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Background check: Profound differences in serum antibody isotypes among C57BL/6 mouse substrains discourage substrain interchanges in immunology experiments

Gene editing technology has revolutionized biological research, allowing swift production of mouse strains with targeted mutations [1,2]. Within a matter of weeks, mutant mice can be produced with a focus on virtually any desired locus and any genetic background to study immune cells and functions. The C57BL/6 mouse is a preferred host due to its well characterized background and plentiful associated research reagents.

Unfortunately, manuscripts that describe knock-out (KO) and knock-in (KI) experiments using C57BL/6 mice often fail to document substrain origins and breeding details. This is despite numerous warnings in previous literature that describe genetic differences between C57BL/6 substrains. Mutations that are mismatched between substrains are plentiful and will influence lymphocyte functions and pathogen control [3–7]. Full differences between substrains are currently unknown, and will amplify with each generation of animals in independent breeding colonies.

We have tested C57BL/6 mice from Jackson (C57BL/6 J) and Envigo (C57BL/6 NHsd) sources for serum isotype profiles, isotypes such as IgA that provide a first line of defense against pathogens in respiratory and intestinal tissues [8]. As shown in Fig. 1, male and female mice differed, confirming previous results [9]. We found that differences between C57BL/6 substrains were profound. For example, mean serum IgA values differed by more than 10-fold when male mice from Envigo and Jackson were compared (Fig. 1A). IgG subclass patterns, key to the control of pathogens such as *Bacillus anthracis* [10], also differed. IgG2b was significantly higher in Envigo mice, as was the IgG2b/IgG1 ratio (Fig. 1E-F). A known mutation in the *Dock2* gene in C57BL/6 N mice that alters B cell development [4] may be responsible for disparate isotype profiles, but a plethora of additional mismatched mutations may also contribute.

Differences in antibody isotypes between substrains will influence virtually any immunology and microbiology experiment performed in C57BL/6 mice. When test and control mice derive from different vendors, phenotypes due to substrain mismatches can be falsely ascribed to the targets of KO or KI experiments, driving false research leads. Errors are sometimes corrected [3,4], but this is rare, and corrections may go unnoticed by newcomers in the field.

The frequency of indiscriminate interchange between C57BL/6 substrains in immunology and microbiology fields is unknown, because policies do not require authors to provide detailed information about mouse origins and backcrossing methods. Backcrossing, when conducted, may be insufficient to match test and control animals, particularly if genes targeted for KO or KI experiments are closely linked to mismatched background sequences [7]. Errors in experimental design and data interpretation will follow.

Fortunately, new technologies including clustered regularly interspaced short palindromic repeats-CRISPR-associated protein (CRISPR-Cas)-based genome editing simplify the targeting of mutations onto desired inbred mouse substrains [1,2]. With these improved methods, researchers may select one substrain for gene-editing experiments, and thereby avoid mismatches between test and control animals. To ensure high-quality experimental design, we propose that new policies be implemented and that authors be requested to document mouse and embryo origins, substrains, and breeding details in future manuscripts. Researchers may then avoid mistakes in data interpretation and prevent false leads in the immunology field.

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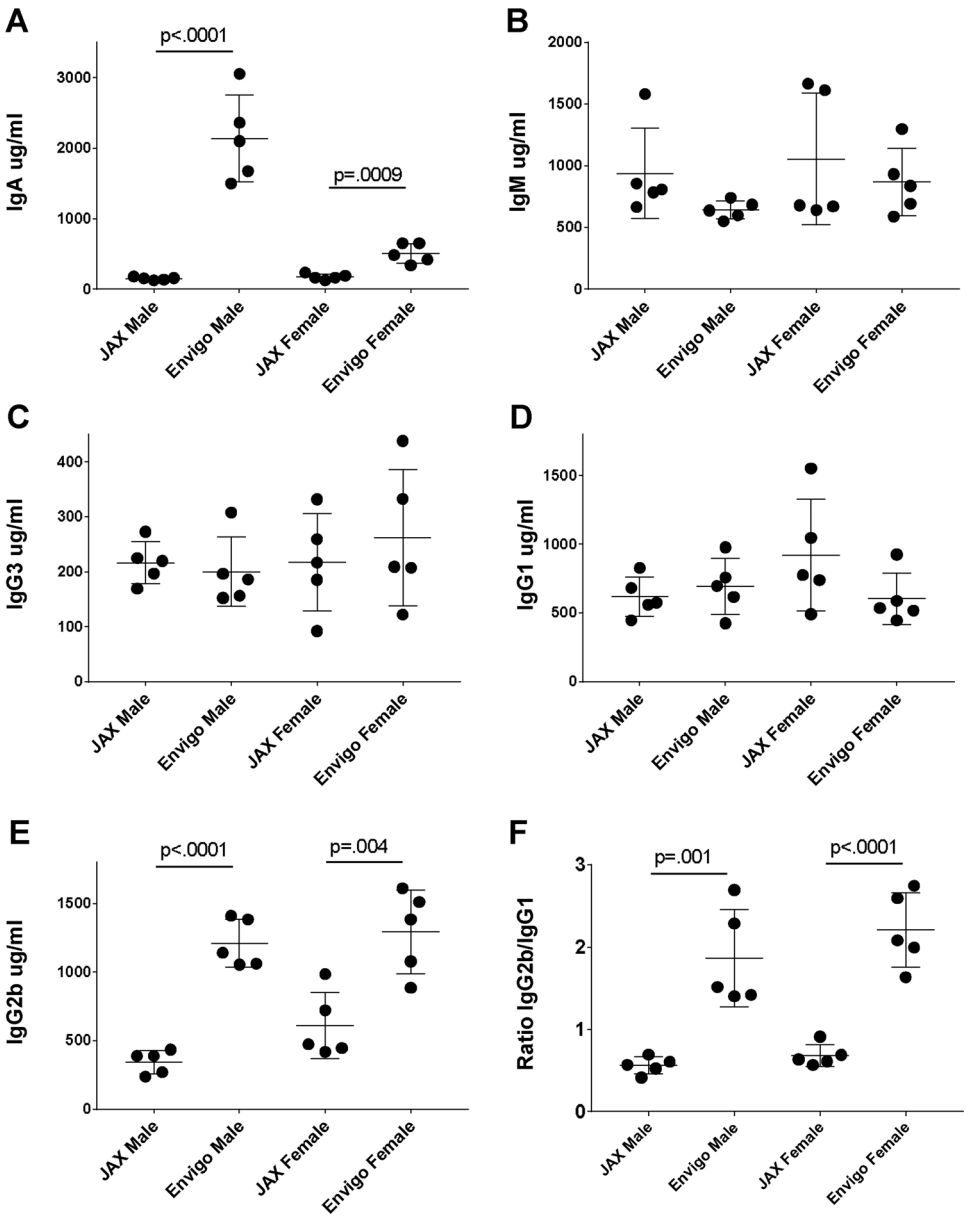

Fig. 1. Significant differences in serum immunoglobulin isotypes between C57BL/6 mouse substrains from Jackson and Envigo vendors.

Results of a representative experiment are shown. C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, Maine) and Envigo laboratories (Somerset, New Jersey). When mice were 8 weeks of age, sera were collected and tested for total immunoglobulin isotypes using Luminex technology. Isotype values (A–E) and the ratio of IgG2b/IgG1 (F) are shown. *Methods:* For isotype testing, sera were diluted 1:25,000 in assay buffer and evaluated for IgM, IgG3, IgG1, IgG2b, and IgA using a MILLIPLEX MAP mouse immunoglobulin isotyping kit (Cat #MGAMMAG-300 K), per manufacturer's instructions. The test also scored IgG2a, but this was not graphed, because the C57BL/6 mouse expresses IgG2c, not IgG2a. Samples were read on a Luminex 200 Multiplexing instrument using xPonent software. Data were processed using Milliplex Analyst software. For calculations, if samples were below or above the limit of detection (LOD), they were assigned the LOD score. Unpaired T tests were used to compare results from different mouse substrains using GraphPad Prism software.

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