Green Tea: A potent Immmunomodulatory Agent

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Introduction

The human immune system is a complex yet systematic network involving several defense mechanisms that warrant our well-being. We are exposed to millions of pathogens and other toxic agents every moment and the miracle of life is that we evade them successfully [1]. The immune defense mechanism interplays at different levels to combat such pathogens. There are specialized cells like different leucocytes, chemokines, cytokines and many more that play an important role in the defense mechanism. Furthermore the different mucosal surfaces with mucus and different secretions serve as an interface between the external and internal environment. These surfaces are vital as they allow different substances, toxins and pathogens to pass through them. The surfaces have specialized receptors and active substances that recognize and inhibit the harmful agents from entering the vital organs. It is here that the concept of immunomodulation becomes important. Immunomodulation refers to the onset of events towards immunostimulation or immunoinhibition, and the choice depends on the nature of the pathogen/agent crossing the mucosal surface as they serve as the gates of entry. A fine orchestrated immune-response is dependent on the presence of pattern recognition receptors (PRR) with pathogen-associated molecular patterns (PAMPs) [2]. Toll-like receptors (TLR) are PRRs which have different subsets that bind with different ligand and are expressed on different cell types. The TLRs are essentially important in combating the pathogens. Once the TLRs are activated by their respective ligands they trigger the transcription factors like NF-κβ which promote the transcription of different proinflammatory cytokines which enhances the immunity [3]. After pathogen detection, TLR signal transmission is initiated through the toll / interleukin 1 receptor (TIR) domain. Many TIRs use the adapter protein MyD88, which activates NF-κβ, activator protein-1 and Map kinases (MAPK) and triggers stream signaling. Therefore, MyD88 is important for inflammation and disruption of TLRs that often lead to chronic pathologic conditions and autoimmune diseases [4].

Due to the advent of different novel pathogens and the varied types of pleiotropism exhibited by them, research today is focussed towards identification of different novel immunomodulatory agents that can efficiently trigger the anti-inflammatory, anti-oxidative, antimicrobial response in the host and control the severity of disease pathogenicity. Many compounds of herbal origin and polyphenols have immunomodulatory action and many phytochemicals exert their action

by targeting the TLR. Reports have documented the medicinal value of Green tea from the leaves of *Camellia sinensis*. The green tea polyphenols (GTPs), especially (–)-epigallocatechin-3-gallate (EGCG) and (–)-epicatechingallate (ECG) can greatly enhance immune response and lower the risk of inflammation in different pathological conditions [5]. Over the past decades green tea has been used for its medicinal value as anti-inflammatory, anti-cancer and anti-oxidative agents. This review focuses on the immunomodulatory potency of the polyphenols of green tea and the recent advancements in this field.

Immunomodulatory effects of Green Tea Polyphenols (GTP)

GTP exerts their immunomodulatory role through the TLRs thereby triggering the downstream signalling cascade. EGCG has been shown to consistently achieve the healthy functioning of several body systems. EGCG polyphenols has shown promising immunomodulatory effects. Reports have documented the inhibition of NF-κβ mediated downstream signalling and MyD88, the adaptor protein required for the activation of TLRs [6]. The anti-inflammatory response of EGCG is mediated by inhibition of STAT-1. The production of IFN-γ by peripheral blood mononuclear cells stimulated by *Staphylococcus aureus* enterotoxin-B was also decreased when treated with EGCG [7]. In vitro studies have shown that EGCG can suppress the maturation of mouse bone marrow derived and human monocyte derived dendritic cells (antigen presenting cells) signifying their potent role in immune modulation. Furthermore EGCG could also prevent UV-mediated immune-suppression [8].

The mechanism of immunomodulation is dependent on the balance of Th1/Th2 response. T lymphocytes are a major source of cytokines [9]. These cells have specific antigen receptors in their cell surface to allow for the detection of foreign viruses. They can also see normal tissue during episodes of autoimmune diseases. There are two main components of T lymphocytes, which are separated by the presence of cell surface molecules known as CD4 and CD8. CD4-producing T lymphocytes are also known as helper T cells, and these are considered to be the major producers of cytokines. This subset can also be divided into Th1 and Th2, and the cytokines they produce are known as Th1-type cytokines and Th2-type cytokines. Th1-type cytokines usually produce inflammatory responses that are responsible for killing the internal intracellular parasites and promoting autoimmune responses. Interferon gamma is the main Th1 cytokine. Excessive inflammation can lead to uncontrolled tissue damage, so there must be a way to deal with this. Th2-type cytokines include interleukins 4, 5, and 13, which are associated with IgE stimulation and eosinophilic responses to atopy, as well as interleukin-10, which has a broad anti-inflammatory response. In excess, Th2 responses will withstand the microbicidal action of Th1 mediators. So the right situation may seem like humans should produce a balanced Th1 and Th2 response, which is a challenge for the immune system. GTPs slightly reduced IFNγ-induced phosphorylation and

STAT1 activity, inhibited the regulation of inducible nitric oxide synthetase (NOS) 2 and NF-κB, and inhibited the uptake and enhancement of TNF-α and IFNγ, all indicating that GTP response inhibits Th1. They may also be involved in blocking signals that allow for the maturation and function of the T-lymphocyte in the Th1 type [10-12]. IL-4, the signature cytokine of the Th2 response may be elevated by EGCG stimulation. Dietary GTPs may lower the IFNγ / IL-4 ratio, the Th1 / Th2 index.

Effect on Cellular Immunity

Reports have documented that GTP attenuated inflammation in IL-2(-/-) mice indicating their probable role as immunomodulators for treating chronic inflammatory conditions [10, 13]. Another study demonstrated that the ratio of CD3+CD4+ to CD3+CD8+ T lymphocytes in the peripheral blood increased in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice following treatment with EGCG, and EGCG reduced expression of inflammatory factors tumor necrosis factor-α and interleukin-6 in serum [14]. Studies have also shown that EGCG inhibited CD4 (+) T cell expansion in response to either polyclonal or antigen specific stimulation. Further, it was demonstrated that EGCG also inhibited Th1 and Th17 differentiation as a result of downregulation of transcription factors like STAT1 and T-bet for Th1, and STAT3 and RORγt for Th17 [15]. Such studies documenting the anti-inflammatory and protective role of EGCG has been shown in several studies using animal models of inflammatory arthritis. Leukocytes have the ability to migrate to intravascular spaces of the tissue to combat micro-organisms. The EGCG is also able to reduce the transmigration of neutrophils through the monolayers of endothelial cells. Studies have demonstrated that EGCG also has the potency to inhibit the neutrophil elastase and repress ROS activity [13]. Thus EGCG, the active principle of GTP has the ability to modulate the cellular immunity.

Effect on Humoral immunity

Humoral immunity is mediated by circulating antibodies. Studies by various groups have shown that polyphenols from green tea can cause a humoral reaction as evidenced by the titre of various antibodies. Studies have reported that polyphenols in green tea cause modulation in immunoglobulin-secreting splenocytes leading to IgG-mediated and IgM-mediated immunity in BALB / C mice immunized with antigens [16]. In the case of polyphenols, mice showed a sharp increase in IgM and IgG antibodies at days 14 and 21, respectively. Mice fed with GTP showed decreased levels of IgG total and type II collagen-specific IgG l in serum and arthritic joints. Studies have clearly shown the use of green tea in controlling the onset of arthritis [17]. In vitro studies also reported the mitogenic activity of splenic B-cells under the influence of green tea polyphenols (EGCG). In addition, EGCG also enhanced the direct plaque-forming cell (PFC) response in red blood cells of sheep, particularly the galloyl group played a key role in this. ECG, EGCG, and theaflavin

digallate (TFDG) have shown significant improvements in the spontaneous proliferation of B-cells [18], albeit with very different potency.

Anti-Oxidation based Immunomodulation

The burden of excessive oxidative stress often leads to cell damage and a deficient immune system. There are a few signs of oxidative stress such as reactive oxygen species (ROS), superoxide, oxygen ions and free radicals [19]. Usually, due to the metabolic activities of the cell, oxidative stress is triggered within the cell but is neutralized by an internal antioxidant that brings balance and aids the proper cell function. During a pathogenic infection or other pathological conditions the immune system tries to eradicate the pathogen which leads to an increased oxidative stress. ROS is used as an indicator of increased physiological oxidative stress [20-21]. However, uncontrolled ROS also damages T-cells, causes inflammation and often poses a risk to the host cell. Therefore, powerful anti-inflammatory and antioxidative agents can help control the severity of a particular pathological condition. Polyphenols of green tea like EGCG have aromatic polycyclic properties that can reduce free radicals and regulate oxidative changes [22]. The protective effect of EGCG is due to its ability to reduce lipid peroxidation, oxidative stress and the production of nitric oxide (NO) radicals by inhibiting iNOS expression.EGCG also promotes excessive production of pro-inflammatory cytokines and mediators, reducing NF-κB and AP-1 activity and subsequent formation of peroxynitrite by NO and active oxygen species. Therefore, EGCG effectively reduces cell damage by reducing the inflammatory response and reducing lipid peroxidation and NO-producing radicals that lead to oxidative stress. Green tea is proposed to be a dietary supplement in preventing heart disease where oxidative stress and proinflammation are the main causes. Studies have shown that tea extract significantly lowers GSH and increases GSSG levels in erythrocytes without G6PD in a dose-dependent manner, but not in normal erythrocytes. Similar dose-dependent responses to (-) - epigallocatechin (EGC) and (-) - epigallocatechin-3-gallate (EGCG), but not to other polyphenols, were observed. In vitro, tea extracts and polyphenols greatly improved the oxidative status of G6PD-deficient erythrocytes, as evidenced by a drop in GSH and an increase in GSSG, methemoglobin, and plasma haemoglobin [23]. The anti-inflammatory cytokine IL-4, leads to the transformation of the body from Th1 to Th2, and reduces growth stress, which has shown remarkable immunity. The anti-inflammatory role of Catechins (bioactive polyphenols in green tea) has been shown to diminish the expression of ICAM-1 and VCAM-1 in dental pulp cells after treatment with GTPs (EGCG and ECG) [24]. Polyphenols also reduced the expression of IL-6 and IL-8 in dental pulp cells exposed to Lipopolysaccharide or Peptidoglycan proving their anti-inflammatory role. ECG also caused DNA oxidative damage in lymphocyte cell nuclei by binding to copper and induction of ROS production [25].

Effects on autoimmune diseases

Autoimmune diseases have become a major threat in the modern world. They are diseases with complex pathology that are affected by genetic, infectious and environmental factors. Nutrition has served as a modifiable factor/environment for prevent /controlling the progression of autoimmune diseases. Autoimmunity is caused by genetic or acquired abnormalities in immunological tolerance or immune regulatory pathways, as well as molecular mimicry of viral or bacterial proteins and defective clearance of apoptotic cell debris. As a result, an abundance of autoantibodies and autoreactive cells may be the primary cause of autoimmunity. Limited amount of studies have documented the role of catechins (the most potent EGCG) of GTP in improving the status of autoimmune diseases. Diseases like rheumatoid arthritis, inflammatory bowel disease (IBD), Sjögren's syndrome [26] and Type 1 diabetes [27] and experimental autoimmune encephalomyelitis (EAE, murine model for human multiple sclerosis [MS]). Polyphenols isolated from green tea (green tea polyphenols, GTPs) has demonstrated anti-inflammatory and anticarcinogenic properties in experimental animals. Studies documented a reduced incidence of arthritis in GTP fed mice as compared to that in control mice without GTP. A significant reduction in the expression of inflammatory mediators such as cyclooxygenase 2, IFN-γ, and tumor necrosis factor-α in arthritic joints of GTP-fed mice indicating their immunomodulatory role in arthritis.

Sjogren's syndrome (SS) is an autoimmune illness that causes inflammatory cell infiltration and loss of function of the lacrimal and salivary glands, resulting in ocular and oral health issues. Molecular, cellular and animal studies have indicated that EGCG can provide protective effects against autoimmune and inflammatory reactions in salivary glands in diseases such as SS. Reports also documented that GTPs reduce autoimmune symptoms in a murine model for human SS and protect human salivary acinar cells from TNF-alpha-induced cytotoxicity. Similar reports demonstrated that in the NOD mouse, a model for human SS, oral administration of green tea extract reduced the serum total autoantibody levels and the autoimmune-induced lymphocytic infiltration of the submandibular glands. It was concluded that GTPs may provide a degree of protection against autoimmune-induced tissue damage in SS, mediated in part through activation of MAPK elements.

Green tea extract and EGCG treatments reduced the expression of Th-17 associated pro-inflammatory genes [interleukin 1 beta (IL-1), IL-6, IL-17A, and tumour necrosis factor alpha (TNF-)], with no detectable morphological defects in the liver and kidney in non-induced and autoimmune uveoretinitis (EAU) mice. The study suggests that GTE consumption can serve as a potent therapeutic agent as well as a food supplement for developing alternative treatments against autoimmune uveitis [28].

Conclusion

Many studies have documented the effect of different phytochemicals in different pathological conditions. Decades of research has focussed to find potent agents that could trigger the immune response so that the severity of the chronic infection can be controlled. Green tea extracts is one of the commonest beverages consumed worldwide. The biologically active polyphenols especially EGCG has shown promising role in triggering and modulating immune response. Different studies have reported the anti-inflammatory and anti-oxidative effect of the EGCG of GTPs, which have significantly made a difference in disease pathogenesis. Compared with individual compounds, green tea extracts showed more great health promoting potential in due to the synergistic effects of different compounds. To date, large-scale efforts have been made both in vitro and in vivo, but the exact mechanisms are still unclear. Therefore, very carefully designed studies are needed to in-depth define strong cellular immune systems and immune responses. In addition, guidelines include specifying specific mechanisms of action of green tea polyphenols, total therapeutic doses, duration of treatment, and outcomes in both in vitro and in vivo models, especially in the setting of inflammatory diseases, are also required to be performed. Whether GTP applies to TLRs with joint or non-binding binding they need further clarification.

Conflicts of Interest: The authors declare no conflict of interest.

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