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The impact of vaccination on RBC alloimmunization in a murine model

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Summary

Emerging data in animal models and humans suggest that pathogen-associated and damage-associated molecular patterns variably impact RBC alloantibody formation. In this study, we tested the hypothesis that vaccinations may enhance immune responses to transfused RBCs. **The** Pneumovax23 vaccine decreased the magnitude of anti-KEL alloimmunization in a murine model, whereas the hepB vaccine did not impact the response; RBC transfusion did not alter immune responses to either vaccine. These data highlight the complexities of the intersection of innate and adaptive immunity and suggest that future studies investigating the pathways through which inflammation impacts alloimmunization are warranted.

Keywords

vaccination; red blood cell; alloimmunization

Red blood cell alloantibodies, induced in some patients through transfusion or pregnancy, can be clinically significant in both settings. Inflammation has been associated with increased rates of RBC alloimmunization in humans[1, 2] and in mouse models[3]. We hypothesized that vaccinations, which may be administered during a clinic visit in close proximity with an RBC transfusion, might provide inflammatory signals capable of driving RBC alloimmunization. We tested this hypothesis in a reductionist murine model, which allows an evaluation of the humoral immune response to a single human blood group protein (KEL) expressed on donor and lacking on recipient RBCs. We selected the Pneumovax23 and the hepatitis B/hepB (recombivax HB) vaccines for study: Pneumovax23 is an adjuvant free polysaccharide vaccine that acts in a T cell independent manner, whereas hepB contains alum (potassium aluminum sulfate) and acts in a T-cell dependent manner.

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PN and JEH designed the experiments; PN and MS completed the experiments; PN, MS, CT, and JEH interpreted the data; PN and JEH wrote the manuscript and all authors edited the manuscript.

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Wild-type (WT) C57BL/6 recipient mice were given an intramuscular injection of Pneumovax23 or saline, and 3-4 hours later were transfused with 1 human equivalent of packed leukoreduced KEL RBCs (*see supporting information for additional methodologic details*). All mice that were transfused generated anti-KEL alloantibodies, as measured using a flow cytometric crossmatch assay (Figure 1A). However, the magnitude of the anti-KEL IgG antibody response was significantly *lower* in mice vaccinated with Pneumovax23 as compared to the unvaccinated controls (Figure 1B). To confirm that the vaccinations themselves were immunogenic, we measured levels of anti-Pneumovax23 IgM antibodies by ELISA 1-week post-vaccination. Regardless of transfusion status, sera from all vaccinated mice had evidence of anti-Pneumovax antibodies (Figure 1C).

We next investigated the effects of the hepB vaccine on KEL alloimmunization. Unlike the blunted anti-KEL responses observed in the Pneumovax23 group, mice transfused in the presence or absence of the hepB vaccine made equally robust anti-KEL responses (Figure 2A). Independent of transfusion status, all vaccinated mice also had evidence of anti-hepB antibodies (Figure 2B).

Emerging data in murine models[4, 5] and in humans[1] suggest that the type of an adjuvant or the type of infection influences alloimmune responses. Thus, we next investigated the impact of viral-like (polyinosinic polycytidylic acid, poly (I:C)) or bacterial (endotoxin lipopolysaccharide (LPS)) inflammation on KEL alloimmunization. Whereas poly I:C significantly boosted the magnitude of the anti-KEL IgG response in a manner similar to what we have previously observed[6], 50 µg of LPS given in the peri-transfusion period did not enhance the response (Figure 2C).

Mechanistic studies of vaccines and their adjuvants[7] highlight the complexities of the intersection of innate and adaptive immunity. The type of adjuvant/antigen combination in a vaccine impacts whether the recipient's immune response will be of the Th1, Th2, or mixed type[8], with alum being the most common adjuvant used in vaccines in the US. Autoimmune sequelae of vaccination[9] have been more thoroughly studied than alloimmune sequelae, though no significant increases in HLA alloimmunization status have been observed in solid organ transplant recipients receiving the seasonal influenza vaccine [10, 11]. A recent study of factor VIII alloantibody responses in a murine hemophilia model reported a decrease in factor VIII alloantibody responses following the influenza vaccine; T-cell migration experiments documented immune deviation away from the alloantigen and towards the influenza vaccine[12]. The blunted anti-KEL responses observed in the current **study after Pneumovax23 may be due** to a similar immune deviation. Notably, no differences in factor VIII alloantibody responses were observed following a different vaccination (MMR)[12], similar to what we observe with the hepB vaccine.

Our experimental design was practical but by no means exhaustive. We selected vaccines that might routinely be given in clinics, with vaccination and transfusion timing that is clinically relevant, with an RBC alloimmunization model that involves a clinically significant antigen. However, we cannot extrapolate the results of this limited study design to all vaccinations, to all vaccination/transfusion timing scenarios, to all blood group

antigens, or to all patient populations. Future studies of the impact of immunization on alloimmunization (and vice versa) may be informative.

In conclusion, we found that Pneumovax23 decreased and hepB did not impact the magnitude of anti-KEL alloantibody responses; RBC transfusion did not impact the humoral immune response to either vaccine. Our findings join data emerging from other studies in mice and humans suggesting that adjuvants, pathogen-associated molecular patterns, and damage-associated molecular patterns variably impact RBC alloantibody formation. A better understanding of these variables is desirable, in working towards RBC alloantibody prevention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Impact of Pneumovax23 vaccine on KEL alloimmunization

(A) Representative histogram of anti-sera crossmatch. (B) Representative anti-KEL glycoprotein IgG responses 4-weeks post-transfusion, with adjusted mean fluorescence intensity (MFI) being the difference between fluorescence intensity of KEL and WT RBC targets. (C) Representative anti-Pneumovax IgM responses 1-week post vaccination. Data in B and C are representative of two independent experiments (n= 4 to 5 mice per group per experiment; each data point represents an animal and bars represent mean $\pm$ s.d.). * $p < 0.05$, ns = no significance; determined by Mann Whitney U test.

Figure 2. Impact of hepB vaccine or other adjuvants on KEL alloimmunization

(A) Representative anti-KEL glycoprotein IgG responses 4-weeks post transfusion. (B) Representative anti-hepB IgM responses 1-week post- vaccination. (C) Representative anti-KEL glycoprotein IgG responses 4-weeks post transfusion, with or without intraperitoneal poly I:C (PIC) or LPS injection. Data in A-C are representative of at least two experiments (n= 3 to 5 mice per group per experiment); one pilot experiment showed lower anti-KEL responses in vaccinated animals. Statistics as per Figure 1, but with Kruskal-Wallis with Dunn's Post-test for C.