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Dual genetic absence of STAT6 and IL-10 does not abrogate anti-hyperglycemic effects of *Schistosoma mansoni* in streptozotocin-treated diabetic mice

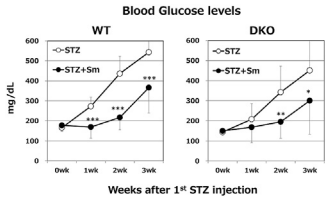
Yoshio Osada [a, *], Tomohiro Fujiyama [a], Naoto Kamimura [a], Tsukushi Kaji [a], Susumu Nakae [b], Katsuko Sudo [c], Kenji Ishiwata [d], Tamotsu Kanazawa [a]

[a] Department of Immunology and Parasitology, University of Occupational and Environmental Health, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan
[b] Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[c] Animal Research Center, Tokyo Medical University, 6-1-1, Shinjuku, Shinjuku-ku, Tokyo 160-0022, Japan
[d] Department of Tropical Medicine, The Jikei University School of Medicine, 3-25-8, Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan

HIGHLIGHTS

- *Schistosoma mansoni* infection reduces hyperglycemia induced by multiple low −dose streptozotocin injection in mice.
- The anti-hyperglycemic effect of *S. mansoni* is not dependent on Treg, STAT6 and IL-10.
- T-cell cytokine modulations are not relevant to the anti-hyperglycemic effects.
- *S. manosni* induces M2-like macrophage activation in the absence of both STAT6 and IL-10.

GRAPHICAL ABSTRACT



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ABSTRACT

Schistosoma mansoni (Sm) is known to exert protective effects against various allergic and autoimmune disorders. It has been reported that this parasite protects NOD mice from spontaneous type 1 diabetes (T1D) and ameliorates streptozotocin (STZ)-induced T1D in wild-type mice. Here, we tried to clarify the anti-diabetic mechanisms of Sm in the latter model. Sm infection partially prevented the degradation of pancreatic islets and hyperglycemia in multiple low-dose (MLD) STZ-treated mice. Neither Treg cell depletion nor genetic absences of IL-10 and/or STAT6 abrogated the anti-hyperglycemic effects of Sm. Among M2 macrophage markers, Arg-1 and Ym1, but not Retnla, remained up-regulated in the pancreatic lymph nodes and in the spleens of STAT6/IL-10 double deficient (DKO) mice. Collectively, it is suggested that Sm exerts anti-diabetic effects on this experimental T1D model via Treg/IL-4/IL-13/IL-10-independent mechanisms. Augmented expressions of Arg-1 and Ym1 in the lymphoid organs adjacent to pancreas may be relevant to the anti-diabetic effects of Sm.

* Corresponding author.
E-mail address: yosada@med.uoeh-u.ac.jp (Y. Osada).

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1. Introduction

According to “hygiene hypothesis”, bacterial and/or parasitic infections protect their hosts from allergic and/or autoimmune diseases. This hypothesis is partly supported by inverse trends of immunological disorders and infectious diseases (Bach, 2002). Parasitic helminths have been reported to protect their experimental hosts against experimental immunological disorders (Osada and Kanazawa, 2010; Osada, 2011; Versini et al., 2015). The mechanisms of helminth-induced protection have been extensively investigated, but not yet fully elucidated. Among various parasitic helminths, a blood fluke (schistosome) is known to exert systemic robust Th2 polarization under egg deposition (Grzych et al., 1991), not only against its own antigens, but also against bystander antigens (Kullberg et al., 1992). In addition, schistosome infections or their antigen administration affect other microbial/parasitic infections, allergic and autoimmune disorders in experimental settings (Osada and Kanazawa, 2011). We have previously reported *Schistosoma mansoni* (Sm) infection reduced the severity of arthritis in collagen-induced arthritis (CIA) (Osada et al., 2009) and in IL-1 receptor antagonist-deficient male mice (Osada et al., 2015).

Type 1 diabetes (T1D) is an autoimmune disease caused by immunological destruction of pancreatic islet β-cells and one of the diseases where the high prevalence in developed societies may be explained by the hygiene hypothesis (Bodansky et al., 1992). Experimental infections or antigen administration of helminths are reported to ameliorate or prevent spontaneous T1D in NOD mice (Ajendra et al., 2016; Berbudi et al., 2016; Versini et al., 2015). The NOD mouse is an ideal T1D model because of its similarity to human T1D; however, lack of rapid synchronous onset renders pathogenic parasites (e.g. schistosome) difficult for use in long-term experiments due to death of the infected mice during the experimental period. Thus, we used a multiple low-dose (MLD) streptozotocin (STZ)-induced T1D model in C57BL/6 mice (Paik et al., 1980) to evaluate effects of Sm. STZ is toxic to pancreatic β-cells (Yamamoto et al., 1981) and MLD administration of STZ induces β-cell destruction via direct damage by the compound and possibly, subsequent immunological mechanisms (Kantwerk-Funke et al., 1991; Herold et al., 1995). Several parasitic helminths (Sm, *Taenia crassiceps* (Tc), *Heligmosomoides polygyrus* (Hp) and *Strongyloides venezuelensis* (Sv)) have been reported to prevent T1D in this model (El-Wakil et al., 2002; Espinoza-Jiménez et al., 2010; Osada et al., 2013; Peres et al., 2013) but the anti-diabetic mechanisms are not fully elucidated. Here, we confirmed the anti-diabetic effects of Sm in STZ-induced diabetes reported by El-Wakil's group and tried to identify essential immunomodulation by Sm for the anti-diabetic effects. We discuss requirement of Th2 cytokines and possible roles of regulatory cells (especially Treg cells and M2 macrophages) in the anti-diabetic mechanisms.

2. Materials and methods

2.1. Ethical approval

All procedures performed in this study involving animals were accepted and conducted in accordance with the Guiding of Principle for Animal Care Experimentation, The University of Occupational and Environmental Health, Japan, and under the control of the Japanese Law for Animal Welfare and Care (No.221).

2.2. Mice and parasites

Age-matched male wild-type (WT) C57BL/6 and gene-deficient mice were used in the animal experiments. Specific pathogen-free WT C57BL/6 mice were purchased from SLC (Hamamatsu, Japan) at 6–8 weeks of age. STAT6-deficient (STAT6KO) mice (Takeda et al., 1996) were kindly provided by Dr. Akira at Osaka University. Interleukin-10 (IL-10) −deficient (IL-10KO) mice (Kühn et al., 1993) were obtained from the Jackson Laboratory (Bar Harbor, ME). STAT6/IL-10 double deficient (DKO) mice were produced by the crossbreeding of STAT6KO mice and IL-10KO mice. All mice used in the experiments were kept in an air-conditioned room (22 ± 2 °C, 55% humidity) with a sufficient supply of food and water. Tail vein bleeding for blood glucose level measurement was performed under inhalational anesthesia with sevoflurane.

A Puerto Rican strain of Sm was maintained using intermediate host snails (*Biomphalaria glabrata*) and male ICR mice. Fresh cercariae were obtained from the infected snails at 5–10 weeks post-infection. ICR mice were infected with the cercariae via tail skin percutaneously. More than 8 weeks later, the infected mice were euthanized and the granulomatous livers were minced and then digested with 1 mg/mL collagenase and 1 mg/mL actinase in 1.7% NaCl (2× physiological saline) solution at 37 °C for 4–6 h. The eggs were purified by filtration through metal mesh. Miracidia were obtained from the eggs under low osmotic pressure in aged tap water and immediately used for infection to the snails.

An intestinal nematode Hp was used for some of the infection experiments. For maintenance of the life cycle, male ICR mice were orally infected with infective larvae of Hp suspended in water. More than 2 weeks later, stools of the infected mice were harvested and the urine was removed by gentle washing. Mashed stools were cultured on rolled wet filter paper partially immersed in tap water. After a 1 week culture, infective larvae were harvested, washed and enumerated, and kept in tap water at 4 °C until used.

2.3. Parasite infections and T1D induction

WT and gene-deficient C57BL/6 mice were infected with 100 cercariae of Sm via tail skin percutaneously. Six weeks later (day 0), mice were intra-peritoneally injected with 50 mg/kg STZ (Wako Pure Chemical Industries, ltd., Japan). STZ was dissolved in a 1:1 mixture of 0.1 M sodium citrate buffer (pH 4.5) and physiological saline, and immediately injected to the mice within 5 min. The mice were fasted for 4 h before each injection. The same dose of STZ was administered once a day for the next 4 days (day 1 to day 4): i.e. 5 times in total (a multiple low-dose model of STZ-induced T1D). Blood glucose levels were measured weekly with a glucometer (Glutest Neo, Sanwa Kagaku Kenkyusho Co., Ltd, Japan). At 3 weeks after the first injection of STZ, the mice were euthanized for sampling. Adult worms were harvested by portal perfusion with 0.45% trisodium citrate in physiological saline and then enumerated. No significant differences in adult worm recovery were observed between WT mice and the gene-deficient mice (data not shown). The spleens were excised and the spleen cells were used for culture experiments for cytokine measurement. Pancreatic lymph nodes (PLN) and small parts of the spleens were snap-frozen in liquid nitrogen and kept at −80 °C for real-time PCR analysis. The pancreases were fixed in 10% neutral buffered formalin for histopathological analysis. In some experiments (Figs. 2 and 3), the tissue samples were excised from euthanized mice 1 week after the first injection of STZ. Regarding Hp infection, mice were infected with 200 infective larvae by oral administration 1 week before the first injection of STZ. There were no significant differences in adult worm recovery between WT and DKO mice (data not shown).

2.4. Treatment of mice with cyclophosphamide or anti-CD25 Ab

In some experiments, the mice were treated with cyclophosphamide (CY) or an anti-CD25 MoAb to deplete regulatory T (Treg) cells. CY was intraperitoneally injected to the mice 1 day before and