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Review Article

The Innate Immunity Defense against Gastrointestinal Nematodes: Vaccine Development

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ABSTRACT

The nematode parasite infects both humans and animals, causing severe infections. Their unusual surface structures, in particular, pose significant challenges to the immune system. Vaccine-induced immunity, mediated by the innate immune system, could be crucial in the development of an adaptive effector response. The purpose of this paper was to provide an overview of recent research on the host's innate immune system, barriers, and cells that respond to parasitic nematodes. This study investigated the nematode-associated molecular patterns that may recognize by host. Given the innate defense is more than just a static barrier against pathogen infections. It can actively contribute as a director of the adaptive immune response, which is ultimately responsible for the rejection of invasions. The role of innate defense against pathogen infections is located in zone of researcher concentration. Some nematode parasites can actively move through tissues, they pose a challenge to the innate immune system. Furthermore, their cuticular surface, which varies with each molting, cannot be phagocytosed. The nematode's thin, carbohydrate-rich surface layer, as well as the chemicals produced by this layer, cause the first contact with the host's innate immune system. Notably, all components of the innate immune response can be activated and play an important role in the adaptive immune effector response.

1. Introduction

Gastrointestinal nematodes are among the most prevalent worms that infest humans[1]. People are frequently exposed to nematode parasites, particularly in countries lacking proper medical services and effective hygiene standards. Nematodes can cause significant injury to infected humans or animals. They can be transmitted through water, food, soil, or close contact with animals. These parasites can cause damage to several tissues and organs by feeding on host tissues or locating larval stages inside organs[2]. In general, nematodes can be avoided by increasing basic hygiene standards. Mild anemia, gastrointestinal pain, diarrhea, decreased cognitive development, or limited growth are all nematode infection symptoms. Furthermore, nematodes infest animals, and controlling gastrointestinal (GI) nematodes is critical to improving animal health and welfare and expanding livestock production[3]. Many common and costly diseases are caused by GI nematodes in food animals such as small ruminants, cattle, pigs, and poultry production systems worldwide. Some gastrointestinal nematode species are sensitive to animals, particularly those with outdoor access and pigs and poultry kept indoors. In lambs, for example, *Haemonchus contortus* can cause substantial death rates[4]. Gastrointestinal nematodes are primarily responsible for chronic infection and concealed subclinical losses, which affect wool growth and quality, milk production, weight loss, and reproductive issues[5]. Farmers must raise their production efficiency to remain competitive as a result of these losses. Infection of livestock with gastrointestinal nematodes has resulted in serious health issues as well as a loss of output. This issue has sparked increased interest in disease control approaches such as anthelmintic medicines, vaccinations, and selective breeding for host resistance. Although with an increased risk of drug resistance among

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gastrointestinal nematodes, adaptive and innate immune responses to these parasites enhance the interests of researchers in studying this issue[6].

Nevertheless, nematodes co-evolved with their hosts to develop mechanisms that prevented excessive immune responses, which enabled them to continue their lives. Incredibly these nematodes produce many different and particular molecules that affect the microenvironment around them, the density of tissues, and the immune system[7]. These parasites have different types of immunomodulatory molecules at different life stages. Additionally, nematodes secrete a variety of miRNAs, immunomodulatory proteins, vesicles, and other molecules, called excretory-secretory (ES) products, to weaken the immune system[8]. Some of these parasite molecules are homologous to host molecules through the expression of miRNAs that target host gene expression or mimic host proteins. In this way, parasites can manipulate immune cell function to their advantage. Hosts must orchestrate their immune response to counter this parasite survival strategies[9]. This response includes maintaining a balance between immunity against helminths and wound healing without disturbing the immune system to the point of inflammation in the body[10]. This review aimed to identify research priorities in animal gastrointestinal nematode control. Numerous cellular and molecular processes can boost immunity response against enteric roundworms, as described in this study. Several helminth species mimic human infections, including *Trichuris muris*, *Nippostrongylus brasiliensis*, *Trichinella spiralis*, and *Heligmosomoides polygyrus*. This paper aimed to review the latest findings in immunoregulation against gastrointestinal nematodes and host protection.

2. Immune system

The mammalian host has evolved a typical immune defense system comprised of eosinophils and mast cells to combat parasitic worms. In this situation, type II immunity, also known as allergic immunity, occurs. Even though most recent immunological revisions have focused on the most recent effector mechanisms that operate throughout immune-mediated rebuttal. The effectiveness of an adaptive immune response is likely mediated by the primary activation of the innate immune system on its first encounter with a pathogen.

2.1. The first line of intestinal defense: The concealed mucus barrier

Body epithelia include the skin, tubular structures, and respiratory, gastrointestinal, and urogenital systems. Epithelia facilitate enzyme digestion of the compound nutrients due to their extended surface areas. Glycocalyx, which lies above epithelial cells, can prevent the attachment of the mucus layer of microorganisms[11]. This barrier is constructed of polymeric, gel-forming mucin. The goblet cells produce giant O-linked glycoproteins as well as other preservative factors. Mucin generation and its attributes are influenced by the immune regulation of goblet cells throughout infection. Mucin is an essential component of innate immune defense that acts as a distrustful barrier between the host and invaders such as parasites[12]. Furthermore, disulfide-linked mucin polymers are lubricants that prevent the epithelial surface from drying out and binding pathogens. As food moves through the gastrointestinal tract, peristalsis movement helps protect it from infectious agents. Studies have shown that several components of the immune system constrain mucus production[13]. Undeniably, the type 2 cytokines, including interleukin-13 (IL-13) and IL-4, play an influential role in the differentiation and proliferation of glycocalyx components, differentiation, and proliferation. Mechanisms such as intestinal motility, pancreatic secretions, gastric juice, intestinal microflora, and bile also inhibit microorganism invasion in the gastrointestinal tract[14]. The Paneth cells below the epithelial stem cells in the small intestine make antibacterial and antifungal peptides known as crypts or α-defensins[15].

2.2. Second line of defense: Innate immunity sentinel cells

2.2.1. Neutrophils

Neutrophils play an essential role in eliminating parasites from the body and entering the parasite bodies through their natural processes[16]. Neutrophil lysosomes contain enzymes, proteins, and peptides converted to an intracellular antiparasitic response[17]. The neutrophils' ability to conceal a variety of toxic substances allows them to kill microorganisms near them. Nitric oxide (NO), hydrogen peroxide (H2O2), and superoxide anion are the most significant toxins produced by neutrophil[18]. NADPH oxidases have these toxic products in the lysosome. The NE (neutrophil elastase) is responsible for causing neutrophil chemotaxis and digesting parasites' bodies[19].

2.2.2. Macrophages

In addition to phagocytosis, macrophages produce toxic free radicals, cytokines such as IL-12 and IFN-γ, and other chemokines[20]. In the gastrointestinal tract, macrophages are mainly in the smooth muscles (muscularis externa), submucosal tissue, and mucosa[21]. While macrophages are generally the most effective against bacterial infections, some nematodes can stimulate these cells directly[22]. Larvae and adults from *Ascaris suum*, *Toxocara canis*, and *Trichinella spiralis* could produce different ES products that may induce alveolar or peritoneal macrophages to produce nitric oxide (NO). *Toxocara canis* ES secretes prostaglandin E2. In the case of *T. spiralis*, the interaction between macrophages and larvae is between the mannose receptors (MR) expressed by the cells and oligosaccharide structures expressed by the larvae[23]. *Acanthocheilonema vitae* produce ES-62, which could modulate macrophage activation via TLR-4, expressed by dendritic cells. IL-13 or IL-4 activate alternately activated macrophages (AAMφ)

but do not upregulate their inducible Nitric Oxide synthase. As well as increasing the expression of the Macrophage receptors and specific chemokines, AAMφ produces Ym1, Fizz, and arginase[24]. Activation of AAMφ by nematodes may happen indirectly. Mast cells produce IL-13 and IL-4 in response to innate immune activation. By stimulating AAMφ, these cytokines would increase the expression of MR, enabling direct interaction with nematode glycoproteins. AAMφ precise role is unknown, although it may function as an effector, suppressor, and repair cells[25].

2.2.3. Dendritic cell

There is no doubt that dendritic cells represent the most potent antigen-presenting cells (APC), and they are the only APCs capable of activating naive T cells. A dendritic cell migrates from the blood to the tissues at the end of its immature state[26]. During macropinocytosis and phagocytosis, dendritic cells engulf many extracellular fluids. Mature dendritic cells migrate to lymph nodes when they encounter a pathogen. The dendritic cell can engage nematodes and their products by receptors such as C-type lectins, mannose receptors (MR), and Toll-like receptors (TLR)[27]. In addition, nematodes can utilize excretory-secretory products to modulate dendritic cell function during larval phases[28].

2.2.4. Mast cell

The vital role of mast cells in the clearance of nematode infections differs from species to species[29]. Activated mast cells produce cytokines such as leukotrienes, IL-5, IL-4, and chemokines, including histamine, heparin, and proteases[30]. During activation, mast cells are degranulated, bind to immunoglobulin E, and crosslink to the FcεR receptors. Histamine secreted by mast cells can activate eosinophils[31].

2.2.5. Eosinophils

Eosinophils and mast cells can penetrate the nematodes cuticle[32]. The granules of eosinophils contain several cationic proteins capable of releasing proinflammatory cytokines, chemokines, and lipid mediators[33]. The numeral of peripheral blood eosinophils increases significantly throughout parasitic infections. This action occurs because of the effect of T helper 2 (Th2) cell-derived IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Eosinophils are engaged by Eotaxin, from blood circulation to inflamed or damaged tissues[34]. The eosinophils become ready by communication with connective tissue matrix proteins like laminin and fibronectin before becoming stimulated by cytokines via receptor-mediated signals. Then, the activated eosinophils release helminthologic or histotoxic reactive oxygen species and granular proteins[35]. A diverse range of cell receptors is present on eosinophils. These receptors enhanced cell signaling, including apoptosis, adhesion, chemotaxis, degranulation, production of cytokines and chemokines, and respiratory burst[36]. These can be strongly associated with eosinophil-mediated tissue inflammatory responses in helminth infection. The most recent experimental studies have indicated that eosinophils can perform as antigen-presenting cells (APCs)[37]. Eosinophils have the aptitude to provide and present an assorted range of parasitic, microbial, and viral antigens[38]. Eosinophils in helminth infections are engaged in tissue inflammatory responses, but their defensive task against tissue-invasive helminths is still arguable[39]. Eeosinophils can be distinguished by bilobed nuclei and four primary granules. The primary granule is the central creation zone of Charcot Leyden Crystal protein (CLC or galectin-10)[40]. There is a possibility that CLC is engaged in the association between eosinophils and the numerous carbohydrate remainders that parasitic worms carry on their surfaces. Cytotoxic granular proteins include eosinophil neurotoxin (EDN), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and significant essential protein (MBP) located in the crystalloid secondary granule nearby several cytokines[41]. Eosinophils, with the assistance of Eotaxin-1, adhesion molecules, and IL-5, could move to the peripheral blood circulation and migrate to particular tissues, particularly the gastrointestinal tract Eotaxin-1[42]. Reactive oxygen species (ROS) are toxic complexes secreted by eosinophils and other toxic granule proteins like EDN, MBP, and ECP. ROS produced by the NOX family of NADPH oxidase and can be activated by the IL-3, IL-5, C5a, GM-CSF, and Eotaxin[43].

2.2.6. Natural killers

Natural killers respond immediately to injured cells and do not require activation. Because of this characteristic, they are considered a sort of cytotoxic lymphocyte, which is highly significant to the innate immune system[44]. Two noble features boost the value of natural killers. First, compared to other innate immune systems, they recognize stressed cells without pre-activation and could respond faster[45]. Second, they play a crucial role in surveillance against tumor[46].

2.2.7 Complement system

The complement system plays an imperative role in innate immunity. The complement system can directly suppress pathogens or stimulate inflammation[47]. Some studies reported that nematodes activate complement systems in different pathways on their surfaces[48] (Figure 1).

2.2.8. Toll-Like receptors

Understanding parasite mechanisms is essential for developing an effective innate immune response[49]. Such identifications could be attributed to a pattern recognition receptor, such as a Toll-like receptor (TLR)[50]. TLRs are

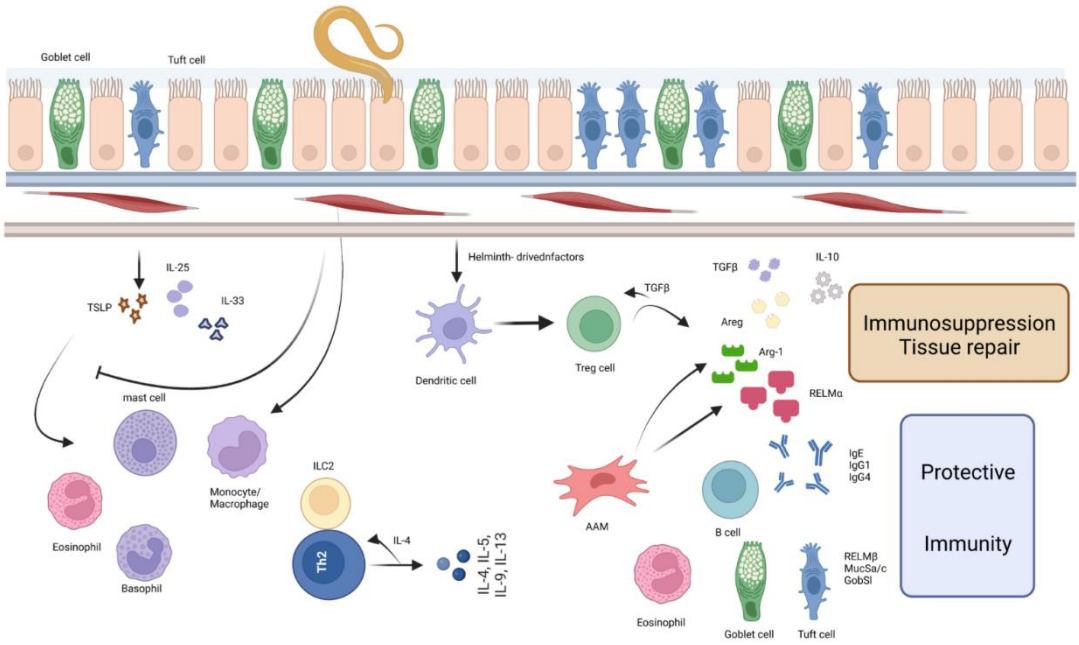

Figure 1. Innate immunity sentinel cells. Mechanisms that gastrointestinal nematodes activate the innate immune system. IL: Interleukin, ILC: Innate Lymphoid Cells, Ig: Immunoglobulin, TGF: Transforming Growth Factor, TSLP: Thymic Stromal Lymphopoietin, Areg: Amphiregulin Regulates. (The authors designed the figure)

important for activating immune cells such as dendritic cells and macrophages by detecting microbes and parasites, according to current research. Toll-like receptors, which play an important role in antigen recognition, are one of the most commonly discovered pattern recognition receptors[51]. TGF- is an immunosuppressive receptor that can improve the environment for nematode survival by reducing gut inflammation[52]. Mice with TLR4 protein mutations had a stronger type II immune response to *Onchocerca volvulus* infection[53]. They were unable to kill the parasite, indicating that TLR4 is required for vaccination-induced immunity. The ES62 glycoprotein of *Acanthocheilonema vitae* stimulates type II immunity while suppressing type I immunity via a TLR4-dependent mechanism mediated by phosphorylcholine (PC). ES62 affects mice lacking TLR4 but not mice lacking TLR2[54] (Figure 2).

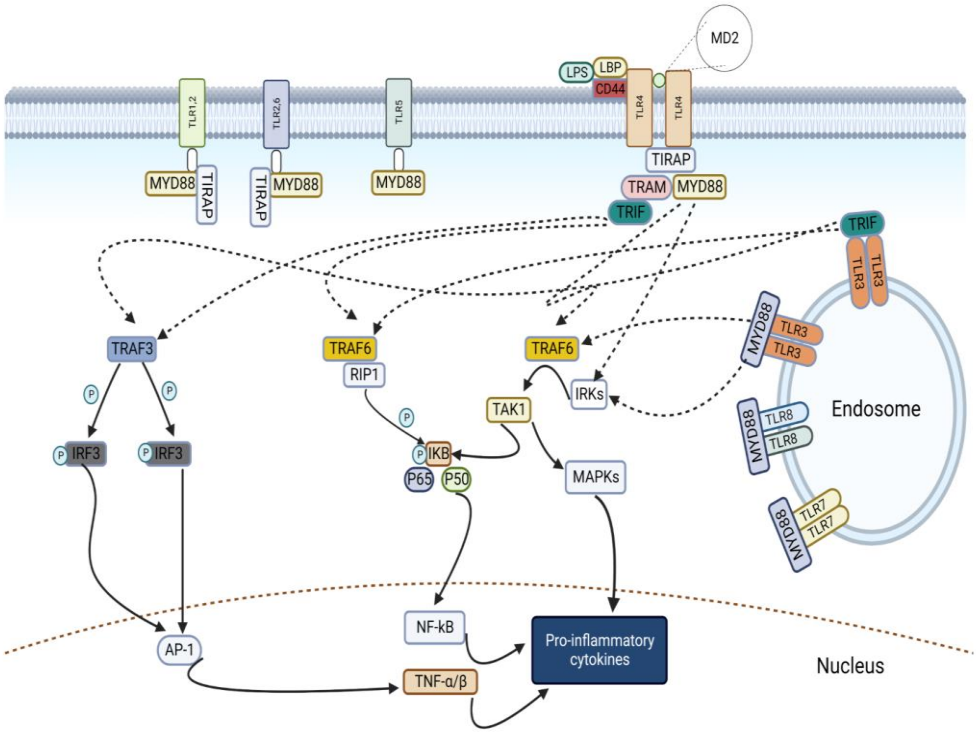

Figure 2. Toll-Like Receptors**.** Mechanisms by which digestive nematodes activate the complement system**.** TLR: Toll-Like Receptor**,** MyD: Myeloid Differentiation, TNF: Tumor necrosis factor, NF-kB: Nuclear factor kappa B, MAPK: Mitogen-activated protein kinase, TAK 1: Transforming growth factor β-activated kinase, IRF: Interferon regulatory factors, RIP: Receptor-interacting protein (The authors designed the figure).

3. Immunogenic nematode excretory-secretory proteins

Some molecules contribute to the parasite's development, colonization, and feeding in the host, often used for making anthelminthic agents and vaccines[55]. Vaccines could produced against worms that excrete some molecules which stimulate a protective immune response against the infection[56].

3.1. The family of venom allergen-like proteins

Venom allergen-like proteins have been extensively studied since many nematodes express this family[57]. These proteins can perform various functions in nematodes, including pro-inflammatory and immunosuppressive functions. There is evidence that human venom allergen-like proteins (VAL) have a similar structure and effect to venom proteins (wasps), which stimulate inflammation or cause allergies in the body[58]. Therefore, examining nematode-derived homologs of VAL proteins is critical to understand better host-nematode interactions that result in excessive pathology. The VAL proteins detected in parasites such as *Teladorsagia circumcincta*, *Brugia malayi*, *Trichinella pseudospiralis*, *Heligmosomoides polygyrus*, and several other parasitic nematodes[59]. Because of the maintained structure of VALs, it could be possible to develop vaccines[60]. Vaccination models with birds and mice have shown that one of the *Brugia malayi* proteins named Bm-VAL-1 is highly immunogenic and promotes antibody stimulation and T-cell feedback in humans[61]. A combination of antigens is becoming more widely recognized as an approach to improving vaccine effectiveness. Vaccination with three VALs from *Heligmosomoides polygyrus* caused antibody production, protecting mice from complicated infections with *Heligmosomoides polygyrus*[62]. VALs act as sterol-binding proteins in infections, but they may bind immunomodulatory molecules like prostaglandins and leukotrienes, which both stimulate and regulate the immune system[63].

3.2. Serine protease inhibitors (serpins)

One of the most conserved families of nematode ES proteins is serpins (Serine protease inhibitors), found in nematodes such as *Haemonchus contortus*, *Anisakis simplex*, and *Brugia malayi*[64]. *Haemonchus contortus* serpins have been shown to reduce blood coagulation *in vitro*. Due to their anti-coagulation properties, serpins likely provide blood-feeding nematodes with an effective feeding mechanism. Microfilariae from *Brugia malayi* secrete serpins in response to the excess circulating of microfilariae in the host's circulatory system[65].

4. vaccines

Because of the limitations of anthelmintic drugs and rising drug resistance, the use of nematode vaccines is growing. Ideally, vaccines would provide long-term protection while leaving no chemical residues. Animal gastrointestinal nematode vaccines have different stages of development. Finding worm antigens in vaccine trials is critical. The Barbervax vaccine contains a microsomal aminopeptidase (H11) enzyme and a galactose-containing glycoprotein complex (H-gal-GP) derived from the gut of *Haemonchus contortus*[66]. Separate vaccination trials with adult nematode somatic extracts, which included a low-molecular-weight protein structure, revealed protective effects. After repeated vaccination with local ASPs (activation-associated secretory proteins) from adult *Coopria oncophora* and *Ostertagia ostertagi*[67], FEC (fecal egg counts) improved significantly in cattle. To improve production and assemblage variability, as well as to reduce production costs, recombinant vaccine antigens would be required for mass-produced vaccines[68]. Despite this, obtaining adequate levels of protection with recombinant antigens is difficult. Recombinant vaccine antigens such as *Pichia pastoris*, *E. coli*, free-living nematodes, or insect cells did not provide complete protection[69]. Some recombinant vaccines' efficacy has recently been approved. *Haemonchus contortus* produces antibodies that protect lambs from synthetic exigent infections as a result of its expression of *Escherichia coli* proteins[70]. Successful vaccines necessitate simple administration procedures that elicit effective immune responses for an extended period of time. To elicit a strong immune response, vaccine antigens can be delivered directly into the mucosal layer. When vaccination antigens were administered to sheep via the intestinal mucosa, variable immunity was obtained[71].

Adjuvants aligned with the delivery route of vaccines are an essential function for immune response. Quil A saponin adjuvant works well with *Teladorsagia circumcincta* antigen cocktail, *Cooperia oncophora*, and *Ostertagia ostertagi* ASPs5[72]. However, combining *Ostertagia ostertagi* ASP with aluminum hydroxide had no protective effect. Adjuvants can direct the immune response towards Th2 (aluminum hydroxide) or Th1 (Quil A), which suggests that a defensive vaccine-induced immune response can vary for different antigens or parasites, even within the same host. More information on the immune mechanisms associated with protection induced by vaccines would help deliver antigens and select adjuvants better. A marketable vaccine requires improvements in the production and delivery of recombinant antigens. Regions and nematode species have significant effect on local farm trials to control diseases and parasite epidemiology[73]. In the future, vaccines may contain antigens from different parasite species or pathogens, and vaccinations could be used in conjunction with other parasite control measures, such as anthelmintic medications[74].

5. Conclusion

Given the innate immune defense not only acts as a static barrier against pathogen infections but also actively contributes as a vital director of the adaptive immune

response, which is ultimately responsible for the rejection of parasite invasion and vaccine-induced immunity. Thus, interest in the innate immune defense against pathogen infections has increased. Cuticular surfaces of parasites that could actively move through the tissues are a unique challenge to the natural immune system. The best candidates for innate immune resistant identification by the host are receptors and galectins that are released by epithelial cells and eosinophils during infection. In nematode infections, the adaptive immune system might provide powerful and specific innate immune effector mechanisms. It can also boost the recruitment and activation of eosinophils and mast cells. Finding resistance genes and appropriate vaccines against different microbial diseases can be effective for recognition of the innate immune defense system's crucial role in resistance to infections.

Declarations

Competing interests

The authors declare that they have no conflicts of interests.

Authors' contribution

Narges Lotfalizadeh wrote the draft of the article. Soheil Sadr, Safa Moghaddam, Mahdis Saberi Najjar, Amin Khakshoor, and Pouria Ahmadi Simab participated in the preparation of the final draft of the manuscript. Hassan Borji participated as a supervisor and assisted in preparing the manuscript. All Authors have read and approved the final version of the manuscript.

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Ethical considerations

Ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors before publication in the present journal.

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