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Molecular mechanisms of hookworm disease: Stealth, virulence, and vaccines

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Hookworms produce a vast repertoire of structurally and functionally diverse molecules that mediate their long-term survival and pathogenesis within a human host. Many of these molecules are secreted by the parasite, after which they interact with critical components of host biology, including processes that are key to host survival. The most important of these interactions is the hookworm's interruption of nutrient acquisition by the host through its ingestion and digestion of host blood. This results in iron deficiency and eventually the microcytic hypochromic anemia or iron deficiency anemia that is the clinical hallmark of hookworm infection. Other molecular mechanisms of hookworm infection cause a systematic suppression of the host immune response to both the parasite and to bystander antigens (eg, vaccines or allergens). This is achieved by a series of molecules that assist the parasite in the stealthy evasion of the host immune response. This review will summarize the current knowledge of the molecular mechanisms used by hookworms to survive for extended periods in the human host (up to 7 years or longer) and examine the pivotal contributions of these molecular mechanisms to chronic hookworm parasitism and host clinical outcomes. (J Allergy Clin Immunol 2012;130:13-21.)

Key words: *Hookworms, virulence factors, immune modulation, vaccines, proteases,* Ancylostoma *secreted proteins*

Abbreviations used
APR: Aspartic protease
ASP: *Ancylostoma* secreted protein
ES: Excretory/secretory
GST: Glutathione-S-transferase
IDA: Iron deficiency anemia
KI: Kunitz-type protease inhibitor
L3: Third-stage larvae
MIF: Macrophage migration inhibitory factor
MMP: Matrix metalloprotease
NAP: Nematode anticoagulant peptide
NIF: Neutrophil inhibitory factor
TIMP: Tissue inhibitor of metalloproteases

The hookworms *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum* infect 576 to 740 million persons worldwide, predominantly in impoverished rural and tropical regions of the world, and together they cause one of the world's most debilitating neglected tropical diseases. Chronic hookworm infection results in long-term pathologic consequences primarily because of continuous intestinal blood loss caused by the feeding activities of these hematophagous parasites. Intestinal blood loss is the major pathologic sequela of human hookworm infection.[1-3] Heavily and even moderately infected subjects with poor underlying iron or protein stores can have hookworm disease, the clinical entity that specifically refers to the microcytic hypochromic anemia or iron deficiency anemia (IDA) that results from hookworms feeding on blood.[1-3] Hookworm-induced blood loss is estimated to be as great as 9.0 mL/d in subjects with heavy infections, with hookworm burdens of 40 to 160 worms sufficient to cause anemia. In school-aged children and adults living in resource-poor areas, where host iron stores are often lower than those in developed countries, there is a well-established relationship between the intensity of hookworm infection, intestinal blood loss, and host anemia. Moreover, children and women in the child-bearing years have the lowest iron reserves and, as such, are the most vulnerable to hookworm-induced anemia.[4,5] The consequences of chronic IDA include not only malnutrition but also the impairment of physical and cognitive development. As such, hookworm infection has a significant effect not only on the health of infected subjects but also on economic productivity and educational achievement in regions where the parasite is endemic.[2]

Although hookworm infection can be treated effectively with medication, reinfection often occurs rapidly after treatment.[6] The

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Terms in boldface and italics are defined in the glossary on page 14.

failure to interrupt transmission, coupled with the widespread prevalence of the disease in resource-poor areas and the potential emergence of drug resistance,[7] suggests that additional control measures are urgently needed.

NATURAL HISTORY OF HOOKWORM INFECTION: MOLECULAR MECHANISMS OF SURVIVAL

Immature hookworm third-stage larvae (L3) are approximately 0.5 mm in length and infect the host by penetrating the skin, a process that takes between 30 minutes and 6 hours depending on the species,[8,9] and invading the circulation, where they are carried to the heart and lungs. The parasites then penetrate the alveolae, migrate to the trachea, and are swallowed, reaching their site of predilection in the gastrointestinal tract (fourth-stage larvae), where they develop into blood-feeding, adult-stage hookworms that live out their parasitic existence for years by attaching onto the duodenal mucosa and consuming host blood to obtain nutrition and sexually reproduce.[10] Pivotal to hookworm survival in the host is a vast repertoire of molecules, mostly proteins, known as excretory/secretory (ES) products, which interact with host proteins and play key roles throughout all aspects of the host-parasite relationship. A comprehensive list of such proteins was previously published.[11]

This review will examine the different types of molecular mechanisms that mediate hookworm survival in the host and that result in disease (Box 1). The role of these molecular mechanisms in the host-parasite relationship will be described, including the more "classical" molecular mechanisms that directly contribute to hookworm-related IDA and malnutrition (eg, protease inhibitors and peptidases associated with blood feeding), as well as more novel and recently discovered mechanisms that have more indirect or as yet undefined roles in the pathogenic process of hookworm infection (eg, molecular mechanisms associated with the establishment of parasitism and the worm's survival within the host). These latter molecular mechanisms constitute the most recent research into the pathogenesis of hookworm disease.

BOX 1. Molecular mechanisms of hookworm disease: A natural history of stealth and virulence (Fig 1)

Key hookworm molecules are critical mediators of the biological processes that determine parasitism and are involved in the following stages of infection within the host:

1. larval activation, host invasion, and tissue migration, including digestion of skin and other tissue macromolecules;
2. nutrient acquisition by anticoagulation and degradation of host serum proteins for food; and
3. neutralization of host defenses through inhibition of host intestinal proteases and immune evasion by modulation of the host inflammation response.[12]

Molecular mechanisms associated with larval activation, skin penetration, and tissue migration

Penetration of the skin of human hosts by hookworm L3 is primarily a chemical process that is mediated by the release of a range of proteolytic enzymes from specialized larval glands. Secretions of *N americanus* larvae possess enzymatic activity belonging to all the known major mechanistic classes of proteases and have the ability to degrade the connective tissue substrates ***collagen***, fibronectin, laminin, and elastin. Furthermore, larval skin penetration can be significantly neutralized only by pepstatin A, an inhibitor of aspartic proteases (APRs), implicating the importance of this class of enzymes in the infection process.[13] The molecule most prominently implicated in this process is the APR *Na*-APR-1 because of its ability to digest skin macromolecules and its presence in larval parasites.[14] ***Hyaluronic acid*** is a major component of the extracellular matrix and is associated with cell adhesion by ligand binding with the CD44 cell-surface receptor.[15] *Ancylostoma* species larvae have been shown to exhibit hyaluronidase activity, which might facilitate their migration through host dermal layers by interrupting cellular adhesion mediated by hyaluronic acid.[16] However, the best characterized protease in larval ES is *Ac*-MTP-1, an astacin-like zinc metalloprotease from the dog hookworm *Ancylostoma caninum*.[17,18] *Ac*-MTP-1 aids larval migration through the skin and

GLOSSARY

COLLAGEN: An insoluble fibrous protein of vertebrates that is the chief constituent of the fibrils of connective tissue (as in skin and tendons) and of the organic substance of bones and yields gelatin and glue on prolonged heating with water.

CR3 RECEPTOR: Also known as CD11b/CD18, an adhesion molecule that is also a receptor for iC3. Deficiency of CD18 in type 1 leukocyte adhesion deficiency results in a lack of β_2-integrin adhesion molecules.

EUKARYOTE: Organisms composed of 1 or more cells containing visibly evident nuclei and organelles.

FIBRINOGEN: A plasma protein that is produced in the liver and is converted into fibrin during blood clot formation.

FLUKE: A flattened trematode worm.

GLUTATHIONE-S-TRANSFERASE: An enzyme that functions to detoxify various xenobiotics by conjugating them with glutathione.

HYALURONIC ACID: A viscous glycosaminoglycan that occurs especially in the vitreous body, the umbilical cord, and synovial fluid and as a cementing substance in the subcutaneous tissue.

HYDROLYZE: To undergo the process of splitting a bond and the addition of a hydrogen cation and the hydroxide anion.

INTESTINAL BRUSH BORDER: An area in the small intestine comprised of microvilli that contain enzymes important for digestion. This area is also where nutrient absorption takes place.

MATRIX METALLOPROTEASE (MMP): Proteases that are involved in degrading the extracellular matrix and in tissue repair. They are induced by proinflammatory cytokines. MMPs are produced by the airway epithelium, and levels are increased in the bronchoalveolar lavage fluid of asthmatic subjects.

PHYLOGENETIC: Relating to the evolutionary history of a kind of organism or a group of genetically related organisms.

SERINE: A nonessential amino acid ($C_3H_7NO_3$) that occurs especially as a structural part of many proteins and is a precursor of glycine.

TRYPSIN: A crystallizable proteolytic enzyme most active in a slightly alkaline medium that is produced and secreted in the pancreatic juice in the form of inactive trypsinogen and activated in the intestine.

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