Endogenous anti-β-glucan antibodies and FcgRIIA (CD32a) single-nucleotide polymorphisms (SNP) as potential predictive biomarkers for the efficacy of Imprime PGG immunotherapy in cancer patients


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Abstract

Imprime PGG (Imprime), a soluble 1.37-β-glucan, is being developed as a novel cancer immunotherapy in conjunction with anti-toxins in several cancer models. Randomized Phase I/II clinical trial of Imprime in the treatment of stage IV non-small cell lung cancer has shown promising efficacy in terms of both objective tumor response and survival. Mechanistic studies have demonstrated that Imprime forms an immune complex with endogenous IgG and IgM anti-β-glucan antibodies in human plasma and primes innate immune cells, including macrophages, monocytes and neutrophils, to kill antibody-targeted cancer cells via a complement receptor 3-dependent mechanism.

In this study, we examined the functional relevance of this Imprime-ABA immune complex might influence patient selection strategies. We have shown that there is a threshold level of ABA required for binding of Imprime-ABA immune complex to the innate immune effectors. In human healthy volunteers (N=143) IgG and IgM ABA concentrations vary from 80 to 1448 ng/ml. Imprime treatment of whole blood from individuals with ABA concentrations above a threshold yields innate immune complex formation that allows both classical and alternative complement pathways leading to complement opsonization of the complex. This complex then binds innate immune cells, triggering the mobilization of cell surface receptors and production of selective cytokines such as IL-8.

We now show that the predominant subclass of endogenous IgG ABA specific to Imprime is IgG2. Additionally, we show that binding of Imprime to immune cells critically involves FcgRIIA, the only Fc receptor capable of binding IgG2 immune complexes. Blocking FcgRIIA significantly inhibits binding to both neutrophils and monocytes in whole blood. This observation was further confirmed by binding of Imprime to HEK293 cells expressing FcgRIIA. In order to evaluate the effect that ABA subclass has on immune response, we purified IgG subclasses from healthy donors with IgG1 or IgG2 added to whole blood of healthy donors with low ABA levels, was able to increase binding, consistent with previous data showing that binding of Imprime is dependent on ABA levels. However, the relative increase in binding due to addition of IgG1 or IgG2 ABA varied among donors. While addition of either IgG1 or IgG2 ABA was equivalent in increasing binding in some donors, IgG2 was more efficacious than IgG2 in other donors. These results led us to look at the role of FcgRIIA at the genetic level.

Methods

Neutrophil and Monocyte Imprime Binding Assays

Neutrophil and monocyte binding to Imprime was assessed in whole blood samples from healthy subjects after incubation with 10 µg/mL of Imprime or vehicle for 30 minutes at 37°C. Surface bound Imprime was determined by staining with a specific antibody (6B4) and an assay using flow cytometry.

Complement Activation and IL-8 Assays

For IL-8 measurement, while blood was incubated for 30 minutes with Imprime (10 µg/mL), for 24 hours with Imprime (10 µg/mL), following incubation, plasma levels of C3a and IL-8 were measured using commercially available ELISA kits.

Preparation of the IgG1 and IgG2 Chimeric

Heavy and light chain binding sequences were isolated from the mouse IgG2a, IgM, and IgG1 heavy chain of the constant regions of human IgG1 and IgM light chains. The (as well as vector DNA) was transfected into 293 cells. Each healthy subject’s serum ABA levels in healthy volunteers were determined by genotyping the individuals previously tested for PMN binding and serum ABA levels using a PCR assay. (A1) donors tested were separated by R131H genotype and data points plotted for IgG2 ABA level, % PMN binding, the same data was correlated with high ABA donors that carry an HH or HR genotype have a lower IgG2 ABA threshold for high PMN binding than RR donors.

Statistical Methods

The statistical significance (p) of the correlation of ABA concentrations with demographic and functional parameters was performed using an unpaired, two-tailed Student’s t-Test. A Pearson coefficient (r) was calculated for all linear regression correlations.

Summary

• Imprime forms an immune complex with ABA endogenous present in human serum
• ABA binding above a certain threshold is required for Imprime to form an immune complex and subsequently get complement opsonized
• Significant differences in high and low ABA donors demonstrate the importance of Imprime and innate immune functional responses
• As compared to binding ABA, levels of IgG2 antibodies correlate more strongly with the total ABA IgA, Binding of Imprime PGG to both neutrophils and monocytes significantly correlated with the relative amount of ABA IgG
• Imprime PGG-ABA immune complex is capable of binding to FcgRIIA in addition to complement receptor 3
• Single nucleotide polymorphism of the FCGR2A gene impacts the extent of binding of Imprime ABA as compared to those with R1131 genotype
• The prevalence and functional relevance of this SNP on Imprime binding to innate immune cells in cancer patients needs evaluating

The data implicate both the concentration of ABA in serum and FCGR2A genotype as critical determinants of the ability of an individual to derive therapeutic benefit from Imprime treatment.

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