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Research Article

Antigen-Specific Immunomodulatory Effects of Transfer Factor

Vetvicka V* and Vetvickova J
Department of Pathology, University of Louisville, USA

***Corresponding author:** Vaclav Vetvicka, Department of Pathology, University of Louisville, 511 S. Floyd, Louisville, KY 40202, USA

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Abstract

The aim of this study was to evaluate the antigen-specific immunomodulatory effects of colostrum and egg yolk-derived transfer factors. All mice were immunized with ovalbumin during the administration period. To evaluate the immunological effects, proliferation, surface markers, antibody formation, serum immunoglobulins and cytokine secretion were measured. The immunomodulatory effects of the Transfer Factor Plus Tri-Factor Formula were shown by evaluating ovalbumin-specific immune cell proliferation, cytokine release, and formation of specific antibodies. Our data showed potentiation of IL-2 and IFN-γ secretion, higher proliferation of spleen cells and increased production of ovalbumin-specific IgG_{2a} antibodies. This study confirmed that these transfer factors have significant potential to modulate specific immune responses.

Keywords: Transfer factors; Cytokines; Antibodies; Immunoglobulins; Proliferation

Introduction

Transfer Factors (TF), sometimes known as Lawrence TF, was originally described and developed by Sherwood Lawrence, who observed that antigen-specific cell-mediated immunity can be transferred from sensitive individuals to naïve individuals via a dialyzable leukocyte extract [1]. At that time, TF represented a revolutionary hypothesis, as the prevailing dogma favored antibodies.

Originally, TF was thought to be present in colostrum material that was smaller than 20 kDa. Later it was better characterized to be around 2.5 kDa. Originally applied by subcutaneous injection, further studies found that long term oral administration yielded positive effects too [2]. Fear of HIV infection via blood-borne products and a scandal involving two Harvard researchers working with TF [3] had a negative impact on further TF research during that period. Despite this setback, new data showing antigen-specific extracts from CD8-positive lymphocytes renewed interest in TF research [4]. More detailed studies followed using different preparations of bovine and murine TF and found the same conserved amino acid sequence in all of these isolates [5]. Current knowledge of TF preparation and characterization allows not only the use of TF prepared from cow colostrum or chicken egg yolks, but also specifically specific TF, such as Staphylococcus aureus-specific TF [6].

The current study is a continuation of the evaluation of the biological effects of Transfer Factor Plus Tri-Factor Formula combining TF isolated from chicken egg yolk (Ovo-Factor) and bovine colostrum (Ultra-Factor XF and Nano-Factor, proprietary filtration method), as well as other ingredients. As the original study found significant effects on phagocytosis, proliferation of splenocytes, cytotoxicity of NK cells and production of some cytokines [7], we expanded our study to further test the potential stimulation of the immune system.

Material and Methods

Animals

Female, 6 week old BALB/c mice were purchased from the BioLASCO (Taiwan). Ten mice/group were used in this study. Each animal was weighed once a week during the study period.

Transfer factor

Transfer Factor Plus Tri-Factor sample was kindly donated by 4Life Research, Sandy, UT, USA. Three different doses, 273 mg/kg/day (low), 546 mg/kg/day (medium) and 1,365 mg/kg/day (high) were used. Individual samples and a negative control (sterile water) were administered daily by oral gavage for 8 weeks.

Sample collection

Blood samples were collected at the end of experiments by heart puncture. Serum was obtained after centrifugation at 2,200 x g for 15 minutes and stored at -30° C. Upon use of isoflurane as anesthesia, spleens were removed and gently ground with fine steel mesh.

Material

Ovalbumin (OVA) and complete and incomplete Freund's adjuvant were purchased from Sigma (St. Louis, MO, USA).

Cytokine analysis

Splenocytes at 1-2x10^6 cells/well concentration were treated with OVA for 72 hrs at 37° C. After centrifugation, supernatants were collected and levels of IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ were evaluated by ELISA using appropriate ELISA kits (Biolegend, San Diego, CA, USA).

Immunization

Mice were immunized with 25μg Ovalbumin (OVA) in complete Freund's adjuvant ip. Four weeks after the supplementation with tested samples. Two weeks later, mice were challenged with a second ip. Injection of the same dose of OVA in incomplete Freund's

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Table 1: Body Weight Changes and Spleen-to-body Weight Ratios.

Group†	NC	TA-L	TA-M	TA-H	Bln
Week	Body weight (g)				
Week 0	17.6 ± 0.7[a]	17.7 ± 0.8[a]	17.5 ± 0.4[a]	17.5 ± 0.6[a]	17.5 ± 0.5[a]
Week 1	18.0 ± 0.7[a]	18.1 ± 0.7[a]	18.1 ± 0.5[a]	18.0 ± 0.6[a]	17.8 ± 0.6[a]
Week 2	18.0 ± 0.8[a]	18.2 ± 0.8[a]	18.4 ± 0.6[a]	18.3 ± 0.6[a]	18.2 ± 0.7[a]
Week 3	18.5 ± 0.9[a]	18.4 ± 1.1[a]	18.8 ± 0.6[a]	18.8 ± 0.6[a]	18.5 ± 0.7[a]
Week 4	19.3 ± 1.0[a]	19.3 ± 1.3[a]	19.6 ± 0.9[a]	19.4 ± 0.7[a]	19.2 ± 0.9[a]
Week 5	19.8 ± 0.9[a]	19.9 ± 1.3[a]	19.9 ± 0.9[a]	19.9 ± 1.0[a]	19.9 ± 1.0[a]
Week 6	20.3 ± 1.0[a]	20.2 ± 1.3[a]	20.5 ± 0.8[a]	20.1 ± 1.1[a]	20.5 ± 1.1[a]
Week 7	20.6 ± 0.9[a]	20.6 ± 1.6[a]	21.0 ± 0.8[a]	20.7 ± 1.0[a]	20.5 ± 0.9[a]
Week 8	20.7 ± 1.1[a]	20.6 ± 1.5[a]	21.1 ± 0.8[a]	20.7 ± 0.9[a]	20.6 ± 0.9[a]
Spleen-to-body weight ratio (%)#					
	1.050 ± 0.190[b]	1.103 ± 0.218[b]	1.136 ± 0.302[b]	1.091 ± 0.143[b]	0.464 ± 0.034[a]

Data are expressed as mean ± Standard Deviation (S.D.) of 10 mice, and were analyzed using one-way ANOVA followed by Duncan's multiple range test. Values with different alphabet letters in same column are significantly different (p<0.05). #Spleen-to-body weight ratios= [Spleen weight (g)/body weight (g)] x 100.
†NC, negative control; TA-L: test article low dose, TA-M: test article middle dose; TA-H: test article high dose; Bln (blank control): normal control, without OVA immunization.

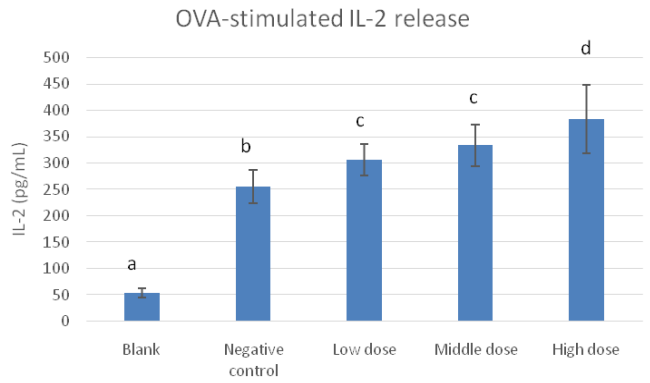

Figure 1: Interleukin-2 (IL-2) stimulation in Ovalbumin (OVA) mice is expressed as mean ± Standard Deviation (S.D.) of 10 mice. Data were analyzed using one-way ANOVA followed by Duncan's multiple range test. Values with different alphabet letters are significantly different (p <0.05).

adjuvant. Anti-OVA antibodies of the IgG_1, IgG_{2a}, and IgE class were determined by an indirect ELISA.

Proliferation

Splenocytes were cultivated in a 95-well plate at a concentration of $1x10^5$ cells/well. After 72 hrs of cultivation with OVA, the proliferation was measured using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) at 490 nm using the following formula:

Stimulation index SI = OD_{490} nm of OVA-stimulated cells/OD_{490} nm of unstimulated cells

Serum immunoglobulins

Whole blood was centrifuged at 2,200 x g for 15 minutes and serum samples were analyzed for levels of OVA-specific IgG_1, IgG_{2a}, and IgE by indirect ELISA using OVA-coated plates and HRP-conjugated anti-mouse IgG_1, IgG_{2a}, and IgE. The amounts of antibodies were calculated after detection of optical density at 450 nm with an ELISA reader and expressed as ELISA Units (EU).

Surface markers

Splenocytes were labeled with fluorescence-conjugated monoclonal antibodies at the concentration of $2x10^5$ cells/well. Antibodies recognizing T lymphocytes ($CD4^+/CD3^+$), cytotoxic T lymphocytes ($CD8^+/CD3^+$), B lymphocytes ($CD19^+/CD45^+$) and NK cells ($PanNK^+/CD45^+$) were used and the individual subpopulations were evaluated by flow cytometry.

Statistics

One way ANOVA followed by Duncan's test were used to statistically analyze the data.

Results

No clinical signs of illness were observed during the study. Mean body weight at the beginning of the study was 16.1-18.9 g, and mean weight at the end of the study was 18.7–23.5 g (Table 1). Shows that the body weight or spleen-to-body weight ratio did not statistically differ among tested groups (Table 1). The growth ratio in each group was about the same. The mean spleen-to-body weight ratio was not statistically different among all studied groups (data not shown). Our data showed no differences in IL-2 and IFN-γ secretion among negative control and treated groups under unstimulated basal level (data not shown).But in the case of OVA-sensitized animals, we found a significant dose-dependent stimulation of IL-2 (Figure 1) and IFN-γ secretion (Figure 2). No significant TF effects were found for IL-4, IL-10 and TNF-α secretion (Table 2). None of the tested conditions changed the production of IL-5 (Table 2). With respect to antibody formation, the production of IgG_1 and IgE was not

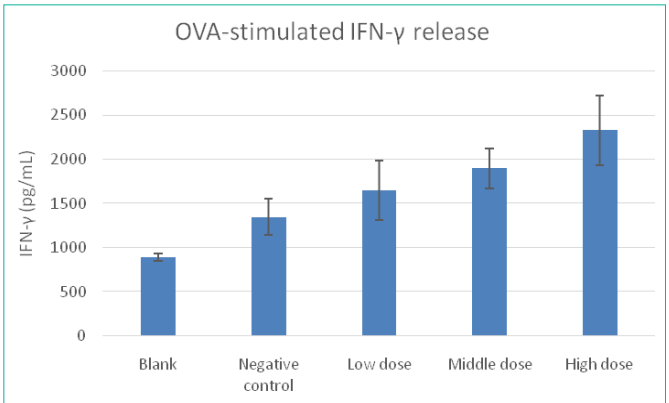

Figure 2: Interferon-gamma (IFN-γ) stimulation in Ovalbumin (OVA) mice is expressed as mean ± Standard Deviation (S.D.) of 10 mice. Values with different alphabet letters are significantly different (p <0.05).

Table 2: Cytokines Release.

Group†	Unstimulated basal level	OVA (25 μg/mL)
IL-4 (pg/mL)		
NC	22.7 ± 1.5[a]	61.1 ± 12.5[a]
TA-L	23.1 ± 2.0[a]	60.3 ± 11.3[a]
TA-M	22.7 ± 1.4[a]	57.7 ± 10.6[a]
TA-H	22.4 ± 1.1[a]	53.3 ± 10.6[a]
Bln	20.9 ± 2.5[a]	49.7 ± 8.6 [a]
IL-5 (pg/mL)		
NC	27.6 ± 3.6[a]	112.8 ± 14.1[a]
TA-L	27.6 ± 4.0[a]	104.8 ± 15.1[a]
TA-M	27.7 ± 2.4[a]	101.3 ± 13.5[a]
TA-H	28.7 ± 3.8[a]	100.8 ± 8.7[a]
Bln	26.4 ± 2.6[a]	95.5 ± 10.4[a]
IL-10 (pg/mL)		
NC	216.2 ± 16.5[a]	1072.2 ± 87.8[b]
TA-L	216.6 ± 21.3[a]	1006.5 ± 95.9[b]
TA-M	217.9 ± 14.0[a]	998.7 ± 69.9[b]
TA-H	216.4 ± 11.6[a]	991.5 ± 90.2[b]
Bln	205.4 ± 15.9[a]	640.0 ± 74.0[a]
TNF-α (pg/mL)		
NC	24.9 ± 3.0[a]	243.5 ± 16.6[b]
TA-L	24.7 ± 3.8[a]	250.9 ± 21.7[b]
TA-M	24.0 ± 3.3[a]	255.3 ± 33.8[b]
TA-H	24.2 ± 4.2[a]	262.2 ± 46.7[b]
Bln	25.0 ± 3.4[a]	107.5 ± 15.8[a]

Data are expressed as mean ± Standard Deviation (S.D.) of 10 mice, and were analyzed using one-way ANOVA followed by Duncan's multiple range test. Values with different alphabet letters in same column are significantly different (p<0.05). †NC, negative control; TA-L: test article low dose, TA-M: test article middle dose; TA-H: test article high dose; Bln (blank control): normal control, without OVA immunization. IL-4: interleukin-4; IL-5: interleukin-5; IL-10: interleukin-10; TNF-α: tumor necrosis factor-α

Table 3: OVA-Specific Antibody Levels.

Group†	OVA-specific antibody (ELISA unit, EU)#	
	anti-OVA IgG1	anti-OVA IgE
NC	1.43 ± 0.06[b]	0.12 ± 0.04[b]
TA-L	1.45 ± 0.06[b]	0.13 ± 0.03[b]
TA-M	1.40 ± 0.04[b]	0.12 ± 0.04[b]
TA-H	1.41 ± 0.13[b]	0.14 ± 0.04[b]
Bln	0.04 ± 0.01[a]	0.05 ± 0.02[a]

Data are expressed as mean ± Standard Deviation (S.D.) of 10 mice, and were analyzed using one-way ANOVA followed by Duncan's multiple range test. Values with different alphabet letters in same column are significantly different (p<0.05). #ELISA units (EU) = (A sample —A blank)/(A positive —A blank). †NC, negative control; TA-L: test article low dose, TA-M: test article middle dose; TA-H: test article high dose; Bln (blank control): normal control, without OVA immunization.

changed (Table 3). However, the production of IgG_{2a} was significantly increased by low, medium and high doses of tested material (Figure 3). Lastly, proliferative response to OVA stimulation was significantly increased when compared to the blank control. Among the OVA-sensitized groups, the proliferation induced by OVA was significantly enhanced in low, medium, and high dose groups in comparison with the negative control (Figure 4).

There were no significant differences among all study groups in immunophenotyping of splenocytes for T lymphocytes ($CD3^+$/$CD45^+$), B lymphocytes ($CD19^+$/$CD45^+$), T4 lymphocytes ($CD3^+$/$CD4^+$), T8 lymphocytes ($CD3^+$/$CD8^+$), and NK cells ($panNK^+$/CD45+) as documented in (Table 4).

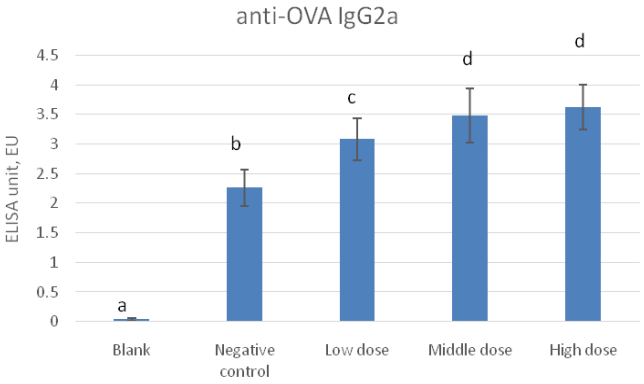

Figure 3: Level of OVA-specific IgG_{2a} antibodies. Data are expressed as mean ± Standard Deviation (S.D.) of 10 mice. Values with different alphabet letters are significantly different (p <0.05). ELISA units (EU) = (A sample —A blank)/(A positive —A blank).

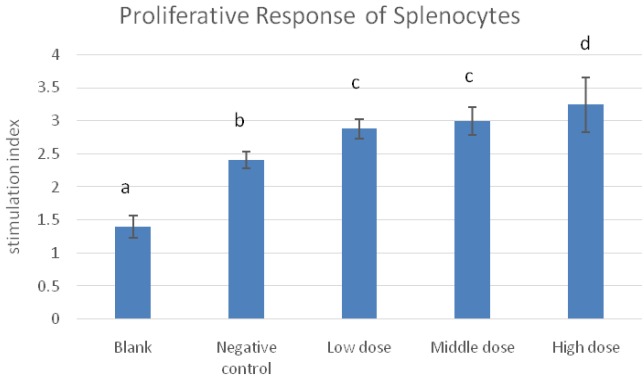

Figure 4: Proliferative response of splenocytes. Data are expressed as mean ± Standard Deviation (S.D.) of 10 mice. Values with different alphabet letters are significantly different (p <0.05). Stimulation Index (S.I.) = OD490 nm of OVA stimulated cells/OD490 nm of unstimulated cells.

Discussion

The use of natural products for the treatment of various diseases is as old as the history of medicine. The first documented history of medicinal use of a plant preparation is a record on Sumerian clay tablets from 4000 B.C. A written Indian document talking about medicinal effects of mushrooms is app. 5000 years old. Indian Ayurveda and Traditional Chinese Medicine can serve as examples of healing trends which have been developed through empirical experience. The population growth in the developing world and the increasing interest in alternative medicine in industrial nations have greatly expanded the interest in natural remedies. At least 20% of adults in the United States have taken a dietary supplement in the past six months and over 4 billion dollars is spent annually on herbal supplements alone.

Transfer factors represent a somehow neglected but still present alternative for the treatment of various diseases. It offers substantial effects as immunotherapy and asa supplement for chemotherapy, as well as in treatment of various health problems [8]. In addition to the direct therapeutic properties, transfer factors also offer protective effects such as hepatoprotection in the case of larval cestode infection [9].

Table 4: Immunophenotyping of Murine Splenocytes.

Group†	Immune cell type (%)				
	T4 cell ($CD4^+$,$CD3^+$)	T8 cell ($CD8^+$,$CD3^+$)	T cell ($CD3^+$,$CD45^+$)	B cell ($CD19^+$,$CD45^+$)	NK cell (PanNK^+,$CD4^+$)
NC	31.33 ± 3.86[a]	10.69 ± 2.02[a]	52.38 ± 4.54[a]	42.81 ± 4.96[a]	5.43 ± 1.04[a]
TA-L	30.87 ± 2.38[a]	9.66 ± 1.92[a]	51.82 ± 4.06[a]	42.52 ± 4.20[a]	5.32 ± 0.77[a]
TA-M	31.43 ± 4.31[a]	10.72 ± 1.72[a]	50.21 ± 4.05[a]	44.17 ± 5.52[a]	5.91 ± 0.77[a]
TA-	31.98 ± 3.76[a]	10.72 ± 2.11	50.35 ± 4.68[a]	44.28 ± 4.56[a]	5.77 ± 0.81[a]
Bln	31.85 ± 3.42[a]	9.73 ± 1.14[a]	49.78 ± 4.00[a]	44.45 ± 4.62[a]	5.94 ± 0.96[a]

Data are expressed as mean ± Standard Deviation (S.D.) of 10 mice, and were analyzed using one-way ANOVA followed by Duncan's multiple range test. Values with different alphabet letters in same column are significantly different (p<0.05). †NC, negative control; TA-L: test article low dose, TA-M: test article middle dose; TA-H: test article high dose; Bln (blank control): normal control, without OVA immunization.

In addition to traditional transfer factors, a version isolated from colostrum and egg yolks has been often used. When eggs from immunized chickens are employed, the extracts have significant results suggesting that this material might be a potential candidate for immunoregulation of various diseases including hepatitis B [10]. Similarly successful was testing of material isolated from colostrum, where feeding of calves resulted in passive transfer of delayed-type hypersensitivity [11]. The current literature clearly shows that TF can be used as an adjuvant to primary treatment for parasitic, viral, bacterial, and fungal diseases. In addition, TF has a potential to answer the challenge from unknown pathogenic agents even before their identification, suggesting both a therapeutic and preventative role of TF (for review see [12]). This study is a follow up of the previous study focused on immunostimulating effects of Transfer Factor Plus Tri-Factor Formula [7]. Next we focused on humoral immunity, as the immunostimulating effects of colostrum are usually based on their high antibody content [13,14], and the presence of multiple immune modulating molecules in the first part, we studied production of cytokines. Our data showed significant dose-dependent stimulation of IL-2 and IFN-γ secretion in OVA-sensitized animals. Other tested cytokines were not affected, which might be beneficial in possible clinical use, as systemic increase of numerous cytokines is not desirable. Next, we evaluated the effects on formation of specific anti-ovalbumin antibodies. Of tested classes of immunoglobulins, only IgG_{2a} production was significantly stimulated by TF.

In conclusion, we found evidence that the effects of supplementation with TF resulted in stimulation of both the cellular and humoral branches of immunity. Clearly, colostrum- and/or egg-derived transfer factors possess an interesting and substantial immunostimulating capacity. This modulation of immune responses can potentially be used in stimulation of defense reactions.

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