Imprime PGG, a novel cancer immunotherapeutic, engages the complement system to prime innate immune effector functions

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Abstract

Background: Imprime PGG (Imprime), an intravenously-administered soluble, beta-1,3,1,6-glucan, is currently in clinical development with tumor-targeting antibodies, antiangiogenics, and checkpoint inhibitors. The fundamental mechanistic rationale for these therapeutic combinations is that Imprime, being a PAMP, primes innate immune effector functions to ultimately inspire an adaptive immune response-based anti-cancer immunity cycle. Imprime forms a tripartite immune complex (IC) comprised of Imprime, naturally occurring anti-glucan antibodies (ABA) and iC3b complement opsonin in subjects with sufficient ABA levels. Ex vivo human and in vivo mouse studies have shown that the innate immune receptor, FcgRIIA, and the pattern recognition receptors, complement receptor 3 (CR3) and Dectin-1, are critical for Imprime’s innate immune responses. However, the contributions of the complement system, a vital component of innate immunity, towards the functional activity of Imprime have not been thoroughly investigated. Imprime-ABA IC activates the classical complement pathway and releases C5a. As C5a is a well-known priming agent, and involved in cross-talk with the other innate immune receptors, we hypothesized that Imprime-induced C5a will engage the C5a-C5a receptor (C5aR) signaling pathway to enhance Imprime binding and innate immune effector functionalities.

Methods: The role of C5a in Imprime-ABA binding to isolated neutrophils was evaluated by: a) adding exogenous C5a; b) using C5-depleted serum, and c) using C5a antagonists (C5aRA). Cytokine production in healthy subjects with sufficient ABA levels were measured 24hrs post-Imprime treatment in the presence or absence of C5aRA by multiplex Luminex assays. The effect of C5a inhibitors was also evaluated in a chemiluminescence-based oxidative burst assay assessing reactive oxygen species (ROS) generated by Imprime-treated neutrophils in response to a tumor-bound cell lymphoma cells. In order to test these endpoints in complement-depleted conditions, the whole blood was washed extensively to remove the plasma.

Results: Addition of exogenous C5a increased the percentage of neutrophils binding to Imprime in a dose-dependent manner. Furthermore, Imprime binding in the presence of C5aRA and C5-depleted serum was significantly reduced. Functionally, C5aRA aborted cytokine production (IL-8, MCP-1, MIP-1alpha, and IL-6) in Imprime-treated blood. Likewise, Imprime-ABA induced ROS in high-ABA blood was greatly inhibited in C5a-depleted serum and could be rescued by replelling complement. C5aRA also inhibited Imprime-induced ROS production. In a non-physiological complement-depleted condition, Imprime bound predominantly via FcgRIIA, resulting in diminished cytokine and ROS responses.

Conclusions: These results collectively demonstrate that Imprime-induced C5a plays a critical role in enhancing Imprime binding and functional responses, potentially lowering the signaling threshold of the other innate immune receptors.

C5a-C5aR axis is critical for Imprime-ABA immune complex-mediated innate immune effector functions, including triggering a series of innate immune activation events that culminate in enhanced T cell activation and cytokine/chemokine production and anti-tumor cytotoxicity via ROS production.

Figure 6. C5a-C5aR inhibitors specifically block Imprime-induced cytokine release.

Figure 7. Imprime-induced neutrophil ROS production against antibody-opsonized tumor cells requires complement. (A) Whole Blood (WB) was treated with vehicle or Imprime (20μg/mL) on ice for 15 minutes. Neutrophils were isolated using negative selection directly from WB (Stemcell technologies) and cells were resuspended at 2-3 x 10^6 cells per well in a 96 well plate. Neutrophils were stimulated in the presence of luminol (10μM) with Raji II cell lymphoma cells that were pre-treated with or without anti-CD20 (50μg/mL) in a 1:50 ratio. luminol (10μM) was read at 30 second intervals corresponding to reactive oxygen species (ROS) production. The panel on the right shows that the Imprime-treated neutrophils also showed enhanced cytokotoxicity when co-cultured with Raji cells that had been labeled with Calcein AM dye with Rituximab at a 50:1 effector to target ratio. Cells were incubated for 3hrs and then the co-culture was stained with a live/dead dye and analyzed by flow cytometry. The inset panel shows that C5aRA does not inhibit IL-8 produced by Imprime-ABA immune complex in the absence of serum.

In vivo Evidence of C5a Production [SC5b-9 Measured as a Surrogate Marker] in HIV and Cancer Subjects

Figure 4. Systemic administration of Imprime results in C5a production. As a surrogate marker for C5a, average plasma levels of SC5b-9 was measured 2-3 hrs post-imprime administration in (A) HIV (N=11) and (B) triple negative breast cancer subjects (N=12) using commercial enzyme immunoassay kits.

Conclusion

- Imprime-ABA immune complex activates classical complement pathway and as a result, releases C5a, a potent complement-activation product
- C5a, a known priming agent of myeloid cells and PMN, acts via C5aR to enhance Imprime binding
- C5a-C5aR axis is critical for Imprime-ABA immune complex-mediated innate immune effector functions, including cytokines/chemokine production and anti-tumor cytotoxicity via ROS production.

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