SUPPLEMENTAL ONLINE DATA

Title: Renal Ischemia-Reperfusion Injury Amplifies the Humoral Immune Response

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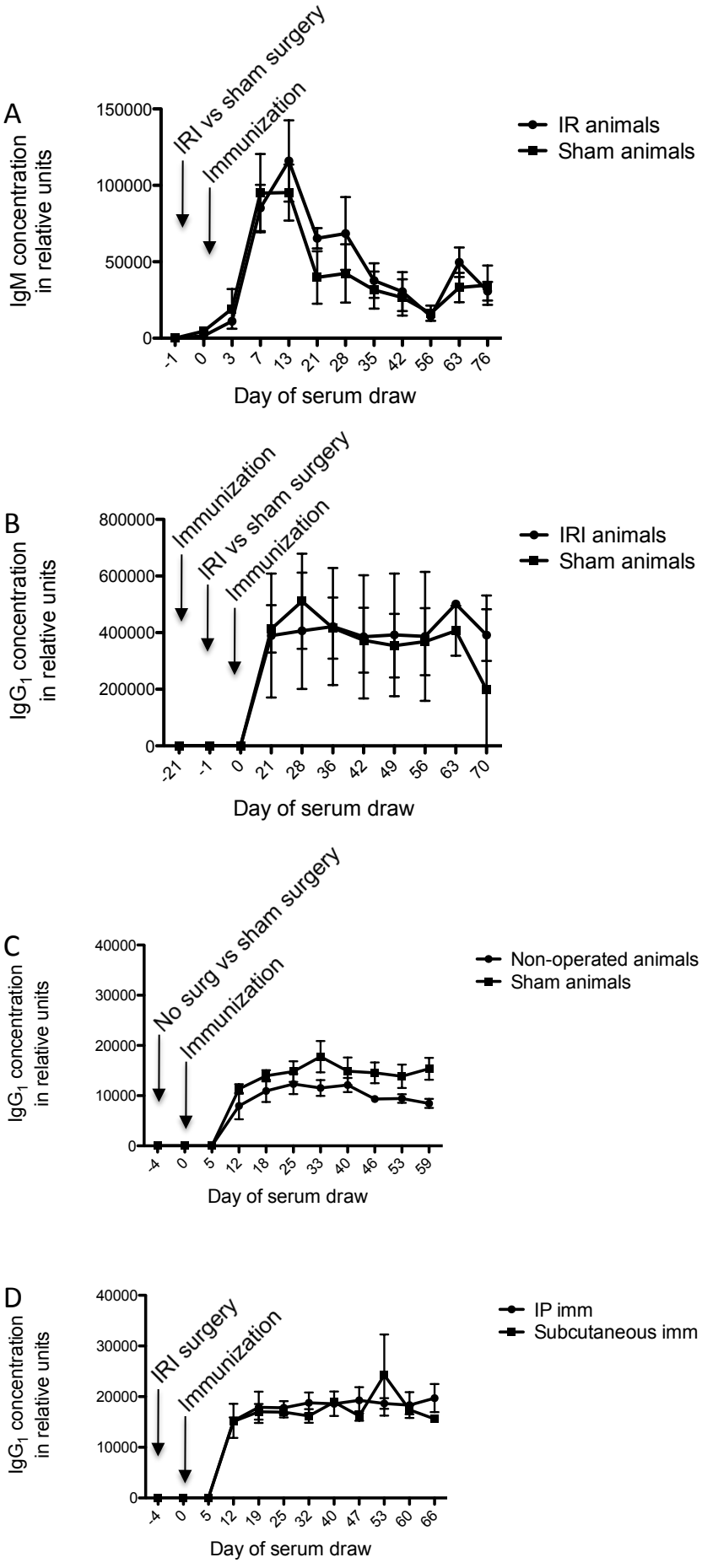


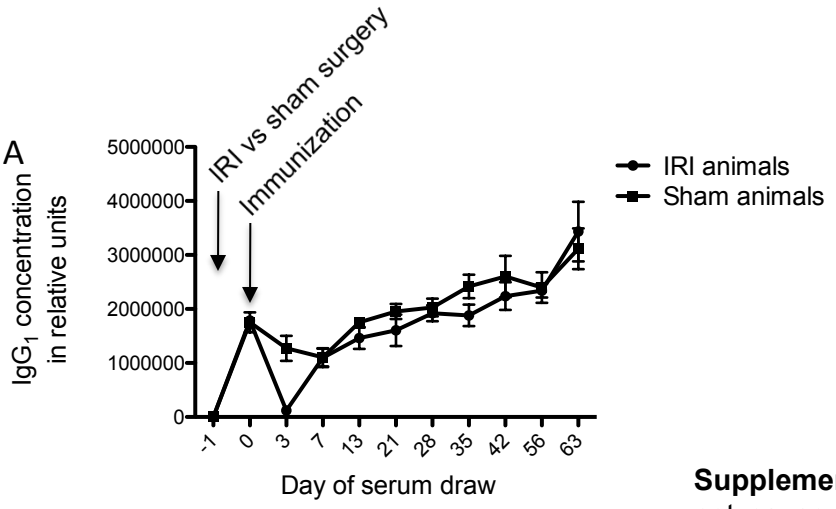
Supplemental Figure 1. Control experiments in immunization.

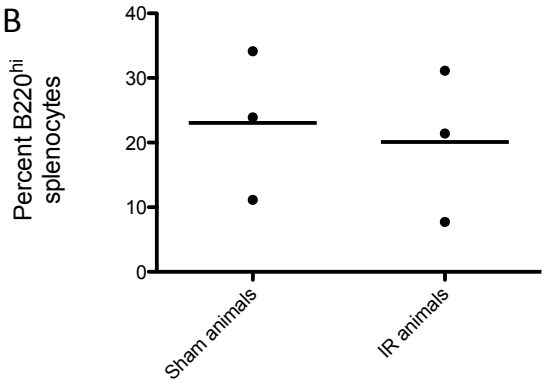
(A) The augmentation effect described above was not observed for antigen-specific IgM. Data shown are from ELISA; n=5 for IRI-treated group, and n=4 for sham-treated group.

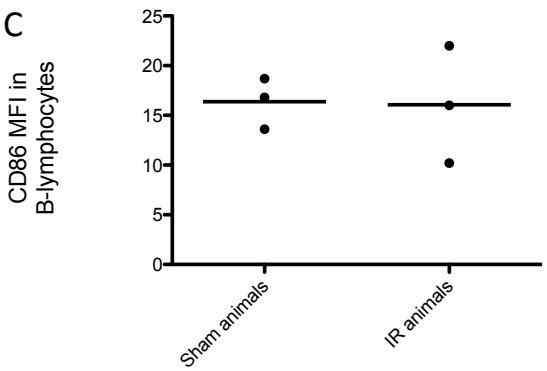
(B) To investigate the effect of IRI on a secondary ("memory") response to immunization, mice were immunized at day -21 and then reimmunized at day 1 (24 hours after surgery). Data shown are from ELISA; n=6 for IRI-treated group, and n=5 for sham-treated group.

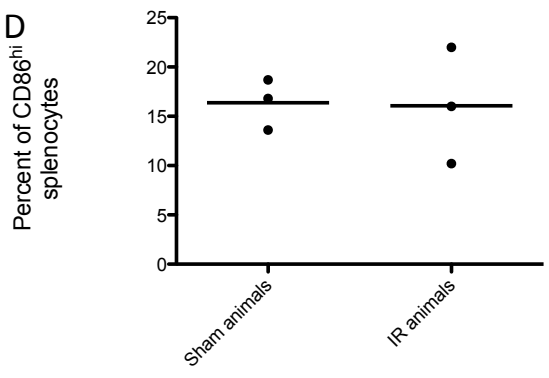
(C) Sham-operated mice were compared to non-operated controls in their IgG_1 response to NP-KLH immunization at day 4. There was an amplification in antigen-specific antibody titer, but not to the same degree as in figure 2A ($p=0.0624$ by mixed model analysis). Data shown are from ELISA; n=5 for non-operated group, and n=5 for sham-treated group.

(D) Next, we subjected IRI-treated mice to IP vs. subcutaneous immunization with NP-KLH at day 4. There was no difference between the two groups. This would indicate that the immunization need not be in the peritoneum for the amplification of humoral immunity to be observed. Data shown are from ELISA; n=3 for IP-immunized group, and n=2 for subcutaneous-immunized group.

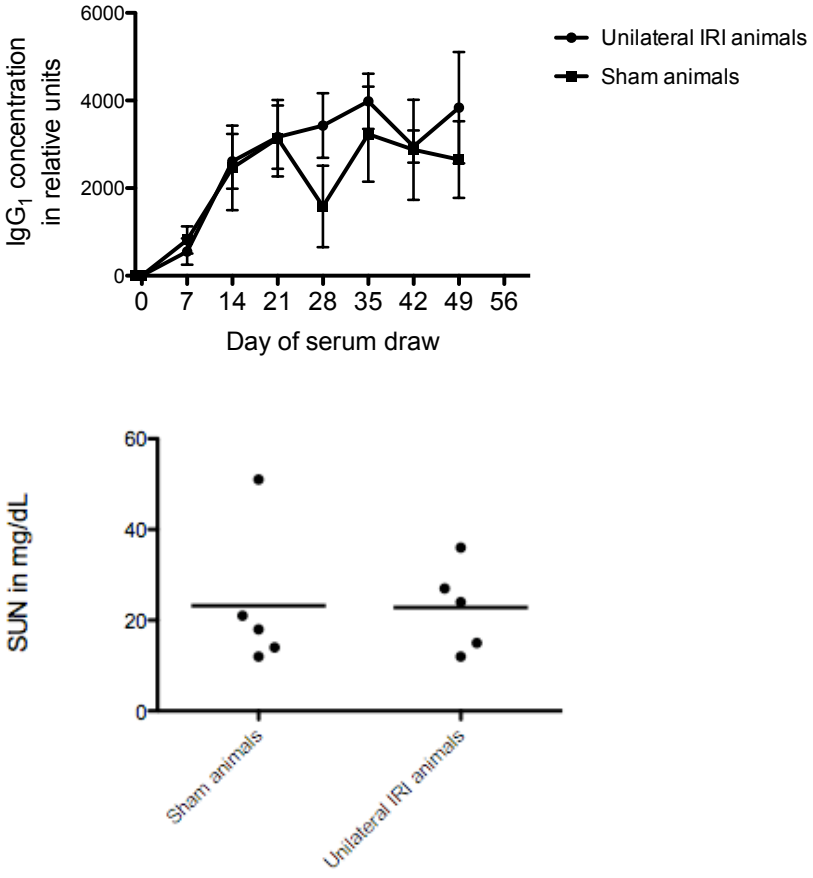




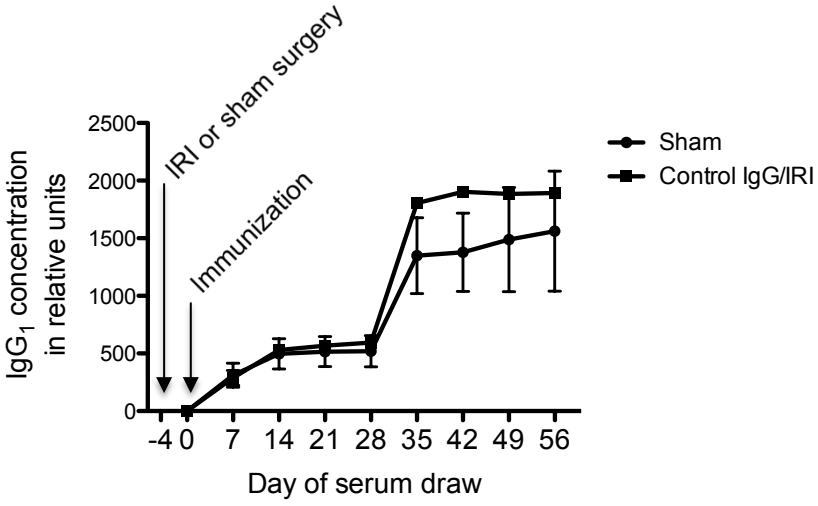




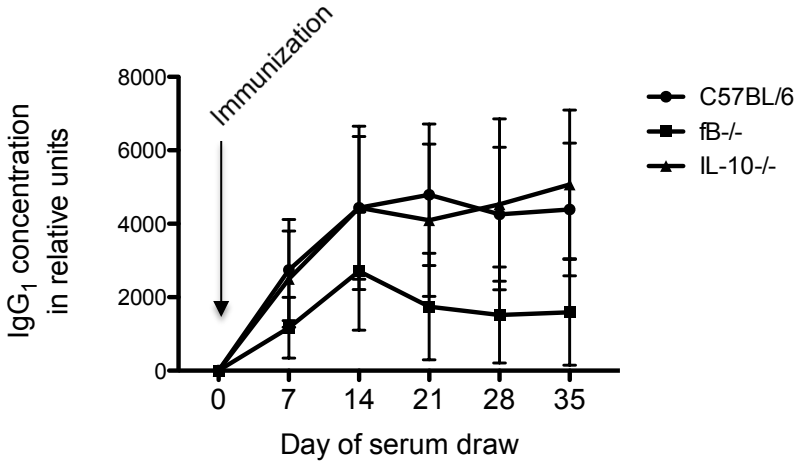
Supplemental Figure 2. IRI does not cause a polyclonal activation of B-lymphocytes.
(A) Total IgG_1 levels (not antigen-specific) were measured. There was no difference between IR- and sham-treated animals in their total IgG_1 levels. Data shown are from ELISA; n=4 for IRI-treated group, and n=4 for sham-treated group.
(B-D) Animals were treated with IR (n=3) vs sham surgery (n=3) and then sacrificed 48 hours after surgery. Splenocytes were analyzed via FACS and it was found that there was no difference in absolute number of $B220^{hi}$ cells (B-lymphocytes), the mean fluorescent intensity (MFI) of CD86 (a marker of B-lymphocyte activation) among $B220^{hi}$ cells, or the percentage of $B220^{hi}$ cells that had increased levels of CD86 expression. All experiments were not statistically significant.



Supplemental Figure 3. Immunization of mice with unilateral IRI. (A) In mice subjected to prolonged (45 minutes) unilateral IRI (n=5) vs sham surgery (n=4), there was no amplification of the NP-specific IgG_1 titers. (B) SUNs were measured in mice subjected to unilateral IRI. The SUNs were not elevated at 24 hours after surgery.



Supplemental Figure 4. IRI in mice injected with control IgG. Monoclonal antibody 171 was used as an isotype control for monoclonal antibody 1379 (figure 6B). In this experiment pre-treatment of the mice (n=5) with the monoclonal IgG did not eliminate the amplification effect in the IRI-treated mice.



Supplemental Figure 5. Immunization of unmanipulated factor B deficient and IL-10 deficient mice. To investigate the effect of complement deficiency and IL-10 deficiency on humoral immune response, we immunized unmanipulated WT (n=4), factor B deficient (n=5), and IL-10 deficient mice (n=4). The factor B deficient mice had reduced NP-specific antibody titers compared to the other 2 groups of mice.