



Inhibition of Jurkat T cell and Peripheral Blood Lymphocyte IL-2 Production by Novel Fungal Extracts

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Introduction

The immune system is composed of many interdependent cell types that produce innate and adaptive responses that collectively protect the body from infection. Often, these cell types depend on the adaptive T-helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines, or more specifically interleukins (Malek 2002). These cytokines are released upon T-cell activation, and chemical messengers allow T-cells to communicate with other cells in the body to initiate immune responses. Interleukin-2, a cytokine important in T-cell activation, aids in T-cell production and the control of effector cells therefore regulating the immune response (Fleischer 2007). In the case of infection, heightened T-cell response is necessary, however, in the case of tissue transplantation, T-cells activity may need to be reduced in order to prevent tissue rejection. There are many immunosuppressive drugs such as Cyclosporine A, an inhibitory drug isolated from a fungal extract, that prevent tissue rejection and has been used in the medical field for many years (Zeng 2007)(Kang 2007). Our goal was to measure IL-2 levels in activated Jurkat cells and peripheral blood leukocytes (PBLs) treated with a variety of wild mushroom fungal extracts. The IL-2 concentrations were quantified using an Enzyme-linked Immunosorbent Assay (ELISA).

Materials and Methods

Jurkat cells were obtained from American Type Culture Collection. Peripheral blood was drawn from human volunteers via venipuncture and peripheral blood lymphocytes were isolated via Histopaque-1077 separation (Sigma-Aldrich). Monocytes were removed by incubation in tissue culture flasks for 30 minutes at 37 C and 5% CO₂. Both Jurkat cells and PBLs were cultured in RPMI 1640 media with a 1X L-glutamine/pen-strep and 10% Fetal Bovine Serum (FBS) and incubated at 37 C and 5% CO₂ (Springael, 2007).

Cells were activated with Concanavalin A (ConA, 2ug/ml) and Phorbol Myristate Acetate (PMA, 50ng/ml) while (CsA, 2ug/ml) served as the inhibiting negative control. Fungal extracts were obtained from Dr. Aaron Monte (Chemistry Department University of Wisconsin-LaCrosse).

Cells were cultured with activators, negative controls and fungal extracts for 24hrs at 37°C and 5% CO₂. IL-2 production was screened via a commercially available IL-2 specific Enzyme-linked Immunosorbent Assay (ELISA) (eBioscience Cat #: 88-7026-88). Ascent software was used to measure sample absorbance at 450nm. Sample values were calculated according to the provided IL-2 standard curve.

Results

Preliminary results show that several extracts demonstrated inhibitory properties when compared to control samples. PBLs cells were inhibited by fungal extracts F307-4 and F307-5. Jurkat cells were inhibited by fungal extracts F42, F64 and F204.

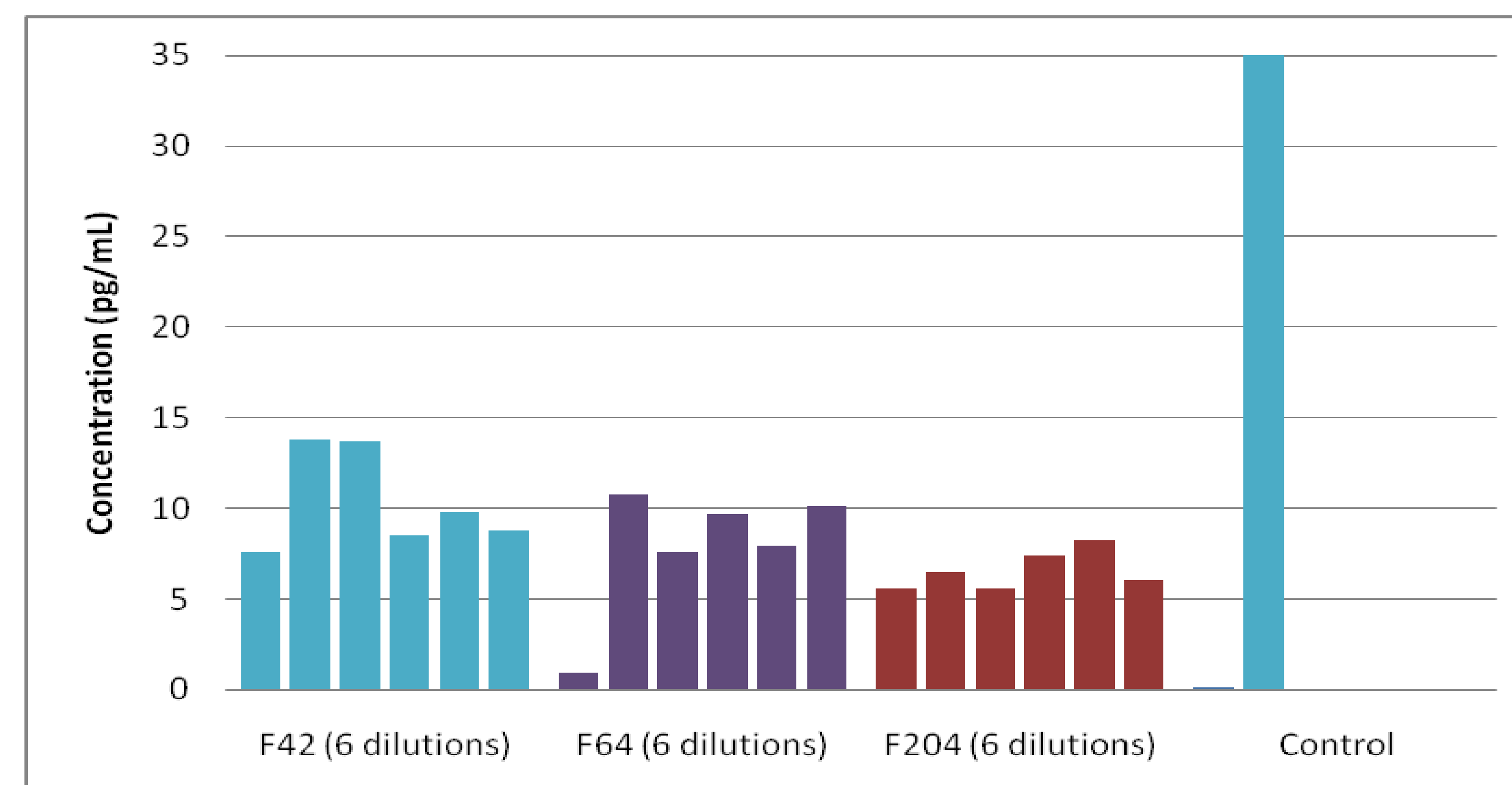


Figure 1. Represents serial dilutions of fungal extracts (F42, 64, 204) added to Jurkat cells activated with ConA and PMA. Samples treated with fungal extracts demonstrated a substantial decrease in IL-2 concentration.

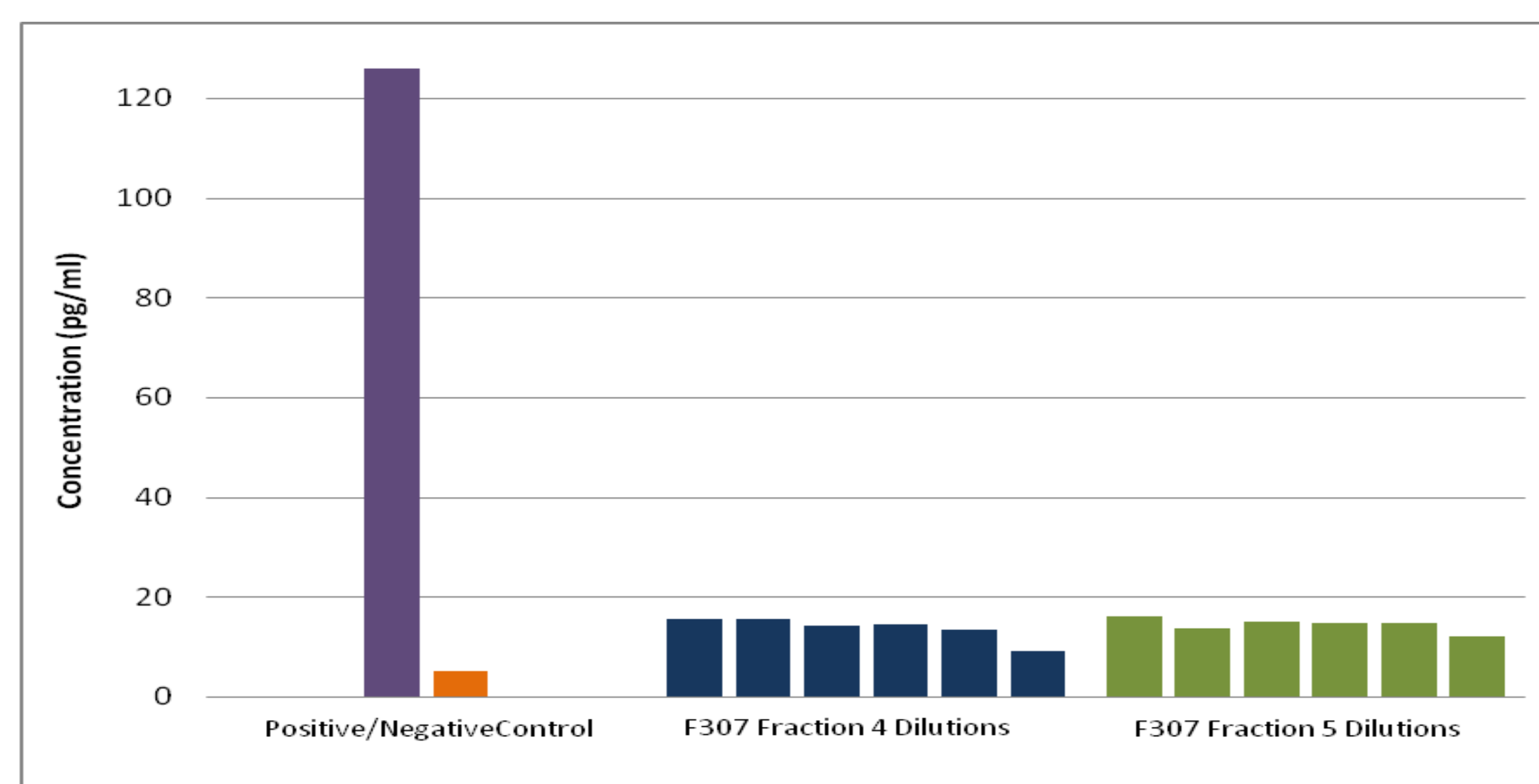


Figure 2. Represents serial dilutions of fungal extracts (F307-4 and F307-5) added to PBLs activated with ConA and PMA. Samples treated with fungal extracts demonstrated a substantial decrease in IL-2 concentration.

Discussion

In this study we began the process of screening fungal extracts to determine potential compounds that would inhibit white blood cell IL-2 production. Initial results showed approximately eight fungal extracts with an ability to sustain inhibition through six 1/10 dilutions (down to 256ng/ml). We propose that further fractionation of the extracts will allow us to identify the compounds contributing to the inhibitory activity. And, it is possible that these extracts may exhibit similar IL-2 inhibitory mechanisms as demonstrated by the well characterized fungal extract, CsA.

Acknowledgments

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Resources

1. Malek, T. R. (2002). T helper cells, IL-2 and the generation of cytotoxic T-cell responses. *Trends in Immunology*, 23(10), 465-467.
2. Fleischer, A., Duhamel, M., Lopez-Fernandez, L. A., Muñoz, M., Rebollo, M. P., Alvarez-Franco, F., & Rebollo, A. (2007). Cascade of transcriptional induction and repression during IL-2 deprivation-induced apoptosis. *Immunology Letters*, 112(1), 9-29.
3. Zeng, C., Zhen, Y., Shu, S., Wu, T., Yi, H., Lian, Z., & Zhao, Y. (2007). The influence of cyclosporin A on lymphocyte attenuator expression. *Journal of Autoimmunity*, 28(4), 234-244.
4. Kang, H. G., Zhang, D., Degauque, N., Mariat, C., Alexopoulos, S., & Zheng, X. X. (2007). Effects of cyclosporine on transplant tolerance: The role of IL-2. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, 7(8), 1907-1916.
5. Springael, C., Thomas, S., Rahmouni, S., Vandamme, A., Goldman, M., Willems, F., & Vosters, O. (2007). Rottlerin inhibits human T cell responses. *Biochemical Pharmacology*, 73(4), 515-525.