The Effects of Phytoncides on Natural Killer Cells in Humans

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Introduction

Forest and natural environments have been enjoyed by humans for thousands of years for the quiet atmosphere, beauty, and clean, fresh air. Up until the Industrial Revolution, humans spent most of their daily lives outside, immersed in the outdoors and in-tune with the cyclical-nature of Earth. However, with the advent of new technologies and factory-production came a cultural shift away from the outdoors, and to an artificial landscape of fluorescent light bulbs, recycled air, and rubber-soled shoes. After spending 99.99% of our existence outside, humanity disconnected from nature and moved indoors.

Since this shift, we have seen a global rise of chronic and infectious disease, some of which were unheard of during the times of outdoor living. History shows us that following the Industrial Revolution, the world saw epidemics of influenza, small-pox and polio- all diseases caused by bacteria and viral infections. What was it about the shift from outdoors to an indoor environment that led to an increase in such diseases? What properties of an outdoor environment lead to healthier human beings? Can outdoor environments, particularly forest environments, have healing properties that humans are unaware of?

In the following pages I will explore the healing characteristics of outdoor and forest environments, and the effects they have on human immune function. I will look at the factors leading to increased immune function while in a forest environment, and the implications of such findings. It is my hope that through this examination, the healing capabilities of nature will be revealed and a 'return to nature' will be considered.

Forest Bathing and Phytoncides

A trip to and time spent in the forest, called "Shinrin-yoku" in Japanese, has been proven to have positive effects on human psychology and physiology. In general terms, Shinrin-yoku is the practice of 'forest-bathing', or making contact with nature and "taking in the atmosphere of the forest" (Park et al., 2007).

A 2006 Japanese study on the physiological effects of Shinrin-yoku found that this practice can effectively relax people's body and mind. The study consisted of 12 male students, broken up into two groups. Each group was sent to immerse themselves in the atmosphere of either a forest or urban area for 20 minutes and the groups switched locations the following day. Salivary cortisol levels and cerebral activity in the prefrontal area were measured before and after each observation period, as well as during the middle of the day. The results indicated that "cerebral activity in the prefrontal area of the forest group was significantly lower" than that of the city group, and the "concentration of salivary cortisol of the forest area group was significantly lower than of the group in the city" area before and after watching each landscape (Park et al., 2007). This study demonstrates the relaxing powers in nature compared to a city environment. By allowing oneself to fully immerse in a natural environment, cortisol levels are lowered and the brain is allowed to relax. But the question remains, what is causing the lower cortisol and prefrontal cortex levels while in a forest environment?

Similar to 'natural aromatherapy', forest bathing practitioners immerse themselves in the natural environment for relaxation, all the while breathing in substances called phytoncides. According to Li (2008), phytoncides are "volatile organic compounds derived from trees." The term phytoncide, coined by Russian biochemist Dr. Boris Tokin in 1937, literally means "exterminated by the plant" (Romanov, 2005). Some of the phytoncides excreted by plants include isoprene, □-pinene, □-pinene and *d*-limonene (Li and Kawada, 2009). Phytoncides are chemically similar to that of essential oils produced by plants and are active substances that are anti-bacterial, anti-fungal, anti-viral and prevent the plants from rotting (Li, 2008). They are produced and emitted

from trees and plants to protect them from harmful insects, animals and germs. Phytoncides give a forest environment its quintessential 'forest smell' and have been proven to purify the air (Romanov, 2005). These chemical compound not only enhance the health of the trees and plants emitting them, but have an effect on human physiology as well.

Phytoncides released from trees decrease the production of stress hormones and overall reduces stress in humans, hence the calming effect being in a forest has on a human (Li, 2008). It is well known that stress inhibits immune function, and that a properly functioning immune system plays "an important role on the defense of bacteria, viruses, and tumors" (Wu and Lanier, 2003 as seen in Li and Kawada, 2009). We can then speculate that forest environments may have beneficial effects on the human immune system by reducing stress and affecting immune-cell production and function.

Human immunity, NK-cells and T-cells

The human immune systems is organized into two overlapping and complementary components: the adaptive immune component and the innate immune component. Adaptive immunity, or acquired immunity, involves a delayed response to a specific antigen. It involves T-lymphocytes (cell-mediated immunity), B-lymphocytes (humoral immunity), and plasma cells, which synthesize and release antibodies (McKinley, O'Loughlin & Bidle, 2012). Innate immunity, or non-specific immunity, is initiated immediately in response to a wide array of substances. It includes the barriers of skin and mucosal membranes that prevent entry (the body's first line of defense), as well as non-specific cellular and molecular defenses (the body's second line of defense) (McKinley, O'Loughlin & Bidle, 2012). Some of these molecular defenses include: neutrophils and macrophages which engulf and destroy unwanted substances, basophil and mast cells which are proinflammatory chemical-secreting cells, eosinophils which target parasites, and NK (natural killer) cells which destroy a wide variety of unwanted cells (McKinley, O'Loughlin & Bidle, 2012).

Natural killer (NK) cells patrol the body in a process called immune surveillance, in an effort to detect unhealthy cells. As part of the body's innate immunity, they seek out and destroy cells including "virus-infected cells, bacteria-infected cells, tumor cells, and cells of transplanted tissue" (McKinley, O'Loughlin & Bidle, 2012). The NK cells make contact with the unwanted cells and kill them by releasing cytotoxic chemicals. These cytotoxic chemicals include "perforin, which forms a transmembrane pore in the unwanted cell, and granzymes, which then enter the cell through the perforin-created pore, initiating apoptosis (cellular death in which the cell shrivels)" (McKinley, O'Loughlin & Bidle, 2012). Natural Killer cells have been reported to kill tumors or virus-infected cells through the release of perfornin, granzymes, as well as granulysin (GRN) (Li, 2008). The function of granulysin (GRN) is to create holes in the target cell and destroy it- similar to granzymes.

Akin to NK cells, T-lymphocytes (T-cells) are an aspect of the body's adaptive immunity component and reside in the blood. They are a type of white blood cell, and are distinguished from other lymphocytes by the TCR (T-cell receptor). T-lymphocytes originate in the red bone marrow and continue to travel to the thymus to complete their maturation ("T" stands for thymus). Here, the T-lymphocytes are tested determine whether their unique TCR receptor is able to bind to antigens that are foreign or 'nonself' (McKinley, O'Loughlin & Bidle, 2012). A process of selection is initiated to determine whether or not the T-lymphocyte recognizes a self-antigen or binds to an MHC molecule (McKinley, O'Loughlin & Bidle, 2012). MHC molecules bind to peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T-cells (Janeway, et al., 2001). Once the process of selection is

completed, the T-cells undergo activation before they can carry out their immune functions. The can be activated into either helper T-lymphocytes (which retain only the CD4 protein) and cytotoxic T-lymphocytes (which retain only the CD8 protein) (McKinley, O'Loughlin & Bidle, 2012). T-lymphocytes have both proteins until the selection process in the thymus. Once activated, helper T-lymphocytes release cytokines to regulate the cells of the immune system and cytotoxic T-lymphocytes release cytotoxic chemicals which induce apoptosis in abnormal cells (McKinley, O'Loughlin & Bidle, 2012).

Natural Killer and T-lymphocytes are both extremely important aspects of the human immune system and their proper functioning is necessary for the maintenance of bodily health. It is good practice to support these molecular defenses in whatever ways we know how, and one way to do this is through forest bathing and exposure to phytoncides.

Human Immune Function and Phytoncides

The roles of NK-cells and T-cells in innate and adaptive immunity are analogous. Their function is to search for and destroy unwanted cells through forced apoptosis. It is these cells that keep bacteria, virus-infected cells, tumors and abnormal cells from wreaking havoc in the body and compromising the immune systems. It is these cells that benefit from phytoncide-exposure.

Since 2005, a series of investigations to study the effects of phytoncide exposure on human immune function through forest bathing have been conducted by measuring "human NK activity, the numbers/proportions of NK and T cells, and perforin-, granzymes A and B (GrA/B)-, and granulysin (GRN)-expressing lymphocytes in human peripheral blood lymphocytes" during visits to forest parks (Li and Kawada, 2009). The subjects of these studies experienced a 3-day/2-night trip to forest areas. Blood and urine were sampled on days 2 (first sampling of the trip) and 3 (second sampling of the trip), and on days 7 and 30 after the trips, to determine how long the effects lasted. The same measurements were made before the trip on a normal working day as a control. Along with sampling lymphocytes in human peripheral blood, the concentration of urinary adrenaline was measured (Li, 2009). Investigators found that there were significant differences in NK activity and in the number of NK cells and T-cells both before and after the trip, indicating that the forest bathing trip significantly increased NK cell activity and the numbers of NK cells and T-cells in the blood samples (Li, 2009). The results also showed that "the forest bathing trips also significantly increased the number of intercellular perforin-, GRN-, and GrA/B-expressing lymphocytes", indicating forest bathing increases NK activity and numbers (Li, 2009).

Another study was conducted with the incubation of NK-92MI cells (an interleukin-2-independent human NK cell line) in the presence of phytoncides, such as □-pinene, *d*-limonene, and essential oils extracted from trees including Japanese cedar (Li, 2009). The NK-92MI cells remained with the phytoncides for 48-144 hours. NK activity and intracellular levels of perforin, GrA, and GRN were measured following incubation. Li (2009) found that "phytoncides significantly increased the cytolytic activity of NK-92MI cells...and significantly increased the intracellular levels of perforin, GrA, and GRN in NK-92MI cells". This data indicates that phytoncides significantly enhances human NK activity, ultimately bolstering human immune function.

These studies also looked at how long these effects lasted after a forest bathing trip, either 1-day or 3-days in length, and whether or not a trip to a place without a forest (a city tourist visit) also increases NK activity. Investigators found:

> that increase in NK activity...and the higher percentage of GRN-, perforin-, and GrA/B-expressing cells lasted for more than 7 days, while the increased NK activity and the higher number of NK cells and GRN- and GrB-expressing cells lasted for 30 days. In contrast, the city tourist visit did not increase NK activity, number of NK cells, or the expression of the selected intracellular perforin, GRN, and GrA/B (Li, 2009).

In fact, Li (2009) found that phytoncides were almost completely absent from city air.

Infectious Disease and Phytoncides

Cities containing little to no trees are completely devoid of phytoncides- nature's aromatherapy. Living air produced by tree and plants have been proven to have beneficial effects on human immune function, and this leads one to wonder about the effects of *not* having exposure to phytoncides either periodically or regularly. Many researchers are studying the correlation between rampant deforestation in parts of the world such as China and Brazil, and the sudden proliferation of infectious diseases originating in these locations.

In one example, according to Wolfe, et al. (2005), deforestation leads to an increase in contact between humans and wildlife, and many infectious diseases are carried by such animals and birds. A greater chance of coming into contact with viral and bacterial pathogens unknown to humans is created with the loss of habitat for wildlife. These pathogens are able to breed and proliferate without the presence of phytoncides to exterminate them. Wolfe, et al. (2005) postulates that diseases such as Ebola virus, Nipah virus, and severe-acute respiratory syndrome (SARS) are a growing threat because of deforestation. There is a theory that the SARS epidemic began due to the complete deforestation of Guangdong Province in China- the believed origin of SARS (Sehgal, 2010). Without trees and plants to produce microbe-destroying phytoncides, infectious diseases are given space to reproduce and spread to areas they are not normally found. It seems that the air-purifying capabilities of phytoncides have kept humans safe from infectious diseases for millennia. However, the question remains, with the consistent destruction of Earth's biosphere where will that leave human's ability to ward off and fight lurking microbes? How can humans defend themselves from bacteria and viruses once eliminated by our plant-allies? What is the fate of humanity if we continue our deforestation practices?

Conclusion

These questions, and many more, circulate through my mind as I research the epidemiological effects our actions are having on Earth. Not only are we wreaking havoc on our bodies through deforestation and ecological destruction, but on our minds as well. In his 2005 book *Last Child in the Woods*, author Richard Louv coined the term *nature deficit disorder*, defined as "a now common disorder where people do not go outside enough, and lose touch with the natural world and all of its wonder". Humanity's disconnection from Earth has deepened to a point of giving it a name, irrevocably instilling it into our awareness and lives. Our disconnection from the forest and phytoncides have lead to the invention of a disorder.

Nonetheless, nowadays it is common for urban- and suburban-dwelling individuals to take day trips or extended vacations in remote areas to ease stress and 'recharge'. The theme of 'returning to the wild' and 'nature appreciation' is gaining popularity with movies such as *Into the Wild* and *Avatar*, and wilderness adventure vacations and travel are gaining momentum as well. Many people tout nature's ability to leave them feeling refreshed and reinvigorated, and an ever-increasing amount of people are choosing nature-immersion activities over urban activities. Human intuition is leading us back to the forest and back to the natural healing properties of

plants- back to phytoncides. It is my hope that humanity will remember our innate connection to nature and rediscover the wondrous curative features of Earth. Let us return to where we came from, and bathe in the medicine provided by our planet. Let us go outside and be around trees and plants, all in the name of health.

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