Comparative Analysis of 3 Experimental Mouse Model for Blood Hematology and Chemistry

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The immune system and neuroendocrine systems are the two key components that maintain bodily homeostasis. Peripheral blood specimens can indicate abnormalities in a body, which often cause various threats to human health, including devastating autoimmune or metabolic diseases. To develop a treatment regimen for such diseases, experimental animal models are indispensable to researchers in academic fields. In this study, we examined the peripheral blood of 3 representative mouse strains (ICR, Balb/c, and C57Bl/6), which are widely used, to investigate whether there is a difference in reference range according to animal model. We performed hematological and chemistry analysis on individuals of both genders. The results of hematology analysis showed that the number of most types of blood cells was lower in ICR than in the other two strains. The results of chemical analysis revealed no specific pattern, but different patterns according to the individual indicator. Although the distinction between ICR and B6 was prominent, differences between Balb/c and B6 were also observed for several indicators. For some indicators, totally different patterns existed between females and males. Conclusively, this study provides the information that 3 experimentally representative mouse models have their own basal levels of blood components, suggesting the importance of a careful choice of a proper mouse model in research into immune or metabolic diseases, to exclude any biases.

Key Words: Mouse model, Blood hematology, Blood chemistry

INTRODUCTION

In the human body, homeostatic balance is elaborately controlled by two key components, which are the immune system and the neuroendocrine system (Reichlin, 1993).

They do not function separately from one another, but work mutually and cooperatively and have intimate connections through a complex network via continuous communication of cytokines, neurotransmitters, hormones, and etc. (Reichlin, 1993). Recently, the importance of such interactions has been become clearer in aspects of disease because abnormalities

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in certain processes often cause various harmful effects on human beings, such as autoimmune or metabolic diseases (Postal and Appenzeller, 2015; Procaccini et al., 2016).

Thousands of researchers in medical fields are intensively working on effective treatment regimens for those diseases. In the process, they are heavily relying on experimental animal models (Rust, 1982; Lee et al., 2015). Various kinds of animals are being used for experiments, which include rodents, such as mice and rats, larger animals, like rabbits and goats, and even primates, such as monkeys. Of those, the mouse is the most commonly used animal model because of advantages including genomic similarities with humans (Mouse Genome Sequencing Consortium et al., 2002). Recent advanced gene editing techniques make them even more attractive to researchers (Yang et al., 2014).

For the purpose of ease and reproducibility of experiments, genetically identical mouse models have been produced, and also various different mouse strains with genetic modifications have been generated (Justice et al., 2011). Each mouse strain has its own unique biological characteristics and so using just one may lead to inaccurate conclusions or interpretations of an experiment. Therefore, it is important that researchers carefully choose the proper mouse model for their purposes.

As metabolites and biochemical molecules in the body are soluble or solubilized, they flow in the body's fluids (Antunes-Rodrigues et al., 2004). Immune cells also move around the whole body to seek and attack an invader via the circulatory system (Israels and Israels, 1999). Therefore, peripheral blood is helpful in detecting abnormalities that occur in the immune and neuroendocrine systems. Although experimental mouse models are widely used in most studies on human diseases, there is still not enough information on the normal blood conditions of the mouse models. In this study, we examined the peripheral blood of 3 representative mouse strains (ICR, Balb/c, and C57Bl/6) with different genetic backgrounds in order to help researchers choose a proper animal model by investigating the reference ranges for blood tests in each.

MATERIALS AND METHODS

Animals

Five week-old female and male ICR, Balb/c, and C57Bl/6 (n=3~10/group) were purchased from Central Lab Animal Inc., and maintained in specific pathogen-free conditions at an animal facility. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University, and performed in accordance with animal ethics regulations. At all times, the mice were carefully observed for any behavioral abnormalities. When the mice reached 11~12 weeks of age, blood tests were performed. Beginning one day before the tests, the mice were fasted for 24 hours.

Blood analysis

Peripheral blood was collected by eye bleeding under inhalation anesthesia. The amount of blood to be collected was 200~300 µl/mouse. Upon sampling, the whole blood specimen was first applied to a hematology analyzer (iDEXX Procyte Dx) and then diluted with normal saline at a 1:1 ratio to apply to a chemistry analyzer (iDEXX Procyte Dx). For blood biochemistry, Chem 17 CLIP (iDEXX procyte Dx) was used. The output results were made into graphs using GraphPad prism 5.0 software.

Statistical significance

Statistical significance was analyzed by Students' *t*-test using GraphPad prism 5.0 software. *P* values under 0.05 were considered statistically significant.

RESULTS

Hematological difference between 3 mouse strains

We obtained peripheral blood from both genders of the each mouse strain, and first applied it to a hematology analyzer. First, we analyzed the concentrations of 3 major categories of immune cells, which are red blood cells (RBCs), white blood cells (WBCs), and platelets. As shown in Fig. 1, the results showed that B6 mice had the highest levels of RBCs in both genders, WBCs in males, and mildly higher

Fig. 1. Hematology analysis for 3 major categories of blood cells. Red blood cells, white blood cells and platelets were analyzed in peripheral blood from both genders of ICR, Balb/c, and B6 mice. Statistical significance (vs control group) * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

levels of WBCs in female, while ICR mice showed slightly higher levels of platelets in females and significantly higher platelet counts in males. Balb/c mice had intermediate levels of these parameters.

Next, we analyzed further RBC-related parameters like reticulocyte number and hemoglobin concentration. Interestingly, Balb/c mice had a markedly higher number of reticulocytes in females, but not in males, while there was no distinction in the concentration of hemoglobin between the 3 strains (Fig. 2A). Then, we analyzed the subpopulations of WBCs (Fig. 2B). B6 showed the highest levels of lymphocytes in both genders, and monocytes in males, while also showing the lowest levels of neutrophils in females and basophils in males. Balb/c mice showed the highest levels of monocytes and neutrophils in females, and lower amounts of eosinophils in females. ICR mice had the lowest number of monocytes in females, and of eosinophils and basophils in males. Overall, B6 mice tended to be rich in lymphocytes and monocytes, whereas Balb/c mice tended to have larger granulocyte populations. ICR mice had patterns that were closer to those of B6 mice.

Biochemical differences between the 3 mouse strains

Then, we examined biochemical molecules in the peripheral blood using chemical analysis. First, we went through a routine checklist (Fig. 3). The results for total protein (TP), albumin, and globulin showed that there was a significant difference between ICR and B6 in males, but such a

phenomenon was not observed in females (Fig. 3A). When ion components like phosphate and calcium were examined, the results showed that B6 mice contained significantly lower concentrations of phosphate in both genders, and of calcium in males, compared to the other 2 strains (Fig. 3B). In contrast, the results of representative metabolites like glucose and cholesterol showed a rather different pattern (Fig. 3B). B6 mice showed a significantly higher glucose concentration in both genders, while ICR mice showed the lowest levels in females, but a similar level as male B6 mice. In contrast, the cholesterol level in the blood was significantly higher in ICR mice of both genders than it was in the other two strains.

Finally, we examined indicators of organ function (Fig. 4). First, representative markers of liver function were analyzed, which are total bilirubin (TB), ALT, and ALKP (Fig. 4A). The results showed that Balb/c mice contained the highest level of TB in both genders, and the highest level of ALKP in females, but the lowest level of ALT in males. Interestingly, ICR and B6 showed a similar pattern except for ALT in males and ALKP in females. There was no obvious difference between the 3 strains in the level of ALT in females and ALKP in males.

Furthermore, we analyzed indicators of other organs, such as the kidney or pancreas, which are BUN or amylase and lipase, respectively (Fig. 4B). The results of BUN analysis revealed a completely opposite pattern between females and males because B6 mice showed significantly higher levels

Fig. 2. Hematology analysis for individual types of blood cells. (A) RBC-related indicators and (B) immune cells belonged to the class of WBCs were analyzed in peripheral blood from both genders of ICR, Balb/c, and B6 mice. Statistical significance (vs. control group) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

in females but the lowest in males when compared to the other 2 strains. In contrast, the results of amylase and lipase testing showed that ICR mice contained the highest levels, Balb/c had the next highest levels, and then B6 had the lowest levels in both genders, except the level of lipase in females, for which B6 mice showed the highest level, similar to ICR.

Overall, B6 mice tended to show distinctively higher levels than did the other strains in hematological analysis, whereas ICR and Balb/c mice tended to show relatively higher levels in chemical analysis. It is notable that there was a significance difference between ICR and B6 mice, rather than between ICR and Balb/c mice.

Fig. 3. Chemistry analysis for routine check-up lists in blood. (A) Protein components (total protein, albumin, globulin), (B) ions (phosphate, calcium), and (C) metabolites (glucose, cholesterol) were analyzed in peripheral blood from both genders of ICR, Balb/c, and B6 mice. Statistical significance (vs. control group) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

The neuroendocrine system releases its products into bodily fluids to function in target tissues (Antunes-Rodrigues et al., 2004). The immune system circulates immune cells via blood vessels to protect the body (Israel and Israel, 1999). Therefore, the systems in a body are connected via

the circulatory system, which is well organized and controlled to ensure cooperative function (Reichlin, 1993). As an imbalance or abnormality in the relationship may cause serious diseases, scientists are attempting to develop effective therapies for human beings. To accomplish this purpose, researchers should carefully choose and use an experimental animal model that can properly reflect humans.

Doctors can predict disease progression primarily from

Fig. 4. Chemistry analysis for indicators of organ function in blood. (A) Indicators for liver function (Total bilirubin, ALT, ALKP), (B) kidney (BUN), and pancreas (amylase, lipase) were analyzed in peripheral blood from both genders of ICR, Balb/c, and B6 mice. Statistical significance (vs control group) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

blood tests of their patients, which normally provide information about immune cell profiles and biochemical components in the blood. In this study, we performed blood tests on 3 representative mouse models, which are ICR, Balb/c, and B6. Although they are all wild type mouse strains, they are known to have their own unique characteristics in terms of reproduction, immunity, and etc. (Altman, 1985). Briefly, ICR is known to be highly reproductive, and so is widely used in stem cell studies (Matsuda et al., 1999). Balb/c is known to have strong Th2-type immune responses, while B6 is known to be produce powerful Th1-type immune responses (Watanabe et al., 2004). Our results demonstrated such differences in hematological and chemical analyses.

The first result, regarding the number of RBCs and WBCs, showed a tendency to be highest in the B6 strain (Fig. 1). As mentioned above about immune function, it was expected that B6 would have more immune-related cells than would the other strains. However, the result that Balb/c mice have

similar levels of immune cells as ICR mice was quite unexpected. Nevertheless, the result about the number of reticulocytes (Fig. 2A) indicated that Balb/c mice were superior in bone marrow function because reticulocytes are immature red blood cells, and their presence in blood indicates whether enough red blood cells are being produced in the bone marrow (Piva et al., 2015). As bone marrow is the location in which B cells develop, our results demonstrated why Balb/c is a useful strain for the study of immune function. The successive results about WBCs also demonstrated the usefulness of Balb/c and B6. The interesting finding was that B6 showed a tendency to have more lymphocytes and monocytes in contrast to Balb/c, which showed a tendency to have more granulocytes (Fig. 2B). This obvious difference between the strains may explain why they evoke different types of immune responses.

Compared to hematological analysis, biochemical analysis showed rather more complex patterns. In most of the regular

checkup items (Fig. 3), B6 showed the lowest levels or a pattern of reduced levels, which was prominent in males. The concentration of TP indicates the amount of albumin and globulin in blood. As proteins are necessary for growth, development, and health, the level of TP is highly related to symptoms like weight loss (Szewczuk et al., 2011). Phosphate and calcium are the most common minerals in a body and are important for bones, nerves, and muscles (Penido and Alon, 2012). In humans, it is known that the phosphate level in blood affects the amount of calcium, and that the two are inversely related (Levine et al., 2014). However, the results in this study using the mouse models showed a correlation between them. This may be an example of a clear discrepancy between human and animal models. Any abnormality in the indicators suggests a problem of organ function, or inflammation in the body. It was interesting that the measurements of glucose were very different even between females and males in the same strain (Fig. 3C). Naturally, the level of glucose in blood is tightly regulated via homeostasis (Kuo et al., 2015) as abnormal level of glucose is the indicator for diabetes (Kim et al., 2013). Therefore, our results suggest that different experimental results can be deduced according to which gender of which strain is used.

The results on indicators of organ function were quite complex and difficult to interpret (Fig. 4). Tests for total bilirubin (TB), ALT, and ALKP are used to diagnose liver dysfunction and/or monitor the progression of treatment of liver disease (Agrawal et al., 2016). However, our tests of the indicators showed no correlation between them (Fig. 4A), which is an issue that requires further study. A BUN test is normally performed with creatinine levels to evaluate kidney function (Takaya et al., 2015). Actually, we also tested creatinine blood levels. However, the data is not shown here because the values were below the limit of detection. Amylase and lipase are enzymes involved in carbohydrate and lipid metabolism, respectively. Normally, amylase and lipase tests are performed at the same time to evaluate pancreatic function (Keim et al., 1998). In this study, the results provided the information that their levels were highest in ICR mice.

From the blood tests, we could determine an individual's general health status. If inflammation or organ damage occurs,

the level of components that circulate in the blood changes. The test animals used in this study were normal wild type mice, and all the values measured in this study were within normal ranges. Therefore, this study provides useful information about the basal metabolic rates of each mouse strain that will allow for a proper choice of an animal model for medical research.

Abbreviations used are

RBC, Red Blood Cells; WBC, White Blood Cells; BUN, Blood Urea Nitrogen; TP, Total Protein; TB, Total Bilirubin; ALT, Alanine Aminotransferase; ALKP, Alkaline Phosphatase

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Conflicts of interest

The authors have no financial conflicts of interest.

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