Role of phytosterols as antivirals and immunomodulators

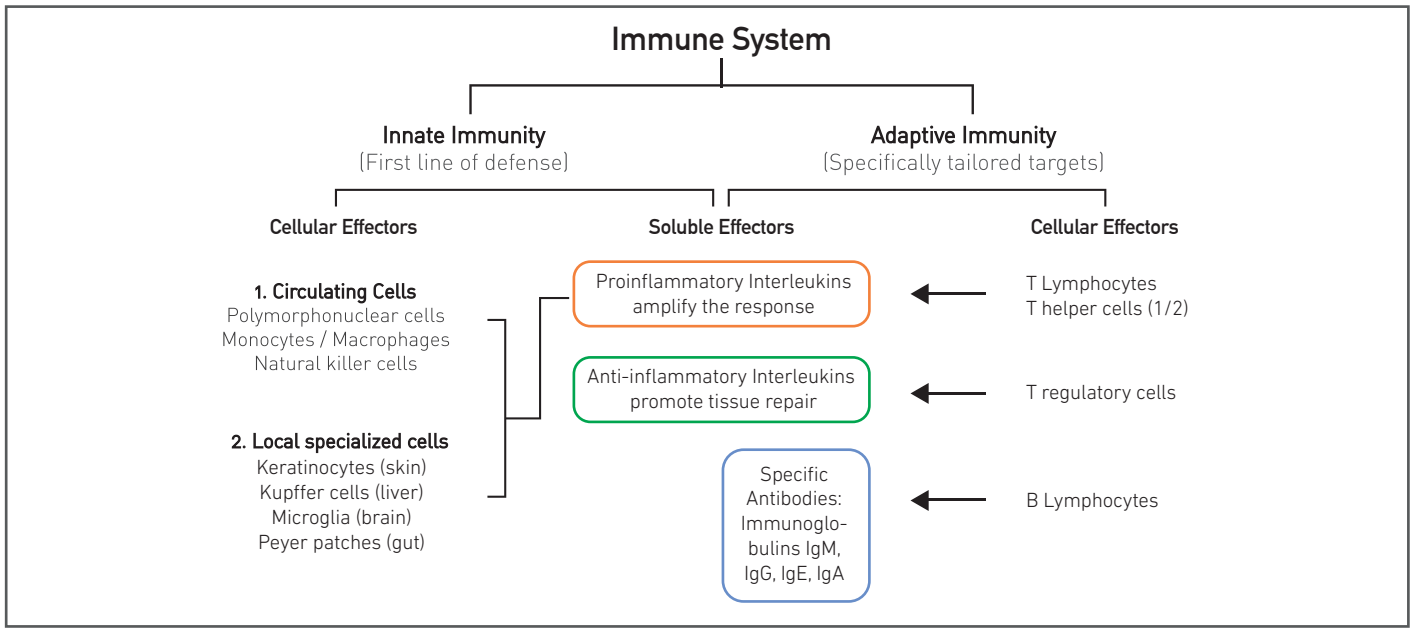
Lipids, especially cholesterol and its plant counterpart phytosterols, are key components of cell membranes, hormone precursors and regulators of many biological processes. In this white paper we will expand on the relation between phytosterols as human immunomodulators and as antivirals.

Introduction

During the past decade there is increasing evidence of the interconnection between metabolic routes of lipids and the control of immunological processes. Accordingly, a healthy immunological status is a consequence of a correct control of energy sources in the body. Specifically, products that aid in the management of cholesterol and triglyceride levels, such as phytosterols, have shown to play a role as well, both through immunomodulatory activities as well as in antiviral activity.

Immunity is provided by a complex array and interactions of specific cells and molecules that contribute different types of barriers and strategies to stop and eliminate a substance, particle or microorganism recognized in our bodies as a foreign agent. An imbalance of its components or functions may cause either an overreaction, as for example in allergies or in the other hand immunodeficiencies, as occurs with some pharmacological treatments. Lifestyle may certainly help condition our immune response to either side, which is why proper eating, sleep and physical activity are essential to balanced immune response.

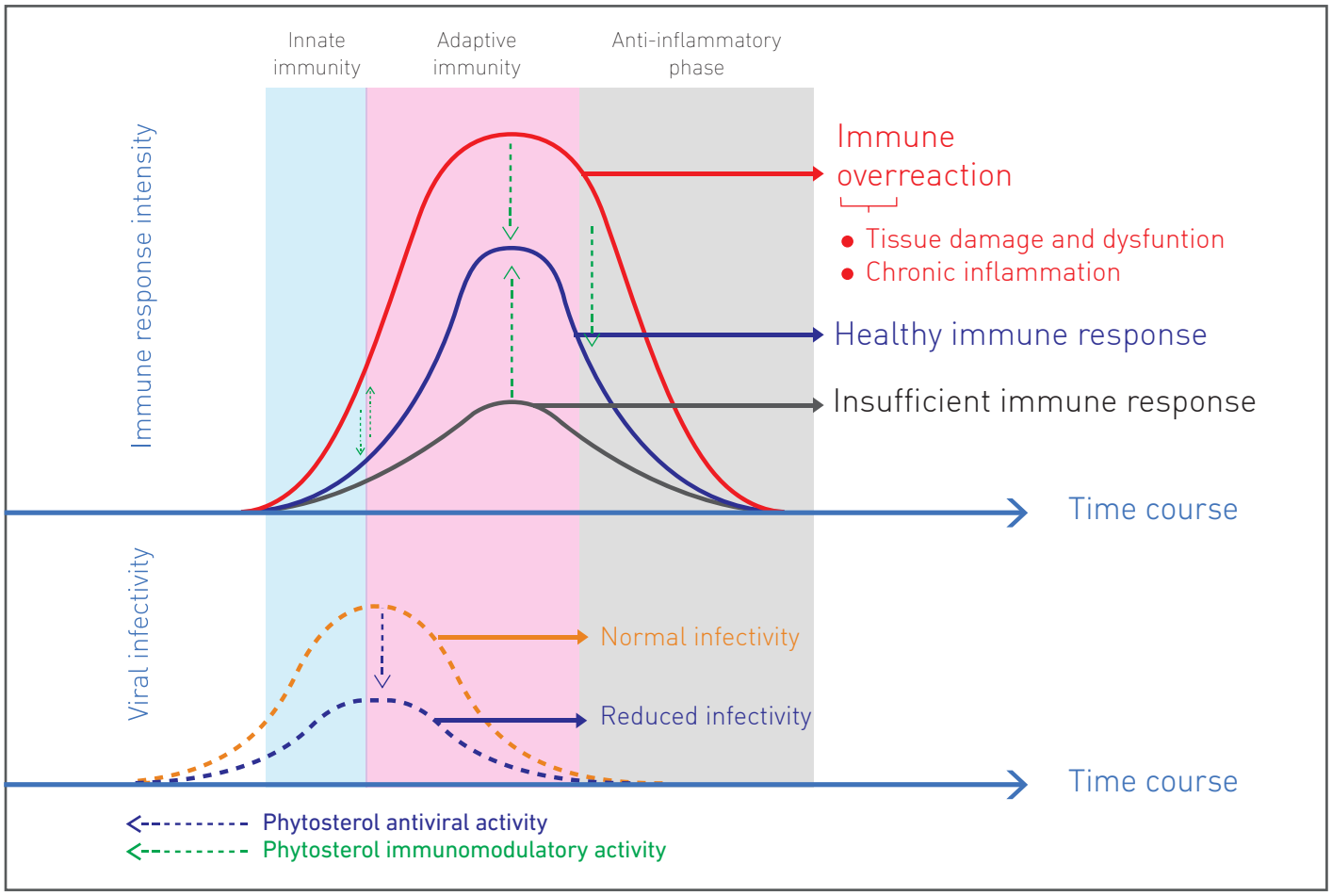
Immune response is categorized as innate or adaptive response (Figure 1).



- *Figure 1: Simplified scheme of the immune system*

Innate immunity is composed of circulating and local tissue specific cells that are continuously surveilling our organism to react against foreign species. A secondary response, the adaptive immunity, may also develop mediated by another subgroup of cells (T lymphocytes and B lymphocytes). After activation, these cells and soluble effectors interact in a complex manner not yet completely unveiled. All these intercellular interactions are mediated through lock and key like recognition of specific molecules at the surface between lymphocytes and/or innate immune cells and by liberating pro or anti-inflammatory mediators generally known as cytokines (interleukins, interferons, prostaglandins, among others).

Phytosterols have been shown to participate in diverse steps of the immune response (Figure 2), such as modulating the innate immune response, equilibrating the function of the adaptive immunity and favoring the resolution of the inflammatory response once started, but also, it has been shown that phytosterols act as antivirals, inhibiting RNA viruses to enter host cells.



● *Figure 2: Schematic immunomodulatory and antiviral effect of phytosterols*

Innate immunomodulation

Various *in vitro* studies have revealed the anti-inflammatory effect phytosterols have in innate immune cells by reducing the secretion of inflammatory cytokines (Ding et al., 2009; Valerio and Awad 2011; Valerio et al., 2013; Kim et al., 2014) and stimulation of antioxidant responses (Vivancos and Moreno, 2005) to suppress prostaglandin synthesis (Awad et al., 2004; Vivancos and Moreno, 2008; Moreno, 2013). Early studies have also shown that phytosterols produce immunomodulatory reactions like T cell proliferation and natural killer cells increased activity (Bouic et al., 1996). Evidence is indicative that phytosterols may influence innate immune system function yet further studies are required to unveil the full complexity of underlying mechanisms.

Adaptive immunomodulation

Adaptive immunity is a much slower process and is mediated by T and B cells (lymphocytes), which are aided by T helper cells: Th1 cells which tend to initiate the response against intracellular bacteria and viruses and Th2 cells which trigger the response against extracellular microorganisms. Another group, T regulatory lymphocytes (Tregs) prevent excessive damage on normal surrounding tissues by soothing the immune response when it is no longer needed.

Th1/Th2 imbalance may occur under determined pathological conditions, predisposing immune response to unfavorable conditions. For example, allergies exacerbate a Th2 response to promote B lymphocyte production of antibodies directed against a specific allergen. On the contrary, a decreased Th1 response is developed by HIV infection, generating immunosuppression.

Of special interest is SARS-CoV-2 which tricks the immune response into a Th2 type response, while a Th1 type response is needed: The virus has been shown to avoid detection by the host immune system by blocking and avoiding recognition by the innate immune response as shown by the absence of IL-12, a key cytokine required to mount a proper Th1 mediated response for intracellular infections (Prompetchara, et al., 2020), leading to severely inflammation known as "cytokine storm" (Mehta, P. et al., 2020). It also induces cell necrosis which misleads the immune system into initiating a Th2 response (Tian, S. et al., 2020; Chen, Y. et al., 2020), signaled by the presence of Th2-polarizing cytokines IL4 and IL-13 (Huang, C. et al., 2020).

Evidence suggests that phytosterols play a role in restoring Th1/Th2 equilibrium by inhibiting the activation of the dominant effector T-cells. *In vitro* and *in vivo* studies have shown that phytosterols favor Th1 lymphocyte cytokines production in Th2 driven immune cells populations (Brull et al., 2012; Kun and Lin, 2013; Lee et al., 2007). Interestingly, obese allergic individuals (Th2 skewed) have shown a better response in antibody production by boosting Th1 mechanisms after phytosterol consumption (Plat et al., 2019). Clinical tests with tuberculosis (Donald et al., 1997) and HIV patients (Breytenbach et al., 1996) have also demonstrated a beneficial immunomodulatory effect of phytosterol consumption. Also, phytosterols may favor attenuation mechanisms preventing excessive reactions soothing the inflammatory response and promoting tissue repair. The latter may be achieved by Treg activation by phytosterols (Kassen et al., 2000).

Antiviral activity

The infectious activity of viruses having a lipid external envelope, as SARS-CoV2, HIV, Zika and hepatitis B, depend on its interaction with a specialized section in the membrane of the cell it infects characterized by being very rich in cholesterol, called lipid rafts. Virus-lipid raft interaction is required both, for virus internalization and, after using the cell machinery for self-replication, the budding of new viruses from the infected cell (Verma, 2009). If cholesterol is decreased from the membrane, viral infectivity is decreased, as demonstrated in cells treated with cyclodextrin, a membrane cholesterol depleting agent, severely decreasing the infectious activity of SARS-CoV (Glende et al., 2008). Consistently, the *in vitro* infectious activity of hepatitis B virus has been blocked by 45% with sitosterol (Parvez et al., 2019). Therefore, pharmacological as well as natural compounds like phytosterols, that regulate plasma and cell cholesterol content, may be a valuable tool to avoid massive viral infection.

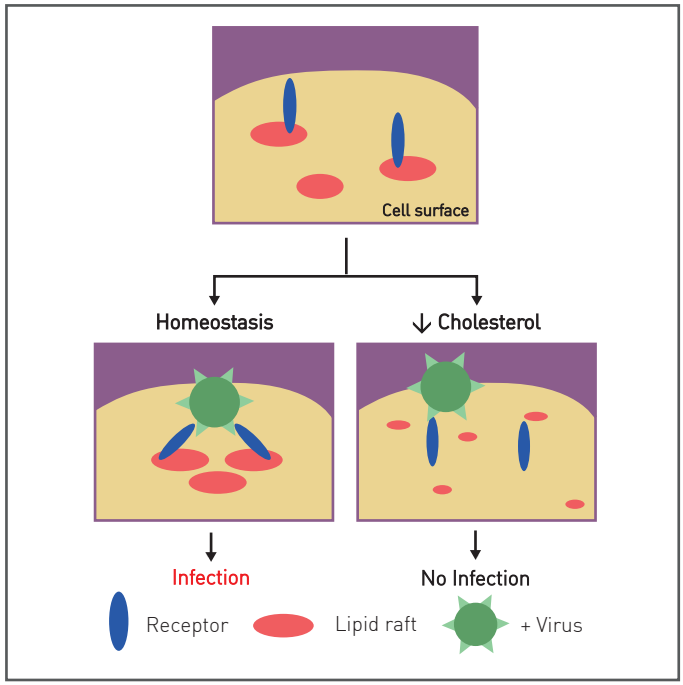


Figure 3: Lipid raft, receptor, and virus interaction

Summary

Increasing scientific and clinical evidence supports the role of phytosterols as favoring a healthy immune response that could aid maintaining a strong organism against viral infections, which is of special interest under the current worldwide situation. The provided information resonates with ancient wisdom of food as medicine, and future research will surely provide more certainty in phytosterols' efficacy in this area.

For future research, the delivery format of plant sterols must be taken into consideration, as commercially available plant sterols may be found un-esterified, esterified with fatty acids and dispersed in water. Cardiosmile, a novel formulation of sub-micron unesterified phytosterols in water, has consistently proven to lower triglycerides as well as cholesterol in healthy (Shaghaghi et al., 2014) and metabolic syndrome (pending publication) patients, unlike sterols esters which have shown conflicting results in their triglyceride lowering effectiveness (Ras et. al., 2014). Triglyceride reduction efficacy points not only to an enhanced modulation of lipidic metabolic routes, which are tightly related to immunomodulation, but also to aiding in the clinical management of metabolic comorbidities.

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