

ALERT: Treating Swine and Avian Flu by Down-Regulating the Inflammatory Response

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The problem with current approaches to defeating Swine Flu, is we have not been able to beat the virus, because it's too good at mutating. Although it might be possible to use different antigenic targets to create a vaccine that would be more universal and effective in the future, we need something now to defend against Swine Flu. We believe the solution is to change how our bodies deal with the virus. Recent discoveries about the anti-inflammatory properties of cannabinoids can provide us with new medicines, which can modify how we respond to these viruses and provide us with effective, non-toxic therapies. This paper provides a theory, with peer reviewed references, that supports the use of cannabinoids to prevent deaths associated with avian flu infections. If the lethality caused by the current swine flu can also be attributed to ARDS, then our proposal can be extended to the current problem.

1.0 Introduction: The Endocannabinoid System

Far-from-equilibrium thermodynamics as pioneered by Nobel Laureate Ilya Prigogine, provides a physical underpinning for all biological processes^{1,2}. An intrinsic characteristic that emerges, and permeates all organizational levels of life, is oscillations of opposing biochemical phenomena, often linked with inflammatory anti-inflammatory processes. In the same manner that temperature in a house varies around the set point determined by a thermostat, countless interacting reactions in human biochemistry oscillate around set points that turn up or down inflammatory responses and associated free radical production³. Evolution has selected the endocannabinoid system as a critical modulator of inflammatory biochemical pathways⁴.

Essentially, inflammation-generated free radicals may be thought of as biochemical friction, and endocannabinoids as the oil of life⁵ in that they reduce this friction. From this perspective, it is easy to understand why the endocannabinoids system has life promoting activities⁶, and why phytocannabinoids (plant derived cannabinoids), by virtue of their ability to mimic endocannabinoids, have therapeutic benefits for such wide range of illnesses, including cardiovascular^{7,8}, neurological^{9,10}, immunological¹¹⁻¹⁴, skeletal^{15,16}, diseases and cancers¹⁷⁻²⁴. They in fact appear to function as anti-aging compounds as indicated by the increased lifespan observed when mice were treated with THC for extended periods of time²⁵. In contrast, knockout mice that lacked the CB1 receptor die prematurely and CB2 knockout mice appear to have a number of associated phenotypes relating to the immune system, cardiovascular system, nervous system, digestive system, and reproductive system²⁶⁻²⁹.

The bird flu is one of the most critical viral diseases to threaten mankind today. Influenza viruses have already killed millions of individuals around the world. The following sections on the bird flu are a logical synthesis of existing knowledge that dramatically shows how important cannabis-based research can be for mankind, and why we have chosen influenza as an early focus of our research efforts. **We feel that the evidence below sufficiently supports the possibility that cannabinoids may save millions of lives that would otherwise be lost due to influenza and HIV infections, and it would be immoral and irresponsible not to determine if our hypothesis is correct.**

2.0 A Brief Introduction Into the Immune System

In order to appreciate the hypothesized life-saving possibilities offered by cannabinoids with respect to the bird flu and HIV, a limited understanding of how the immune system works is necessary. Upon infection, the infectious agent and damaged host tissue release chemical signals that serve as markers so that neutrophils, the foot soldiers of the immune system, can find their way to the invading pathogens. These specialized white blood cells bring with them a formidable array of biochemical weaponry including specialized receptors (TLRs), known as toll receptors that recognize molecular patterns on various pathogens. Bound TLRs activate neutrophils to produce highly inflammatory bacteriocidal chemicals such as hydrogen peroxide and sodium perchlorate. Additionally, neutrophils phagocytize the invaders. The neutrophils die young, lasting only a few days. The debris field is subsequently cleaned up by additional, late to arrive, phagocytic cells, monocytes and macrophage. The immune process thus far described is known as the innate immune system. We inherit it and are born with it functioning. The high levels of free radicals and other cytotoxic agents produced during the innate response create a lot of collateral damage. To overcome this damage problem, evolution has selected an additional more targeted, less inflammatory immune process known as the acquired response.

The acquired immune response takes pieces of the phagocytized pathogens and presents them on the surface of phagocytic cells in order to generate a specific response via the collaborative action of T and B cells that ideally kill pathogens and pathogen infected cells with a more specific targeted, less inflammatory response directed by B and T cell receptors.

2.1 Pathology associated with an Excessive Inflammatory Response

Today, most people in first world countries die from age-related illnesses³⁰. One hundred years ago, people in the same countries died predominantly of infectious diseases. The proinflammatory arm of the human immune system has evolved to play a critical role in fighting many infectious diseases. However, the inflammatory responses and associated free radical production appear to be at the heart of age-related illnesses including neurological disorders, cardiovascular disease, autoimmune diseases, and cancers³¹.

Man has changed the world in which we live in a manner that – for now – has greatly increased our lifespan. Improvements in public health, for example, have resulted in dramatic increases in the health of the human population. However, these changes have occurred too rapidly for the evolution of our immune system to keep pace with changing environmental demands. We live cleaner today, and in general appear to need lower levels of inflammation for control of most infections. Since the endocannabinoid system plays a critical role in up-regulating the anti-inflammatory arm of the immune system, phytocannabinoids can play a natural role in bringing man's immune system up-to-date by reducing the levels of immune generated inflammation, i.e. resetting the inflammatory thermostat.

It is important to keep in mind that different infections elicit different types of immune responses. There is an ongoing evolutionary battle between our immune system and pathogens. While many illnesses are exacerbated by an excessive inflammatory immune response, this type of response is required to control infection of tuberculosis, Legionella pneumophila, and Leishmania. The use of cannabis for these types of infections could be lethal as indicated by animal models³², because some types of infections actually require the pro-inflammatory response for their survival as most recent studies indicate is the case with HIV.

2.2 Avian Influenza (Bird Flu)

The Problem:

The bird flu is one of the most dangerous viral diseases to threaten mankind today³³. The main

source of fear is that mutated viruses will be acquire the capacity to transfer not only from wild birds to domestic birds and then to people, from man to man³⁴ and result in a worldwide pandemic. North Americans may be particularly vulnerable to this threat as a result of the migratory route over Canada taken by many wild birds³⁵.

This danger is underscored by the recent outbreak of the avian flu on the Canadian turkey farms that resulted in the killing of thousands of birds³⁶. The Canadian press recently reported that Baxter International's European facility in Austria mistakenly provided materials that were contaminated with the deadly avian H5N1 strain of influenza to a research company that subsequently sent samples to other European countries³⁷. When samples were injected into animals, the unexpected death that resulted led to investigations that identified the deadly strain as the problem.

This error could easily have resulted in a pandemic that would have killed millions. The magnitude of the threat posed by the avian flu to humanity was recently further emphasized by studies showing an unexpected rise in resistance to currently used antiviral medications³³. The bird flu, should it mutate to efficiently infect humans, will kill many millions in one season.

The Solution:

The lethality associated with bird flu infections in humans is very high (63%)³⁸. Based on animal studies, it appears that the bird flu elicits a proinflammatory immune response that is many times greater than that which results from infections by other influenza strains both in the lungs³⁹ and the brain⁴⁰. The apparently excessive proinflammatory immune response results in the lethal development of adult respiratory distress syndrome (ARDS) and multiple organ failure⁴¹. We hypothesize that a life-saving down-regulation of the excessively high proinflammatory response to the bird flu may be accomplished by orally ingesting an appropriate dose of phytocannabinoids without impairing immune control of the virus (resetting the inflammatory thermostat). Smoking or vaporizing cannabis will not work, and in fact could make things worse since using the pulmonary route will promote an added degree of inflammation.

There are typically two phases to any immune response. Initially, the innate arm of the immune system responds by initiating acute inflammation and free radical-induced cell killing. This general, non-specific response is then turned down as the more targeted acquired immune response kicks in. A successful immune response is characterized by the control of infection in a manner that minimizes harm to the infected organism. This goal is difficult and complex to accomplish. Both genetic and environmental factors, as well as chance, determine the outcome of a given infection.

The immune-cell-driven functions of the innate immune system are very inflammation dependant, and as a consequence produce collateral damage to surrounding tissue. Neutrophils, monocytes and macrophage are migratory cells that travel to the site of infection and initiate an innate immune response. Ultimately, these same cell types are also responsible for the transition to the acquired response as a result of antigen uptake and presentation. Current thinking suggests that the monocytes release MCL-1 attractant protein that binds the chemokine receptor CCR2 on a novel dendritic cell subset that produces TNF and iNOS (Tip)⁴², which during later stages of infection promote antigen specific T cell responses⁴³.

There are numerous studies that demonstrate the capacity of cannabinoids to down regulate the cascade of pro-inflammatory immune responses. Neutrophil⁴⁴ and monocyte migration is inhibited by activating CB2 receptor⁴⁵. Similarly, cannabinoids reduce the response to pro-inflammatory chemokines and cytokines⁴⁶ including TNF⁴⁷⁻⁴⁹. Most relevant to our proposal, the effect of THC on influenza induced lung inflammation has been examined^{50,51}. These studies demonstrated that THC could prevent influenza induced lung epithelial cell death even though there was an increase in viral load.

In order to appreciate the significance of these findings the thermostat model is helpful. The inflammatory thermostat of *Homo sapiens* was set over the past hundreds of thousands of years. Humans lived short dirty lives. A strong inflammatory response was essential. Under some circumstances, such as occurs with influenza infection in a cleaner modern world, the inflammatory thermostat may be set too high. As a result, rather than protecting us, our immune system is killing us. Biology is never simple. The influenza virus is itself cytolytic and therefore destructive of respiratory epithelial cells, and our defenses are complicated⁵². The question therefore becomes what kills first the virus or the immune system? The probable, but complex answer is that the outcome will depend the idiosyncratic biochemical balances of an individual, past exposures, and their genetics.

Case Study

What counts is the way in which the complicated dialog between an infectious agent and its host comes together to promote survival or death. Can an individual reduce inflammation and its lethal consequences while still controlling the viral infection? We have a limited but significant answer. Steve Kubby has inoperable, metastasized pheochromocytoma. He is the sole long-term survivor of this illness, having had it for 35 years. His only medication has been Cannabis. Recently, when he came down with serious case of the common flu, he treated himself with "homemade" cannabis extract-based lozenges instead of smoking cannabis. His symptoms were much milder than what normally occurs when he has had the flu and smoked cannabis in the past. Turning down immune modulated inflammation did not harm him and in fact appears to have been beneficial.

Today it is essential to determine if our lozenge is effective in reducing bird flu associated deaths. We feel that this work is particularly important since people are continually coming down with new variants of the influenza virus, such as the bird and swine strain currently threatening humanity. Because of the intrinsic high degree of variability that is built into the influenza virus, it's only a matter of time before this problem becomes even more serious. We hope to provide a cost-effective, and safe solution to this threat, which could literally kill millions. It is critical that work start as soon as possible. When the "Spanish flu" broke out in 1918, more people died from it than from World War I.

1. Nicolis, G. & Prigogine, I. *Exploring Complexity: An Introduction* (W.H. Freeman & Company, 1989).
2. Melamede, R. J. Dissipative Structures and the Origins of Life. *Interjournal Complex Systems* 601 (2006).
3. Melamede, R. Harm reduction--the cannabis paradox. *Harm Reduct J* 2, 17 (2005).
4. Burstein, S. H. & Zurier, R. B. Cannabinoids, Endocannabinoids, and Related Analogs in Inflammation. *AAPS J* (2009).
5. Melamede, R. J. Endocannabinoids: Multi-scaled, Global Homeostatic Regulators of Cells and Society. *Interjournal Complex Systems* 1669 (2006).
6. Zimmer, A., Zimmer, A. M., Hohmann, A. G., Herkenham, M. & Bonner, T. I. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl. Acad. Sci. U S A* 96, 5780-5785 (1999).
7. Mach, F. & Steffens, S. The role of the endocannabinoid system in atherosclerosis. *J. Neuroendocrinol.* 20 Suppl 1, 53-57 (2008).
8. Pacher, P. & Hasko, G. Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion injury and preconditioning. *Br. J. Pharmacol.* (2008).
9. Hosking, R. D. & Zajicek, J. P. Therapeutic potential of cannabis in pain medicine. *Br J Anaesth* (2008).
10. Rog, D. J., Nurmikko, T. J. & Young, C. A. Oromucosal delta9-tetrahydrocannabinol/cannabidiol for neuropathic pain associated with multiple sclerosis: an uncontrolled, open-label, 2-year extension trial. *Clin Ther* 29, 2068-2079 (2007).
11. Cabral, G. A., Raborn, E. S., Griffin, L., Dennis, J. & Marciano-Cabral, F. CB(2) receptors in the brain: role in central immune function. *Br. J. Pharmacol.* (2007).
12. Eisenstein, T. K., Meissler, J. J., Wilson, Q., Gaughan, J. P. & Adler, M. W. Anandamide and Delta(9)-

- tetrahydrocannabinol directly inhibit cells of the immune system via CB(2) receptors. *J. Neuroimmunol.* (2007).
13. Klein, T. W. & Cabral, G. A. Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. *J Neuroimmune Pharmacol* **1**, 50-64 (2006).
 14. Verhoeckx, K. C. et al. Unheated Cannabis sativa extracts and its major compound THC-acid have potential immuno-modulating properties not mediated by CB1 and CB2 receptor coupled pathways. *Int Immunopharmacol* **6**, 656-665 (2006).
 15. Ofek, O. et al. Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proc. Natl. Acad. Sci. U S A* **103**, 696-701 (2006).
 16. Idris, A. I. et al. Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. *Nat. Med.* **11**, 774-779 (2005).
 17. Blazquez, C. et al. Cannabinoids inhibit glioma cell invasion by down-regulating matrix metalloproteinase-2 expression. *Cancer Res.* **68**, 1945-1952 (2008).
 18. Widmer, M., Hanemann, O. & Zajicek, J. High concentrations of cannabinoids activate apoptosis in human U373MG glioma cells. *J. Neurosci. Res.* (2008).
 19. Ramer, R. & Hinz, B. Inhibition of cancer cell invasion by cannabinoids via increased expression of tissue inhibitor of matrix metalloproteinases-1. *J. Natl. Cancer Inst.* **100**, 59-69 (2008).
 20. Preet, A., Ganju, R. K. & Groopman, J. E. Delta(9)-Tetrahydrocannabinol inhibits epithelial growth factor-induced lung cancer cell migration in vitro as well as its growth and metastasis in vivo. *Oncogene* (2007).
 21. Velasco, G. et al. Cannabinoids and gliomas. *Mol. Neurobiol.* **36**, 60-67 (2007).
 22. Ligresti, A. et al. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther* **318**, 1375-1387 (2006).
 23. Guzman, M. et al. A pilot clinical study of Delta(9)-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. *Br. J. Cancer* (2006).
 24. Caffarel, M. M., Sarrío, D., Palacios, J., Guzman, M. & Sanchez, C. Delta}9-Tetrahydrocannabinol Inhibits Cell Cycle Progression in Human Breast Cancer Cells through Cdc2 Regulation. *Cancer Res.* **66**, 6615-6621 (2006).
 25. NIH. NTP Toxicology and Carcinogenesis Studies of 1-Trans-Delta(9)-Tetrahydrocannabinol (CAS No. 1972-08-3) in F344 Rats and B6C3F1 Mice (Gavage Studies). *Natl Toxicol Program Tech Rep Ser S* **446**, 1-317 (1996).
 26. Buckley, N. E. et al. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur. J. Pharmacol.* **396**, 141-149 (2000).
 27. Kunos, G. & Batkai, S. Novel physiologic functions of endocannabinoids as revealed through the use of mutant mice. *Neurochem Res* **26**, 1015-1021 (2001).
 28. Buckley, N. E. The Peripheral Cannabinoid Receptor Knockout Mice: an Update. *Br. J. Pharmacol.* **153**, 309-318 (2008).
 29. Springs, A. E., Karmaus, P. W., Crawford, R. B., Kaplan, B. L. & Kaminski, N. E. Effects of targeted deletion of cannabinoid receptors CB1 and CB2 on immune competence and sensitivity to immune modulation by {Delta}9-tetrahydrocannabinol. *J. Leukoc. Biol.* (2008).
 30. Walczak, J. S., Price, T. J. & Cervero, F. Cannabinoid CB(1) receptors are expressed in the mouse urinary bladder and their activation modulates afferent bladder activity. *Neuroscience* **159**, 1154-1163 (2009).
 31. Balaban, R. S., Nemoto, S. & Finkel, T. Mitochondria, oxidants, and aging. *Cell* **120**, 483-495 (2005).
 32. Newton, C. A., Klein, T. W. & Friedman, H. Secondary immunity to Legionella pneumophila and Th1 activity are suppressed by delta-9-tetrahydrocannabinol injection. *Infect. Immun.* **62**, 4015-4020 (1994).
 33. Khanna, M., Kumar, P., Choudhary, K., Kumar, B. & Vijayan, V. K. Emerging influenza virus: a global threat. *J Biosci* **33**, 475-482 (2008).
 34. Jeong, O. M. et al. Experimental infection of chickens, ducks and quails with the highly pathogenic H5N1 avian influenza virus. *J Vet Sci* **10**, 53-60 (2009).
 35. Anekonda, T. S. & Adamus, G. Resveratrol prevents antibody-induced apoptotic death of retinal cells through upregulation of Sirt1 and Ku70. *BMC Res Notes* **1**, 122 (2008).
 36. Times, A. Two more farms under quarantine. (2009).
 37. D., Z. Vaccine maker's snafu sparks pandemic scare. *World Net Daily* (2009).
 38. Baskin, C. R. et al. Early and sustained innate immune response defines pathology and death in nonhuman primates infected by highly pathogenic influenza virus. *Proc. Natl. Acad. Sci. U S A* **106**, 3455-3460 (2009).

39. Chan, M. C. et al. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir Res* **6**, 135 (2005).
40. Korteweg, C. & Gu, J. Pathology, molecular biology, and pathogenesis of avian influenza A (H5N1) infection in humans. *Am. J. Pathol.* **172**, 1155-1170 (2008).
41. To, K. F. et al. Pathology of fatal human infection associated with avian influenza A H5N1 virus. *J. Med. Virol.* **63**, 242-246 (2001).
42. Aldridge, J. R. J. et al. TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proc. Natl. Acad. Sci. U S A* **106**, 5306-5311 (2009).
43. Pamer, E. G. Tipping the balance in favor of protective immunity during influenza virus infection. *Proc. Natl. Acad. Sci. U S A* **106**, 4961-4962 (2009).
44. McHugh, D., Tanner, C., Mechoulam, R., Pertwee, R. G. & Ross, R. A. Inhibition of human neutrophil chemotaxis by endogenous cannabinoids and phytocannabinoids: evidence for a site distinct from CB1 and CB2. *Mol. Pharmacol.* **73**, 441-450 (2008).
45. Montecucco, F., Burger, F., Mach, F. & Steffens, S. The CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. *Am J Physiol Heart Circ Physiol* (2008).
46. Di Filippo, C., Rossi, F., Rossi, S. & D'Amico, M. Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. *J. Leukoc. Biol.* **75**, 453-459 (2004).
47. Rajesh, M. et al. CB(2) cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. *Br. J. Pharmacol.* (2007).
48. Rajesh, M. et al. Cannabinoid-2 receptor stimulation attenuates TNF {alpha}-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol* (2007).
49. Facchinetti, F., Del Giudice, E., Furegato, S., Passarotto, M. & Leon, A. Cannabinoids ablate release of TNFalpha in rat microglial cells stimulated with lipopolysaccharide. *Glia* **41**, 161-168 (2003).
50. Buchweitz, J. P., Karmaus, P. W., Williams, K. J., Harkema, J. R. & Kaminski, N. E. Targeted deletion of cannabinoid receptors CB1 and CB2 produced enhanced inflammatory responses to influenza A/PR/8/34 in the absence and presence of {Delta}9-tetrahydrocannabinol. *J. Leukoc. Biol.* (2007).
51. Buchweitz, J. P., Karmaus, P. W., Harkema, J. R., Williams, K. J. & Kaminski, N. E. Modulation Of Airway Responses To Influenza A/PR/8/34 By Delta-9-Tetrahydrocannabinol In C57BL/6 Mice. *J Pharmacol Exp Ther* (2007).
52. Topham, D. J., Tripp, R. A. & Doherty, P. C. CD8+ T cells clear influenza virus by perforin or Fas-dependent processes. *J. Immunol.* **159**, 5197-5200 (1997).