Nuclear option prevents hyperinfection in the *Strongyloides* worm war

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Humans are fighting a grinding war against parasitic nematodes. More than 100 species of nematode parasites are known to infect humans. Additionally, 1.5 billion people are infected by soil-transmitted helminths worldwide that cause an annual disease burden of 5 million years lost due to disability (YLD) (1). This disease burden is greater than the annual disease burdens of malaria (4 million YLD) and HIV/AIDS (4.5 million YLD). The soil-transmitted parasite infections crush human health, productivity, and educational development (2). Currently, there are no fully effective vaccines against nematode parasites, and in the absence of adequate sanitation, drug treatment is the main method of control. Despite the numerous soil-transmitted parasitic infections, there are only a few drugs to treat them. Treatment is based on three main classes of drug (anthelmintic): the benzimidazoles (albendazole and mebendazole), the macrocyclic lactones (ivermectin), and the nicotinic compounds (pyrantel). There are concerns about the regular use of these three classes of compounds for mass dose administration because of the potential for development of drug resistance (3). There is a real and urgent need for novel therapeutic approaches to be developed to control these parasites. An innovative model and original therapeutic approach is described in PNAS by Patton et al. (4), who show that the nematode parasite, *Strongyloides stercoralis* (5), which causes a deadly hyperinfection in immunocompromised humans, can be accurately modeled in NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice, and wonderfully, can be controlled by a DAF-12 nuclear receptor agonist, Δ7-dafachronic acid. This approach provides a novel strategy for treating these lethal hyperinfections and perhaps other parasitic nematodes.

Strongyloides stercoralis is a soil-transmitted nematode, currently infecting some 30–100 million people in countries like Vietnam, Central America, and Africa in rural areas where there is limited sanitation. Nematode parasites infect their host by means of a tough third larval stage (L3) that develops after two molts from egg hatching. Once the L3 have gained entry to their host, another molt is triggered, and the parasite adapts to the new host environment and continues to grow to become an adult. There are similarities between the infectious L3 stages of parasitic nematodes and the resistant dauer stage of the model nematode, *Caenorhabditis elegans*: they are thought to be equivalent. In *C. elegans* the nuclear receptor, DAF-12, is triggered by dafachronic acids (6), a process that inhibits the transition to the resistant dauer and promotes continued normal growth. The DAF-12 nuclear receptors are also found in other parasitic nematodes and are conserved in *S. stercoralis* (7), as are the dafachronic acid signaling molecules (8).

Humans and mice are both initially infected by the third-stage *S. stercoralis* larvae (Fig. 1*A*, L3i), which penetrate the skin, often through uncovered feet. These L3i larvae migrate to the intestine, where they develop into adult females that reproduce parthogenically. In the intestine, females produce eggs that hatch and develop as first-stage larvae, also in the intestine. The L1 larvae (Fig. 1*B*) are then released and voided in the feces, or remain within the intestine to develop as L3a, autoinfective third-stage larvae that burrow into the large intestine to start a new cycle of infection within the same host. This self-infection, which is continuous, allows the *S. stercoralis* parasite infection to last for years if undetected. Unfortunately, if these infected people become immunosuppressed, for example by glucocorticoids or if they develop HIV/AIDS, the worms multiply and cause a hyperinfection, which is life-threatening. The hyperinfection is characterized by increased parasite numbers, dissemination of the parasites to different regions and tissues of the body, along with a systemic distribution of gut bacteria (septicemia).

Patton et al. (4), show that this human hyperinfection condition can be closely modeled in NSG immunocompromised mice when triggered by treatment with the glucocorticoid, methylprednisolone, and that Δ7-dafachronic acid, an agonist of the DAF-12 nuclear receptor, limits the development of hyperinfection. Their observations are both remarkable and important because they illustrate the significance of a nuclear receptor in

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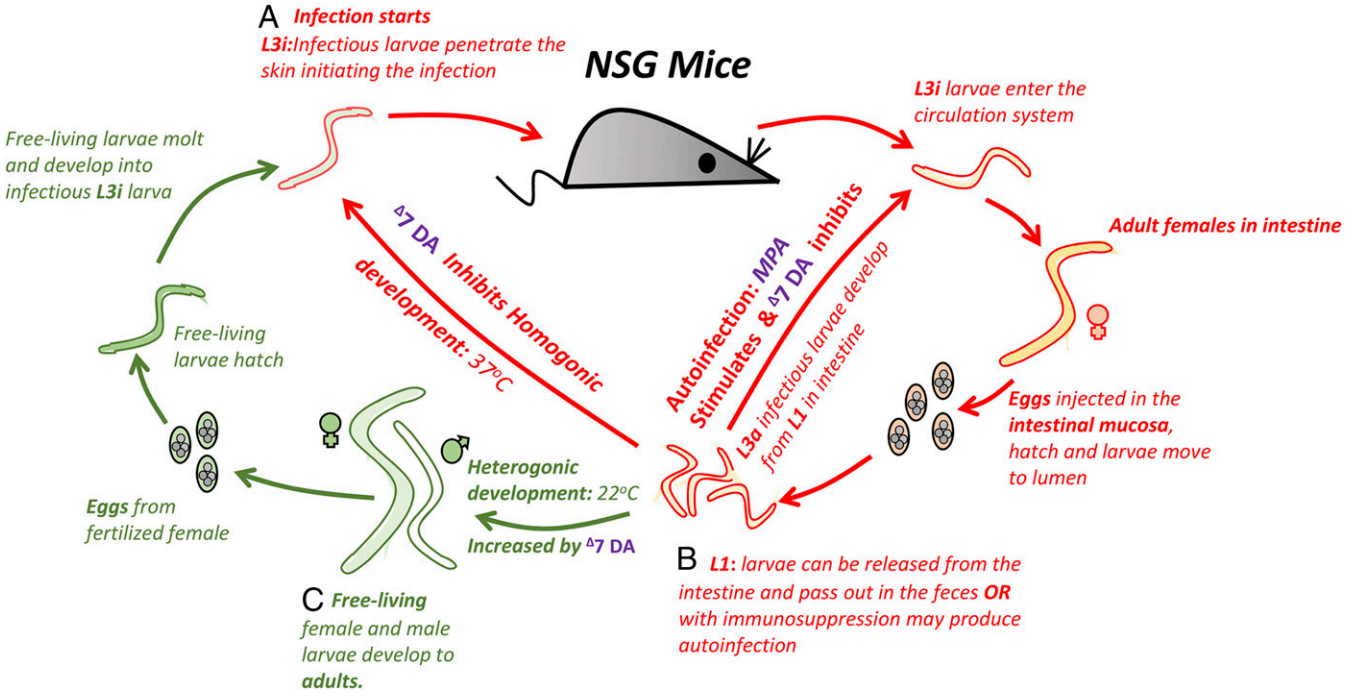

Fig. 1. Diagram of the life cycle of *S. stercoralis* in NSG mice illustrating the inhibition by methylprednisolone (MPA) and inhibition by Δ7-dafachronic acid of autoinfection. (*A*) The infection of mice through the skin by the L3i larval stage. The larvae pass into the circulating system and eventually reach the intestine after passing through the lungs and being coughed up and swallowed. They mature in the gastrointestinal tract of females that produce eggs parthogenically. (*B*) These eggs hatch in the intestine as L1 larvae, either to be voided in the feces and undergo heterogonic development to male and female adults outside the host or, alternatively, the L1 larvae remain in the intestine and undergo homogonic development to infectious L3i larvae that invade the peri-anal skin or invade the large intestine to maintain an autoinfection via L3a larvae. Methylprednisolone treatment stimulates autoinfection. Δ7-dafachronic acid inhibits autoinfection. (*C*) The free-living L1 larvae that have undergone heterogonic development to female and male worms outside the host mate and produce eggs. The eggs then hatch as free-living larvae that develop to L3i infectious larvae and complete the life cycle.

nematode parasite disease, and they describe the first mouse model that recapitulates the full range of the human *Strongyloides* hyperinfection disease characteristics. This NSG mouse model can, in future studies, be humanized with human blood stem cells to allow studies of the human immune response to *S. stercoralis* (9). The NSG model must provide all of the necessary signals and growth factors for *S. stercoralis* for it to follow its usual life cycle. We can wonder then, what are features of the NSG mouse that allow the full gamut of the disease features of *S. stercoralis* to occur? In wild-type mice, two components of the innate immune responses are involved in dealing with the parasite—the first depends on eosinophils (10, 11) and the other depends of neutrophils and macrophages (12)—but what are other factors that limit or permit hyperinfection? These factors can now be investigated in the NSG mouse model. The role of methylprednisolone in promoting hyperinfection from the limited intestinal infection is also noteworthy. How is this produced? A possibility is that the glucocorticoid inhibits a remaining component of the NSG immune system, which might be in the intestine. The well-defined effects of glucocorticoids on the intestine and intestinal barrier (13) and the L3a translocation through the intestine occur during hyperinfection, suggesting that this could be a mechanism that is involved. Glucocorticoids do not suppress the DAF-12 receptor of either *C. elegans* or *S. stercoralis*, but as reported here by Patton et al. (4), Δ7-dafachronic acid will inhibit hyperinfection in a dose-dependent manner. In *C. elegans* activation of the nuclear receptor, DAF-12, by Δ7-dafachronic acid converts the development from the dauer stage to the continuous growth stages. In *S. stercoralis* Δ7-dafachronic acid inhibits the formation of L3i that are passed out into the feces. Patton et al. (4) show here that L1 recovered from the infected gerbils, when exposed to Δ7-dafachronic acid and incubated at 37 °C, develop into free living female worms rather than L3i (Fig. 1*D*). Given the effect of Δ7-dafachronic acid inhibiting the formation of L3i and therefore L3a, we can see now why it has a very significant effect inhibiting hyperinfection in methylprednisolone-treated NSG mice.

The report and observations of Patton et al. (4) for the first time describe a mouse model that mimics the full range of the human disease characteristics of *S. stercoralis*. The study also demonstrates the very beneficial effects of Δ7-dafachronic acid treatment that is known to activate the DAF-12 nuclear receptor and is shown here to suppress the *S. stercoralis* hyperinfection induced by immunosuppression. A feature of major significance reported is the targeting of the DAF-12 nuclear receptor, which has the potential to suppress all stages of nematode parasite development related to autoinfection. The work has medical and agricultural significance that prompts the use of the nuclear option for developing advanced therapeutic weapons for the defeat of nematode parasites in the worm wars.

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