“PROBLEMATIC” ABSTRACT Third-stage larvae of parasitic nematodes, which, in most species are the infectious stages for the mammalian host, including humans, of whom more than 3.5 billion may be infected worldwide, share common behavioral, morphological and developmental characteristics with the developmentally arrested dauer larvae of the free- living nematode Caenorhabditis elegans. It is proposed that molecular regulation of the transition from free-living to parasitic forms of parasitic nematodes and C. elegans dauer larva development regulation are similar. Significantly for the present study, it has been shown that in C. elegans, one of the key factors regulating the dauer transition is the insulin-like receptor kinase DAF-2. The parasitic nematode Haemonchus contortus has an insulin-like receptor (Hc-daf-2), which displays significant homology to insulin receptors in both vertebrates and invertebrates and is predicted to contain conserved structural domains. Examination of the parasite by RT-PCR showed Hc-daf-2 transcription in all life stages. An important proteolytic motif was identified in the predicted peptide sequence of Hc-DAF-2 and is consistent with the HIR (human insulin receptor), suggesting that it could be involved in the formation of the insulin receptor complex. To test this, comparison of the patterns of expression between Hc-daf-2 and Ce-daf-2 was performed with reporter constructs fusing the Ce-daf-2 or Hc-daf-2 promoter to the coding sequence of gfp. These were microinjected into the N2 strain of C. elegans, and establishment and examination of transgenic lines were performed. These showed similar patterns of expression in amphidial/head neurons for both genes, which may be related to sensation and signal transduction, which are important processes in host finding by infective parasitic nematode larvae. For further functional analyses of Hc-daf-2, heterologous genetic complementation studies were attempted in the CB1370 daf-2 mutant strain of C. elegans. These studies revealed that this mutation can be partially rescued by Hc-daf-2. Taken together, these data support the hypothesis that Hc-DAF-2 plays a crucial role in the transition from the free-living state to parasitism.

“FIXED” ABSTRACT  Parasitic nematodes may infect 3.5 billion humans world-wide. These nematodes are most infectious at the third-stage of larvae development. It is proposed that un this stage, the larvae share common behavioral, morphological and developmental characteristics with the developmentally arrested dauer larvae of the free- living nematode Caenorhabditis elegans. Significantly for the present study, it has been shown that in C. elegans, one of the key factors regulating the dauer transition is the insulin-like receptor kinase (Ce-daf-2). The parasitic nematode Haemonchus contortus has an insulin-like receptor (Hc-daf-2) which displays significant homology to Ce-daf-2 and is predicted to contain conserved structural domains. Examination of the parasite showed Hc-daf-2 transcription in all life stages. In this study, the patterns of expression between Hc-daf-2 and Ce-daf-2 were compared. Similar patterns of expression in amphidial/head neurons were found for both genes. We hypothesize that this be related to sensation and signal transduction, which are important processes in host finding by infective parasitic nematode larvae. For further functional analyses of Hc-daf-2, heterologous genetic complementation studies were attempted in the CB1370 daf-2 mutant strain of C. elegans. These studies revealed that this mutation can be partially rescued by Hc-daf-2 and support the hypothesis that Hc-DAF-2 plays a crucial role in the transition from the free-living state to parasitism.

“PROBLEMATIC” SPECIFIC AIMS

As part of the adult Strongyloides stercoralis (SS) life cycle (LC), female SS lay eggs in the intestinal mucosa that hatch into rhabditiform larvae, which are shed in the stool. Caused by the parasitic nematode (pn) SS, and being characterized by extreme hyperchronicity with infected individuals being diagnosed decades after leaving the endemic environment, human SS affects ~100 million people globally. It has been shown by Schad et al. (19) that maintenance of hyperchronic SS may be by a process unique to SS in which parasite larvae develop precociously to successive generations of parasitic females in the same host, which is called autoinfection (18). It has been shown that in most cases, senescent parasitic females are gradually replaced with new individuals through a continuous process of tightly regulated low-level autoinfection (9, 10). However, these chronic, clinically latent infections, in patients immunosuppressed by corticosteroid therapy (CT) or underlying HTLV-1 infection, become unregulated, resulting in a fulminant often-fatal hyperinfection (8). Clearly, a better understanding of mechanisms initiating and maintaining autoinfection by SS is critical for preventing disseminated strongyloidiasis in at-risk patients.

Hyperchronic strongyloidiasis can be modeled experimentally in infected dogs by administering low dose CT, and it is widely assumed that such autoinfection is driven primarily by steroid suppression of immune responses that would normally clear the parasite (10). However, our preliminary data are supportive of the fact that steroid-induced autoinfection results from direct action by the drug on a parasite-intrinsic steroid signaling pathway. First, we have shown that autoinfection by SS in immune-deficient NSG mice requires exogenous (hereafter ‘medicinal’) CT. Second, our multidisciplinary team, which includes the PI, a medicinal chemist, a statistician, and members of the PIs laboratory, including graduate students and post-docs, has amassed compelling evidence that endogenous steroid signaling regulates larval development in SS. Specifically, we have shown that DAF-12, a corticosteroid-class nuclear hormone receptor (NHR) signaling pathway that regulates larval development in Caenorhabditis elegans (CE), is conserved in SS. Moreover, dafachronic acids (DAs), natural ligands of the CE receptor DAF-12, regulate crucial developmental events when applied exogenously to SS (11).

Therefore, we will characterize the action of medicinal steroids or their host metabolites with the parasite homolog of DAF-12 during the process of autoinfection by SS. We will also evaluate how parasite-intrinsic NHR signaling relates to very low levels of autoinfection the relationship of this autoinfection to host immunity. We know that disseminated hyperinfection is observed in immunocompromised patients in the absence of CT. Therefore, in Aim 2, we will determine if the residual innate immune effectors of NSG mice prevents NHR- dependent autoinfection in the absence of medicinal steroids. Evidence that medicinal steroids or their metabolites function as ligands for endogenous NHR signaling in SS to promote autoinfection will constitute a milestone supporting translational studies where compounds identified as agonists or antagonists of SS NHR signaling in an existing high-throughput screen will be prioritized for in vivo testing. Prioritized compounds will be tested for efficacy in preventing autoinfection in gerbil and/or NSG mouse models of autoinfection. Milestones indicating success will be three or more lead compounds that clear hyperchronic SS infection. Our specific aims are to:

SPECIFIC AIM 1: Characterize the interaction of medicinal steroids or their host metabolites with SS during autoinfection. To this end, we will determine a) the effect of medicinal CT of young SS larvae on the frequency of autoinfection, b) whether medicinal steroids act as direct ligands for SS NHR signaling c) whether the DA-synthetic enzymes of CE are conserved in SS, and d) whether bile acid precursors in steroid-treated hosts are substrates for nematode DA-synthetic enzymes.

SPECIFIC AIM 2: Investigate the roles of remaining immune functions in the NSG mouse in regulating autoinfection. We will assess remaining components of immune functionality in the NSG mouse (neutrophils, basophils, and eosinophils) for their role in autoinfection in non-steroid treated mice.



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SPECIFIC AIM 3: Identify hits from an existing high throughput screen (HTS) for compounds that agonize or antagonize the SS NHR Ss-DAF-12. Using cell-based assays in a multi-well format we will screen small molecule libraries for hits that interfere with autoinfection.

SPECIFIC AIM 4: Advance HTS hits from Aim 3 as appropriate to testing in in vivo models of autoinfective strongyloidiasis. Hits from the HTS will be assayed for ability to prevent autoinfection in a well-characterized model of autoinfective strongyloidiasis in gerbils and in the NSG mouse model.

SPECIFIC AIM 5: Develop new in vivo models for testing. We will explore whether other animal models are also appropriate for testing hits from HTS.

“FIXED” SPECIFIC AIMS

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