pilot study show that age substantially influences the failure rate of CID treatment using TF. In older people, it is easier to improve the clinical condition than CID: this may be related to the diminished number of lymphocytes, however, a placebo effect cannot be totally excluded.

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ORALLY ADMINISTERED HSV-SPECIFIC TRANSFER FACTOR (TF) PREVENTS GENITAL OR LABIAL HERPES RELAPSES

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Forty-four patients, suffering from genital (22) and labial (22) herpes were orally treated with HSV-1/2-specific transfer factor(TF). TF was obtained by in vitro replication of a HSV-1/2-specific bovine dialysable lymphocyte extract. Treatment was administered bi-weekly the first 2 weeks, and then weekly for 6 months, most patients received 2-3 courses. The total observation period for all patients before treatment was 26660 days, with 544 relapses, and a relapse index of 61.2, whereas the cumulative observation period during and after treatment was 16945 days, with a total of 121 relapsing episodes and a cumulative RI of 21.4 ($P<0.0001$). Results were equally significant when the 2 groups of patients (labial and genital) were considered separately. These observations confirm previous results obtained with the bovine HSV-specific TF, and warrant further studies to establish HSV-specific TF as a choice of treatment for preventing herpes recurrences.

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EFFECT OF ANTI-HERPES SPECIFIC TRANSFER FACTOR

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Using a blood cell separator, lymphocytes were collected from otherwise healthy convalescents suffering from herpetic infections. A specific anti-herpes dialysate (AH-DLE) was prepared from the lymphocytes, using standard procedures. Patients with recurrent herpetic infections were treated with a single dose of the dialysate, at the initial signs of herpetic infection (group A), in two doses (group B) or in three doses (group C). A total number of 37 patients (29 women, 8 men, age range 15-73 years) were treated. No improvement was observed in 7 patients (18.9%), whilst 7 patients did not manifest any exacerbation of their herpetic infection in the course of the one-year follow-up. The remaining 62.2% of the patients showed a marked improvement: decrease of the frequency and/or duration or relapses. Before AH-DLE administration, the mean number of herpes relapses in this group of patients was 12 p.a.. After therapy, the number of relapses decreased to 3.5 p.a.. No statistically significant difference was observed between groups A and B. The least favourable results were registered in group C. However, this group included 6 female patients extremely resistant to the previously therapeutic attempts, including inosiplex, non-specific DLE or acyclovir. Thus, even in this group, the therapy was successful in 50% of the patients.

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EFFICACY OF TRANSFER FACTOR IN TREATING PATIENTS WITH RECURRENT OCULAR HERPES INFECTIONS

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Recurrent ocular herpes is an insoluble problem for the clinician. As cellular immunity plays an important role in controlling herpes relapses, and other studies have shown the efficacy of HSV-specific transfer factor (TF) for the treatment of herpes patients, an open clinical trial was undertaken in 134 patients (71 keratitis, 29 kerato-uveitis, 34 uveitis) suffering from recurrent ocular herpetic infections. The mean duration of the treatment was 358 days, and the entire follow-up period 189121 before, and 64062 days after TF treatment. The cell-mediated immune response to the viral antigens, evaluated by the lymphocyte stimulation test (LST) and the leucocyte migration test (LMT) ($P<0.001$), was significantly increased by the TF treatment. The total number of relapses was decreased significantly during/after TF treatment, dropping from 832 before, to 89 after treatment, whereas the cumulative relapse index (RI) dropped, during the same period, from 13.2 to 4.17 ($P<0.0001$). No side effects were observed. It is concluded that patients with relapsing ocular herpes can benefit from treatment with HSV-specific TF.

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TRANSFER FACTOR IN CHRONIC MUCOCUTANEOUS CANDIDIASIS

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Fifteen patients suffering from chronic mucocutaneous candidiasis were treated with an in vitro produced TF specific for Candida albicans antigens and/or with TF extracted from pooled buffy coats of blood donors. CMI of the patients was assessed using the LMT and the LST in presence of candidine. The aim of the study was the clinical evaluation of TF treatment and the incidence of positive tests before, during, and after therapy. Immunological data were matched using the Chi square test. 87 LMT were performed for each antigen dose and, at the dilution of 1/50, 58.9% (33/56) tests were positive during non-treatment or non-specific TF treatment. On the contrary 83.9% (26/31) were positive during specific TF treatment ($P<0.05$). In the LST, a significant decrease of thymidine uptake in the control cultures in presence of autologous or AB serum was observed when patients were matched according to non-treatment, and both non specific ($P<0.05$) and specific TF treatment ($P<0.01$). Only during specific TF treatment was a significant increase of reactivity against the Candida antigen at the highest concentration noticed when compared with the period of non specific treatment ($P<0.01$). Clinical observations were encouraging: all but one patient experienced significant improvement during treatment with specific TF. These data confirm that orally administered specific TF, extracted from induced lymphoblastoid cell-lines, increases the incidence of reactivity against Candida antigens in the LMT. LST reactivity appeared not significantly increased with respect to the periods of non treatment, but was significantly increased when it was compared to the non-specific TF treatment periods. At the same time, a clinical improvement was noticed.

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USE OF TRANSFER FACTOR FOR THE TREATMENT OF RECURRENT NON-BACTERIAL FEMALE CYSTITIS (NBRC): A PRELIMINARY REPORT

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Results of conventional treatment of female non-bacterial recurrent cystitis (NBRC) are discouraging. Most patients show an unexpected high incidence of vaginal candidiasis, while their cell mediated immunity to Herpes simplex viruses (HSV) and Candida antigens seems impaired, and it is known that the persistence of mucocutaneous chronic candidiasis is, mainly, due to a selective defect of CMI to Candida antigens.

Twenty nine women suffering of NBRC, and in whom previous treatment with antibiotics and non-steroid anti-inflammatory drugs was unsuccessful, underwent oral transfer factor (TF) therapy. TF specific to Candida and/or to HSV was administered bi-weekly for the first 2 weeks, and then once a week for the following 6 months. No side effects were observed before treatment. The total observation period of our cohort was 24379 days, with 353 episodes of cystitis recorded and a cumulative relapse index (RI) of 43. The observation period during and after treatment was 13920 days with 108 relapses and a cumulative RI of 23 ($P<0.0001$). It, thus, seems that specific TF may be capable of controlling NBRC and alleviate the symptoms.

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SOME PROPERTIES AND PROTECTIVE ACTIVITY OF SPECIFIC DLE AGAINST SALMONELLA CHOLERA SUIS INFECTION

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From a rabbit lymphoid tissue, twice immunized with a Salmonella ch. suis vaccine, it was obtained a dialysable leucocyte extract (DLE) (m.w. 10000 Da ; protein content 1.14 mg/ml; content of ribose 2.7 mg/ml; A260/A280 ratio 2.17 and pH 6.8). By gel filtration on Sephadex G-25, six peaks were obtained and activity was found in peak IV. The activity of the extract was determined by a dermo-application test (DAT) on 10 cows. The protective effect was tested by challenge with Salmonella ch. suis and Salmonella dublin pathogen strains on white mice intraperitoneally treated with DLE. The DAT proved to be positive in 8 of the 10 cows. When applied on white mice, it induced a high specific protective effect against Salmonella ch. suis (70%), but not against Salmonella dublin infection.

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DIALYSABLE LYMPHOCYTE EXTRACT (DLYE) IN INFANTILE ONSET AUTISM: A PILOT STUDY

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40 infantile autistic patients were studied. They ranged from 6 years to 15 years of age at entry. 22 were cases of classical infantile autism; whereas 18 lacked one or more clinical defects associated with infantile autism ("pseudo-autism"). Of the 22 with classic autism, 21 responded to transfer factor (TF) treatment by gaining at least 2 points in symptoms severity score average (SSSA); and 10 became normal in that they were main-streamed in school and clinical characteristics were fully normalized. Of the 18 remaining, 4 responded to TF, some to other therapies. After cessation of TF therapy, 5 in the autistic group and 3 of the pseudo-autistic group regressed, but they did not drop as low as baseline levels.

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AN ATTEMPT TO INHIBIT THE COURSE OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) BY SUPPRESSOR FACTOR

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Forty amyotrophic lateral sclerosis (ALS) patients were treated with suppressor factor. The therapy led to the normalization of the immunoregulatory index in approximately two thirds of the patients. The responder patients had a better clinical response, i.e. the degenerative process slowed down or it was even arrested. This favourable effect was accompanied with a significant increase in the patients' life span. When the therapy had no effect on the CD8 cells, it was discontinued. Stopping the therapy led to disease progression and death; thus, in some patients, therapy was carried out despite its failure to increase the CD8 cell numbers. Substantial clinical improvement was noticed in these patients. The mean survival of patients with ALS was 2-3 years, whereas ALS patients treated with the suppressor factor survived on the average more than 5 years.

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DIALYZABLE LYMPHOID EXTRACT (DLE) FROM MICE RESISTANT TO STZ-INDUCED DIABETOGENESIS CAN INTERRUPT THE PROGRESS OF DIABETES IN STZ-TREATED CD-1 MICE

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DLE was prepared from the minority of euglycemic CD-1 mice, previously injected with STZ, and was administered to hyperglycemic CD-1 male mice 1, 2 and 3 weeks after completion of multidose STZ. Mice treated with DLE derived from 2 x 107 (1X) or 108 lymphocyte equivalents (lymph.equ.) were significantly less hyperglycemic than the saline treated controls ($P<0.001$). The effects of DLE remained evident for more than 10 weeks after the final DLE treatment. Mice treated with DLE prepared from diabetic mice (hg DLE) developed a somewhat more rapid onset of hyperglycemia than the STZ treated control animals, although this effect did not achieve statistical significance ($P=0.1$). This DLE was absorbed on a rat insulinoma cell line (RIN), which contains interspecies cross-reacting islet antigens, and compared to the unabsorbed DLE. Mice treated with hg DLE preabsorbed on RIN cells, showed a slower onset of hyperglycemia. DLE prepared from euglycemia mice and the RIN-absorbed fraction were equally capable of preventing hyperglycemia ($P<0.05$).

In order to determine whether the DLE effects were genetically restricted, DLE was prepared from BALB/c mice, normally resistant to the diabetogenic effects of multidose STZ, both before and after STZ treatment. STZ primed CD-1 mice treated with 3 weekly doses of 2 x 10^7 lymph. equ. of untreated BALB/c derived DLE, STZ treated BALB/c derived DLE, and STZ treated CD-1 DLE were all less hyperglycemic than the control mice, who received saline ($P<0.001$). However, mice treated with CD-1 DLE were less hyperglycemic than the mice given BALB/c derived DLE ($P<0.05$). These effects were relatively long-lived.

Mice that were given the >3,500 Dalton fraction of CD-1 DLE were significantly less hyperglycemic than either the control mice or those treated with the 3,500 Dalton fraction of CD-1 DLE ($P<0.05$). Effects remained evident for more than 3 months after the last dose of DLE. Pancreatic tissue from the mice treated with the >3,500 Dalton fraction of CD-1 derived DLE revealed slightly more islets of a slightly greater size with less surrounding inflammation than either control mice or mice treated with the <3,500 Dalton fraction of DLE.

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PROFILES OF CYTOKINE PRODUCTION IN RECIPIENTS OF TRANSFER FACTORS

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Transfer factors (TF) are proteins that transfer the ability to express cell-mediated immunity from immune donors to non-immune recipients. The mechanisms of these effects have not been defined. The experiments described in this report were undertaken to test the hypothesis that a mechanism through which the beneficial effects of TF are expressed in clinical situation is through "education" of the immune system to produce certain cytokines in response to antigenic stimulation. BALB/c mice were sensitized to Herpes simplex virus (HSV) either by sublethal systemic or cutaneous infections by administration of a HSV-specific TF. One week later their spleen cells were collected and single cell suspensions were stimulated in vitro with irradiated HSV or concanavalin A. Culture supernatants were collected and assayed for content of IL-2, IL-4, IL-10 and IFN-g.

Spleen cells from infected mice responded to concanavalin A and to HSV by secreting large amounts of IL-2 and IFN-g, modest amounts of IL-10, and no IL-4. Transfer factor recipients produced similar cytokine profiles in response to concanavalin A. These mice, however, responded to HSV by secreting IFN-g, but no IL-2. Thus, TF treatment selectively affects cytokine production in response to antigenic stimulation.

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THE EFFECT OF DLE FRACTIONS ON GM-PROGENITORS OF HAEMATOPOIETIC STEM CELLS IN VITRO

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Dialysable leucocyte extracts (DLE) prepared from buffy coats of human blood, potentiates the effect of Colony-stimulating factor (CSF) on the growth of granulocyte-macrophage colony forming cell (GM-CFC) colonies in vitro. This relative increase of the number of colonies is apparent when diluted CSF (present in lung conditioning medium) as a control, and DLE, in a wide range of concentrations are added to the culture of mouse bone marrow cells. Fractionation of DLE on Amicon membranes revealed that the activity resides in molecules of 0-5kD. Molecules 5-10kD have no potentiating effect. DLE and its fractions (0-5kD, 0-1kD), except fractions 0-500 D and 5-10kD, when added undiluted i.e. at the initial concentration, exerted a suppressive effect: colonies are not formed despite the presence of CSF. In a pilot experiment, it was shown that DLE is able to stimulate colony-forming activity of earlier progenitors of erythroid cells (BFUe), under the influence of erythropoietin.

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DIALYSABLE LEUCOCYTE EXTRACT (DLE) REDUCES LIPOPOLYSACCHARIDE-INDUCED TUMOUR NECROSIS FACTOR SECRETION IN HUMAN LEUCOCYTES

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Dialysable leucocyte extract (DLE), obtained from lysed leucocytes, provide clinical effectiveness in a broad spectrum of diseases. Tumour necrosis factor (TNF) is raised in AIDS patients leading to increasing in human immunodeficiency virus (HIV) replication in vitro [1,2]. Whereas progression to AIDS in asymptomatic HIV infected individuals is retarded under treatment with DLE. In the present study we tested the DLE effect in vitro on both TNF biological activity (cytotoxicity) in L929 cells and its induction by lipopolysaccharide (LPS) in human monocytes as well as in whole blood from healthy donors. When monocytic cells were simultaneously exposed to LPS and DLE during a period of 5 1/2 hours, the induction of TNF was strongly diminished. The same inhibitory effect of DLE on TNF induction was observed when LPS was added to the culture medium prior to DLE. No significant effect of DLE on TNF-mediated cytotoxicity, even in the presence of the highest concentrations of DLE tested, was detected. DLE treatment of whole human blood regulates responses to LPS: simultaneous in vitro expose to endotoxin provokes a remarkable decrease (4- and 1.6-fold) of TNF release. In pre-incubation experiments, TNF production was largely reduced or completed abrogated. These results could, in part, explain the in vivo observed effect, when under treatment with this extract, the progression to AIDS of HIV-infected individuals was retarded. The results suggest that 'natural' substances like DLE may be important immunomodulators in inflammatory diseases.

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INFLUENCE OF A DLE-EXTRACTED LYMPHOCYTIC SUPPRESSOR FACTOR ON CSA-INDUCED IMMUNOSUPPRESSION

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From dialysable leucocyte extracts (DLE) we have purified a hydrophilic low-mol. wt. factor (about 1 kDa) which we have named lymphocytic suppressor factor (LSF) as it is able to suppress antigen- and mitogen-induced lymphocyte transformation and to prolong allograft survival in C57b/6N mice (H-2b) transplanted with fully mismatched skin from C3H/HeN mice (H-2k). At the molecular level LSF acts by inhibiting DNA replicational and transcriptional processes in activated lymphocytes, isolated rat hepatocyte nuclei, and cell-free systems. Amino acid analysis indicates that LSF is a peptide composed of Asp, Glu, Ser, Thr, Ala, Gly, Arg and probably Met, with the N-terminus blocked, possibly by pyroglutamic acid. When combined "in vitro" with cyclosporine A (CsA), LSF increased about 20 times the potency of CsA in inducing suppression of mitogen-stimulated lymphocytes. In C57b/6N mice with skin graft from C3H/HeN mice and undergoing immunosuppression with CsA (50 mg/kg/day), the splenocyte LSF content increased about 5 times. However, LSF values returned to normal in mice recovering normal responsiveness due to progressive withdrawal of CsA. These data show that LSF has an important role in the development and maintenance of CsA-induced immunosuppression. We suggest that, by influencing DNA replicational and transcriptional processes of lymphocytes, LSF may play a role also in the onset and progression of retro-viral diseases including AIDS.

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IN VITRO STUDIES DURING LONG TERM ORAL ADMINISTRATION OF SPECIFIC TRANSFER FACTOR

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153 patients suffering from recurrent pathologies, i.e. viral infections (keratitis, keratouveitis, genital and labial herpes) uveitis, cystitis, and candidiasis were treated with in vitro produced transfer factor (TF) specific for HSV-1/2, CMV and Candida albicans. The cell-mediated immunity of seropositive patients to HSV-1/2 and/or CMV viruses was assessed using the leucocyte migration inhibition test (LMT) and lymphocyte stimulation test (LST) in presence of the corresponding antigens, and the frequency of positive tests before, during and after TF administration was studied. The data were stratified per type of test, antigen and the recipients' pathology, and statistically evaluated. For the LMT, a total of 960 tests were carried out for each antigen dilution, 3 different antigen dilutions were used per test. 240/960 tests (25.4%) were found positive during non-treatment or treatment with unspecific TF, whereas 147/346 tests (42.5%) were found positive when the antigen corresponding to the specificity of the TF administered to the patient was used ($P<0.001$). When the data were stratified following pathology, a significant increased incidence of positive tests during specific treatment was also observed ($0.0001<P<0.05$). In the LST (1174 tests), a significant increase of thymidine uptake was observed in the absence of antigen (control cultures), during treatment with both specific and unspecific TF, but also in the presence of antigen and/or autologous serum during specific TF administration ($P<0.0001$). TF administration also significantly increased the soluble HLA class I antigens level, in 40 patients studied to this effect.

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