Trends in Immunotherapy (2021) Volume 5 Issue 2.1: Special Issue - Research Progress in Immunotherapy in China
doi:10.24294/ti.v5.i2.1.1365

ORIGINAL RESEARCH ARTICLE

The effects of isolates and immune function on hematologic parameters of Blastocystis infection rats

Ting Deng, Juan Li*, Xiaohua Li, Xiaobo Li, Yiming Yan*

School of Basic Medicine, Gannan Medical University, Ganzhou 341000, Jiangxi Province, China. E-mail: lily_lij@163.com (Juan Li); yym2008101@163.com (Yiming Yan)

ABSTRACT

Objective: To define a complex of changes in hematologic parameters associated with subtypes (ST) of Blastocystis sp. infections and the status of immune function in Sprague Dawley (SD) rats, and lay the foundation for Blastocystis hominis pathogenesis research. **Methods:** 5 isolates of ST1, ST3 and ST7 were used, including 1 isolate of ST1 from symptomatic patient, 2 isolates of ST3 and ST7 from symptomatic patients and asymptomatic carrier separately. Immune compromise model was set up using dexamethasone (DEX) and infection models with 5 isolates of ST1, ST3 and ST7, and then examined the hematologic changes post infection 15 days using fully automatic hematology analyzer sysmex xe-2100. **Results:** The results showed that infections of Blastocystis STs leaded to the increase of platelet indexes including MPV and PDW except ST3 isolated from asymptomatic carrier only with PDW increase and the higher values of PLT in ST7 isolated from asymptomatic carrier compared with the controls in the immune competence status ($P < 0.05$). However, the infections of Blastocystis ST7 isolated from symptomatic patient gave rise to higher values of WBC, LYMP, EO, MCV and RDW-SD while lower values of NEU% compared with the controls in immune compromise status ($P < 0.05$). Meanwhile, higher values of WBC and LYMP and lower NEUT% values were observed in ST1 infections compared with the controls ($P < 0.05$); lower NEUT values in ST1 infections and controls compared with ST3 and ST7 respectively were observed ($P < 0.05$); the infection of ST3 isolated from symptomatic patient resulted in higher values of MCV and RDW-SD while the asymptomatic isolate of ST3 only had higher RDW-SD ($P < 0.05$). **Conclusion:** The virulence of Blastocystis sp. isolated from symptomatic patient is higher than that of the identical subtype one isolated from asymptomatic carrier. The infection of ST7 isolated from symptomatic patients may result in the most distinct hematologic changes among STs, and then followed by ST1 symptomatic isolate. And the severity of Blastocystis sp. infection may be mediated by the immune status of host.

Keywords: Blastocystis; Immune Function; Hematologic Parameters

ARTICLE INFO

Received 16 August 2021
Accepted 28 September 2021
Available online 6 October 2021

1. Introduction

Blastocystis is a protozoan that lives in the intestines of humans, mammals, birds, mice and reptiles. The infection rate is about 3%–5% in developed countries and about 60% in developing countries[1]. Many intestinal symptoms and skin diseases are related to Blastocystis infection[1–3]. The most common intestinal symptoms of Blastocystis infection are diarrhea and abdominal pain[4], and nonspecific symptoms include nausea, vomiting and flatulence[1,4,5]. Based on small subunit rRNA gene analysis, 13 subtypes have been found[6]. Among them, 9 types (ST1–ST9) are found in humans[7,8]. Recent studies have suggested that the pathogenicity of Blastocystis is related to subtypes[9,10], but the results are still controversial[2,11]. Some reports believe that the symptoms of ST1 infection are highly correlated with

subtypes, suggesting that ST1 has potential pathogenicity[12]. ST3 is the most common subtype in human epidemiological investigation, having potential pathogenicity according to some studies[5,10].ST7 is common in Asia[1], but rarely reported in the West[13], and most are isolated from patients with symptoms[5]. It has been suggested that Blastocystis is an opportunistic pathogenic protozoan, because immunosuppressed people are prone to infection and show symptoms. However, the role of this protozoan in health and disease is unknown.

It is known that many pathogens such as bacteria, viruses and fungi can cause changes in hematologic parameters[14–16]. There are also some reports on the changes of hematologic parameters caused by parasitic infection[17–20], but there is little study on the changes of hematologic parameters caused by Blastocystis infection. It is reported that dexamethasone can cause apoptosis of thymus, spleen and lymph node cells and significantly inhibit the immune system[21]. In this paper, dexamethasone was used to establish an immunosuppressive animal model to explore the relationship between the changes of hematologic parameters and isolates and immune function of Blastocystis infection rats.

2. Materials and methods

2.1 Collection of isolates and capsules

Five isolates, HC07-12 (ST1), HC09-01 (ST3), HC08-03 (ST3), HC07-03 (ST7) and HC09-05 (ST7), were used in this experiment. Among them, HC07-12, HC08-03 and HC07-03 were isolated from diarrhea patients in the First Affiliated Hospital of Gannan Medical College, and the rest were isolated from asymptomatic physical examination personnel. These isolates were conserved and passaged in the laboratory. The trophozoites in the LES medium of Blastocystis hominis were inoculated into the cystic medium after preliminary centrifugation. At the peak of capsule maturation on the 6th day, the capsules in the cystic medium were separated with lymphocyte separation solution. The capsules were treated with distilled water and stored at 4 ℃ for standby.

2.2 Experimental animals, feed, and experimental environment

60 SPF-grade SD rats, male, with a body mass of (100 ± 10) g, were purchased from Hunan SJA Laboratory Animal Co., Ltd., (animal certificate No.: 4300016942). All animals were fed in a single cage for 1 week with mixed formula feed (provided by the Experimental Animal Center of Gannan Medical College), ate and drank freely. Change drinking water and feed every day, keep the living environment of rats ventilated and clean, observe their conditions of eating, drinking, activity and defecation, and start the experiment after confirming the health of rats.

2.3 Establishment of the dexamethasone immunosuppression model

60 SD rats were adaptively maintained for 1 week and randomly divided into two groups, namely the immunosuppression group and immunonormal group. Immunosuppressive rats were added with dexamethasone at 0.25 mg·kg^{-1}·d^{-1} in drinking water for 10 days, while the rats in the immunonormal group were routinely housed.

2.4 Infection experiments

On the 11th day after immunosuppression modeling, the immunosuppression group and immunonormal group were randomly divided into 6 groups, respectively. The infection group was orally fed with 1000 capsules of HC07-12, HC09-01, HC08-03, HC07-03 and HC09-05 respectively, and the control group was orally fed with the same amount of normal saline (0.85% NaCl). Feces of all rats were collected every other day after gavage and placed in LES medium containing 10% calf serum[22].Typical vacuolar or granular type found by microscopic examination was positive for infection.

2.5 Collection of blood samples and determination of hematologic parameters

On the 16th day of infection, the rats in the two groups fasted food for 24 hours and water for 12 hours. Weigh and inject 10% chloral hydrate into the standard abdominal cavity of rats according to 0.3 mL·100 g^{-1}. Fix the limbs of anesthetized rats, collect blood from their hearts and inject it into heparin sodium anticoagulant tubes for examination.

The automatic hematology analyzer sysmex xe-2100, produced by Sysmex Corporation, was used to determine hematologic parameters by the impedance method.

2.6 Statistical analysis

Express hematologic parameters as $\bar{x} \pm s$, analyze data by SPSS 14.0 two-way ANOVA, and plot with prism 5.0.

3. Results

The results of changes in hematologic parameters caused by infection of different isolates under normal immune function and immunosuppression are shown in **Figures 1–10. Figure 1** shows that the PDW value of Blastocystis infection rats in the immunonormal group were higher than that in the control group, which was statistically significant ($P < 0.05$). **Figure 2** shows that except HC09-01 isolated from asymptomatic carriers, the MPV value of other Blastocystis infection rats in the immunonormal group were higher than that in the control group, which is statistically significant ($P < 0.05$).

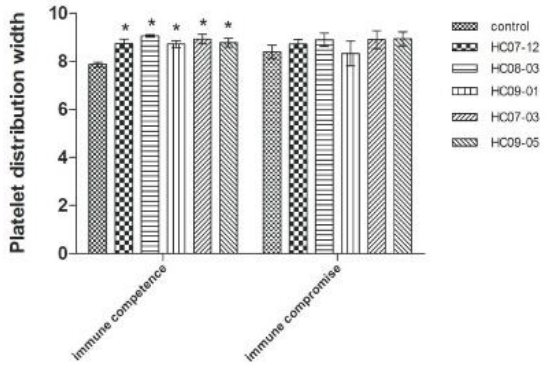

Figure 1. Effect of different isolates of Blastocystis infection on PDW of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

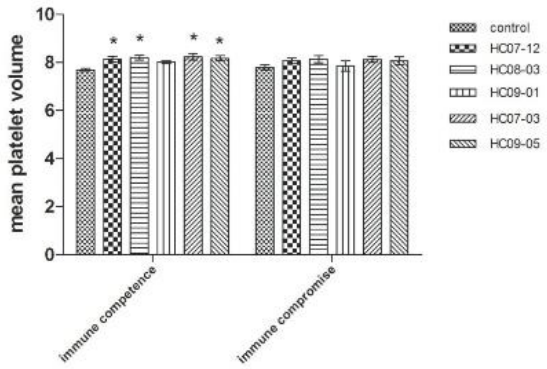

Figure 2. Effect of different isolates of Blastocystis infection on MPV of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figure 3 shows that ST7 HC09-05 isolated from asymptomatic carriers leaded to the PLT value of rats with normal immune function was higher than that of rats in the control group, which was statistically significant ($P < 0.05$).

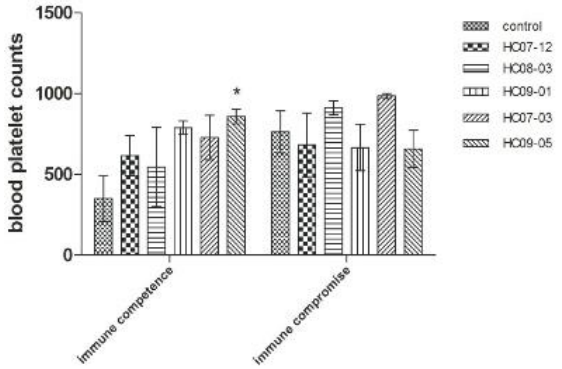

Figure 3. Effect of different isolates of Blastocystis infection on PLT of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figures 4 and **5** show that the WBC and LYMP values of rats in the immunosuppression group were higher than those in the control group ($P < 0.05$) due to the isolation of ST7 HC07-03 and ST1 HC07-12 from symptomatic patients.

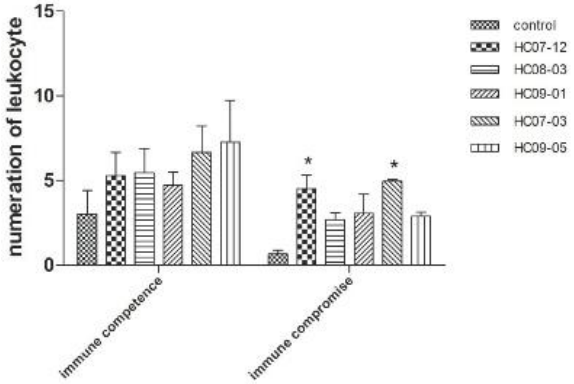

Figure 4. Effect of different isolates of Blastocystis infection on WBC of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

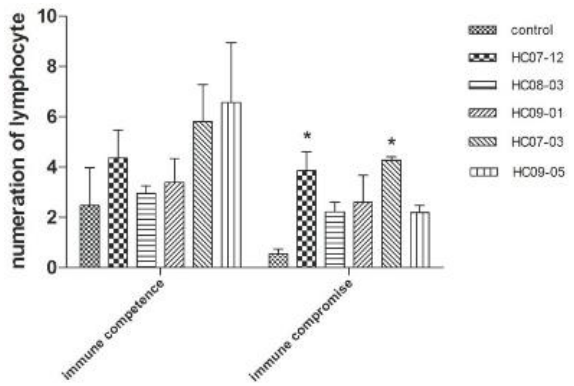

Figure 5. Effect of different isolates of Blastocystis infection on LYMP of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figure 6 shows that the infection of ST3 HC09-01 isolated from asymptomatic carriers resulted in the increase of NEUT value in the immunonormal group ($P < 0.05$). The infection of ST3 HC08-03 from symptomatic patients and ST3 HC09-01 and ST7 HC09-05 isolated from asymptomatic carriers resulted in the increase of NEUT value in the immunosuppression group.

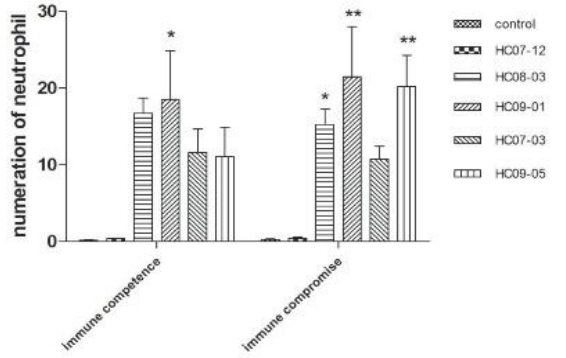

Figure 6. Effect of different isolates of Blastocystis infection on NEUT of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figure 7 shows that ST1 HC07-12 and ST7 HC07-03 isolated from symptomatic patients resulted in lower NEUT% of immunosuppressive rats than in the control and other subtypes ($P < 0.05$).

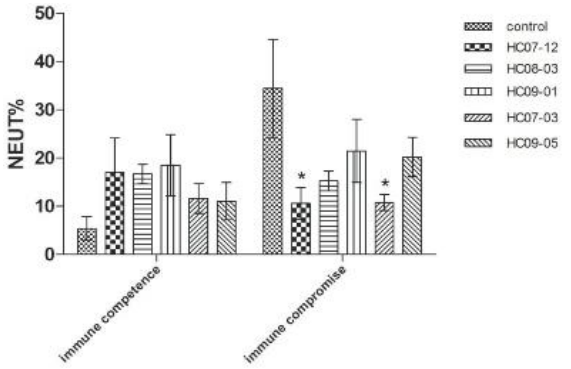

Figure 7. Effect of different isolates of Blastocystis infection on NEUT% of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

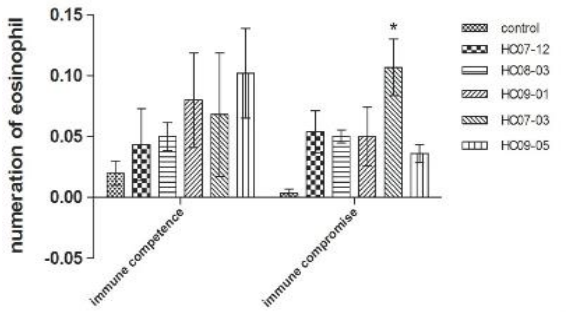

Figure 8. Effect of different isolates of Blastocystis infection on EO values of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figure 8 shows that ST7 HC07-03 isolated from symptomatic patients resulted in higher EO values of immunosuppressive rats compared with those of the control group ($P < 0.05$).

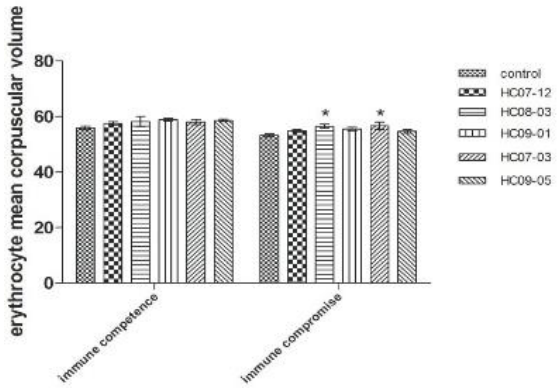

Figure 9. Effect of different isolates of Blastocystis infection on MCV values of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figure 9 shows that ST7 HC07-03 and ST3 HC08-03 isolated from symptomatic patients resulted in higher MCV values of immunosuppressive rats than those of the control group ($P < 0.05$).

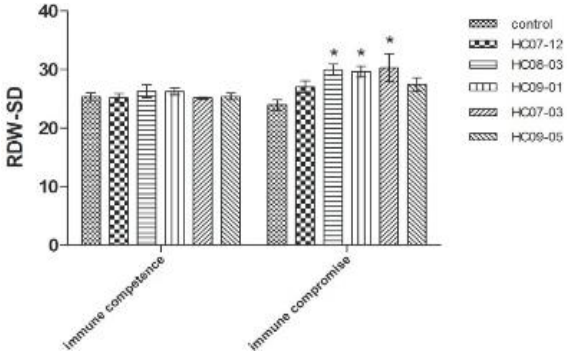

Figure 10. Effect of different isolates of Blastocystis infection on RDW-SD of SD rats with different immune functional states.
Note: * comparison with the control group, $P < 0.05$.

Figure 10 shows that the infection of ST7 HC07-03 and ST3 HC08-03 isolated from symptomatic patients and ST3 HC09-01 isolated from asymptomatic carriers was higher than that of the control group ($P < 0.05$).

4. Discussion

Blastocystis is a widely distributed intestinal parasitic protozoan of humans and many kinds of animals. Although Blastocystis infection is common, its pathogenesis is still controversial. This is because Blastocystis is not only distributed in symptomatic patients, but also in asymptomatic peo-

ple[12,23], and Blastocystis has genetic diversity, and pathogenic strains coexist with non-pathogenic strains[23,24]. The effects of different subtypes and different Blastocystis isolates in the same subtype on hematologic parameters in immunonormal and immunosuppressive rats were studied. Five isolates of subtypes ST1, ST3 and ST7 were used in this study. One isolate of each subtype was isolated from symptomatic patients, and ST3 and ST7 were isolated from asymptomatic carriers. In this study, it was found that under the condition of normal immune function, the infection of each isolate of Blastocystis only caused the overall or partial increase of platelet parameters PDW, MPV and PLT, suggesting that Blastocystis infection has the tendency to lead to coagulation, and its exact mechanism is not clear. Blastocystis infection releases proinflammatory factors such as IL-6, IL-8 and cysteine protease, which may have a potential mechanism to activate coagulation reaction. Blastocystis infection causes changes in the parameters of erythrocyte and leukocyte lines under immunosuppression, but it varies according to the strain. Isolation of ST3 and ST7 strains from symptomatic patients resulted in MCV and RDW-SD changes in immunosuppressive rats, suggesting that infection with strong toxic isoforms ST3 and ST7 may cause large cellular anemia, consistent with clinical reports that Blastocystis infection can cause iron deficiency anemia[25].

The infection of ST7 isolated from symptomatic patients led to the increase of WBC, LYMP, NEUT and EO, and the decrease of NEUT%, which may be due to the increase of neutrophil number, but the increase proportion is less than that of WBC, LYMP and EO. The infection of ST1 isolated from symptomatic patients resulted in the increase of WBC and LYMP, but the decrease of NEUT and NEUT%. The infection of ST3 isolated from asymptomatic carriers increased the NEUT value of rats in the immunonormal group and immunosuppression group, while the infection of ST3 isolated from symptomatic patients only increased the NEUT value of rats in the immunosuppression group. Neutrophils are the most important movable phagocytes in mammals. As the first innate immune cell to reach the infected site, it plays an important role in initiating innate immunity, inflammatory response and specific immune response. In this study, it was found that the number and proportion of neutrophils decreased due to the infection of ST1 with strong virulence, while the neutrophils increased due to the infection of ST3 with weak virulence. These results suggest that neutrophils may play an important role in immunity against Blastocystis infection. ST1 subtype Blastocystis may have virulence factors to kill neutrophils, but the exact mechanism needs to be further explored. This study found that the infection of ST3 isolated from asymptomatic carriers increased the number of neutrophils, but whether it could improve other natural immune functions, whether it was a normal member of intestinal microecology, and whether it had clinical application value still need to be confirmed by further research.

5. Conclusion

In conclusion, the virulence of the isolates from symptomatic patients was stronger than that of the same subtype isolated from asymptomatic carriers. The virulence of ST7 isolated from symptomatic patients was the strongest, resulting in the most extensive changes of hematologic parameters, followed by ST1, whose characteristic change was the decrease of neutrophils. The severity of Blastocystis infection also depends on the host's immune function status. Hosts with normal immune function are not pathogenic or only show a tendency to increase platelet parameters; immunosuppressive hosts can result in changing parameters of white blood cells and red blood cells.

Conflict of interest

The authors declare no potential conflicts of interest.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 30860254), and the Natural Science Foundation of Jiangxi Province (No. 20132BAB2050551).

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