Review Article

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REVISITING SWARNAPRASHANA THROUGH THE LENS OF GUT MICROBIOTA THEORY: A CRITICAL REVIEW

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ABSTRACT

A vast and intricate colony of commensal bacteria lives in the mammalian GI tract. Over millennia, this gut microbiota has co-evolved with its host and benefits it in a variety of ways, including but not limited to digestion, nutrition synthesis, detoxification, protection from infections, and immune system control. Numerous methods have been utilised to show that signals from the gut microbiota are essential for the immune system's development. Concept of Swarnaprashana in Ayurveda and its role in immunity can be understand on the basis of theory of effect of gut microbiota on immunity as honey in the Swarnaprashana acts as a source of various micro-organism as it formed as a result of mixture of nectars from various flowers. This honey and clarified butter containing Swarnaprashana acts as a source which maintains microbiota which upon stimulation triggers the immune response at subclinical level without causing any kind of infection and improving the response for the second time infection and also increase the number of immunoglobulins in the immune system.

Keywords Swarnaprashana, immunity, microbiota, bacteria.

INTRODUCTION

Immunity is a well-known word that explains the importance of one's defence mechanism that is vital for the maintenance of healthy life. The immune system is now being regarded as an equally important system with other vital system in the human body. The immune system activities decide the health status of the person. If the immune system over functions it results in various auto-immune disorders and if the system fails to work it causes repeated infections of various bacteria's, viruses etc that otherwise would have been neutralized by the body defence cells. The Gut microbiota is the term for the complex community of commensal bacteria that are present in human intestine and it benefits the human body in a variety of ways, including but not limited to digestion, nutrition synthesis, detoxification, protection from infections, and immune system control. Various studies have confirmed the role of gut microbiota in the maintenance of both innate and acquired immunity.

Ayurveda provides various concept of Vyadhikshmatva (Immunity) or the internal strength and is termed as the acquired capacity of the body to halt the process of a disease or alter the course of an already manifested disease towards a lesser extent. Acharya Kashyapa has narrated the concept of Lehana (electuary) in Lehana Adhaya which is the process in which child is given gold preparations to booster his immunity, called Swarnaprashana. Swarnaprashana is a method in which Swarna Bhasma along with Madhu (honey), Ghrita (clarified butter) and various herbs, are taken in liquid state, semi-solid or paste orally. According to Acharya Kashyapa method for Swarnaprashana includes titrating Swarna along with honey, clarified butter and water on pre-washed stone with the child facing East direction and is made to lick the compound. Acharya Kashyapa further explained the reward for the administration of Swarna (Gold) in children for:

Medhavardha (Improving Intellect), Agnivardha (Promoting digestion and Metabolism)

Balavardha (Promoting immunity and Physical Strength), Ayushyam (Promoting Longevity)

Mangalam (Auspicious), Punyam (Virtuous), Varnya (Improves Complexion), Vrishya (Fertility), Grahapaham (Protection against infectious organism). [1]

UNDERSTANDING GUT MICROBIOTA AND IMMUNE SYSTEM

Gut Microbiota and Immune Homeostasis

The importance of signals obtained from gut microbiota for immune system development has been shown using a variety of methods. A potent method for demonstrating the significance of the microbiota in determining both innate and adaptive immunity is the use of germ-free (GF) models, in which animals are raised in sterile environments and hence have never been exposed to any microbes. [2] Alternately, altering the microbiota through antibiotic treatment or microbial reconstitution also offers important proof for the microbiome's function in immunological homeostasis. [3] These methods are also helpful for figuring out how the microbiota plays a part in autoimmunity, which will be covered in a later section. One important point is that the gut microbiota has a significant impact on both the systemic immune responses as well as the local intestinal immune system. We will discuss how gut microbiota influences innate and adaptive immunity to attain immunological homeostasis in this section.

Microbiota and innate immune homeostasis

An important characteristic of intestinal APCs, which co-evolved with the microbiota, is their capacity to defend the body against

infection while preserving immunological tolerance to the typical gut flora. For instance, compared to splenic DCs activated under comparable conditions, dendritic cells (DCs) of Peyer's patches (lymphoid nodules implanted in the gut wall) produce large quantities of interleukin-10 (IL-10). [4] When they come into contact with microbial stimuli under homeostatic settings, gut macrophages, like DCs, adopt a distinct phenotype known as "inflammation anergy," which refers to the noninflammatory profile of intestinal macrophages. [5] For instance, in response to microbial stimuli like Toll-like receptor (TLR) ligands, a group of microbe-associated molecular patterns, intestinal macrophages do not create pro-inflammatory cytokines. [6] Numerous studies offer concrete proof of the gut microbiota's crucial function in controlling the growth of APCs. In GF animals, there were fewer intestinal but not systemic DCs, and Escherichia coli mono-colonization was sufficient to attract DCs to the intestines in GF animals. [7] Furthermore, it has recently been demonstrated that microbe-derived ATP stimulates a subset of DCs that have CD70 and CX3CR1 on their surface, which in turn triggers the development of Th17 cells. [8]

Microbiota and adaptive immune homeostasis

The acquired immunity is a type of immunity that develops after exposure to a suitable agent and is regulated by the activity of CD4 T cells. The mucosa of intestine is being made up of epithelium and basement membrane and between these two layers lies Lamina propria, a loose connective tissue. This Lamina propria is the source of intestinal CD4 T cell, which upon stimulation gets converted into 4 subtypes: Th1, Th2, Th17 and Treg (regulatory). All the subtypes require particular transcription factor and a particular species of bacteria to cause the differentiation. [Figure 1]

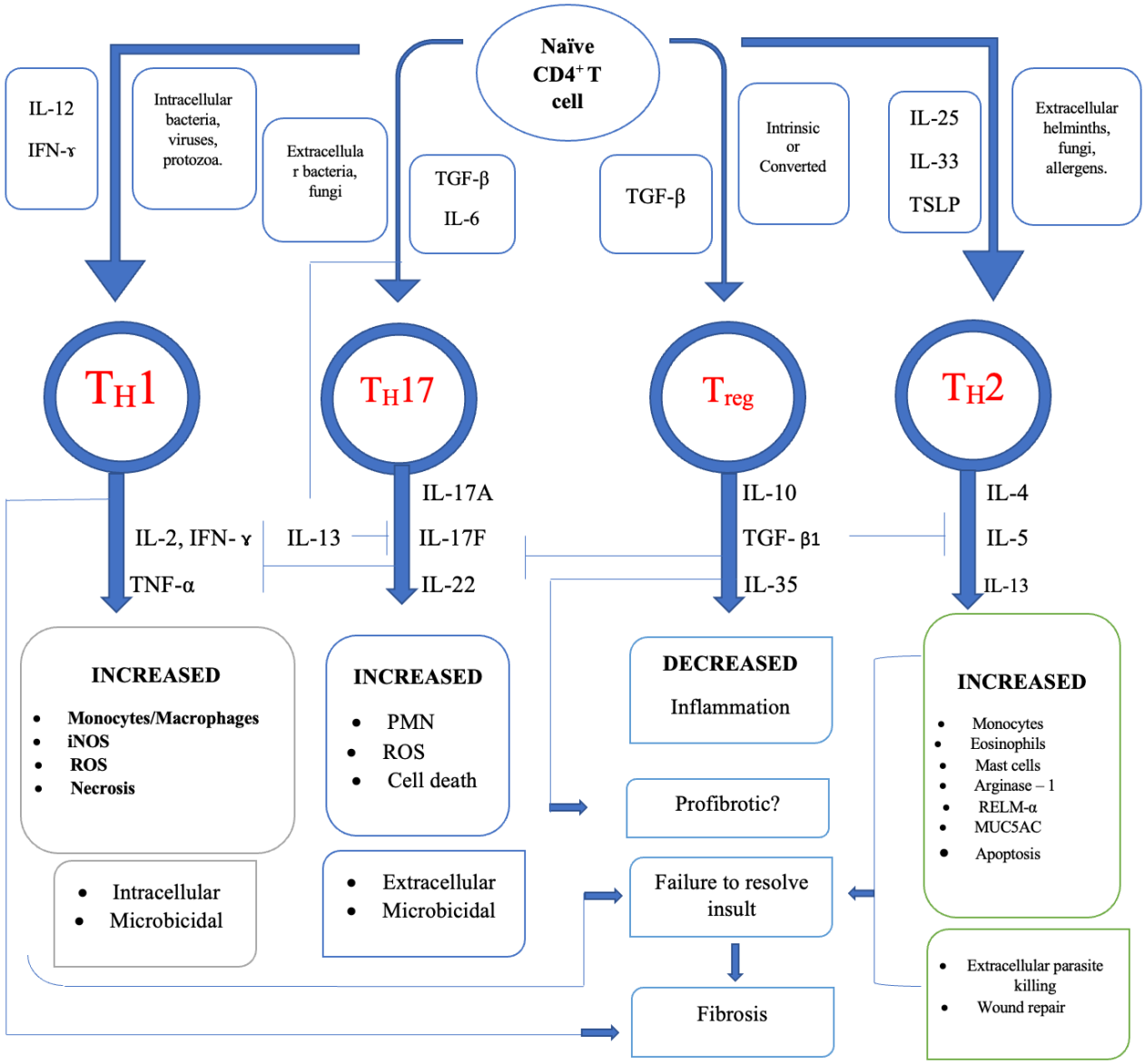

Figure 1: The four main cell types that can develop from naive CD4+ T cells are Th1, Th2, Tregs, and Th17. A transcription factor specific to each lineage must be induced in order for it to differentiate. Each lineage secretes a unique (set of) cytokines after differentiating.

Th2 cells are crucial for the host's defence against parasite infections and Th1 cells are essential for the host's protection against intracellular microbial infection. Immune tolerance is mediated in part by Treg cells, whose malfunction can result in autoimmune diseases. Both inside and outside the intestine, the gut microbiota is crucial for the development of CD4+ T lymphocytes. Macpherson AJ *et.al* explained through its study on germ free model that there was significant reduction in number of

LP CD4 T cells in germ free models of mice as compared to a normal mouse. [9] Mazmanian SK et.al confirmed in his study that splenic and mesenteric lymph nodes were not having lymphocytes zones in the germ-free models as compared to a normal mouse and also demonstrated that the polysaccharide A (PSA) molecules produced by Bacteroides fragilis cause the emergence of a systemic Th1 response. [10] Atarashi K *et.al* demonstrated that clostridial species, particularly those from clusters IV and XIVa, can facilitate the development of colonic Tregs. [11]

The intraepithelial region of the gut contains the majority of the intestinal CD8+ T lymphocytes. Intestinal CD8+ T cells in germ free animals were less numerous and less cytotoxic, indicating that signals from the microbiota are crucial for preserving the population and functionality of intestinal CD8+ T cells. [12] Wei B *et.al* explains that the gut microbiota plays a crucial role in conditioning CD8 T cells, so that is controls the activity of other peripheral immune cells, such as marginal zone B cells, plasmacytoid dendritic cells, and invariant natural killer T cells. [13]

Gama Delta T cell is known to be a connecting link between innate and acquired immunity. The concentration of Gama Delta T cells is more prominent in intestinal intraepithelial lymphocytes as compared to lymph nodes. Gama Delta T cell functions its activity in expressing CD8 T cells and perform lytic activity. Kawaguchi-Miyashita M *et.al* explains in his work that the absence of commensal microbiota has little effect on the amount of Gama Delta T cell but it does affect the cytolytic activity of these cells as seen on germ free model of mice. [14]

The Peyer's patches are the primary sites of gut-associated B lymphocytes, the majority of which are plasma cells that are the source of immunoglobulin (Ig) A. Daily production of IgA is thought to be 0.8 g per metre of intestine, which is significantly more than the sum of all other immunoglobulin classes. [15] Crabbé PA *et.al* confirmed in his experiment that there were lower amount of IgA and fewer plasma cells in the colon of germ-free mice as a result of the Peyer's patches' number and cellularity being drastically reduced in germ free animals. It can be confirmed from the experiment that commensal microbiota can be regarded as driving force for the appropriate production of immunoglobulins. [16]

Swarnaprashana and Gut Microbiota

Effect of Honey on Microbiota

Internal administration of honey explains the concept of Prebiotics to improve the gut microbiota. Prebiotics are food components that give specific probiotic genera a fermentable carbohydrate substrate, enhancing the host's health by regulating the balance of bacteria in the gut. [17] Pancreatic amylases and brush-border enzymes like dextrinases and glucoamylases are essentially incapable of breaking down the prebiotic carbohydrates. Prebiotics are crucial for positively altering the colonic microflora, which may have become less dominated by lactobacilli and bifidobacteria as a result of several circumstances, including medication, chronic stress, and normal ageing. The capacity of prebiotics to inhibit the development and activity of infections and other unwanted microorganisms is a reliable sign of their efficacy. Numerous fermentable substances from the meals that should be digested instead end up at the large intestine's aperture. [18] Since their metabolism is totally saccharolytic and lacks any proteolytic activity, lactobacilli and bifidobacteria, which ferment the non-digestible oligosaccharides, are regarded as helpful bacteria. End products of anaerobic saccharolytic fermentation include gases and short-chain fatty acids (SCFA), which are anti-inflammatory, inhibit pathogens, regulate appetite, and reduce the risk of cancer and cardiovascular diseases, in addition to the growth in bacterial cell mass and the release of energy. [19] These end products of fermentation results in the proper maintenance of the gut microbiota which upon stimulation is capable of producing antibodies and provide immunity. Honey has antibacterial elements and perhaps prebiotic oligosaccharides, which together may increase the probiotics' ability to fight infections. Other claimed advantages include greater levels of SCFA, improved probiotic persistence in the GI tract, and better resistance to infections, in addition to raising the viable cell count. [20]

Effect of Ghrita on Microbiota

Ghrita (clarified butter) is a basic constituent of Swarnaprashana. It can be used in a simple plain form or can be fortified with medicated drugs like Vacha (*Acorus calamus* L.), Brahmi (*Bacopa monnieri* (L.) Wettst.), Shankhpushpi (*Convolvulus pluricaulis* Choisy) etc which are having nootropic properties. Medicated clarified butter can serve both the purpose of medium as well as source of medicaments. Unlike other oils, clarified butter contains butyric acid; a short chain fatty acid which gives distinct flavour and help in digestion. Beneficial microorganism in intestinal convert fibre of clarified butter into butyric acid and that forms the source of energy and intestinal wall support. Once the intestinal walls are supported and protected, the intestine continues to be a source of antibodies which maintains the immunity. Studies have confirmed that people with unhealthy digestive tracts do not produce butyric acid. [21] Research also suggests that the production of killer T cells in the gut get adequate support from production of butyric acid and as a result strong immune system. [22]

Effect of Swarna Bhasma on Microbiota

Swarna Bhasma is the basic part of Swarnaprashana. All the advantages which Swarnaprashana presents is predominantly the result of Swarna Bhasma. Since ancient times gold was used as a source of medicines and with the advancement of time evenly prepared small particles of gold are used in medicines. The immunomodulatory nature of gold is widely accepted which confirms its role in the maintenance of immunity. K Hashimoto *et.al* explained in his study that at pharmacologically appropriate concentrations, either gold sodium thiomalate (GST) or auranofin (AUR) inhibited the proliferation of human T cells and the production of interleukin 2 by Jurkat cells. It was investigated in vitro to see if gold compounds may inhibit protein kinase C (PKC) that had been partially separated from Jurkat cells because protein kinase C (PKC) is known to have a role in the activation of T cells. In contrast to AUR, which did not significantly inhibit PKC at pharmacologically relevant concentrations, GST was found to significantly inhibit PKC in a dose-dependent manner. These results suggest that the immunomodulatory properties of GST may result from its capacity to decrease PKC activity. [23]

Associated with age

The introduction of solid food causes a secondary shift in the gut microbiota, which causes substantial alterations in the microbiome shortly after lactation. Up to the age of about 2-3 years, the infant is exposed to low bacterial diversity and a high rate of microbial flux. The gut microbiota is developing throughout this window, and alterations have been related to a higher risk of autoimmune disorders and metabolic issues in later life. [24] The gut microbiota stabilises after about 3 years, keeping relative proportions of species but making compositional changes more difficult to impose. In this approach, the early years of seeding and building the core gut bacteria profile may be crucial for the host's health as an adult. Environmental influences, such as nutrition and antibiotics, but also host metabolic and

immunological homeostasis disruption, can still have an impact on or disturb microbial composition after the age of three. [25]

Dose of Swarnaprashana

According to Ayurvedic Pharmacopoeia of India, adult dose of Swarna Bhasma is 15mg to 62.5mg. Dose for Swarna Bhasma can be calculated by using young's formula. If a 1-year-old child weighs 10kg then daily dose of Swarna Bhasma will be ranging from 1.15mg - 4.8 mg. An average of 2.975mg of Swarna Bhasma can be given in a day to a 1year old child. By using similar formula dose for children up to 16 years can be calculated.

Duration of Swarnaprashana

Again, there is a great deal of debate on the length and nature of duration for administration of Swarnaprashana. because the precise length of the treatment is not stated in a clear-cut manner. According to Acharya Kashyapa, Swarnaprashana for one month and six months has advantages for higher mental processes. In present era daily administration of Swarnaprashana is difficult due to fast moving life of both parents and children. So, a monthly dose that is not listed anywhere is the method used in a modified form of Swarnaprashana. This periodic administration with a set interval between doses may have the effect of a booster dosage and provide the immune system ample time to become sensitised. From the time of birth to the Kumara Avastha (until the conclusion of age of 16), this can be given. A day in month which coincide with Pushya Nakshatra (holy star) have been adopted to keep regularity as Swarnaprashana is changed to suit the convenience of people. The general public is sensitive to these difficulties, and a holy day provides emotional comfort. Acharya Sushruta also made it very clear that administering all Swarna preparations on the day of Pushya-Nakshatra maximises their effectiveness and potency.

Acharya Kashyapa explains that 1 month use of Swarnaprashana makes the child Param Medhavi (highly intelligent), while 6-month continuous use make it Shruta Dhara (improves memory and grasping power). If we count days for 6 months it accounts for roughly 180 days. Monthly administration of Swarnaprashana on a fixed date will last for 15 years. So, it can be explained that maximum duration of Swarnaprashana can be up to 15 years if provided on monthly basis. At the same time prolonged use of Swarnaprashana nourishes the Dhatu (major structural components of the body) and up to 16 years of age according Acharya Charaka, Dhatu are Apparipakva i.e. immature. So, to make them timely mature and proper Swarnaprashna can be used till 16 years of age. Also, in the new born period if the child is derived from breast feed, it can be advised for Swarnaprashana as it is a source of a variety of important fatty acids, glycoproteins, saturated fatty acids, lipoproteins, folic acid, and vitamins. All these are essential for proper brain development of the child. So, it can be said that Swarnaprashana mentioned by Acharya Kashyapa can be used since birth up to 16 years depending upon the situation of the child.

Safety of Swarna Bhasma

This study, which describes the toxicity aspect of Swarna Bhasma, used three distinct doses of the herb: a low dose (3 mg/kg), a medium dose (15 mg/kg), and a high dose (30 mg/kg). It was given to male and female rats separately over the course of 90 days. After 45 and 90 days of Swarna Bhasma treatment, several haematological, biochemical, and histological tests were performed. With a dose up to 30 mg/kg, which is around 10 times more than the therapeutic level for humans, Swarna Bhasma's toxicity was evaluated in the rat model. After 90 days, all Swarna Bhasma treated groups had higher neutrophil counts in male rats than the control group in terms of haematological markers. This change, however, was not statistically significant. Additionally, in female rats treated with Swarna Bhasma, neutrophil counts at equal doses did not result in the same alterations. Therefore, even at a dose that was far higher than the therapeutic dose for humans, haematology measures in rats did not suggest any negative effects of Swarna Bhasma. Most significantly, the biochemical parameter alterations were well within acceptable bounds and the typical range. Under the current experimental setup, the histopathological changes seen at a dosage of 30 mg/kg are typically seen in laboratory rats of this strain and age. The Swarna Bhasma therapy had no effect on the animals' feed intake, body weights, or organ weights. As a result, it can be said that Swarna Bhasma is safe up to a level of 30 mg/kg (10 times the therapeutic dose) based on the rat study. [26]

Additionally, a prior study found no evidence of any negative effects from Swarna Bhasma up to 13.5 mg/kg dose in Wistar rats.[27] From these studies it can be said that administration of Swarna Bhasma is safe if prescribed in an appropriate amount.

DISCUSSION

Swarnaprashana is the process in which Swarna Bhasma (Gold) is administered to the recipient in the media of Madhu (Honey) and Ghrita (clarified butter). Swarna Bhasma is proven to be having an effect in the improvement of the immune response. [28] The role of honey and clarified butter in Swarnaprashana can be explained by its effect on the gut microbiota. Honey is a fermented food that is prepared from nectars of different flowers while clarified butter is a butter fat produced from cow's milk. The properties of honey depend upon the pollen or flower from which the nectars are extracted by the honey bees. Honey contains both monosaccharides and oligosaccharides. [29] Monosaccharides like Glucose and Fructose are immediate source of energy and provide suitable input for metabolism that results in proper growth of an individual. On the other hand, oligosaccharides undergo saccharolytic reaction without any proteolytic activity in the presence of bacteria in the gut like lactobacilli and bifidobacteria, which ferment the non-digestible oligosaccharides. This anaerobic reaction results in the formation of short chain fatty acids and few gases that provides suitable media for growth of gut microbiota and also acts as anti-inflammatory and limits different type of infection. [30] As the gut microbiota remains intact it results in sub-clinical infection of the intestine which further trigger Peyer's patches in the intestine to release immunoglobulins from the B lymphocytes. These immunoglobulins remain in the circulation and provide protection from various infections and result in improvement in the immunity of the individual. At the same time various species of the gut microbiota are essential for the differentiation of T cell into subtypes like Th1, Th2, Th17 and Treg. All these T cells are crucial aspect in maintenance of immune system of an individual.

Various Acharyas advised the administration of Swarnaprashana to be started as early as possible through Lehana Sanskara or continue at least up to age of 1 year as by Acharya Indu. The reason behind this is explained through the concept of development of gut microbiota.[31] Gut microbiota is found to be slowly developing after ingestion of solid food that is Ksheeranada stage in Ayurveda. As the child advances to take solid food there is secondary shift from milk to solid food that result in much more different exposure to its gut. This exposure results in the growth of different varieties of bacteria in the gut of the child. The development of such varieties of bacteria depends upon the cultural food of the family that are commonly taken by the family members and is now a part of its core. So, it can be said that first year i.e. Ksheeranada Avastha is crucial for the development of gut of a child and the administration of Swarnaprashana during this period is beneficial for the

development of gut microbiota which provides the child with proper immune response.

Both honey and clarified butter after complete digestion results in the formation of oligosaccharides and Butyric acid respectively. These end products act as substrate for the maintenance of microbiota. As microbiota is confirmed to be source of antibodies, it plays an important role in maintenance of immunity. Swarna Bhasma directly plays an important role in immunity by stimulating peritoneal macrophages. So, honey and clarified butter are not merely a medium for administration of Swarna Bhasma, but they are also having direct effect on immunity through their action on gut microbiota.

CONCLUSION

From the available studies it can be concluded that there is significant role of gut microbiota on immune system. The gut microbiota is crucial for the modulation of the immune response and its maintenance is an important aspect to remain disease free. Concept of Swarnaprashana is always advised to improve the immune status of the child. Honey and clarified butter in the Swarnaprashana can be explained to be beneficial in improving the immune system by its action in maintaining gut microbiota. Both honey and clarified butter upon digestion yields products which provides maintenance to the gut microbiota and thereby explaining its role in immunity. This can be concluded that administration of Swarnaprashana is an immunomodulatory drug as it regulates the gut microbiota.

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