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Peptides with immunomodulatory properties (Imuno TF®) increase the frequency of the CD8$^+$ T cell population *in vitro*

Peptídeos com propriedade imunomodulatórias (Imuno TF®) aumentam a frequência da população de células T CD8$^+$ *in vitro*

Péptidos con propiedades inmunomoduladoras (Imuno TF®) aumentan la frecuencia de la población de células T CD8$^+$ *in vitro*

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Abstract
Imuno TF® is a food supplement composed of oligo- and polypeptides with roles in the immune system. It has been previously demonstrated that Imuno TF® positively regulated Th1 cytokines while decreasing Th2 cytokines (reduced secretion of IL-10 and increased secretion of IL-6 and TNF-α). Here we aimed to investigate the actions of Imuno TF® on the frequency of stimulated CD8$^+$ and CD4$^+$ T-cell populations and their cytokine productions. Human lymphocyte cultures were used for that, and IL-2, IFN-γ, IL-4, IL-5, IL-7, IL-13, and IL-35 were quantified by ELISA and RT qPCR. The frequency of CD4$^+$ and CD8$^+$ populations was investigated through flow cytometry. We observed an increased frequency of CD8$^+$ T-cells after the combined stimulation of cells with Imuno TF® and ConA compared to controls. No differences were observed regarding the frequency of CD4$^+$ T-cells. In addition, a significant increase in IL-2, IL-7, and IFN-γ levels was observed, while IL-4, IL-5, and IL-13 presented reduced levels. No alterations were observed in IL-35 levels. Our results suggest that the Imuno TF® can potentially increase the CD8$^+$ T-cell population by positively regulating cytokines associated with Th1 response and increased IL-7 levels while reducing Th2 cytokine-mediated immune responses.
Keywords: Dietary supplement; Immune system; Immune peptides; Th1 response.

Resumo

Imuno TF® é um suplemento alimentar composto por oligo e polipeptídios com funções no sistema imunológico. Foi anteriormente demonstrado que o Imuno TF® pode regular positivamente as citocinas de perfil Th1 enquanto reduzia os níveis das citocinas Th2 (reduzindo a secreção de IL-10 e aumentando a secreção de IL-6 e TNF-α). Nosso objetivo foi investigar as ações do Imuno TF® na frequência de populações de células T CD8+ e CD4+ estimuladas e suas produções de citocinas. Culturas de linfócitos humanos foram utilizadas para isso, e IL-2, IFN-γ, IL-4, IL-5, IL-7, IL-13 e IL-35 foram quantificados por ELISA e RT qPCR. A frequência das populações $CD4^+$ e $CD8^+$ foi investigada por citometria de fluxo. Observamos um aumento na frequência de células T $CD8^+$ após a estimulação combinada de células com Imuno TF® e ConA em comparação aos controles. Não foram observadas diferenças em relação à frequência de células T $CD4^+$. Além disso, foi observado aumento significativo nos níveis de IL-2, IL-7 e IFN-γ, enquanto IL-4, IL-5 e IL-13 apresentaram níveis reduzidos. Não foram observadas alterações nos níveis de IL-35. Nossos resultados sugerem que o Imuno TF® tem o potencial de aumentar a frequência da população de células T $CD8^+$ regulando positivamente as citocinas associadas à resposta Th1 e aumentando os níveis de IL-7, ao mesmo tempo que reduz as respostas imunes mediadas por citocinas com perfil Th2.

Palavras-chave: Suplemento alimentar; Sistema imunológico; Peptídeos imunológicos; Resposta Th1.

Resumen

Imuno TF® es un complemento alimenticio compuesto por oligo y polipéptidos con funciones en el sistema inmunológico. Anteriormente se demostró que Imuno TF® puede regular positivamente las citocinas del perfil Th1 al tiempo que reduce los niveles de citocinas Th2 (reduciendo la secreción de IL-10 y aumentando la secreción de IL-6 y TNF-α). Nuestro objetivo fue investigar las acciones de Imuno TF® sobre la frecuencia de poblaciones de células T $CD8^+$ y $CD4^+$ estimuladas y su producción de citoquinas. Para ello se utilizaron cultivos de linfocitos humanos y se cuantificaron IL-2, IFN-γ, IL-4, IL-5, IL-7, IL-13 e IL-35 mediante ELISA y RT qPCR. La frecuencia de las poblaciones $CD4^+$ y $CD8^+$ se investigó mediante citometría de flujo. Observamos un aumento en la frecuencia de células T $CD8^+$ después de la estimulación combinada de células con Imuno TF® y ConA en comparación con los controles. No se observaron diferencias en relación con la frecuencia de células T $CD4^+$. Además, se observó un aumento significativo en los niveles de IL-2, IL-7 e IFN-γ, mientras que IL-4, IL-5 e IL-13 mostraron niveles reducidos. No se observaron cambios en los niveles de IL-35. Nuestros resultados sugieren que Imuno TF® tiene el potencial de aumentar la población de células T $CD8^+$ al regular positivamente las citoquinas asociadas con la respuesta Th1 y aumentar los niveles de IL-7, al tiempo que reduce las respuestas inmunes mediadas por citocinas con perfil Th2.

Palabras clave: Suplemento dietético; Sistema inmunitario; Péptidos inmunes; Respuesta Th1.

1. Introduction

Natural-derived products or metabolites have always been used for diverse health applications, including immunity strengthening. In recent years, this has been gaining even more momentum, as pandemics caused by MERS and SARS-CoV-2 have aroused – and several natural compounds show antiviral activity and properties that help the organism keep the normal function of the immune system (Othman et al., 2021). Within this context, one such product is Imuno TF®, a nutritional, natural-derived supplement composed of oligo- and polypeptides fractions from porcine spleen, known by the scientific community as transfer factors (TF) due to the biological activity on immune regulation (Ferreira et al., 2020).

Previously, our group has characterized Imuno TF® and showed that it is composed of oligo- and polypeptides constituted of 163 different peptides (Polonini et al., 2021). Such peptides have potential roles in the immune system pathways. Additionally, no mutagenic effect was observed for this product, and the LD50 was 5000 mg kg-1 body weight, which characterizes it as a safe product to be used by the oral route (Polonini et al., 2021). Subsequently, we demonstrated that Imuno TF® positively regulated Th1 cytokines, decreasing Th2 cytokines. Specifically, it showed effects on the increased secretion of IL-10, an anti-inflammatory cytokine, and decreasing secretion of IL-6 and TNF-α, pro-inflammatory cytokines. It additionally showed a reversing impact on the hypersecretion of IL-17 (Rocha Oliveira et al., 2021). Both studies have helped us understand the mechanism of action of Imuno TF® on the immune system, which has been clinically shown to improve the recovery of patients with COVID-19 (Hernández et al., 2021).

In the present investigation, we aimed to extend the knowledge of the actions of Imuno TF® on the immune system. For this, we focused on the effect of Imuno TF® stimulation on CD4+ and CD8+ T-cell frequency in vitro and the cytokines

produced by these cell populations.

2. Methodology

2.1 Ethical Aspects

This study was carried out after approval by the local Ethics Committee of the Anhembi Morumbi University (approval number: 4.142.254), and written consent was obtained from each volunteer.

2.2 Human lymphocyte culture

The experiments were conducted using human lymphocytes from peripheral blood circulation. The human lymphocytes were isolated from fresh heparinized venous blood via centrifugation over Ficoll gradients (Histopaque-1077; Sigma-Aldrich, Gillingham, United Kingdom), according to the manufacturer's instructions. The lymphocytes were cultured in RPMI-1640 supplemented with 10% FBS, L-glutamine (2mol/L), penicillin (100U/mL), and streptomycin (100 mg/mL). Cells were distributed at the rate of 1×10^6 per well in a 96-well plate and stimulated with 2.5µg/mL concanavalin (ConA) 2 hours before or concomitantly with Imuno TF® (10µg/mL) at 37° C, in a 5% CO2 humidified atmosphere for 24 hours. Each treatment represented 4 wells in the plate (n = 4 per treatment).

2.3 Cytotoxicity evaluation of Imuno TF® by MTT assay

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, and phosphate-buffered saline (PBS) were obtained from Gibco BRL (Grand Island, CA, USA). The 3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, Mo., USA). Imuno TF® was supplied by Infinity Pharma Brasil (Campinas, SP, Brazil), a Fagron company (Rotterdam, The Netherlands). A stock solution of Imuno TF® was prepared for all the experiments by dissolving the compound directly in culture media at 0.1-1000 µg/mL. The cell viability of control and Imuno TF®-treated human lymphocytes was measured using a standard MTT assay. Briefly, 5 × 104 viable cells were seeded into clear 96-well flat-bottom plates in RPMI 1640 medium supplemented with 10% FBS and incubated with different concentrations (0.1-1000µg/mL) of Imuno TF® for 24h. Then, 10 µL/well of MTT (5 mg/mL) was added, and the cells were incubated for 4h. Following incubation, 100µL of 10% sodium dodecyl sulfate (SDS) solution in deionized water was added to each well and left overnight. The absorbance was measured at 595nm using a microplate reader (Molecular Device).

2.4 Cytokines quantification by ELISA

Cells were stimulated for 2 hours with LPS or Con A, treated or not with Imuno TF® (10µg/mL) for 24h, as described above (item 2.2). Then, the culture medium was harvested, and the quantification of secreted Th1 cytokines (IL-2 and IFN-γ), Th2 cytokines (IL-4, IL-5, and IL-13), Treg cytokine (IL-35), and Interleukin 7 (IL-7), were performed using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

2.5 Reverse transcription-quantitative PCR (RT qPCR) of cytokine genes

To enrich the analyzes regarding cytokine production, the expression of cytokine messenger RNA was also analyzed. According to the manufacturer's instructions, total RNA was converted to cDNA using a SuperScript® III RT kit (Invitrogen, Carlsbad, CA). The RNA concentration was detected using a NanoDrop 2000 (Thermo Fisher Scientific, Inc.). GAPDH was used as the internal control. The thermocycling conditions were as follows: 95˚C for 10 min followed by 35 cycles of 95˚C for

15 sec and 55˚C for 40 sec. The 2 ΔΔCq method was used to quantify the relative gene expression levels of the target genes. Serial dilutions generated relative standard curves, and all samples were run in triplicates. The table below indicates the sense and antisense sequences of primers used in the qRT-PCR analysis. The primers used to quantify cytokine gene expression are described in Table 1.

Table 1 - List of primers that were used to quantify cytokine gene expression.

Gene	Primer
GAPDH [18]	Forward 5′-CGGTGTGAACGGATTTGGC-3′
	Reverse 5′-GTGAGTGGAGTCATACTGGAAC-3′
IFN-γ [19]	Forward 5′-ATGAAATATACAAGTTATAGC-3′
	Reverse 5′-TTACTGGGATGCTCTTCGACCTCGAAACAGCAT-3′
IL-2 [19]	Forward 5′-GAATGGAATTAATAATTACAAGAATCCC-3′
	Reverse 5′-TGTTTCAGACCCTTTAGTTCAG-3′
IL-4 [20]	Forward 5´-AAAGAACACAAGCGATAAGGAA-3´
	Reverse 5´- TTCAGTGTACTCTTCTTGATTTCATT-3´
IL-5 [24]	Forward 5´- AGCTGCCTACGTGTATGCCA-3´
	Reverse 5´-GCAGTGCCAAGGTCTCTTTCA-3´
IL-13 [23]	Forward 5'-GCTCCTCAATCCTCTCCTGTT-3'
	Reverse 5'-GCAACTTCAATAGTCAGGTCC-3'
IL-7 [36]	Forward 5′-GAGTGACTATGGGCGGTGAGAG-3′
	Reverse 5′-GATGCTACTGGCAACAGAACAAGG-3′
IL-35 (IL-2p35/Ebi3) [25]	(IL-12 p35) Forward 5´-CTGCATCAGCTCATCGATGG-3´ (EBi3) Forward 5´-TGTTTCCCTGACTTTCCAGG
	(IL-12 p35) Reverse 5´- CAGAAGCTAACCATCTCCTGGTTT-3´ (EBi3) Reverse 5´-GGGGCAGCTTCTTTTCTTCT-3´
	Reverse 5′-ACATTTGCCGAAGAGCCCTCAG-3′

Source: Authors.

2.6 FACS analysis: CD8⁺ and CD4⁺ T-cell measurement

After the isolation and culturing, the cells were stained with specific antibodies conjugated to fluorochromes and subsequently analyzed by multiparametric flow cytometry in BD FACS Lyric equipment. For cell staining, the 96-well plate (containing 1×10^6 cells per well) was washed twice with 200µL of FACS Buffer (Biolegend) per well. Then, 25 µL of FACS buffer containing 1% of anti-human CD45RA/PECy7, 1% of anti-human CD3/PerCP, 1% of anti-human CD8/APC, and 1% of anti-human CD4/FITC was added to the wells and incubated for 30 minutes at 4°C, protected from light. After incubation, the cells were washed twice with 175 µL of FACS buffer and resuspended in 2% paraformaldehyde. For each sample/well, 100,000 events were acquired on a FACS Lyric flow cytometer (BD Biosciences), using the FACSuit software (BD Biosciences). Frequency analysis of lymphocyte populations was performed using the FlowJo software, version 10.8.1.

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2.7 Statistical analysis

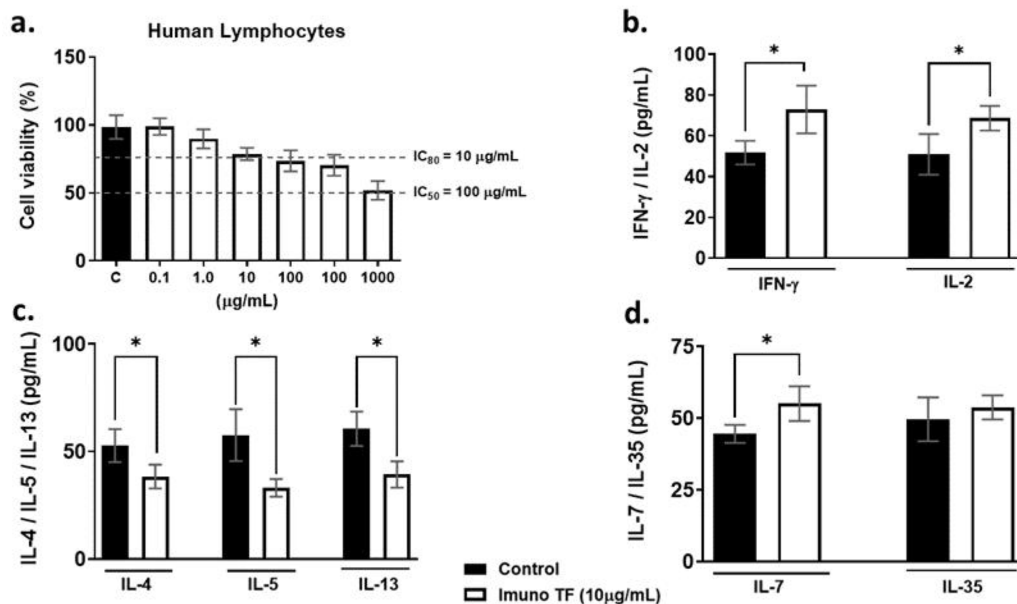
The results were expressed as the mean ± standard error of the mean (SEM) from three independent experiments. Student's *t*-test evaluated paired data. One-way analysis of variance (ANOVA) was used for multiple comparisons. A *p*-value < 0.05 was considered significant.

3. Results

3.1 Cell viability and cytokines secretion

To determine the optimal concentration without compromising cell viability, the MTT test was performed with dose-response of Imuno TF®-mediated effects on cell proliferation. The concentration of 10 µg/mL was defined as the study dose. Figure 1a shows the results for human lymphocyte viability. Figure 1b shows the significantly increased levels of IL-2 and IFN-γ (*$p<0.05$) in the supernatant of human lymphocytes treated with Imuno TF® (10 µg/mL). In contrast to the secretion of Th1 cytokines, Th2 cytokines (IL-4, IL-5, and IL-13) were reduced on the supernatants of human lymphocyte cultures (*$p<0.05$). Figure 1c shows the influence of Imuno TF® on TH2 cytokine secretion. The secretion of IL-7 and IL-35 was evaluated and represented in Figure 1D. Imuno TF® treatment increased the levels of IL-17 (*$p<0.05$) compared to control cells. The secretion of IL-35 showed no significant differences between treatment and controls.

Figure 1 - (a) Effects of Imuno TF® on human lymphocyte viability. (b) Evaluation of the secretion of Th1 cytokines by human lymphocytes. (c) Th2 cytokines secretion by human lymphocytes. (d) Effects of Imuno TF® on IL-7 and IL-35 secretion from human lymphocyte cell cultures. All experiments treated cells with 10 µg/mL of Imuno TF® for 24 hours. The data shown are representative of three independent experiments. The values are expressed as mean ± SEM (n=3). ∗$p < 0.05$ indicates statistical difference vs. control (non-treated cells).



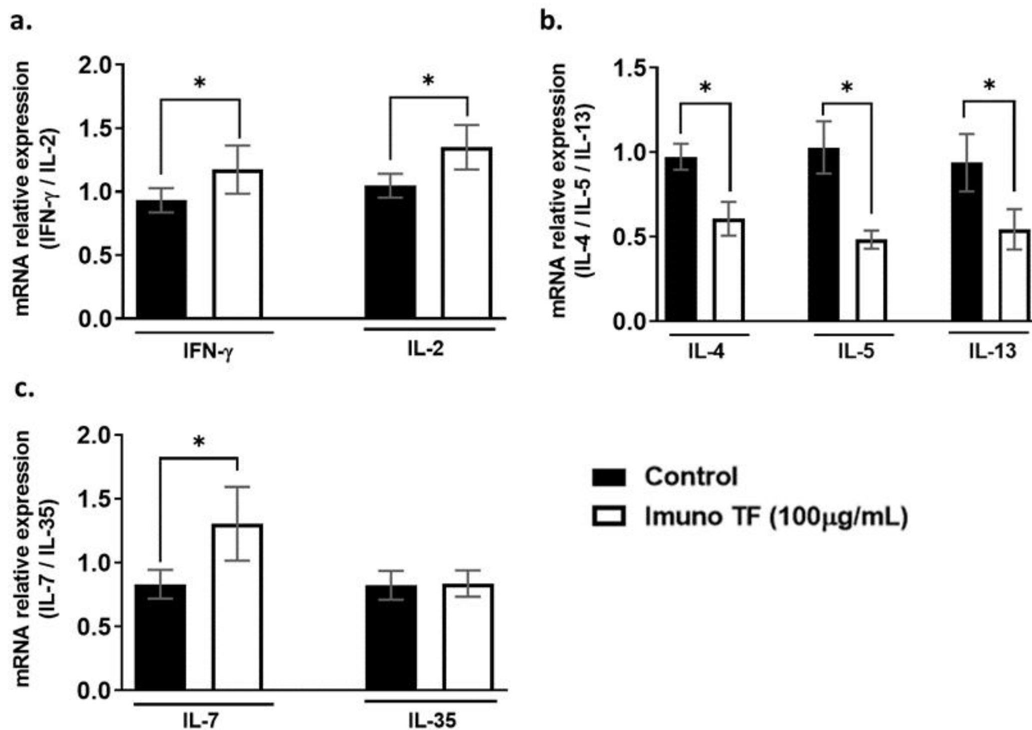
Source: Authors.

3.2 Quantification of mRNA levels by RT-PCR of Th1, Th2, Th7, and Treg cytokines

The influence of Imuno TF® on the modulation of mRNA levels of the evaluated cytokines is depicted in Figure 2. Figure 2A shows a significant increase (*$p<0.05$) concerning the cytokines of the Th1 response. The cytokines of the Th2 response are essentially altered in human lymphocytes, and Figure 2b shows a significant reduction (*$p<0.05$) after treatment

with ImunoTF®. Figure 2c indicates that IL-7 was significantly increased in lymphocyte supernatant cultures (*p<0.05). The secretion of IL-35, on the other hand, showed no significant change after the treatment of human lymphocytes.

Figure 2 - Effects of Imuno TF® (10 µg/mL) on mRNA levels of cytokines from human lymphocytes. Levels of mRNA by RT-PCR: (a) Th1: IFN-γ and IL-2 cytokines; (b) Th2: IL-4, IL-5, and IL-13 cytokines; and (c) IL-7 and IL-35 cytokines. Cells were treated for 24 hours. The data shown are representative of three independent experiments. The values are expressed as mean ± SEM, and ∗p<0.05 indicates statistical difference vs. control (non-treated cells).

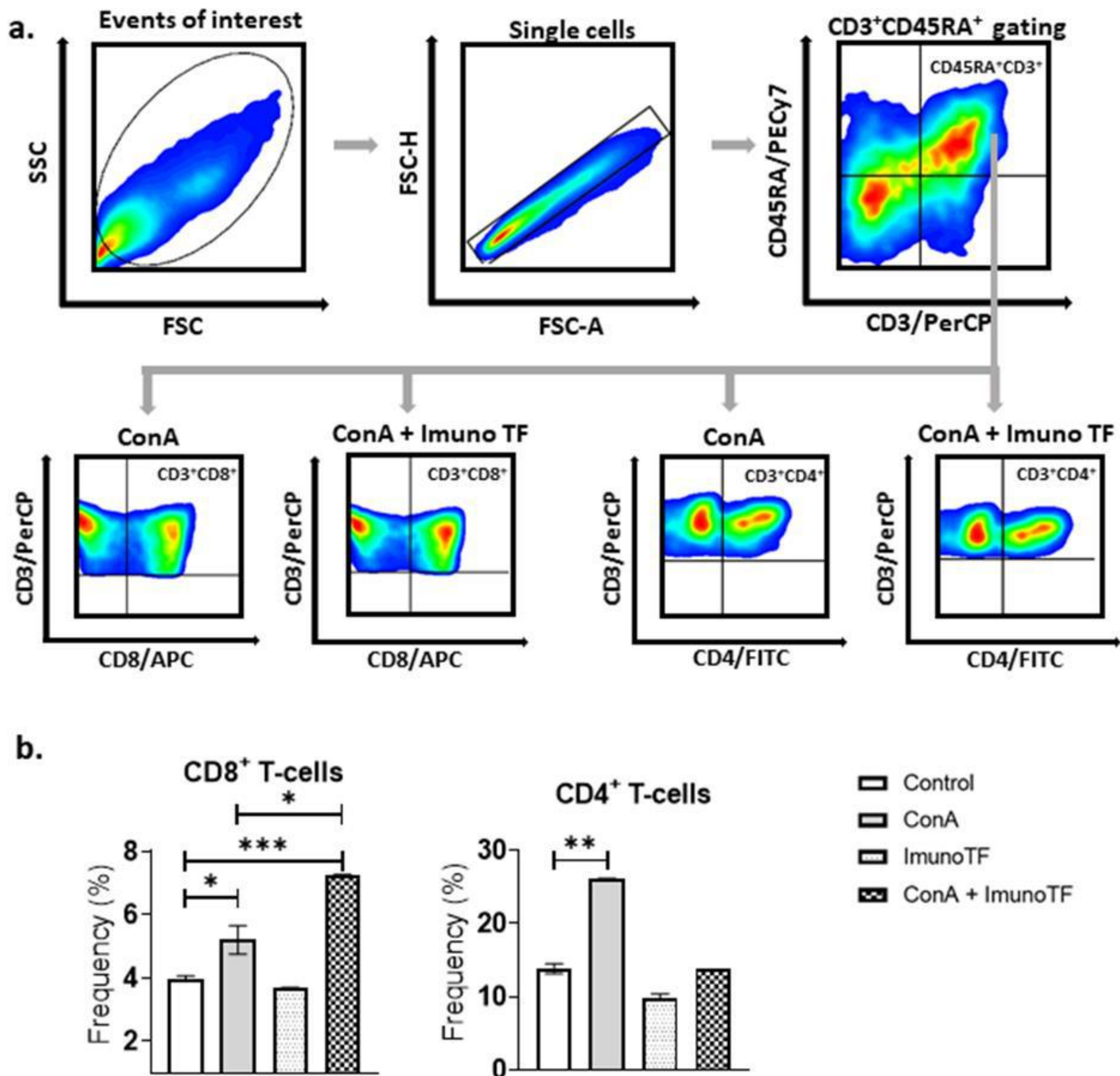


Source: Authors.

3.3 Influence of Imuno TF® on the CD4⁺ and CD8⁺ T lymphocyte populations

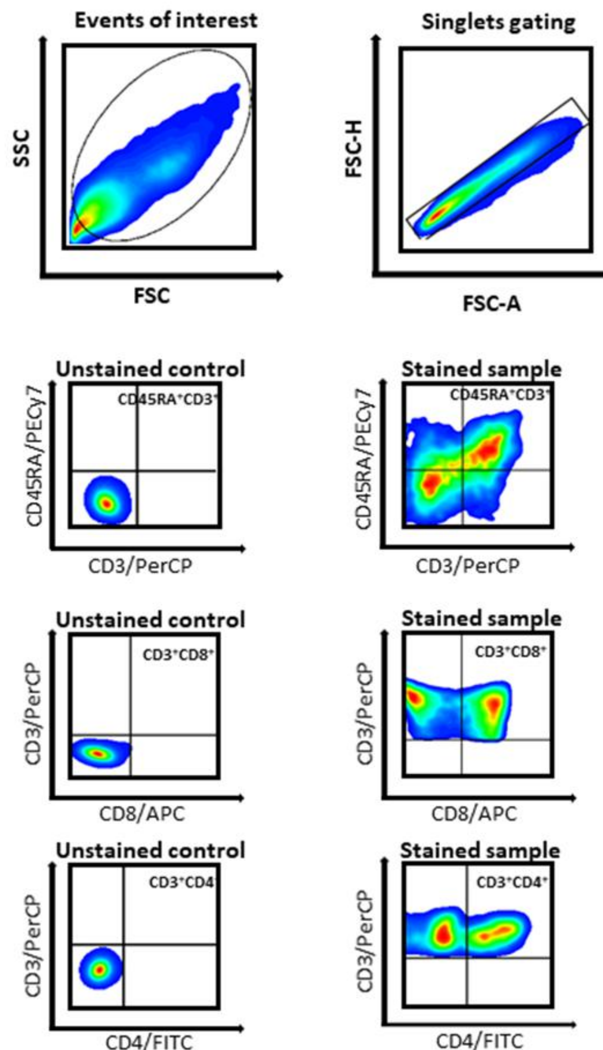
Figure 3 shows the multiparametric flow cytometry analysis to verify the effects of Imuno TF® on the $CD4^+$ and $CD8^+$ T lymphocyte populations. The $CD8^+$ T-cell population showed a significantly increased frequency after simultaneous treatment with ConA and Imuno TF® in comparison to cultures stimulated by ConA only. Regarding the $CD4^+$ population, no differences were observed after stimulation with ConA and the concomitant therapy with Imuno TF®. In complement, Figure 4 shows unstained controls of flow cytometric analysis, which are important controls to define the limit between the natural fluorescence of the cells and the fluorescence corresponding to the antibodies used.

Figure 3 - Multiparametric flow cytometry analyzes to assess the influence of the Imuno TF® on the $CD4^+$ and $CD8^+$ T lymphocyte populations. (a) Gating strategy for the selection of T $CD4^+$ and T $CD8^+$ cells, showing the previous gating of events of interest, singlets, and T $CD45RA^+CD3^+$ cells emphasizing the effects of Imuno TF® (10µg/mL) combined with ConA (2,5µg/mL) on the $CD8^+$ lymphocytes population as compared to cells treated only with ConA (2,5µg/mL). (b) Comparisons among treatments (mean ± standard errors) were analyzed by one-way ANOVA with Tukey's test. Values were considered statistically significant when *p <0.05 and ***p<0.0001.



Source: Authors.

Figure 4 - Unstained controls of multiparametric flow cytometry analysis for evaluating human $CD4^+$ and $CD8^+$ T-cell populations.



Source: Authors.

4. Discussion

In recent years, transfer factors have been characterized and accepted as a natural option for applications in clinical conditions where the immune response plays a leading role (Ferreira et al., 2020; Polonini et al., 2021), such as leukemia associated with viral infections, hepatitis C, candidiasis, and HIV, among others (Steele et al., 1980; Masi et al., 1996; Raise et al., 1996; Tsai et al., 1997; Milich et al., 1998). Aiming to clarify the mechanism of action of Imuno TF®, in 2021, our group demonstrated that this transfer factor regulated mRNA transcription and cytokine secretion associated with Th1 and Th2 responses (Rocha Oliveira et al., 2021). It is known that the production of IL-2 and IFN-γ is associated with the cytotoxicity of $CD8^+$ T cells and regulation of the development and proliferation of $CD8^+$ T-cells (Whitmire et al., 2005; Woodward et al., 2013; MacDonald et al., 2021). Studies using viral infection models have indicated that IL-2 signaling during the primary response is crucial for inducing effector cytotoxic T-cell differentiation and developing $CD8^+$ memory T-cells (Bachmann et al., 2007; Mitchell et al., 2010).

In this context, and seeking to elucidate other points in the mechanism of action of Imuno TF®, the results presented here demonstrate that treatment with the compound could significantly increase IL-2 and IFN-γ cytokines and the mRNA

levels of these proteins, in contrast to the secretion of the cytokines as IL-4, IL-5, and IL-13 (related to a Th2 response). Regarding IL-7 and IL-35 cytokines, the treatment with Imuno TF® increased IL-7 levels, but not IL-35. The significant increase in IL-7 levels sheds light on another hypothesis about the mechanism of action of Imuno TF® since studies suggest that IL-7 may play essential roles in the maintenance of $CD8^+$ T cells after an antigenic stimulus (Rubinstein et al., 2008; Sercan et al., 2015). IL-7 has shown efficacy as an antiviral agent, and IL-7 therapy has been demonstrated to re-establish lymphocyte count and activity, bringing potential benefits for treating oncological, infectious diseases, and immunosenescence (Mackall et al., 2008; Nguyen et al., 2017; Barata et al., 2017; Laterre et al., 2020). On the other hand, the suppressive capacity of IL-35 on effector T-cell responses that are used as an explanation for its immunomodulatory actions and autoimmune disorders (Olson et al., 2013; Bello et al., 2018; Su et al., 2018; Jiang et al., 2019) was not altered by treatment with Imuno TF®. Therefore, the absence of Imuno TF® influences IL-35 secretion, allowing us to suggest that the mechanism of Imuno TF® is mainly the activation of the Th1 response associated with increased IL-7 secretion.

Our ELISA and RT-qPCR experiments showed that IL-5 levels are reduced after Imuno TF® treatment, and it has already been demonstrated that virus-induced $CD8^+$ T-cells can produce IL-5 and induce airway eosinophilia (Coyle et al., 1995). Considering that we observed an increase in the frequency of $CD8^+$ T cells, it is interesting to note that the compound also demonstrated to increase cytotoxicity and prevent IL-5-mediated eosinophilic responses, which could be harmful to the host.

As shown in Figure 3, treatment with Imuno TF® on lymphocyte populations indicated that the $CD8^+$ T-cell is favored by stimulation and treatment with ConA and Imuno TF®, simultaneously. No significant changes were seen in the $CD4^+$ T-cell population after stimulation with ConA and treatment with Imuno TF®. The results suggest that Imuno TF® can enhance an immune response orchestrated by Th1 cytokines (IL-2 and IFN-γ), associated with an increase in IL-7. Of note, IL-7 along with STAT5 has been shown as necessary for optimal cytotoxic T lymphocyte activity and T cell survival and proliferation (Vranjkovic et al., 2011). Thus, the small but significant increase in the frequency of $CD8^+$ T-cells *in vitro* was likely supported by the elevated levels of IL-7 observed in our experiments. In this context, Imuno TF® could contribute not only to stimulation and maintenance but also to the re-establishment of the functions of $CD8^+$ T-cells since we could observe a reduction in both tasks and amplitude of responses of these lymphocytes in some pathological situations (Brooks et al., 2021), as demonstrated in infectious processes, such as COVID-19 (Rha and Shin, 2021).

5. Final Considerations

In this study, aspects related to the mechanism of action of Imuno TF® were evaluated, especially its effects on the $CD8^+$ T cell population and cytokines directly involved in the development of these lymphocytes. Taken together, our results demonstrate that the Imuno TF® increased the frequency of $CD8^+$ T-cells by positive regulation of cytokines associated with Th1 response along with increased IL-7 levels. The aim we had in carrying out the investigations presented here was achieved, as we now know a little more about how immunomodulatory peptides such as Imuno TF® can affect immunological responses. However, it is important to emphasize that further studies, with *in vitro* and *in vivo* approaches, are necessary to elucidate the immune action mechanisms of this compound systemically. Also, new studies in different pathological contexts may reveal important therapeutic roles for the compound addressed in this study.

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Author Contributions

CRO: Conceptualization, Data curation, Investigation, Methodology (MTT assay; ELISA; RT qPCR), Validation, Visualization, Writing – original draft, Funding Acquisition, Project administration. **VLK**: Data curation, Investigation, Methodology (Human lymphocyte culture; FACS analysis), Validation, Visualization, Writing – original draft, Writing – review & editing. **RPV**: Conceptualization, Investigation, Visualization, Writing – original draft, Funding acquisition. **AF**: Conceptualization, Investigation, Visualization, Funding Acquisition. **FRSO**: Investigation, Methodology (MTT assay; ELISA; RT qPCR; Human lymphocyte culture), Validation. **FVL**: Methodology (FACS analysis), Validation, Visualization. **FA**: Conceptualization, Investigation, Visualization, Validation, Writing – original draft, Funding acquisition. **HP**: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Funding Acquisition. The authors' methodological expertise can be confirmed in each author's ORCID (letters in bold correspond to the initial letters of the author's names).

References

Bachmann, M. F., Wolint, P., Walton, S., Schwarz, K. & Oxenius A. (2007). Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections. *Eur J Immunol*. 37(6), 1502-12. 10.1002/eji.200637023

Barata, J. T., Durum, S. K. & Seddon, B. (2019). Flip the coin: IL-7 and IL-7R in health and disease. *Nat Immunol*. 20(12), 1584-1593. 10.1038/s41590-019-0479-x

Bello, R. O., Chin, V. K,. Abd Rachman Isnadi, M. F., Abd Majid, R., Atmadini Abdullah, M., Lee, T.Y., Amiruddin Zakaria, Z., Hussain, M. K. & Basir, R. (2018). The Role, Involvement and Function(s) of Interleukin-35 and Interleukin-37 in Disease Pathogenesis. *Int J Mol Sci*. 19(4), 1149. 10.3390/ijms19041149

Brooks, D. G., Tishon, A., Oldstone, M. B. A. & Mcgavern, D. B. (2021). Prevention of CD8 T Cell Deletion during Chronic Viral Infection. *Viruses*. 13(7), 1189. 10.3390/v13071189

Coyle, A. J, Erard, F., Bertrand, C., Walti, S., Pircher, H. & Le Gros, G. (1995). Virus-specific CD8+ cells can switch to interleukin 5 production and induce airway eosinophilia. *J Exp Med*. 181(3), 1229-33. 10.1084/jem.181.3.1229

Ferreira, A. O., Polonini, H. C. & Dijkers, E. C. F. (2020). Postulated Adjuvant Therapeutic Strategies for COVID-19. *J Pers Med*. 10(3), 80. 10.3390/jpm10030080

Hernández, M. D., Urrea, J. & Bascoy L. (2021). Evolution of COVID-19 patients treated with a combination of nutraceuticals to reduce symptomatology and improve prognosis: a multi-centered, retrospective cohort study, *J Clin Rev C Rep.* 6:662–701. org/10.33140/JCRC.06.07.01

Jiang, H., Zhang, T., Yan, M. X. & Wu, W. (2019). IL-35 inhibits CD8+ T cells activity by suppressing expression of costimulatory molecule CD28 and Th1 cytokine production. *Transl Cancer Res.* 8(4), 1319-1325. 10.21037/tcr.2019.07.30

Laterre, P. F., François, B., Collienne, C., Hantson, P., Jeannet, R., Remy, K. E. & Hotchkiss, R. S. (2020). Association of Interleukin 7 Immunotherapy With Lymphocyte Counts Among Patients With Severe Coronavirus Disease 2019 (COVID-19). *JAMA Netw Open.* 3(7), e2016485. 10.1001/jamanetworkopen

Macdonald, A., Lam, B., Lin, J., Ferrall, L., Kung, Y. J., Tsai, Y. C., Wu, T. C. & Hung, C. F. (2021). Delivery of IL-2 to the T Cell Surface Through Phosphatidylserine Permits Robust Expansion of CD8 T Cells. *Front Immunol.* 12:755995. 10.3389/fimmu.2021.755995

Mackall, C. L., Fry, T. J. & Gress, R. E. (2011). Harnessing the biology of IL-7 for therapeutic application. *Nat Rev Immunol.* 11(5), 330-42. 10.1038/nri2970

Mais, M., De Vinci, C. & Baricordi, O. R. (1996). Transfer factor in chronic mucocutaneous candidiasis. *Biotherapy*. 9(1-3), 97-103. 10.1007/BF02628665

Milich, D. R., Chen, M. K., Hughes, J. L. & Jones, J. E. (1998). The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. *J Immunol.* 160(4), 2013-21.

Mitchell, D. M., Ravkov, E. V. & Williams, M. A. (2010). Distinct roles for IL-2 and IL-15 in the differentiation and survival of CD8+ effector and memory T *cells. J Immunol.* 184(12), 6719-30. 10.4049/jimmunol.0904089

Nguyen, V., Mendelsohn, A. & Larrick, J. W. (2017). Interleukin-7 and Immunosenescence. *J Immunol Res*. 2017:4807853. 10.1155/2017/4807853

Olson, B. M,, Sullivan, J. A, & Burlingham, W. J. (2013). Interleukin 35: a key mediator of suppression and the propagation of infectious tolerance. *Front Immunol*. 4:315. 10.3389/fimmu.2013.00315

Othman, S. I., Nayel, M. A., Alwaele, M. A., Al Fassam, H., Abu-Taweel, G. M., Altoom, N. G., Almalki, A. M., Allam, A. A., Alturki, A. M. & El-Shabasy, R. M. (2021). Immunology and controlling of coronaviruses; the current enemy for humanity: A review. *Int J Biol Macromol.* 193(Pt B), 1532-1540. 10.1016/j.ijbiomac.2021.10.216

Polonini, H., Gonçalves, A. E. S. S., Dijkers, E., & Ferreira, A. O. (2021). Characterization and Safety Profile of Transfer Factors Peptides, a Nutritional Supplement for Immune System Regulation. *Biomolecules.* 11(5), 665. 10.3390/biom11050665

Raise, E., Guerra, L., Viza, D., Pizza, G., De Vinci, C., Schiattone, M. L., Rocaccio, L., Cicognani, M. & Gritti, F. (1996). Preliminary results in HIV-1-infected patients treated with transfer factor (TF) and zidovudine (ZDV). *Biotherapy*. 9(1-3), 49-54. 10.1007/BF02628656

Rha, M. S. & Shin, E. C. (2021). Activation or exhaustion of CD8+ T cells in patients with COVID-19. *Cell Mol Immunol*. 18(10), 2325-2333. 10.1038/s41423-021-00750-4

Rocha Oliveira, C., Paula Vieira, R., De Oliveira Ferreira, A., De Souza Schmidt Gonçalves, E., Polonini, H. (2021). Immunoregulatory effects of Imuno TF® (transfer factors) on Th1/Th2/Th17/Treg cytokines. *J Clin Exp Imm.* 6:421–431. 10.1101/2020.11.06.371435

Rubinstein, M. P., Lind, N. A., Purton, J.F., Filippou, P., Best, J. A., Mcghee, P. A., Surh, C. D., Goldrath, A. W. (2008). IL-7 and IL-15 differentially regulate CD8+ T-cell subsets during contraction of the immune response. *Blood*. 112(9), 3704-12. 10.1182/blood-2008-06-160945

Sa, Q., Woodward, J. & Suzuki, Y. (2013). IL-2 produced by CD8+ immune T cells can augment their IFN-γ production independently from their proliferation in the secondary response to an intracellular pathogen. *J Immunol.* 190(5), 2199-207. 10.4049/jimmunol.1202256

Sercan Alp, Ö., Durlanik, S., Schulz, D., McGrath, M., Grün, J. R., Bardua, M., Ikuta, K., Sgouroudis, E., Riedel, R., Zehentmeier, S., Hauser, A. E., Tsuneto, M., Melchers, F., Tokoyoda, K., Chang, H. D., Thiel, A. & Radbruch, A. (2015). Memory CD8(+) T cells colocalize with IL-7(+) stromal cells in bone marrow and rest in terms of proliferation and transcription. *Eur J Immunol.* 45(4), 975-87. 10.1002/eji.201445295

Steele, R. W., Myers, M. G. & Vincent, M. M. (1980). Transfer factor for the prevention of varicella-zoster infection in childhood leukemia. *N Engl J Med.* 303(7), 355-9. 10.1056/NEJM198008143030702

Su, L. C., Liu, X. Y., Huang, A.F . & Xu, W. D. (2018). Emerging role of IL-35 in inflammatory autoimmune diseases. *Autoimmun Rev.* 17(7), 665-673. 10.1016/j.autrev.2018.01.017

Tsai, S. L., Liaw Y. F., Chen, M. H., Huang C. Y. & Kuo G. C. (1997). Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology*. 25(2), 449-58. 10.1002/hep.510250233

Vranjkovic, A., Crawley, A. M., Patey, A. & Angel, J. B. (2011). IL-7-dependent STAT-5 activation and CD8+ T cell proliferation are impaired in HIV infection. *J Leukoc Biol*. 89(4), 499-506. 10.1189/jlb.0710430

Whitmire, J. K., Tan, J. T. & Whitton, J. L. (2005). Interferon-gamma acts directly on CD8+ T cells to increase their abundance during virus infection. *J Exp Med.* 201(7), 1053-9. 10.1084/jem.20041463