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Letter to the Editor

Development of Antihuman IgG Antibodies and Hematologic Deficits But Not Clinical Abnormalities in C57BL/6 Mice after Repeated Administration of Human Intravenous Immunoglobulin

Dear Editor,

In 2012, we published a study in *Comparative Medicine*[2] investigating whether repeated administration of intravenous immunoglobulin (IVIG) to mice would induce serum sickness. IVIG is prepared from plasma immunoglobulins from thousands of healthy donors and is used to treat a wide range of autoimmune, inflammatory, and immunodeficiency disorders. Its effects on experimental animal models of some of these disorders have been investigated, but no studies were found to indicate whether its repeated administration to mice would produce serum sickness. In our study, male C57BL/6 mice (n = 27) were administered Gammagard (Baxter Healthcare; 1 g/kg weekly by intraperitoneal injection for 6 wk), and body weight, temperature, and renal function (BUN and creatinine) were monitored. CBC with blood smear examination were performed before treatment, and on treatment days 21 (6 d after the third IVIG injection) and 43 (6 d after the sixth IVIG injection). Lactated Ringers solution was given as volume replacement after blood draws. Each animal served as its own control, comparing data between pre- and posttreatment values for statistical significance. The investigation was approved by Beaumont Hospital's animal care committee. The mice remained healthy despite a transient increase in BUN on treatment day 35, 6 d after the fifth IVIG injection. However, there were statistically significant hematologic alterations. Hemoglobin was decreased on treatment day 21 compared with its pretreatment value, and hemoglobin, RBC, platelet, and lymphocyte concentrations, hematocrit, and percent monocytes were decreased on treatment day 43 compared with their pretreatment values. We attributed these changes to the influence of IVIG.

We have now completed a follow-up study whose goal was to determine the mechanisms responsible for these hematologic deficits. In this study, C57BL/6 mice received 1 g/kg of Gammagard or the equivalent volume of vehicle (5% dextrose in water) once per week. The experimental design differed from the first study in several ways: distinct treatment and control (vehicle-treated) groups (n = 20 mice per group) were used, female mice were used to avoid fighting-related injuries, intervals between blood draws were longer (27 to 44 d), and the number of IVIG injections was increased to 12. In addition to pretreatment blood sampling, only one posttreatment CBC with blood smear examination was performed; this was done on the day after the last IVIG injection. All blood samples were obtained from facial vessels, whereas in the first study the second posttreatment sample for CBC and blood smear examination, taken on treatment day 43, was obtained by cardiocentesis as a terminal procedure. In the follow-up study, flow cytometric analysis was performed after the third IVIG injection to compare deposition of mouse C3 and human IgG on mouse RBCs between IVIG- and vehicle-treated mice, and serum bilirubin levels were compared between the 2 groups after the seventh IVIG injection. Postmortem studies were performed on bone marrow paintbrush smears from femurs and on formalin-fixed bone marrow and spleen specimens.

All mice remained clinically normal throughout the study. In contrast to the first study, hematologic deficits specifically associated with IVIG treatment were not found in the second study. There were differences between pretreatment and posttreatment values, most of which achieved statistical significance, for both groups for the hematologic parameters other than hematocrit. These differences resulted from increases in posttreatment total and individual WBCs, and decreases in platelet concentrations, in the posttreatment samples. Using pooled or Satterthwaite t tests the mean change for each of these parameters was found to be the same in both groups with the exception of monocyte percentages, whose posttreatment increase was greater in the IVIG-treated mice (P = 0.024). Therefore the changes between pre- and posttreatment values were most likely due to day-to-day variation in these measurements rather than to a specific effect of IVIG. There were no significant differences in pretreatment hematologic parameters between the two groups except for monocyte percentage, which was higher in the control group (P = 0.025). Perhaps most importantly, there were no significant differences for posttreatment values between the two groups for any of the hematologic parameters. Marked platelet clumping was apparent in all blood smears. Flow cytometric studies on blood samples taken after the third IVIG injection found a slight but statistically significant (P = 0.002) increase in C3+ RBCs in IVIG-treated compared with vehicle-treated mice (mean ± SD: IVIG-treated mice, 0.60 ± 0.18%, vehicle-treated mice, 0.41 ± 0.24%), and no deposition of human IgG on mouse RBC from either group. Serum bilirubin levels taken after the seventh IVIG treatment were similar between groups. No differences were seen in bone marrow or splenic hematopoietic cellularity (histologic sections) or in proportions and maturation of myeloid and erythroid lineages (cytologic preparations) between IVIG- and vehicle-treated mice. Posttreatment blood smears from IVIG-treated mice showed increased RBC rouleaux formation. Increased numbers of Mott cells (plasma cells filled with immunoglobulin-containing cytoplasmic vesicles) were observed in bone marrow smears from the IVIG-treated mice, consistent with chronic immune stimulation.

In the first study, the average daily volume of blood loss of the mice was 19.7 µL, compared with 3.5 µL per day for IVIG-treated mice in the present study. Although the average daily blood volume removed in the first study was less than the maximum recommended by NIH guidelines[3] (21 µL from a 30-g mouse), the greater blood volume taken in the first study may have contributed to the conflicting results between the studies with regard to decreased hematocrit, with erythropoiesis being unable to maintain hematocrits due to the greater rate of blood removal in the first study. Although platelet clumping detected by blood smear examination in the second study indicates that platelet concentration data obtained from facial vessel samples were not reliable, the different routes of blood collection in the first study between pretreatment samples (obtained from facial vessels) and day 43 posttreatment samples (obtained by cardiocentesis) may have contributed to the decrease in platelets at the latter time point. Cardiac collection could have increased platelet activation, resulting in increased clumping and a consequent decrease in their detectable concentrations. The different routes of blood sampling between pretreatment samples and day 43 posttreatment samples may also explain the leukocyte decrease in the first study, as

cardiac sampling has been associated with lower total WBC and lymphocyte concentrations compared to other sampling sites.[1,4]

We conclude, based on the results in the present study, that chronic IVIG administration to mice is unlikely to induce significant hematologic deficits.

Sincerely,

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2. **Loeffler DA, Smith LM, Klaver AC, Brzezinski HA, Morrison EI, Coffey MP, Steficek BA, Cook SS.** 2012. Development of antihuman IgG antibodies and hematologic deficits but not clinical abnormalities in C57BL/6 mice after repeated administration of human intravenous immunoglobulin. Comp Med **62:**31–36.
3. **National Institutes of Health. Office of Animal Care and Use. Animal Research Advisory Committee.** [Internet]. Guidelines for Survival Bleeding of Mice and Rats. [Cited 17 March 2014]. Available at: http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf
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Editors' Note

Linda A Toth and Ravi J Tolwani

In this issue, we are publishing a letter to the editor from Loeffler and colleagues, authors of the article entitled "Development of Antihuman IgG Antibodies and Hematologic Deficits But Not Clinical Abnormalities in C57BL/6 Mice after Repeated Administration of Human Intravenous Immunoglobulin."[1] The article, which appeared in our February 2012 issue (Comp Med 62:31–36), investigated whether repeated administration of intravenous immunoglobulin (IVIG) to mice would induce serum sickness. Since then, the authors have completed a follow-up study with the goal of determining the mechanisms responsible for these hematologic deficits. The authors, however, were not able to replicate their original results and have concluded that chronic IVIG administration to mice is unlikely to induce significant hematologic deficits. Following their discovery, the authors contacted our office to enquire about a mechanism to disseminate these new results.

We would like to commend the authors for making the readership aware of their new findings based on more recent results. We hope the authors' initiative serves as an example of commitment to scientific integrity for future investigations.

Reference

1. **Loeffler DA, Smith LM, Klaver AC, Brzezinski HA, Morrison EI, Coffey MP, Steficek BA, Cook SS.** 2012. Development of antihuman IgG antibodies and hematologic deficits but not clinical abnormalities in C57BL/6 mice after repeated administration of human intravenous immunoglobulin. Comp Med **62:**31–36.

Letters to the Editor

Letters discuss material published in *CM* in the previous 3 issues. They can be submitted through email (journals@aalas.org) or by regular mail (9190 Crestwyn Hills Dr, Memphis, TN 38125). Letters are not necessarily acknowledged upon receipt nor are the authors necessarily consulted before publication. Whether published in full or part, letters are subject to editing for clarity and space. The authors of the cited article will generally be given an opportunity to respond in the same issue in which the letter is published.

Erratum

In the article entitled "Facilitating Multimodal Preclinical Imaging Studies in Mice by Using an Immobilization Bed." (**Geoffrey S Nelson, Jessica Perez, Marta V Colomer, Rehan Ali, Edward Graves.** 2011. Comp Med **61:**499–504), the name of one author was inadvertently published incorrectly.

The authors should appear as: **Geoffrey S Nelson, Jessica Perez, Marta Vilalta, Rehan Ali, Edward Graves.**