VERSITA

DOI: 10.2478/afmnai-2013-0002

ACTA FACULTATIS MEDICAE NAISSENSIS

UDC: 616-097:616.995.1

Scientific Journal of the Faculty of Medicine in Niš 2013;30(3):117-122

Review article ■

Immune Response in Infections Caused by Helminthes

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SUMMARY

The first line of defence in parasitic infection is the innate immune system. On the other hand, adaptive immune system posseses numerous mechanisams of hummoral and cellular immunity.

Cellular immunity in a helminth infection is characterised by Th2 immune response. Considering the fact that the aim of a parasite is not to kill its host, the majority of parasites are highly addapted to the life inside the host, and succesefully avoid or limit its deffences. A special signifficance of the parasite as a potential pathogen is its possibility to escape immunity. Numerous helminths are releasing different substances that are acting as lymphocyte suppressors and macrophage inactivators and they are capable of destroying antibodies. They have a possibility of camouflage, sequestration and surface shell peeling with the aim to avoid immune response. Latest research in the field of immunology has revealed the significance of CD40 co-stimumlating protein of antigen presenting cells in the immune response to parasitic infection.

Immune response in the course of parasitic infestion is important in pathogenesis of helminthioses.

Key words: **helminth, immunity, parasitic infection**

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INTRODUCTION

Helmithoses are human infections caused by cestodes-tape-worms (*Taenia spp., Hymenolepis spp., Echinococcus spp.,..*), trematodes-flukes (*Fasciola hepatica, Dicrocelium spp.,...*) and nematodes-roundworms (*Ascaris spp., Trichuris spp., Strongyloides spp., Enterobius spp., Trichinella spp., Toxocara spp., Dirofilaria spp.,* ...) that can be parasites of the gut, blood, lymphatic vessels and different organs and tissue of the human body (1).

Humans can be infected by ingestion of infective forms of the worm, by active penetration of infective larvae into the skin or transmision can happen by intermediate insect host. In nature, many helmithes have a very complex life cycles. Consequently, immune response of the host organism is a complex process, too. Immunity includes both innate and adaptive response. The innate immunity is the first line of defense (1).

Protection mechanisms of nonspecific immunity to helminths are still not fully understood. However, it is known that they include, as well as in infections caused by other infective agents, physical and chemical barriers in the host organism (2). Humoral responses are important for elimination of extracellular parasites that can live inside blood vessles, other body fluids or in the gut. Cellular immunity in helmintic infection is characterized by Th2 immune response.

Besides the activation of both adaptive and innate immune responses, and controlling of parasite multiplication, in some helminthic infections human immunity can generate the establishment of „concomitant immunity". In this circumstances, the defense mechanisms do not eliminate the initial helmintic infection, however, they set and allow resistance of the host to infection with a new worm of the same species. For example, immunocompetition between two dirofilariae (as suggested by laboratory infections), results in the block of *D. immitis* development, when infection with this species follows that with *D. repens* (3).

Immunity in helminthoses

The first line of defence against helminth is followed by the activation of eosinophils. Eosinophils contain granules, with the substances that are toxic for a parasite (reactive oxygen metabolites, alkilic proteins, eosinophylic neurotoxin, leucotriens, growth factors, enzymes), that are capable to damage helminth's cuticula and destroy it (2).

Antigen presenting cells (APC) play an important role in the innate immune response, because they are capable of recognizing numerous molecular patterns presented on pathogens, called pathogen-associated molecular patterns (PAMPs). In the recent years, it has been known that APC can recognize these PAMPs through Toll-like Receptors (TLRs) and NOD-like receptors (NLRs). These receptors induce signaling through the pathways responsible for inflammatory cytokines production. TLRs are type 1 transmembran proteins that are receptors with a primary role as sensors in the innate immuny response. They can direct the response of the innate immunity. TLRs are present at the surface of many cells in different combinations. For examle, at the surface of dendritic cell (DC), macrofague, neutrophil, endothelial cell and limphocyte. This specific but distinct pattern of expression is a special mechanism that secures different responses to different types of pathogens. The binding of TLRs triggers a series of signals that eventually lead to nuclear factor-κB (NF-κB) activation causing the inflammation. TLRs posses a common conservated domain (TIR) located intracellularly. Once this domain is activated, it starts a signal that travels over five different adaptor molecules resulting in activation of NF-κB – dependant pathway and interferon regulatory factor (4). After the interaction with the specific ligand, TLRs recruit adaptor protein to his TLR domain. NF- κB is a significant regulator of transcription and it is consisted of five subunits p50, p65, p52, RelB and c-Rel. Two of these enable transport to the nucleus where NF- κB binds with the DNA. Once it is inside the nucleus, NF- κB regulates the production of more than 150 genes responsible for coding cytokines, Ag receptors, apoptosis, and host defense. Both sensory and effector functions of TLRs are involved during immune response to pathogens. The production of proinflamatory cytokines and the increase of co-stimulatory potential of APC is probably the host immediate response to the presence of pathogen. Recognition of concrete pathogen by TLR makes these reactions possible (5). TLRs can react in various ways of recognizing the presence of pathogen. Every TLR possesses a unique and specific role in creating such immune response. Helminths can both activate (to a small degree) and negatively regulate TLRs (to a much larger degree). However, prolonged exposure to helminths (and their antigens) creates the situation where reaction of innate imunity cells can be delayed. This negative regulation by TLRs results in decreased production of proinflamatory cytokines whose role is to protect and prevent pathological processes. The compromised expression and function of TLRs can have negative consequences in the response to other pathogens, such as bacteria and viruses. The interaction between tissue helminths and innate immunity is important to explore, because it can reveal the role of TLRs and provide new areas of study for therapy and vaccine development that may involve alterations in TLRs expression and function (6).

Eosinophilic response in helminthic infections is determined by the immune response of the host, but also by distribution, migration and the helminth maturation. The level of epsinophilia mainly corelates to the intensity of tissue infestation with larval or adult forms of parasite. In chronic forms of infection local eosinophilia can be present. The absence of eosinophilia occurs when helminth is boundled inside the tissue (intact echinoccoc cyst, granuloma with differente forms of *Dirofilaria*

spp. intraluminal presence of *Ascaris spp*. in the intestine) (7-11). Intermitent licking of the fluid from echinoccocous cyst can lead to the transient eosinophilia associated with hypersensitive reaction type 1 (anafilactic reaction). Prolonged hypereosinophilia is present in strongiloidosis, filariasis, and in children with *Toxocara canis* infection (8-10, 12-14).

On the other hand, numerous mechanisams of hummoral and cellular immunity are involved in the defence of a host organism (2). Although specific antibodies are mechanisms of hummoral immunity, they are not always efficient in the defence. The presence of these antibodies has a diagnostic value, and often correlates with the activity of the parasite in the human body (7-15). Serodiagnosis of helminthic infections is extremely important in the case of echinococcosis, trichinosis, toxocariosis, infections that have extremely high seroprevalence in Serbia. Although immunodiagnostic tests can only determine the reactivity of the host to the presence of helminth antigens, the use of sensitive and specific commercial kits as a noninvasive method, especially in case of tissue helminthoses, is recommended.

Particularly significant during helmintic infection of the digestive system is IgE production. These antibodies induce mastocyte degranulation that can cause numerous changes in the intestine physiology and structure of the intestine epithelium. This leads to the production of large amounts of fluid, electrolytes and mucus secretion, smooth muscle contraction, vascular and epithelial permeability increase, as well as eosinophile and mastocyte recruitment (16). These changes result in elimination of adult or larval form of helminth from the gastrointestinal tract before the parasite binds to the mucosa or reaches the tissue (17). At the surface of mucosa, IgA helps in neutralisation of metabolic enzymes produced by parasite, disrupting the way that parasite feeds. By releasing microbicide substances, even phagocytes can contribute to the defence from helminths (18).

Cellular immunity in helmintic infection is characterised by Th2 immune response. CD4+ TH2 lymphocytes are producing IL-4, IL-5 and IL-10 (18-19). IL-4 stimulates the production of IgE antibodies that can bind to the surface of helminth and helps eosinophiles to recognize it and destroy it. These antibodies can bind to the mastocytes too, activating them to produce citokynes and induce inflammation. On the other hand, IL-5 and IL-13 react by stimulating maturation and activation of eosinophiles (19). T-regulatory cells can produce IL-10 that has an inflamatory effect and it is possible to have a role in a Th2-like response, too. IL-4 and IL-13 can activate macrophages in alternative way of activation (18-20). Milbourne and Howell proved that *F. hepaticais* secreting the supstance simmilar to IL-5 that is probably responsible for the local and systemic eosinophilia during fasciolosis (5). Nitric oxide (NO), that is toxic for a helminth, is released by macrophages activated through IFN-γ and TNF-$\acute{\alpha}$. This mechanism is best described in infection with *Shistosoma spp.* and *Fasciola spp*. (21).

The latest research in the fiield of immunology reveales the significance of CD40 in the immune response to parasitic infection. CD40 is a co-stimulating protein expressed on antigen presenting cells, and its role is to activate these cells (22, 23). Its counter receptor CD154 (CD40 ligand) is expressed on CD4+T cells (24). The interaction between CD40 and CD 154 controlls many aspects of the celular and humoral immunity (25).

CD40-CD154 signaling in the course of helmintic infection has a role not only in type 1 cytokine response, but also in IFNγ-independent autophagy as well as in stimulating protective type 2 cytokine response. The latest studies have announced the possibility that CD154 polymorphism determines susceptibility to helmintic infection, and may, in future, be used as a therapy (26).

Mechanisms of host immunity avoidance by some helminthes

Despite its immunogenity, helminths can survive inside the host for a long period of time. They have developed several pathways to avoid immune response. It has long been known that helminth is a multicellular organism that is in advantage over the immune system of the host, due to its size and motility (4, 27, 28). All helminths are releasing great amount of antigenic material. This material inside the human body can literarly confuse the immune system or deplete immune potential locally (14).

In addition, numerous helminths release different substances that act as lymphocyte suppressor and macrophage inactivator and can destroy antibodies.

Cestodes can prolong their life inside the host by producing anticomplementary factors. In that way they protect its cuticula from lyses (4).

Other example is *Fasciola spp*. (*F. spp.*) escaping the immune response in different ways: *F. gigantica* produces suproxide dismutasis and Glutation-S- transferasis that can neutralise superoxide toxic radicals. *F. hepatica* releases catepsin L-proteasis that binds to IgE and IgG antibodies involved in antibody-dependant cell-mediated cytotoxicity (ADCC). *Schistosoma spp.* produces the so-called „Schistosomes apoptosis factor" that induces apoptosis of CD4+ T lymphocites using the Fas receptor (5). Worms of this genera can absorb Fc fragment of immunoglobuline and MHC molecules of the host. That is how they can camouflage themselfs and hide its surface Ag.

Filarie contain serum albumins-like antigens in its cuticule, acting as a mask for hiding from the antibodies (8-10, 17).

Avoiding of specific immuny reactions can be through sekquestration. *Echinoccus spp*., *Taenia solium* and *Trichinella spiralis* inhabits the isolated parts of the human body that are unreachable to the immune sys-

tem (central nervous system or a muscle tissue). *Echinoccus spp*. forms cysts that complicates the contact of the immune system components with the parasite (7, 11, 17, 29).

Schistosoma mansoni and other intestinal parasites can periodically peel its glicocalics. It is how they free from the attached antibodies, but also their own antigens. These released antigens are used as a bait to attach emerged antibodies (17).

Damage cased by the immune response to helminthes

Immune response is very important in pathogenesis of parasitic disease. In some parasitoses, the immune response has a key role in a tissue damage. It is very difficult to distinguish the tissue damage that is a consecuence of a parasite presence from the damage made by agents secreted during processes of the host immune response (4). The procedures with the aim of diagnosis tissue parasitoses include histopathological analysis. Benefits of biopsy and histopathological analysis are certainly in the detection and identification of parasites and therefore these methods represent the „golden standard". However, the problems and disadvantages of these procedures include the possibility of morphological destruction of the parasite that can be damaged due to host immune reactivity. Recently, the use of the molecular methods for the diagnosis of biopsy specimens has allowed the detection and identification of parasites in case of the presence of minimal amounts of helminths, as well as the presence of significant damage of the parasite (15, 30).

It is also known that immune response to helminthes posses some of the same signals and mediators as the human body damage repair system (31).

In the course of helmintic infection, Th2-like response is present. Th2 cells, macrophagues, eosinophiles and fibroblasts agglomerate and they form granuloma. Even though the aim of granuloma is to limit the dissemination of parasite, chronic stimulation of the immune system leads to organ damage. In some infections, the circulating immune complexes can be formed, and later accumulate in the small blood vessels, which results in the damage (20). The pathogenesis of *Shistosoma mansoni* infection is the best example of interaction between the direct tissue damage, made by the presence of the parasite itself, and the indirect tissue damage, which is the consequence of the immune system activity. Granuloma, created as the product of hypersensitivity to the presence of *Schistosoma spp.* eggs inside the liver of the host, induces the obstruction of the liver small blood vessels, which leads to severe fibrosis, and in some cases liver failure (5).

Inflammatory reactions, caused by the immune system activity, can appear in the skin, liver, lungs, small intestine, central nervous system and eye. Systemic disorders such as eosinofilia, oedema, and joint pain reflect a local allergic reaction to the parasite. Pathological changes such as villous atrophy that can appear due to inflammation in the early stage of *Strongyloides spp.* and *Trichinella spp.* infection can disrupt mucosa permeability in the small intestine and shorten a time of protein absorption lading to a severe protein loss. These indirect tissue changes contribute to the chronicity of helmintic infection. The fact that many helmintic parasites live for long can explain the irreversibility of inflammatory changes that can lead to functional changes. The example of these irreversible changes is liver ducts hyperplasia in chronic parasitic liver infection, massive fibrosis in chronic shistosomiasis and skin atrophy in onchocercosis (3, 29, 32).

CONCLUSION

Considering the fact that the aim of a parasite is not to kill its host, the majority of parasites are highly addapted to the life inside the host, and can succesefully avoid or limit its deffences. A special significance of the helminthes is its possibility to escape immunity. That is why the immune response to the parasite is not efficient enough to eliminate the parasite, allowing the development of chronic infection.

Funding

This work was financially supported by the Serbian Ministry of Education, Science and Technological Development, grant No 31060 and grant No 175034.

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IMUNOLOŠKI ODGOVOR KOD INFEKCIJA IZAZVANIH HELMINTIMA

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Sažetak

Pored urođene otpornosti, u borbi protiv parazita, organizam domaćina raspolaže i specifičnim humoralnim i celularnim mehanizmima zaštite.

Celularna imunska reaktivnost u toku helmintoza uspostavlja se preko Th2 imunskog odgovora. Imajući u vidu činjenicu da cilj parazita nije da ubije svog domaćina, većina parazita je visoko adaptirana na život unutar domaćina i uspešno izbegava ili ograničava njegove odbrambene sposobnosti. Helminti, paraziti čoveka, produkuju određene supstance koje deluju kao supresori limfocita i inaktivatori makrofaga i sposobni su da izvrše destrukciju produkovanih antitela. Takođe, helminti mogu maskiranjem, sekvencioniranjem i gubitkom eksponiranih antigena kutikule da izbegnu mehanizme odbrane nosioca. Najnovija istraživanja na polju imunologije otkrivaju značaj CD40 ko-stimulirajućeg proteina antigen prezentujućih ćelija u okviru imunološkog odgovora u toku parazitske infekcije.

Pored zaštitne uloge, imunološki odgovor u toku parazitske infekcije značajno utiče i na patogenezu helmintoza.

Ključne reči: **helmint, imunološki odgovor, parazitska infekcija**