Methylprednisolone acetate induces, and \( \Delta 7 \)-dafachronic acid suppresses, *Strongyloides stercoralis* hyperinfection in NSG mice

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*Strongyloides stercoralis* hyperinfection causes high mortality rates in humans, and, while hyperinfection can be induced by immunosuppressive glucocorticoids, the pathogenesis remains unknown. Since immunocompetent mice are resistant to infection with *S. stercoralis*, we hypothesized that NSG mice, which have a reduced innate immune response and lack adaptive immunity, would be susceptible to the infection and develop hyperinfection. Interestingly, despite the presence of large numbers of adult and first-stage larvae in *S. stercoralis*-infected NSG mice, no hyperinfection was observed even when the mice were treated with a monoclonal antibody to eliminate residual granulocyte activity. NSG mice were then infected with third-stage larvae and treated for 6 wk with methylprednisolone acetate (MPA), a synthetic glucocorticoid. MPA treatment of infected mice resulted in 50% mortality and caused a significant \( >10 \)-fold increase in the number of parasitic female worms compared with infected untreated mice. In addition, autoimmune third-stage larvae, which initiate hyperinfection, were found in high numbers in MPA-treated, but not untreated, mice. Remarkably, treatment with \( \Delta 7 \)-dafachronic acid, an agonist of the parasite nuclear receptor Ss-DAF-12, significantly reduced the worm burden in MPA-treated mice undergoing hyperinfection with *S. stercoralis*. Overall, this study provides a useful mouse model for *S. stercoralis* autoinfection and suggests a therapeutic strategy for treating lethal hyperinfection.

\*Strongyloides stercoralis* | hyperinfection | NSG mice | glucocorticoid | dafachronic acid

The parasitic nematode, *Strongyloides stercoralis*, infects an estimated 100 million people. Humans acquire infections through skin penetration by infective third-stage larvae (L3i), which undergo development as they migrate to the intestine, where they molt into pathogenic female worms. Eggs released by female worms hatch in the intestine, and first-stage larvae (L1) may be released in the feces, or they may develop into autoinfective third-stage larvae (L3a). L3a are able to penetrate the bowel and initiate a new infection cycle in the primary host (1). Autoinfection allows *S. stercoralis* infections to persist in an individual undetected for decades (2, 3). Host symptoms are typically mild and nonspecific and include manifestations such as abdominal pain and diarrhea. However, infected individuals treated with glucocorticoids or infected with human T cell lymphotropic virus type 1 (HTLV-1), may develop hyperinfection syndrome. *S. stercoralis* hyperinfection is characterized by significantly increased parasite burden, the presence of L3a, widespread dissemination of parasites, and translocation of gut bacteria as the L3a penetrate the intestinal walls. Hyperinfection can be life threatening, with mortality reaching 87% when untreated (4–6).

* S. stercoralis* naturally infects humans, primates, and canines (7). Gerbils are susceptible to *S. stercoralis* infection, with all parasitic stages developing. Hyperinfection ensues when gerbils are treated with the glucocorticoid methylprednisolone acetate (MPA) (8). Wild-type (WT) mice are only susceptible to the early larval stages of the *S. stercoralis* life cycle and develop both innate and adaptive responses to control the infection. Multiple components of the murine immune response, including neutrophils, eosinophils, macrophages, and the complement cascade, contribute to the immune control of *S. stercoralis* (9). Eosinophils can kill L3i in collaboration with complement factor C3b (10, 11) whereas neutrophils require C3b and alternatively activated macrophages to effectively kill *S. stercoralis* (9, 11–13). While WT mice are resistant to the complete life cycle of *S. stercoralis*, severely immunocompromised SCID mice support the development of limited numbers of adult worms and L1 (14). This observation suggests that B and/or T cell responses are needed for complete clearance of the parasites from the tissue of WT mice (14).

A highly immune-compromised strain of mouse, NOD.Cg-Pkdcm2J2rgm1W/Il2rgtm1Wjl/SzJ (NSG), has been developed, with profound improvement in the immunodeficiency of wild-type SCID mice (15). The NSG strain, that recapitulates all forms of human strongyloidiasis. Even in severely immunocompromised NSG mice, glucocorticoid treatment was required for autoinfection, raising intriguing questions about the mechanism of glucocorticoid action. Notably, administering a nematode-derived steroid, \( \Delta 7 \)-dafachronic acid, which acts through a receptor with *S. stercoralis* to regulate parasite development, significantly diminished autoinfection in glucocorticoid-treated NSG mice. This opens the possibility of a new chemotherapy for hyperinfective strongyloidiasis, targeting the parasite's own steroid hormone mechanisms.

**Significance**

The intestinal parasite *Strongyloides stercoralis* infects an estimated 100 million people. This nematode’s unique ability to autoinfect its host enables it to persist for decades undetected and to progress to a potentially fatal hyperinfection that often is induced by glucocorticoid treatment. We report a mouse model, involving the NSG strain, that recapitulates all forms of human strongyloidiasis. Even in severely immunocompromised NSG mice, glucocorticoid treatment was required for autoinfection, raising intriguing questions about the mechanism of glucocorticoid action. Notably, administering a nematode-derived steroid, \( \Delta 7 \)-dafachronic acid, which acts through a receptor with *S. stercoralis* to regulate parasite development, significantly diminished autoinfection in glucocorticoid-treated NSG mice. This opens the possibility of new chemotherapy for hyperinfective strongyloidiasis, targeting the parasite’s own steroid hormone mechanisms.


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defects in the adaptive and innate immune responses (15). NSG mice are derived from the NOD/ShiLtJ background and carry multiple alleles reducing the function of the innate immune system, including defects in both macrophages and dendritic cells, as well as in the complement cascade (16, 17). The SCID mutation confers loss of both T and B cell function (18). In addition, NSG mice have a null mutation of the common gamma chain gene, which disrupts signaling of six different interleukins (19). Monocytes and neutrophils are present in NSG mice although their functional capacity is currently unknown. Both macrophages and dendritic cells are also found in NSG mice but are functionally compromised due to deficiencies in cytokine signaling and other genetic defects of the NOD/ShiLtJ background (17, 20). Numbers of eosinophils in NSG mice have not been determined (15).

The Caenorhabditis elegans nuclear receptor DAF-12, which regulates the switch between dauer and continuous reproductive development (21), is conserved in S. stercoralis (22). The natural ligands of C. elegans DAF-12, the dafachronic acids (23), have the capacity to signal through homologs of DAF-12 in S. stercoralis and Ancylostoma caninum, stimulating resumption of development by L3i of these parasites (24, 25) and suppressing morphogenesis of S. stercoralis L3i in both the postparasitic and post-free-living generations (24, 25). The ability of 7-dafachronic acid (Δ7-DA) to perturb L3i development in diverse clades of parasitic nematodes bolsters the potential of the DAF-12 signaling pathway as a chemotherapeutic target (24).

Based on the fact that SCID mice are somewhat susceptible to S. stercoralis infection, it was hypothesized that NSG mice would be fully susceptible to the infection. Susceptibility of NSG mice to infection with S. stercoralis was compared in the present study with the susceptibility of B6.CB17-Prkdcscid/SzJ (SCID) (18), NOD/ShiLtJ (NOD) (16, 21), and NOD.CB17-Prkdcscid/J (NOD/SCID) (17) mice. A second study hypothesis, based on the current accepted dogma that hyperinfection develops in humans due to the absence of a functional immune response (1, 5, 6, 26, 27), was that NSG mice would spontaneously develop hyperinfection. Finally, we asked whether the naturally occurring NSG mice would spontaneously develop hyperinfection. As NSG mice have a defect in complement activity (17), we hypothesized that increased susceptibility of NSG mice to S. stercoralis might be due to a deficiency in formation of complement component C3b. C3 levels measured in serum from naive NSG and C57BL/6J mice were found to be equivalent (Fig. S1A). Serum recovered from NSG and C57BL/6J mice was used in an in vitro parasite killing assay. Macrophages and neutrophils from C57BL/6J mice killed the larvae if serum from NSG or C57BL/6J mice was added to the cultures. If the sera were heat-inactivated to destroy complement, parasite killing did not occur, indicating that both sera were sufficient sources of complement for killing of the larvae (Fig. S2).

Flow cytometry was performed (29) on both blood and spleen cells from uninfected mice comprising each of the five strains tested in this study to determine the numbers and percentages of innate immune cells. C57BL/6J and SCID mice had equivalent numbers of eosinophils and neutrophils. Neutrophils were also unaffected in NOD, NOD/SCID, and NSG mice (Table S1) while eosinophils were absent from NOD mice and the derivative strains NOD/SCID and NSG (Table S1). Based on morphological differential cell analysis, eosinophils were found in the bone marrow of NSG mice (Fig. S3) but not in the peripheral blood.

**Results**

**Susceptibility of Immunodeficient Mice to Infection with S. stercoralis.** C57BL/6J, NOD, SCID, NOD/SCID, and NSG mice were infected s.c. with S. stercoralis L3i. Six weeks postinfection, mice were necropsied, and parasites were collected from all body tissues. All stages of the parasite were absent from C57BL/6J and NOD mice. Similar numbers of adult worms were recovered from SCID (6 ± 6) and NOD/SCID (7 ± 3) mice, and a significantly greater number were recovered from NSG mice (50 ± 21) (Fig. L4). Parallel to the adult worm recoveries, L1 were recovered in low numbers from SCID (2 ± 3) and NOD/SCID (1 ± 2) mice and in significantly greater numbers from NSG mice (240 ± 554) (Fig. L8). The number of L3 recovered from SCID (158 ± 123) mice was significantly lower than either NOD/SCID (585 ± 480) or NSG (557 ± 543) mice (Fig. L1C). All larvae recovered from the tissues were within the same size range, indicating that the larvae were all L3i and not L3a (Fig. L1D and Fig. S7A).

Killing of S. stercoralis larvae by the innate and adaptive response in mice depends on C3b (28) in collaboration with macrophages, neutrophils, and eosinophils (10–12). As NSG mice have a defect in complement activity (17), we hypothesized that the increased susceptibility of NSG mice to S. stercoralis might be due to a deficiency in formation of complement component C3b. C3 levels measured in serum from naive NSG and C57BL/6J mice were found to be equivalent (Fig. S1A). Serum recovered from NSG and C57BL/6J mice was used in an in vitro parasite killing assay. Macrophages and neutrophils from C57BL/6J mice killed the larvae if serum from NSG or C57BL/6J mice was added to the cultures. If the sera were heat-inactivated to destroy complement, parasite killing did not occur, indicating that both sera were sufficient sources of complement for killing of the larvae (Fig. S2).

Flowering of the intergenic region of parasitic nematodes bolsters the potential of the DAF-12 signaling pathway as a chemotherapeutic target (24).
Starting at 3 wk postinfection, there were greater numbers of L3 recovered from the MPA-treated infected mice, attaining statistical significance at 5 wk postinfection (Fig. 2D). At 6 wk postinfection, 248 ± 187 L3 were recovered from infected NSG mice and 3,459 ± 1,784 from infected mice treated with MPA. Within the L3 population, L3i could be distinguished from L3a based on size (Fig. 2E) and shape of the tail (Fig. S7A). The L3i has a bifid tail whereas the L3a has a bluntly pointed tail (Fig. S7B). L3a were not observed in untreated infected NSG mice; however, they did develop in infected mice treated with MPA beginning week 3 and continuing through week 6, with increasing frequency each week within the L3 population (Fig. 2 E and F).

**MPA Does Not Alter Switching by Postparasitic** *S. stercoralis* **L1 Between Homogonic and Heterogonic Developmental Alternatives.** We tested the hypothesis that MPA incites autoinfection by direct action on *S. stercoralis* by determining whether MPA can alter the course of development of postparasitic first-stage larvae (PPL1) in culture. PPL1, which are eliminated from the host in the feces, are capable of developing either to free-living males and females (heterogonic development) or directly to the L3i (homogonic development). When the UPD strain of *S. stercoralis* is reared at 22 °C, the heterogonic pattern of development predominates; homogonic development by PPL1 can be induced by raising the culture temperature to 37 °C (30).

MPA did not elicit a significant departure from the predominant pattern of heterogonic development seen in controls developing at 22 °C (Fig. 3A). MPA also had no effect on the pattern of homogonic development seen in control cultures reared at 37 °C (Fig. 3B). In contrast, treatment with Δ7-DA, a DAF-12 agonist, eliminated the small proportion of L3i due to homogonic development seen in control cultures developing at 22 °C (Fig. 3A). Moreover, at 37 °C, Δ7-DA treatment completely prevented infective L3i homogonic development seen in controls and instead induced all of the worms to develop into heterogonic fourth-stage larvae (L4) and free-living adults (Fig. 3B).

**Orally Administered Δ7-DA Disrupts ** *S. stercoralis** **Hyperinfection in MPA-Treated NSG Mice.** Given the ability of Δ7-DA to suppress L3i formation in postparasitic worms, we next tested dafachronic acid’s effects on hyperinfection in NSG mice. NSG mice were infected with L3i and treated with MPA. Starting at 2 wk postinfection and continuing for the remainder of the experiment, mice were treated with 1 and 10 μM Δ7-DA added to the

![Fig. 2. Effect of methylprednisolone acetate (MPA) treatment on NSG mice infected with *Strongyloides stercoralis*.](image)

**Fig. 2.** Effect of methylprednisolone acetate (MPA) treatment on NSG mice infected with *Strongyloides stercoralis*. (A) Survival of NSG mice infected with *S. stercoralis* and treated with MPA. Mice were infected with 5,000 *S. stercoralis* L3i and treated with 0.5 mg MPA. Data presented are means ± SDs (*P ≤ 0.05) of the number of adults recovered from infected mice and controls. (B) L1 recovered at 2 through 6 wk postinfection (n = 5 for vehicle and n = 10 for MPA-treated mice). (C) L3i and L3a, based on size, recovered from MPA-treated NSG mice 2 to 6 wk postinfection. (D) L3i and L3a, based on size, recovered from MPA-treated NSG mice 2 to 6 wk postinfection. (E) Distribution of L3i and L3a within the L3 population recovered 2 to 6 wk postinfection. L3i control worms were isolated from coprocultures as size reference. (F) Number of L3i and L3a, based on size, recovered from MPA-treated NSG mice 2 to 6 wk postinfection.

with increased numbers of circulating neutrophils. Additionally, intravascular and parenchymal larvae were noted in one mouse in this group; severe necrosuppurative pleuritis with Gram-negative bacilli was also noted in this mouse. Intestinal lesions in these mice included a mild neutrophilic and histiocytic enteritis with abundant larvae and bacterial overgrowth. At the time of death, two mice had larvae invading the gastrointestinal tract, heart, lungs, testis, and skeletal muscle (Fig. S6A and B). Bacteria were also observed in the heart, kidney, and lungs (Fig. S6C and Table S2).

Beginning at 2 wk postinfection, the number of adult worms recovered from the intestines of infected mice treated with MPA was significantly higher than seen in untreated infected mice. The highest number of adults was seen at week 6 (708 ± 684) (Fig. 2B). MPA-treated mice had significantly higher numbers of larvae invading the gastrointestinal tract, heart, lungs, testis, and skeletal muscle (Fig. S6A and B). Bacteria were also observed in the heart, kidney, and lungs (Fig. S6C and Table S2).

![Fig. 3. Proportions of *Strongyloides stercoralis* L3i and heterogonic fourth-stage larvae (L4) or free-living adults (FLAd) developing at 22 °C (A) or 37 °C (B) in cultures treated with 1 μM methylprednisolone acetate (MPA), 1 μM Δ7-dafachronic acid (Δ7), or ethanol control. Data are means ± SD of three biological replicates with total sample sizes for treatment or control groups ranging from 111 to 192 parasites (*P ≤ 0.05). ND, not detected.](image)
drinking water. At necropsy 6 wk postinfection, mice treated with Δ7-DA in the absence of MPA had parasite numbers equivalent to controls (Fig. 4). In contrast, in mice treated with MPA and Δ7-DA, there was a significant dose-dependent decrease in worms recovered at 6 wk postinfection compared with treatment with MPA alone (Fig. 4). This dose-dependent effect of Δ7-DA was particularly notable in the L3 population. Total L3 recoveries trended lower in mice treated with 1 μM Δ7-DA and MPA and were significantly lower in mice treated with 10 μM Δ7-DA and MPA compared with mice treated with MPA alone (Fig. 4C). When these L3 were analyzed morphologically (Fig. S7) and partitioned into L3i (Fig. 4D) and L3a (Fig. 4E), it was apparent that this decline in total L3 could be attributed to a significant decrease in both L3i and L3a in mice treated with MPA and 10 μM Δ7-DA (Fig. 4E). These results demonstrate that Δ7-DA has a beneficial therapeutic effect in a mouse model of strongyloidiasis hyperinfection.

Discussion
In this study, we describe an NSG mouse model that is susceptible to infection by *S. stercoralis* and importantly can be induced into a lethal hyperinfection with glucocorticoid treatment. Thus, this model constitutes an inbred murine strain recapitulating the full spectrum of *S. stercoralis* infection that is observed in humans and therefore should have broad application in characterizing this infectious disease. The NSG mouse model also has the potential to be humanized, reconstituted with human hematopoietic stem cells, enabling the study of the human immune response to *S. stercoralis* infection (17). *S. stercoralis* infections in humans are extremely long-lived (2, 31), through a process of autoinfection, whereby L1 develop in the intestine into L3a that penetrate the wall of the lower ileum, colon, or the skin of the perianal region, whereby L3a enter the circulation, travel to the lungs, as well as other routes, and then to the small intestine, thus repeating the life cycle. This maintains the parasite for decades in the human host, with infection levels moderated to limit pathogenicity. We conclude that NSG mice are susceptible to infection with *S. stercoralis* due to an absence of a functional immune response and the presence of cues and growth factors required for the parasite to complete its life cycle. When NSG mice were treated with the glucocorticoid MPA, the large number of adult worms was stimulated to hyperproduce L1, of which a percentage developed into L3a. Some of these L3a completed a systemic migration similar to L3i and matured into adults in the intestines, which increased the intestinal worm burden. Unlike L3i, the L3a do not penetrate the skin but migrate out of the intestine, carrying with them bacteria that can potentially induce fatal systemic infections.

What properties of the NSG mouse permit its susceptibility to *S. stercoralis* infection? NSG mice are characterized by profound deficiencies in both innate and adaptive immunity (15). In WT mice, two parasite-killing mechanisms have been identified in the innate immune response; one depending on eosinophils (10, 11) and the other on collaboration between neutrophils and macrophages (12). Importantly, the activity of both mechanisms is compromised in NSG mice, contributing to the susceptibility of this strain. The innate and adaptive immune responses also require complement factor C3b (28). NSG mice lack a functional complement cascade (32) but still maintain serum levels that are equivalent to those occurring in WT mice. Furthermore, serum derived from NSG mice was shown to be a sufficient source of C3b in vitro killing assays. Thus, we conclude that the defect in innate immunity in NSG mice that allows *S. stercoralis* to complete its life cycle is not in complement activation but rather in cell activity.

Although NSG mice support the complete life cycle of *S. stercoralis*, they do not allow formation of significant numbers of L3a or show any common signs of hyperinfection. This is surprising in view of the widely held hypothesis that hyperinfection occurs when there is a deficiency in the immune response (1, 5, 6), which may explain the prevalence of hyperinfections that are induced by glucocorticoid and HTLV-1 exposure (33).

Because neutrophils are one of the few immune effector cells remaining in NSG mice that are known to kill *S. stercoralis*, we considered whether these cells might be responsible for blocking the development of hyperinfection. However, treatment with an anti-Ly6G monoclonal antibody that depleted circulating and splenic neutrophils from NSG mice did not affect the typical profile of a *S. stercoralis* infection. This observation suggests that neutrophils do not suppress hyperinfection by *S. stercoralis* in NSG mice.
The necessity of glucocorticoid treatment to incite hyperinfection in the NSG mouse as it does in humans naturally leads to the question of what the target of MPA action might be. A plausible explanation is that glucocorticoid suppression of the immune system is required. However, ablating residual immune mechanisms from NSG mice that are known to control S. stercoralis infection in other strains failed to incite hyperinfection in non-MPA-treated animals. These findings prompt two alternative hypotheses. One is that glucocorticoids or their host metabolites promote hyperinfection through an as yet unknown component of immune function or other physiological mechanism in the host that regulates hyperinfection by the parasite. A potential target of glucocorticoid activity may be the intestine. The intestinal mucosa contains high levels of the immunosuppressive cytokines TGFβ and IL-10 that are produced not only by the regulatory T cells and dendritic cells, but importantly also by epithelial cells (34) that are still present in the NSG mouse. Given the profound effects glucocorticoids have on the intestine and in protecting the intestinal barrier (35), and the fact that L3a translocation across the intestinal barrier is required for hyperinfection, it is reasonable to speculate that the intestinal milieu plays an important role in steroid-induced hyperinfection. A second hypothesis is that glucocorticoids incite hyperinfective strongyloidiasis by direct action on the parasite, as envisioned by Genta (36), independent of host-based mechanisms. Demonstrating that glucocorticoids can signal through an appropriate nuclear receptor in the parasite would bolster this alternative hypothesis; however, experiments to date have failed to demonstrate such signaling and so fail to support the hypothesis of direct effects of glucocorticoids on S. stercoralis. Notably, immunosuppressive steroids fail to activate the DAF-12 nuclear receptor that is found in both C. elegans and S. stercoralis (22). In contrast, we found that Δ7-dafachronic acid, which is a known agonist for DAF-12, suppressed glucocorticoid-induced hyperinfection (see next paragraph). This finding is significant because Sx-DAF-12 is homologous to the nuclear receptor that governs switching between dauer and continuous developmental fates in C. elegans L3 (23). Furthermore, results of the present in vitro developmental switching experiment indicated that MPA cannot promote direct development by postparasitic S. stercoralis L1 to free living L3i and so failed to support the alternate hypothesis. However, this result does not discount that MPA promotes the development of L1 to the L3i stage. There presently is no method that promotes development of S. stercoralis L3a in vitro.

The most consistent effect of dafachronic acid on Strongyloides development, and the one most relevant to the present study, is its capacity to suppress formation of L3i, which are similar in form to L3a, in the progeny of both free-living adults of Strongyloides papillosus (37) and S. stercoralis (23, 24) and in the progeny of parasitic female S. stercoralis (24) that are voided in mice and other laboratory animals. In J Helminthol 56:23–26.


Materials and Methods

All animal experiments were conducted in compliance with the guidelines set forth by the Institutional Animal Care and Use Committees (IACUC) at Thomas Jefferson University, the University of Pennsylvania, and the University of Texas Southwestern Medical Center. UPD-strain Strongyloides stercoralis L3i were obtained from commercially available suppliers as previously described (38). Five thousand L3i were injected s.c. into mice. Necropsy consisted of analysis of all body tissues for the presence of parasites. L3i and L3a were differentiated based on lengths of fixed worms. Murine macrophages and polymorphonuclear leukocytes (PMNs) were derived as previously described and used in in vitro killing assays (12). Serum from C57BL/6J and NSG mice was used as a source of complement. Then, 0.5 mg of anti-Ly6G antibody, RB6-BCS, was injected i.p. twice per week for 6 wk to eliminate neutrophils. Mice were treated with 2 mg of methylprednisolone acetate (MPA) once per week for 6 wk by i.p. injection. Δ7-DA was synthesized as described (39). Mice were inoculated s.c. with L3i and then given weekly i.p. injections of MPA for 6 wk. Beginning in week 3 and continuing through week 6, Δ7-DA was administered to mice continuously at concentrations of 1 μM and 10 μM in their drinking water along with the weekly MPA injections. Mice consumed 5 to 7 mL/d of water, which results in a delivery dose of ~0.1–1.0 mg·kg−1·d−1 of Δ7-DA, respectively. L1, isolated from the intestines of gerbils inoculated with L3i in the absence of MPA treatment, were transferred to DMEM with either MPA or Δ7-DA and cultured at 37 or 22 °C. Differential counts, of L3i and worms having developed to heterogonic fourth-stage larvae or free-living adults, were done 24 h after culture inoculation for steroid-treated worms. Statistics and molecular, biological, and pharmacokinetic analyses were performed as described in SI Materials and Methods.

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