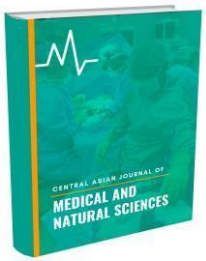

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Article

Regulatory Roles of microRNAs in The Immune Response to Entamoeba Histolytica Infection

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Abstract: Entamoeba histolytica, the protozoan parasite responsible for amebiasis, remains a significant global health concern, especially in developing regions. The host immune response to E. histolytica involves a complex interplay between innate and adaptive immunity, including the activation of macrophages, neutrophils, and the production of cytokines such as IL-1β, IFN-γ, and TNF-α. Recent research has revealed that microRNAs (miRNAs) small non-coding RNAs that post-transcriptionally regulate gene expression play crucial roles in modulating these immune responses during infection. This review highlights the current understanding of miRNA-mediated regulation of host immunity against E. histolytica. Specific miRNAs, such as miR-155, miR-146a, and miR-21, have been shown to regulate inflammatory signaling pathways including NF-κB and MAPK, thereby influencing cytokine production and immune cell activation. Additionally, miRNAs can modulate the expression of Toll-like receptors and other pattern recognition receptors, which are critical for the early detection of the parasite. Dysregulation of miRNA expression during infection may contribute to excessive inflammation or immune evasion by the parasite. Understanding the miRNA–immune interaction offers insights into host-pathogen dynamics and opens new avenues for therapeutic interventions. Targeting key miRNAs may represent a novel strategy to enhance host defense or limit tissue damage in amebiasis. Overall, this review emphasizes the emerging regulatory roles of miRNAs in shaping the immune landscape during E. histolytica infection and underscores their potential as biomarkers and therapeutic targets in parasitic diseases.

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1. Introduction

Entamoeba histolytica is the protozoan parasite that causes intestinal amebiasis, which may turn into severe dysenteric colitis and lead to an estimated over 40,000 deaths per year in developing countries. Prior to infection, amebic trophozoites first adhere to the intestinal epithelium surface and then trigger a host immune response to initiate invasive disease [1]. In the infection process, host factors recognize and attempt to clear E. histolytica; however, parasitic immune evasion mechanisms respond and attempt to overcome host immunity [2]. The ultimate outcome of amoebic infection may shift from asymptomatic colonization, the formation of secured amoebic reservoirs, moderate intestinal pathology, or life-threatening dysentery colitis, depending on all infection parameters. Of note, robust intestinal inflammation, quantified by heavily infiltrated

inflammatory cells and mucosal damage, is significantly correlated with dysentery severity [3].

The mammalian immune system is comprised of highly evolutive mechanisms to recognize invading pathogens and mount appropriate immunological responses. In mammals, the immune response to parasites initially relies on innate immune barriers and cells, such as epithelial cells, natural killer cells, macrophages, neutrophils, and dendritic cells (DCs) [4]. Subsequently, helper T cells, interleukin (IL)-4, IL-13, and IL-25, which induce immune polarizations towards M2 macrophages and Th2 cells, determine protective immunity against parasitic infections. The mammalian genome encodes diverse classes of small noncoding RNAs, including the well-studied class of microRNAs (miRNAs), which control gene expression by targeting messenger RNA (mRNA). The functions of miRNAs in innate immune cells and the response to various infectious or inflammatory stimuli has been widely investigated; however, studies regarding miRNA-mediated regulatory mechanisms in host immunity to protozoan infection are still limited [5].

2. Background on Entamoeba histolytica

A quasi-experimental design with pre- and post-intervention assessments was carried out in the present study which was conducted from 28 November 2024. The research was conducted at the Azadi Teaching Hospital in RCU and ICU. Azadi Teaching Hospital opened in 1985 and is located on the north side of Kirkuk. A non-probability, purposive sampling of Nurses working at RCU and ICU was chosen to collect representative data in Azadi teaching hospital. ". Total sample is)60) nurses' data were collected through pre and post. The Nurses are exposed to the nursing education program all sample have proximately the same demographic characteristics. Through an extensive review of the relevant literature, by researcher, and after identifying information according to the objectives of scientific research, a questionnaire and checklist was constructed for the purpose of study using the self-report technique.

Entamoeba histolytica is a parasitic protozoan that infects the human intestinal tract and is the causative agent of amoebiasis, a disease in which diarrhea, dysentery, and intestinal hemorrhage can occur [6]. E. histolytica is the only pathogenic Entamoeba in humans and is responsible for considerable morbidity and mortality, particularly in developing nations. Additionally, amoebiasis is the world's second-leading cause of parasitic death after malaria. Entamoebas are prominent as a genus of protist that is both ecologically and evolutionary important [7].

Entamoeba histolytica is a parasite of humans, and it is transmitted by the fecal-oral route, through the ingestion of cysts. Upon ingestion, trophozoites are released from the cysts in the small intestine and migrate to the large intestine. The trophozoites can either replicate locally, resulting in an asymptomatic carrier state, or invade the colon wall, resulting in dysentery. Features associated with pathogenicity include adherence to host cells and tissue, colonic invasion, immune evasion, and cytotoxicity to immune and non-immune cells [8]. Cyst formation is not universal among Entamoeba species. E. histolytica and E. invadens are the only two pathogenic species that transiently encyst. E. histolytica cysts are environmental stages, and in sewage they can remain viable for months. Hence, adherence, colonic invasion, and immune evasion are required to be present in freshly excysted trophozoites. This suggests that there is some residual expression of virulence factors at the excysted trophozoite stage [9].

2.1. Epidemiology and Public Health Impact

Entamoeba histolytica is a protozoan parasite belonging to the Entamoeba genus that infects humans, being an important cause of intestinal and/or extra-intestinal illness. Environmental contamination with Entamoeba cysts is the only route of transmission. The cysts sporulate in the intestine and release trophozoites, which adhere to and invade

colonic epithelial cells. There, they replicate by binary fission. Invasive secreted virulence factors cause tissue destruction and a wide inflammatory response [10].

Entamoeba histolytica is the causative agent of amoebiasis, an important cause of morbidity and mortality worldwide. Entamoeba histolytica cysts recovered from feces are the only resistant form that can survive the harsh conditions of the external environment. Therefore, the contamination of water, soil, and vegetables with human feces allows the transmission of amoebic infection. Freshwater sources are also widely exposed to effluents from sewage treatment plants [11]. Outside the host, the infective cysts can remain viable for prolonged periods, and the low rates of cysts found in the environment make it difficult to get an accurate epidemiological picture of their origins. As a consequence, many areas of the world are exposed to E. histolytica as a public health problem, including developing countries with inadequate sanitation systems. Nevertheless, there are also infested local outbreaks in developed countries. Asymptomatic carriers are the main source of the infection [12].

Entamoeba histolytica can be cultivated in axenic culture, and a genomic-proteomic approach was recently developed for this parasite. There are available tools of reverse genetics, including a growing number of plasmids for the construction of gene expression vectors and recombinlog stem virus expression vectors. These tools can potentially deliver genetic material into amoebae in vivo, as demonstrated via the efficient inhibition of protein expression by specific RNA interference . There are also some other available tools, including a yeast two-hybrid system and numerous antibodies. The availability of molecular tools to E. histolytica has fast-tracked the research in characterizing individual genes or classes of virulence factors, and gene regulation and host-pathogen interaction mechanisms [13].

2.2. Pathogenesis and Virulence Factors

Entamoeba histolytica is a protozoan pathogen that infects the human colon and causes amoebiasis, a disease characterized by dysentery. The pathogenesis of amebic dysentery is complex and mainly involves adherence of the organism to epithelial cells, proteolytic degradation of extracellular matrix molecules making way for invasion, and cell killing. The interaction of E. histolytica with host epithelia includes pre-adhesive events, such as de-novo glycosylation of the trogocytosed host membrane, which generates a “host-derived” glycocalyx that shields the pathogen from immunity and reinforces virulence factor activity [14].

E. histolytica adheres preferentially to fucosylated oligosaccharides, with the Gal/GalNAc lectin being the main receptor-recognizing molecules. The lectin consists of two subunits and has two functional non-reducing ends that, in addition to sugar-specific binding sites, bind EHSGless proteins that perform other functions such as apoptosis induction. The adhered pathogen induces cytoskeletal rearrangements, production of nitric oxide, and release of proinflammatory cytokines. These responses increase migration and recruitment of leukocytes, secretion of mucus, and production of prostaglandins by a crotonyl-CoA carboxylase-beta supplying the COX pathway that generates PGE2 [15].

This cytokine in turn increases production of IL-10. In addition, activated macrophages exert pro-inflammatory effects leading to increased production of IL-12 that promotes Th1 responses, crucial for protection against amebic colitis. Responses to E. histolytica vary from the propagation of inflammation leading to immunity or the generation of a permissive environment allowing for tissue injury and new opportunities of reinfection [16].

3. Overview of the Immune Response

The immune system is a complex network of cells, tissues, and organs that work together to protect the body from harmful invaders. The immune response begins when an invader is detected by the immune system, which can include a variety of mechanisms

and players. The innate immune response is the first line of defense against invading pathogens and is immediate, nonspecific, and generally effective during the initial 4–96 hours of infection. Pattern recognition receptors (PRRs) on innate immune cells such as macrophages, dendritic cells (DCs), and natural killer (NK) cells recognize pathogen-associated molecular patterns (PAMPs)/danger-associated molecular patterns (DAMPs) on invading pathogens. PRRs are a group of receptors such as Toll-like receptors (TLRs), C-type lectins, NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and AIM2-like receptors (ALRs). Upon activation, PRRs initiate classical signaling cascades to activate transcription factors (TFs), release proinflammatory cytokines such as TNF-α and IL-6, and induce inflammatory responses [17]. DCs play a key role as antigen-presenting cells and bridge the innate and adaptive immune systems. The adaptive immune response is a secondary immune response that develops hours to weeks after infection. It is highly specific for the antigen, effective but slow, and can develop immunological memory after the resolution of infection. The adaptive immune response is mediated by T and B lymphocytes, which recognize specific peptide antigens presented by MHC Class I and II molecules, respectively. Activation of T lymphocytes induces robust immunity involving cellular mechanisms mediated by CD8+ T cells and humoral mechanisms mediated by CD4+ T cells. Knowledge of the immune response against a specific pathogen is crucial for designing effective prophylactic vaccines and immunotherapeutics [18].

Entamoeba histolytica is a protozoan parasite that infects the colon and causes a disease known as amoebic dysentery or amoebiasis. It is a significant health problem in many developing countries. There is currently no effective vaccine available, and it is thought that a better understanding of the immune and inflammatory response to E. histolytica may help in vaccine design. Detailed studies using both cell culture models and animal models have been carried out on the immune and inflammatory response to E. histolytica infection [19]. E. histolytica infection is accompanied by both innate and acquired immune responses. Many players of innate and acquired immunity have been identified, including macrophages and neutrophils of the innate immune response and antibody secretory B cells and CD4+ T cells of the acquired immune response. The cellular and soluble factors involved in these immune responses have also been identified. Initiation and regulation of these immune responses play an important role. A better understanding of the initiation and regulation of immune responses to E. histolytica infection is crucial to designing better therapeutic strategies [20].

3.1. Innate Immunity

The majority of those infected with E. histolytica have asymptomatic forms of the disease. In this case, mucosal immune responses strongly suppress parasite passage into the gut lumen, thereby restoring homeostasis. Mucosal effector mechanisms include mucins and locally secreted IgA, among others. The capacity to respond to E. histolytica infection by inducing the differentiation of effector Th1, Th2, and Th17 cell subpopulations is extremely important for slowing its development into full-blown disease. However, in some individuals, potent mucosal immune responses fail to suppress parasite proliferation, resulting in cytotoxic cascades that damage gut epithelium, as well as intestinal microbiota and secondary tissues. Such responses are mediated by effector (i.e., classical) Th1 cells and cytotoxic CD8+ T cells. The latter inoculate massive inflammatory responses, which include TNF-α-induced nitric oxide production, leading to secondary tissue injury [21].

Intestinal epithelial cells are the portal through which the parasite invades the intestinal mucosa and accesses its dwelling site. Detection of the cysts by the intestinal epithelium is important for parasite infectivity. Once ingested, cysts are subject to a battery of host defense mechanisms. Cysts that survive gastric acidity and are attacked by bile salts enter the intestine, where high amounts of mucus should inhibit pathogenesis. Yet, E. histolytica cysts are resistant to these mucolytic stresses. Thus, other hurdles need to be

overridden [22]. One likely candidate is the action of toll-like receptors (TLRs). E. histolytica virulence triggers an innate immune response through TLR-2-, TLR-4-, and TLR-6-dependent pathways, then initiating E. histolytica clearance. The outcome of removing individual TLRs is different, which suggests that parasites with a broader or narrower range of virulence factors can invade the mucosa. Different bacteria can either benefit or counteract the toll-like receptor (TLR) or Th1 responses, depending on their propensity to penetrate deeper epithelium layers in the presence of intestinal damage. Moreover, alterations in patterns of initial microbiota colonization seem to determine the establishment of protective immunity to subsequent E. histolytica and Entamoeba dispar infection [23].

3.2. Adaptive Immunity

The interplay between the innate and adaptive immune response is one of the principal mechanisms responsible for defense against infections. This defense mechanism is complex and includes both specific and nonspecific immune responses. The latter (innate immune response) is the first to save the host from invasion by pathogens and is basically formed by a well-defined set of soluble factors and cells. Conversely, the specific (or adaptive immune response) is complex and more slowly engaged, although this immune response ultimately destroys the primary focus of infection. The adaptive immune response to Entamoeba histolytica is mainly elicited by CD4+ T-lymphocytes and mediated by the production of cytotoxic cellular factors and antibodies directed against both secreted and surface-expressed E. histolytica molecules [24].

In the absence of adequate immunity to prevent primary infection, subsequent reinfection can occur. The adaptive immune response targeted against E. histolytica may be influenced by earlier infection with Giardia lamblia, although very little is known about the effect of giardiasis on subsequent amoebic dysentery. Several studies suggest that infection with G. lamblia increases the severity of disease upon infection with E. histolytica and exacerbates amebic colitis. Immunocompetent patients previously infected with G. lamblia can develop amoebic dysentery. Abdominal symptoms in children with acute G. lamblia infection improved four days after treatment, but stool antigen tests were still positive for E. histolytica. There is a report presenting a case of nosocomial G. lamblia infection in a 69-year-old man, with diarrhea, fever, abdominal pain, and bloody stool as major findings, compatible with amoebic dysentery. Based on serologic and stool examinations, underlying concomitant infection with E. histolytica was identified [25].

4. Role of microRNAs in Immune Regulation

While exploring the role of giardians in the immune response to E. histolytica infection, the first step was the transcription profiling of mammalian miRNAs regulated by E. histolytica infection. The infection of E. histolytica on day 3 caused downregulation of a variety of ligan miRNAs, which conversely elicited upregulation on invader assaying. The deep sequencing datasets of miRNAs showed differential expression of contamination in pre- and post-infection amebas. Validation was performed for select candidate miRNAs with statistical analysis. Three confirmed regulatory miRNAs of E. histolytica were further investigated in action. miR-28-3p and miR-30-5p induced downregulation of mRNA target, while miR-499-5p resulted in upregulation [26].

It was previously noticed that certain miRNAs displayed unexpected expression change direction. This functional miRNA should be of a regulatory capacity to contest the weathering assault of host. Thus, the folding of more miRNA by E. histolytica was investigated. From four miRNA libraries from the infection condition of 0, 1, 3, 6, and 24 hr post-infection, numerous novel miRNA candidates were discovered. Indirect target miRNA was predicted using experimental bioinformatic approaches. Selected target genes were verified, including possibility regulatory network exploration [27].

To explore cellular targets of host miRNAs, genome-wide analysis of the predicted regulatory network was constructed. Integrative bioinformatics analysis suggested potentially implicated pathways similar to scRNA-seq, a slew of regulatory miRNAs that might be involved in astute immunity against E. histolytica are of interest. Among which thnis25a4-4-5p is extremely homologous to the target pads on cleavage sites, indicating a highly regulatory capacity, therefore warranting into more detail studies. Lastly, some open questions and future perspectives of miRNAs study were proposed. Intestinal is a major human pathogenic protist which causes amebiasis. Histolysin, a pore-forming toxin produced by E. histolytica tries to penetrate through immune defense. Complement led to lysing and phagocytic upregulation. Meanwhile, triggering of host shRNA showed one the regulatory roles of ligand miRNAs in host defense against distressing perturbation like infection [28].

4.1. Mechanisms of microRNA Action

MicroRNAs (miRNAs) are a large family of small non-coding RNA molecules, ranging from 19 to 25 nucleotides in length, that play key roles in gene regulation by targeting messenger RNAs (mRNAs) for cleavage or translational silencing. These processes occur in both a sequence-specific manner and in a concentration-dependent fashion, and involve a complex cascade of molecular events in which the enzyme Drosha cleaves primary miRNA (pri-miRNA) precursors into approximately 70-nucleotide-long hairpin structures known as miRNA precursors or pre-miRNAs in the nucleus. Pre-miRNAs are then transported to the cytoplasm by Exportin-5, and are further processed by Dicer to yield miRNA duplexes. Finally, the endogenous miRNA strand is loaded into Argonaute proteins to form the miRNA-induced silencing complex (miRISC), which binds to complementary sequences in the target mRNA, and mediates the repression of target expression. Bases 1 to 7, and 2 to 8 (seed region) of the miRNAs interact with the target sites, and this interaction often requires complementarity with a 6–8-nucleotide long sequence at the target's 3′ untranslated region (UTR) [29].

miRNAs were first discovered in 1993 in the nematode Caenorhabditis elegans and their vertebrate counterparts were subsequently identified in 2000 in mouse and human. These first descriptions launched an entire new area of research resulting in the identification of more than 700 human miRNAs to date, with hypotheses that they affect translation of almost 30% of human genes. Moreover, the presence of these small regulatory non-coding RNAs was described for other organisms and their genomes, including viruses and bacteria, fungi, and parasites, including the Apicomplexa phylum. Parasites were shown to secrete miRNAs and/or other extracellular vesicles harboring miRNAs, which in turn were shown to modulate the host response, and some examples include species such as Trypanosoma brucei brucei, Toxocara canis, Toxoplasma gondii and Cryptosporidium parvum [30].

4.2. MicroRNAs in Immune Cell Differentiation

MicroRNAs are a class of small, non-coding RNA molecules that regulate gene expression by negatively modulating post-transcriptional regulation. MicroRNAs are formed by the cleavage of precursor molecules having a double-stranded structure. Biogenesis begins with transcription by polymerases II and III, which produce pri-microRNAs. After being processed into small hairpin structures by the Microprocessor complex, it is exported to the cytoplasm by Exportin5 protein. In the cytoplasm, Terminal deoxynucleotidyl transferase (TUTase) and Dicer proteins eliminate the 3' overhangs and process the hairpin structure in a 5' to 3' direction. The microRNAs are incorporated into the RNA-Induced Silencing Complex (RISC), which assembles around Argonaut proteins and orchestrates microRNA transportation to their targeted mRNA molecules [31].

MicroRNAs are known to participate in a variety of physiological processes, such as proliferation, differentiation, and organogenesis, but their role in the immune system is particularly intriguing. Immune response elicited by infection is integral for the survival of multicellular organisms. Host-parasite interactions have co-evolved across millions of

years, leading to the establishment of various immune responses on both sides. Once a mammalian host is infected by Entamoeba histolytica, a diverse array of molecules that comprise the innate immune response are synthesized by the host. These include the chemokines IL-8 and CCL20, inflammatory cytokines like TNF-α, IL-1, IL-6, and IL-12, and also antimicrobial populations of microbicidal nitric oxide (NO)-producing macrophages. However, resolution of the infection also requires appropriate regulation of inflammation to avoid tissue damage. Precise regulation of immune responses is orchestrated by both ceRNAs (long non-coding RNAs and gene transcripts) and microRNAs (miRNAs; small RNAs) [32].

5. MicroRNAs and Entamoeba histolytica Infection

In the study of microRNAs and E. histolytica, the genome of the parasite has been analyzed in search of candidates that might code for miRNAs. Preliminary results have shown that there are probable miRNAs that fulfill the basic requirements to be classified as such, but further analysis of in vitro and in vivo functional assays is required. AMOEBIC DYSENTERY IS THE SECOND LEADING CAUSE OF DEATH BY PARASITE IN AN INFECTED HOST. The peripheral immune response can be influenced by many modulatory mechanisms. These initial events of the systemic immune response can dictate the trajectory of both the invasive disease associated with E. histolytica and the concomitant intestinal and extra intestinal disease associated with co-infection by other pathogens, such as bacteria, viruses and fungi. The different E. histolytica non-coding RNAs reported recently could be a complementary pool to modulate the host's defenses during the genesis of systemic infection [33].

The first experimental approaches with the putative new E. histolytica miRNAs actively seek target genes in the host in order to start to understand a part of this pool of players in the interaction of host– parasite. Entamoeba histolytica harbors plenty of putative regulatory miRNAs with capacity to modulate the host's immune response. Recent bioinformatics approaches have generated a candidate list of E. histolytica pre-miRNAs that could fulfill the criteria to be classified as such. These candidates are currently under evaluation using comparative phylogenomic and functional analyses, using in silico and in vivo approaches with cells of the immune response directed to kill the pathogen. The results of these approaches will shed light on the complex relationship between amebas and the mammalian host in the first hours of infection [34].

5.1. Altered microRNA Expression Profiles

The pathogenic parasite Entamoeba histolytica, the causal agent of amoebic dysentery, disrupts the intestinal epithelial barrier and invades the colonic mucosa, leading to intestinal lesions and even extraintestinal abscesses. The intense inflammatory response of the host is important for pathogen control, which is characterized by the release of inflammatory mediators, including cytokines that induce a Th1 response. However, an uncontrolled inflammatory response mediated by pro-inflammatory cytokines exacerbates the disease. Therefore, the regulation of the inflammatory response is pivotal for preventing colitis and controlling E. histolytica. Increasing evidence suggests that microRNAs (miRNAs), which act as post-transcriptional regulators of gene expression, play an important role in different biological processes, including innate immune responses to various pathogenic infections [35].

In the past, the regulatory roles of miRNAs in the host immune response to E. histolytica infection were poorly understood. Co-culturing a mouse macrophage-like cell line with trophozoites resulted in altered miRNA expression profiles. A total of 60 miRNAs were detected in cells, of which 38 miRNAs were upregulated and 22 were downregulated in miRNA expression upon infection. The most interesting altered miRNAs were proposed to be related to inflammatory responses to E. histolytica infection. The cellular physiological functions, including immune responses, cellular proliferation, apoptosis,

differentiation of CD4+ T cells, and signal transduction, of host cells can be controlled by these aberrantly expressed miRNAs. Moreover, some altered miRNAs were also predicted to target mRNA transcripts of pro-inflammatory cytokines and chemokines involved in the Th1 response, indicating that these altered miRNAs can regulate E. histolytica-induced inflammatory responses [36]. In addition, a novel regulatory role of let-7d-5p functions as a pro-inflammatory signal. E. histolytica induced the upregulation of let-7d-5p in the host, which increased the production of TNF-α and IFN-γ and promoted amoebic infections. Let-7d-5p exerts its function by targeting two members of the miRNA processing machinery, leading to enhanced expression of participants in the TNF-α pathway and transcriptional upregulation of pro-inflammatory cytokines [37].

5.2. Functional Studies of Specific microRNAs

Studies have shown that the inhibition of pre-miR-430 function by a 25-Nucleotide inhibitor hairpin pre-miR-430 could inhibit the cell cycle of E. histolytica by type 16 with specific timing in cell counting. Pre-miR-430 hairpin transfected E. histolytica enhances E. histolytica cell cycle through the phosphorylation of histone H4, which is the target of miR-430. Pre-miR-430 is predicted to target E. histolytica proteins that respond to host innate immunity through the Wnt pathway. In vitro E. histolytica culture with conditioned medium from the innate immune response of THP-1 and macrophages activates the maturation of pre-miR-430, through the inhibition of its host target, which is predicted to encode a protein that promotes the unconventional secretion of TNF-alpha. Pre-miR-430 transfection into E. histolytica reduces levels of the predicted target protein, promoting the secretion of TNF-alpha. In vitro co-culture of THP-1 cells with specific E. histolytica expressing high levels of pre-miR-430 promotes the secretion of TNF-alpha and is suggestive of a new mechanism of host-parasite interaction that enhances the virulence of E. histolytica [38].

The second microRNA that has been further characterized is pre-miR-191, but some others microRNAs that are consistently identified in sequencing-based and hsa-miR-210 paragons also have evidence of biological activity. With the modulation of the host response to E. histolytica cysts, which are the biocysts difficult to eliminate and source of new infections, some dedicated efforts in the aim of obtaining a large database on the contents and biological activity of mucosubstances excreted by the trophosites of E. histolytica being conducted [39]. Analysis of sequences deposited in databases including both naked sequences or ESTs from available assemblages enabled the selection of the most robust coding sequences for tentative genes whose expression oscillates with the E. histolytica-host response times. E. histolytica small RNAs although limited have since been a topic of interest because of the direct evidence of biological activity from various laboratories over a decade and evidence for regulatory roles in higher eukaryotes [40].

6. MicroRNA-Mediated Pathways in Immune Response

MicroRNAs (miRNAs) are critical small non-coding RNA molecules that regulate global and gene-specific gene expression. miRNAs modulate translation primarily by interacting with messenger RNAs (mRNAs), promoting mRNA decay and translational silencing. A single miRNA can regulate multiple mRNAs and/or pathways. By achieving moderate inhibition of mRNAs, miRNAs help cells achieve dynamic gene expression. Tissue-specific miRNA expression indicates their involvement in cellular development and differentiation. Research has suggested that several miRNAs might be defined as "the miRNA signature," influencing the type of malignancy and its aggressive behavior in various cancers. In other words, these miRNAs have diagnostic, prognostic, and therapeutic potential, particularly in cancers. Some miRNAs are proposed to be considered biomarkers for specific stages of clinical melanomas [41].

Many studies on miRNA biogenesis and activity regarding their most titan activity in cancer have received much attention in the last decade. However, relatively low research

focus has been made to date on other groups of miRNAs broadly classified as parasite-derived miRNAs (Pde-miRNAs) and their immunomodulatory activity during parasites' very early stages of infection in host tissues. Pde-miRNAs are truly evolutionarily conserved long nucleotide sequences found in exosomal bodies' RNA fraction, secreted by parasites. These miRNAs have potential to be developed as HPV biomarkers and are also evaluated for their therapeutic potential as an anticancer agent. In addition, some consensus host miRNAs have herbal and dietary plant origin with anti-helminthic property [42].

6.1. Target Genes and Signaling Pathways

AmiR-29b mRNA target identification using a computational approach was performed. Genes involved in the GO terms "Cellular Response to Type I Interferon," "Intracellular Pathogen Response," and "Negative Regulation of Immunity" were less expressed in the presence of amiR-29b that many of these genes had one or more amiR-29b match sites in the UTR regions. Defense Response to Bacillus Thuringiensis Toxin (BTX) MRNA target genes were identified with luciferase assays and mutagenesis analysis [43]. In the luciferase assay, ectopic expression of the DENV-2 targeting amiRNA reduced the luciferase activity of the reporter gene. Mutation analysis showed that the binding to the target sequence containing the "AA" mutations enhanced luciferase activity, confirming that the predicted sequence was targeted by DENC-2 in a sequence-specific manner. The intracellular response of miR-29b and DENV-2 were analyzed. It was 50% of DENV-2 decrease in the presence of ectopically expressed amiR-29b. Vice versa, ectopic expression of its target, BTX, resulted in the 50% reduction in amiR-29b. In a co-expression assay, between miRNA and its target siRNA both pathways were validated by RT-qPCR assay and luciferase assays. The predicted target IDs and targets of root hair male miRNAs through comparison to available iPAST and miRBase v19. The robust postulation with high combinatorial score indicating strong interaction of miRNA and target is used to identify targets [44]. The predictions and interactions using the miRanda algorithm were validated. Many novel miRNAs and a huge number of targets from rice root hair males were discovered. The expression was high in the root hair and older fibril and insignificant in other types of maturity. The comprehensive analysis indicated high diversity of miRNAs and targets as well as their high complexity. In-silico evaluations were performed to identify the conserved miRNAs that would be effective for control of E. histolytica. The study opens opportunities for further in-silico evaluations of miRNA targets and designing miRNA-modifying chemistries and inhibitors for treatment of other protozoan infections [45].

6.2. Impact on Cytokine Production

In recent years, the role of miRNAs in immune regulation gained increased interest. miRNAs appear to play a role in controlling the immunological balance between cytokines. This is crucial in E. histolytica-infected THP-1 cells, presenting important information about the miRNAs involved. Knowledge about similar host gene responses in E. histolytica infections is scarce and is thus a priority area for future research [46].

Macrophage polarization is dependent on the local cytokine milieu which in turn influences the production of additional soluble mediators. In THP-1 macrophages, E. histolytica infection increased IL-10 and decreased IL-12 production. An increase of miR-255 and a decrease of miR-4324 precede the cytokine change. In this scenario, a functional target is described regarding IL-10 induction, suggesting a role for miR-155 and additional unidentified miRNAs in controlling IL-12 production. Ramos THP-1 cells (which do not respond with IL-12) and the miRNA target discovery approach suggest that a miR-155-dependent and possibly a miR-4324-independent mechanism are operating [47].

miRNA impact in the production of IL-10 and IL-12 was observed after stimulation with E. histolytica-derived mAb 5-3-2. Prior to this cytokine change, large numbers of other miRNAs were differentially expressed in Ramos THP-1 cells and may be involved. This

presents opportunities for complementary research using miRNA arrays and expression libraries. Other infectious agents induce distinct or shared miRNA responses. E. histolytica has some commonalities with E. coli and Staphyloc minuta, which are of particular interest due to the mRNA responses and the distinct miRNA approach. In contrast, serum-starved human monocyte-derived macrophages had little overlap with BCG- or LPs-triggered immunity. Surprisingly, many mitogen-responsive miRNAs were not affected in EHEC-infected macrophages [48].

7. Interactions Between microRNAs and Immune Cells

The first evidence of miRNA involvement in the regulation of E. histolytica infections in humans came from a study showing that E. histolytica-induced pro-inflammatory cytokines were regulated by miRNAs in colorectal epithelial cells. MiR-21 (up-regulated) repressed E. histolytica-induced IL-8, while let-7i (down-regulated) promoted E. histolytica-induced IL-18 expression [49]. Following this study, a comprehensive analysis of the roles that miRNAs exerted in the regulation of the immune response to E. histolytica infection was performed using a novel small RNA sequencing approach to identify the miRNA expression profile during infection and provide mechanistic insight into the regulation of inflammation by identified miRNAs [50].

A signaling pathway triggered by the binding of E. histolytica cysteine proteinase (EhCP) to the Toll-like receptor 4 (TLR4) was shown to be essential for the production of pro-inflammatory cytokines in vitro and in vivo. In response to E. histolytica infection, miR-126-3p was down-regulated both in vitro and in vivo, while IL-6 and IL-1β were up-regulated [51]. Using the simultaneous detection of the expression of these two miRNAs and cytokines during infection upon TLR4 or miR-126-3p post-transcriptional repression, it was revealed that the ELANE gene was transcriptionally activated through the TLR4-mediated NF-κB pathway by TTP sequestration mediated by miR-126-3p–dependent p85α degradation, stabilizing the pro-inflammatory cytokines IL-1β and IL-6. These findings showed that the pathogens could trigger miRNA loss-of-function responses through the functional modulation of miRNAs to regulate host gene expression during infection [52].

Another study demonstrated the important background knowledge of miRNA-target gene interactions for the rational design and functional assessments of miRNA-related drugs. Latest developments in the miRNA delivery system have set the foundation for new drug development. Moreover, many miRNA-target gene regulatory axes have been reported to be associated with the regulation of cytokine expression, providing useful information for further understanding miRNA-target gene interactions to facilitate the establishment of novel therapeutic agents targeting miRNAs. Lastly, recent bioinformatics tools for the prediction of miRNA-target gene interactions and analyses of miRNA-target gene networks are also reviewed [53].

7.1. Dendritic Cells

Dendritic cells (DCs) have an important role in the immune system by capturing, processing, and presenting antigens to naïve T helper cells, thus influencing the adaptive immune response. In mammals, three main types of DCs have been described: dendritic cells conventional (cDCs), which come from lymphoid progenitors and have more common characteristics among species and tissues; dermal dendritic cells that are located in the dermis in a close relationship with other cells; and plasmacytoid dendritic cells (pDCs) [54]. DCs are characterized by the expression of specific surface markers, such as CD1a, CD5, CD11c, and CD209, involved in antigen uptake and particular signaling pathways, which lead to several functions such as variable production of IL-12, TNF-α, IL-15, IL-18, IL-27, and act as a chemokine gradient to enhance T cell proliferation. In terms of types, cDCs can be subdivided into several phenotypically and functionally distinct subsets defined by surface markers, tissue localization, and transcription factor expression, believing to have unique roles in immunity and homeostasis. Dendritic cells are the most

potent antigen presenter cells, bridging innate and adaptive immunity [55]. The maturation of DCs is crucial for their function. Dendritic cells can capture, degrade, and present exogenous antigens and transit through lymphatic vessels to the regional lymphoid organs, resulting in T cell activation. Dendritic cells have a unique surface phenotype and tissue distribution, controlling T cell effector functions, such as Th1/Th2 polarization and regulatory T cell generation [56]. In the rat tonsil microenvironment, the number of mDCs may stimulate effector memory T cells and promote a Th2-type immune response, whereas pDCs may counteract Th2 activity and inhibit IgE production. In addition to T cell differentiation, recent studies have begun to elucidate the function of lymphoid DC subsets in driving T cell clonal expansion and the generation of long-lived TCM and TM cells during antiviral immune responses. Both CD4+ and CD8+ DCs interacted with CD4+ and CD8+ T cells through both direct DC-T cell interactions and the production of soluble mediators such as IFN-γ and IL-10 [57].

7.2. T Cells

Apart from antigen presenting cells (APCs), T lymphocytes are crucial cells of the adaptive immune response, modulating and regulating the immune response to previously encountered pathogens. Upon activation, naïve T cells can differentiate into functionally distinct T helper cell subsets, including the T helper (Th)1, Th2, Th17, and regulatory T cells [58]. In the Th1 pathway, interleukin (IL)-12 and IFN-γ drive the T-bet transcription factor, secreting TNF-α and IFN-γ with a regulatory role in the host immune response. IL-4 and the GATA-3 transcription factors drive Th2 responses, producing IL-4, IL-5, and IL-13, which are critical against external pathogens. On the other hand, IL-6 and TGF-β promote the differentiation of naïve T lymphocytes to Th17 cells that secrete IL-17 in the form of homo or heterodimers (p17), as well as IL-21, IL-22, and TNF-α [59]. Initially classically associated with autoimmune diseases, Th17 cells also present protective roles to ensure the clearance of intracellular bacteria such as Mycobacterium tuberculosis or fungal infections and, in turn, with some parasitic infections. On the contrary regulatory T (Treg) cells have the transcription factor forkhead box P3 (Foxp3) that produces IL-10 and TGF-β. Treg cells can differentiate from naïve T cells in the thymus or in the periphery microenvironments upon recognition of antigens [60].

Lately, an additional level of post-transcriptional regulation mediated by microRNAs (miRNAs) that interact with the 3′-untranslated region (UTR) of target mRNAs causing their degradation or translational repression has been found. miRNAs are small, endogenous, and non-coding RNAs with 18–24 nucleotides found in every domain of life that play a role in tissue-specific, temporally restricted, and developmentally dependent gene expression (process referred to as gene silencing) [61]. Since the discovery of the first known miRNAs lin-4 and let-7 in Caenorhabditis elegans, it has been recognized that they are evolutionarily conserved in all animals and plants, affecting development, differentiation, hematopoiesis, metabolism, and the immune response. They can also act as onco-miRs or tumor suppressors in the pathogenesis of many cancers [62].

7.3. B Cells

In addition to its effect on macrophages, the miR-155 has also a direct effect on the development of B cells. Silencing of the miR-155 in human B lymphoma cells up-regulates the expression of CD80 and CD86 molecules, as well as the levels of IL-10 and sCD23, in response to a TLR-9 agonist. In vivo, miR-155-deficient mice showed elevated levels of CD80 expression, IgM secretion and IL-10 production after immunization with TNP-Ficoll. These results suggest that miR-155 not only takes part in the immune response in the myeloid lineage, but it could also have a role among the B cells [63].

On the other hand, let-7 has effects on B-cell proliferation and IgM secretion. For instance, when Raji cells are transfected with a let-7 inhibitor, the proliferation in response to the polyclonal B-cell stimulation increases, as well as IgM secretion in response to both T-dependent and T-independent antigens, indicating a post-transcriptional regulation of

let-7 on B-cell responses. Additionally, several cytokines, such as IL-4, IL-10 and IL-21, known to affect B-cell activation and differentiation, suppress let-7 family expression in human B cells [64]. Two target genes, TGFβRIE and IL-2, were found to be regulated by the let-7 family, indicating that it has a role in modulating B-cell proliferation and differentiation downstream of several cytokine signaling pathways [65].

IL-10, interferon α (IFNα) and transforming growth factor β (TGFβ) were found to be responsible for decreased levels of let-7. Let-7 family knockdown results in enhanced NF-κB activation, and inhibition of NF-κB rescues let-7 family expression and limits IL-10 production, indicating that let-7 acts to control IL-10 production by inhibiting NF-κB signaling [66]. Furthermore, IL-2 and TGFβ are also targets of let-7, suggesting that the let-7 family could also have other regulatory functions in this pathway. In chronic lymphocytic leukemia (CLL), loss of let-7 contributes to the increased IL-10 production by B cells, which is involved in the maintenance of the leukemic phenotype and could provide a therapeutic target for CLL [67]

8. Therapeutic Implications

E. histolytica, an intracellular enteric parasite of humans, provokes clinical manifestations ranging from mild diarrhea to fulminant dysentery with colonic ulceration. However, a proportion of infected individuals remains asymptomatic. This phenomenon is probably due to a combination of host and parasite determinants, and in this regard, the immune response becomes a decisive aspect in the outcome of diseases associated with this parasite [68]. The immune system of the host must detect the parasite not only in the intestinal lumen but also in other tissues, such as the liver and lungs. To attain protection, the immune response of the host should be oriented toward the efficient elimination of this parasite, while avoiding collateral damage to the host's own tissues. It has either been suggested that, in a proportion of infected people, a Th1 immune response enables protection against amebic dysentery or the establishment of chronic infection and the development of colonic asymptomatic carriers, characterized by an intense cytotoxic Th2 immune response [69].

Importantly, these findings indicate that the perpetuity of E. histolytica in the host is not only due to the escaping strategies displayed by the parasite but also to the suppression pursued by host cells. Thus, there is an urgent need to better understand the pathways regulating the immune response against this parasite. microRNAs (miRNAs) are short, non-coding RNAs (18–25 nucleotides in length) that commonly regulate gene expression by hybridizing to the 3′-UTR region of target mRNAs, leading to their translational repression and/or degradation [70]. In recent years, the accumulating evidence on the regulatory role of miRNAs in the immune response to viral, fungi, bacteria, and parasitic infections has opened this new area of research as a promising field to better understand the role of endogenous miRNAs on the immune response to E. histolytica infections [71].

The purpose of the present review is to summarize the current knowledge about the miRNAs that modulate the immune response to E. histolytica infections in hosts with different clinical outcomes (normal and dysenteric), as well as their associated target mRNAs. Although this information is scarce, the findings described may open new avenues to better understand how miRNAs modulate both the innate and adaptative immune responses to these parasites. Moreover, these miRNAs could be considered as therapeutic tools to better control infections caused by this parasite, as well as the sequelae associated with amebic dysentery [72].

8.1. MicroRNA-Based Therapeutics

Entamoeba histolytica is a protozoan parasite that causes amoebiasis, an intestinal amoebic dysentery, which affects millions of people in developing countries. E. histolytica products induce immune responses (IS), including the production of coinflammatory and regulatory mediators. In the last decade, the role of microRNAs (miRs) as modulators of

IS to E. histolytica has gained relevance. These small, non-coding, single-stranded RNAs regulate gene expression by base-pairing with cognate mRNA targets. Functional studies of miRs in immune cells or tissues are often difficult, but investigational approaches using antagomirs or reversable locks have been employed. The widespread role of miRs in regulating fundamental processes, such as cell proliferation, differentiation, and apoptosis, has led to the investigation of their use as therapeutic agents in several diseases [73].

Several E. histolytica miRs with functional roles in the modulation of the IS to the parasite have been described so far. These studies include miR-fm1 and miR-fm4, which have been shown to regulate the expression of Muc2 fat, a key player in IS against E. histolytica and other protozoan parasites. Target predictions for some E. histolytica miRs have been proposed based on homology with other protozoans, e.g., E. invadens miR-183p target genes, which encode proteins with roles in recognition, invagination, and adherence to epithelial cells [74]. All new miRBs described in E. histolytica are encoded in fragmented genes, and most show evidence of expression targeting conserved bicoid, putative EGF, and NB. The evolutionary history of other miRBs in E. histolytica and closely-related species such as E. invadens has been analyzed, and several machines for analysing their validity for co-transcription have been proposed. Their deep conservation and tissue-specific expression suggest a key role for controlling transcript production in a developmental context [75].

Is miR-203, miR-92/miR-335, and miR-181? are involved in letting IS progression from the TH2/dex S phase to the TH1/dex G1 phase. Their target genes are TH1-transcription factors and catalyzing cytokines of a TH1 inflammatory response. E. histolytica-released proteins or active trophozoites inhibit miR-9a, miR-124 and miR-136, leading to up-regulation of Ccr2 and numeric recruitment of monocytes to the gut. The potential role of miR-pre39 could be directly involved in the recruitment of eosinophils [76].

8.2. Challenges in Clinical Application

Translation of therapeutic oligonucleotides into drugs has always imposed a daunting task. In practice, oligonucleotides often present modest pharmacologic properties. For miRNA-based drugs, major challenges arise directly from the properties of the compounds. Most importantly, quantum dots (QDs) are a specific class of semiconductors nanomaterials that are widely studied due to their unique optical and electronic properties, which are strongly size-dependent. QDs are currently extensively used in biomedical applications in vivo imaging, drug delivery, photothermal therapy, bioimaging. Highly luminescent, QDs could also serve as saturation probes and be used to study miRNA with fluorescence turn-on approach. Suitable targeting ligands or specific absorption of complementary oligonucleotides are abundant and flexible for other applications. Besides, studies of QD-miRNA could also significantly reduce the costs and time used in development of miRNA blockers and enhance their physicochemical properties [77].

miRNAs are short and easily synthesized oligonucleotides, exhibiting fluorescence turn-off. Moreover, stabilization promotes their drug development. Further efforts to introduce optimized stability promoting moieties in miRNA blockers so that they are not degraded in biological samples. However, prior to launching development of this class of drugs, it is necessary to ensure the efficacy and specificity of the candidates. The simple introduction of targeting moieties does not guarantee specificity of the eventual QDs. Therefore, pretesting of miRNA isolation and capture efficiencies would also give insights into the necessary conditions for QD efficiencies. It is likely that the chemistry used in Surface Passivation is crucial and should therefore be accurately delineated in pretesting [78]. Colonization of humans by Entamoeba histolytica is restricted to the gastrointestinal tract, where the protozoan can live asymptomatically or cause amebic dysentery and in severe cases, extraintestinal disease. Its capacity to produce disease relies on virulence determinants that are endowed in part by a multifaceted arsenal of cysteine proteases.

These proteolytic enzymes, excreted into the exterior milieu, can carry out critical pathogenic functions [79].

Regulation of gene expression in eukaryotic cells occurs primarily at the posttranscriptional level and can be mediated by numerous small RNA genomics in addition to traditional transcription factors. In the majority of the eukaryotic species examined, however, a lesser-studied class of small RNAs, microRNAs, is likely to be the most abundant in terms of the number of genes. About 200 million years ago, a few eukaryotic clades experienced a profound expansion of small RNA-encoding loci. Some of these species are now heavily populated with multiple families of primate-specific small ncRNAs [80].

9. Conclusion

E. histolytica is an important pathogen that causes colitis and dysentery. Colonization by E. histolytica can lead to an asymptomatic carrier state, self-limiting colitis, or dysentery, probably due to the presence of different pathogenicity factors and host immune factors. Understanding the genetic basis of host adaptation and pathogenicity in E. histolytica, along with advances in computational biology, has made it possible to study the evolution of the amoebas that infect humans, domesticated animals, and free-living environments. Current evidence suggests that E. histolytica diverged from a common ancestor of E. dispar and E. moshkovskii≈80 million years ago. Recent advances in genome sequencing have revealed complex gene families associated with a range of important processes and traits, including pathogenicity, phagocytosis, exocytosis, cell motility, antigenic variation, differentiation, and horizontal gene transfer. Future understanding of E. histolytica evolution will come from an increasing number of human genome sequences and annelid metagenome fragments from free-living habitats of Entamoeba. Characterization of E. dispar, E. moshkovskii, and E. invadens will provide insight into the evolution of virulence in the host-adapted E. histolytica. E. histolytica infections were recorded in classic texts, but the first specialized studies did not appear until very recently. They focused on the biology of the organism, implications for host parasite relationships, and attempts to develop a vaccine. Extensive literature searches were undertaken, especially regarding the relationship of Escherichia coli with polyps in the colon and salmonellae with enteric fever. The early literature contains a number of interesting post mortem observations of amoebic ulcerative colitis (E. histolytica infection) and a disquisition of action and passage of a specific antibiotic through the gut. The structural or histological changes in the colonic mucosa are well illustrated in the earlier literature and provide a basis for interpreting some of the later investigations on the local immune response. Because the early examinations emphasized the gross pathological changes, a late arrival related to the hybridoma method of monoclonal antibody production may seem out of context. However, this technique is being increasingly used and with its ability to provide pure, single component antibodies able to differentiate between specific antigens in E. histolytica.

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