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Short communication: Efficacy of albendazole in *Echinococcus multilocularis*-infected mice depends on the functional immunity of the host

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ABSTRACT

Alveolar echinococcosis (AE) is a deadly parasitic disease that requires lifelong treatment with albendazole. Development of host immunity is pivotal with regard to the clinical outcome of AE, but its influence on conventional albendazole treatment is unknown. Using T-cell deficient athymic nude mice, we demonstrated that functional immunity is required for albendazole to be efficacious against murine AE. These results call for attention given the increasing number of immunocompromised patients with AE.

1. Introduction

Alveolar Echinococcosis (AE) is a life-threatening disease caused by the metacestode stage of the fox tapeworm *Echinococcus multilocularis*. In Europe and Canada, AE is recognized as an emerging disease (Gottstein et al., 2015a) and was ranked as the most important food-borne parasitic disease in Europe (Bouwknegt et al., 2018). The only curative treatment is surgical resection of the whole parasite tissue, if possible. Alternatively, lifelong treatment with the benzimidazole-carbamates mebendazole or albendazole (ABZ) has to be followed (Kern et al., 2017).

AE is characterized by a chronic disease course with a weak inflammatory response. Metacestodes form slowly growing micro-cysts (or vesicles) that modulate and escape the host immune response in a tumor-like fashion (Gottstein et al., 2015b). The immune status of the host plays a crucial role in determining the outcome of AE, both in the murine model (Boubaker et al., 2015; Emery et al., 1998; Godot et al., 2003; Liance et al., 1998; Pfister et al., 1989; Wang et al., 2018, 2017, 2015), as well as in human AE patients (Harraga et al., 1999; Jenne et al., 1998; Schmid et al., 1995). Briefly, during *E. multilocularis* infections in humans, a Th2-oriented immunity is basically associated with increased susceptibility to disease leading to chronic AE, while Th1 cell activation has been linked to protectivity, which may even yield aborted ("died-out") forms of infection (Vuitton, 2003; Vuitton et al., 2006). Experimental murine AE is characterized, as studied in spleen or lymph node cells, by an initial Th1 response at early infection stage and gradually switches to a mixed Th1/Th2 profile, characterized by the concomitant presence of IL-12α, IFN-γ and IL-4 (Wang et al., 2014). $CD4^+CD25^+$ T regulatory cells (Tregs) play a critical role in human AE by blunting immune responses to specific antigens, or by suppressing the secretion of proinflammatory cytokines, especially through interleukin (IL)-10 and transforming growth factor beta1 (TGF-β1) (Tuxun et al., 2012). Moreover, increased $CD4^+CD25^+$ Tregs were also observed in peritoneal cells of mice intraperitoneally (i.p.) infected with *E. multilocularis*, and depletion of $Foxp3^+$ Tregs led to an improved control of *E. multilocularis* infection (Wang et al., 2018).

However, the relationship between the immune response and benzimidazole treatment is still poorly understood. Human patients' records suggest that the periparasitic immune response gradually increases throughout ABZ-treatment (Ricken et al., 2017), but confirmatory placebo-controlled studies are lacking. Patients with cystic echinococcosis (CE), the disease inflicted by *E. granulosus* (Zhang et al., 2012), displayed a shifted immune response towards a Th1 profile after benzimidazole treatment. Moreover, patients with CE and low CD4 counts showed poor response to ABZ treatment, and continuous ABZ therapy was ineffective in HIV-infected patients with CE and low CD4 counts (Dumitru et al., 2015). Thus, the immune system might not only play a general role in the outcome of AE infections, but may also be involved in the degree of efficacy of ABZ treatment.

With increasing incidences of AE and the rising use of immunomodulatory therapies against other co-morbidities (Chauchet et al.,

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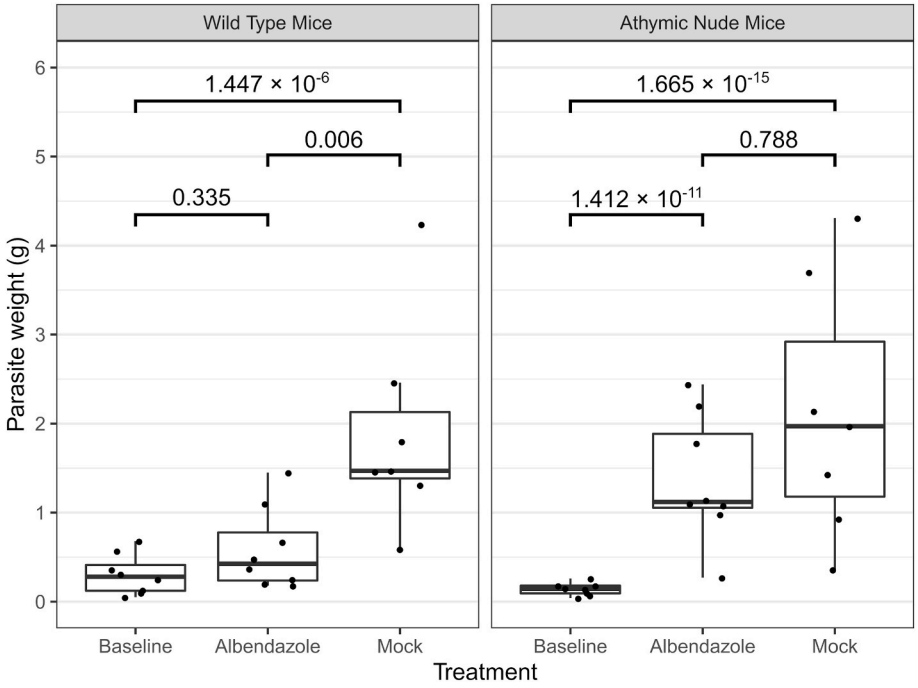

Fig. 1. Effect of albendazole treatment on metacestode cyst weights in immunosuppressed athymic nude and WT mice experimentally infected with *E. multilocularis*. "Baseline" shows the parasite weights in mice before treatment initiation, "Albendazole" and "Mock" show the parasite burdens in the groups that were treated with albendazole or mock-treated, respectively. Dots show individual measurements over summarizing box plots. Brackets show p-values after correction for multiple comparisons.

2014; Lachenmayer et al., 2019), a better understanding of the role of the immune response during AE treatment is urgently needed. We thus addressed the question whether a functional immune system is required for ABZ to be efficacious against AE in a murine model.

2. Methods, results and discussion

We chose a model of athymic nude mice, which are immunodeficient due to lack of mature T cells, and their heterozygous wild type (WT) counterparts as controls. Crl:NU(NCr)-Foxn1nu mice (n = 24) and WT (n = 24) (Charles River Laboratories, Sulzheim, Germany) were housed in a standard temperature- and humidity-controlled environment. The study was performed in accordance with the recommendations of the Swiss Guidelines for the Care and Use of Laboratory Animals (TschV, SR 455) and approved by the Commission for Animal Experimentation of the Canton of Bern (approval no. BE112/17).

At the age of 8 weeks, all mice underwent (secondary) intraperitoneal infection with *E. multilocularis* metacestodes (isolate Svalbard (Knapp et al., 2012) from *in vitro* cultures) as previously described (Rufener et al., 2018). Four weeks after infection, 8 athymic mice, and 8 WT mice (all untreated; baseline group) were euthanized by CO_2 inhalation, and their parasite tissue was resected and weighed, as previously described (Gorgas et al., 2017). Athymic mice hereby exhibited a slightly, but not significantly ($p = 0.150$), lower parasite mass than WT mice (Fig. 1). We consider this fluctuation to range among normal biological differences also observed among naturally infected wild life rodents (Gottstein et al., 1996).

The residual mice were randomly allocated into four treatment groups with 8 animals each: (I) WT control, mock-treated with corn oil; (II) WT ABZ, treated with ABZ suspended in corn oil (200 mg/kg); (III) athymic control, mock-treated with corn oil; (IV) athymic ABZ, treated with ABZ as group II. All animals were treated by gavage (100 μl), from 4 weeks post infection on for a total of 7 weeks. The treatment was given on 5 consecutive days per week, after which the animals were allowed to recover. This protocol has previously been shown to be sufficient to halt further parasite growth by treatment with albendazole, and it contributes to the 3R principle (Gorgas et al., 2017; Rufener et al., 2018). At the end of the experiment, all mice were euthanized, and their parasite mass was determined. The effect of treatment and mouse type on parasite weight was assessed by generalized linear model with gamma error and log link function. Statistical analysis was performed with the R software (R Core Team, 2019) and packages ggplot2 (Wickham, 2009) and multcomp (Hothorn et al., 2008).

The mock-treated WT mice displayed larger parasite mass than their baseline ($p = 2.79$ E−06) as well as their ABZ treated counterparts (Fig. 1, $p = 0.006$). WT mice treated with ABZ exhibited a slightly higher parasite burden compared to the baseline group, but the difference was not significant ($p = 0.335$, Fig. 1). Thus, parasite growth took place in mock-treated WT mice, but not significantly in ABZ-treated ones. In accordance, a parasitostatic effect of ABZ treatment was shown previously in a murine model of AE (Gorgas et al., 2017). When re-injected into BALB/c mice, the resected parasite tissue from both WT groups (groups I and II) grew into proliferative metacestodes confirming their viability.

Athymic nude mice treated with ABZ and mock-treated ones showed a significantly larger parasite mass than the baseline group. There was no significant difference ($p = 0.788$) in the parasite burden in ABZ-treated athymic mice when compared to mock-treated athymic mice (Fig. 1).This demonstrates that ABZ treatment did not reduce the parasite growth in athymic nude mice, in contrast to WT mice. Same as observed in WT mice, parasite tissues from both athymic mouse groups (groups III and IV) were viable. The resected metacestode tissue of all treatment groups was also examined by scanning electron microscopy, as described before (Küster et al., 2011). No differences between the samples were observed, and in all of them the development of protoscoleces was observed (Supplementary Figure).

Mock-treated WT and nude mice harbored similar amounts of parasite ($p = 0.999$). The parasite burden in ABZ-treated WT mice was lower than in nude mice. Albeit this difference was statistically not significant ($p = 0.092$), the trend further confirms that ABZ is less efficacious in nude than in WT mice.

T-cell immunity helps controlling AE (Vuitton et al., 2006). Conversely, immunosuppression favors the re-growth of larval remnants and the formation and growth of *E. multilocularis* metastases (Vuitton et al., 2006). We show here that immunosuppression of athymic mice impairs the activity of ABZ treatment against AE. A functional adaptive immunity is therefore necessary for ABZ to be efficacious against murine AE.

It remains unclear which immune mechanisms are responsible for the efficacy of ABZ against AE. Further studies, including specific cell adoptive transfer of different immune cell populations into athymic mice, are underway and might improve the current treatment options in the future.

Author statement

Conceptualization: JW, NM, AH, BG, BLS.
Experiments and data analysis: JW, NM, RR, AH, BLS.
Writing of manuscript: JW, NM, RR, AH, BG, BLS.

Declaration of competing interest

The authors declare no commercial or financial conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exppara.2020.108013.

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