Isolation and characterization of an immune complex of Imprime PGG®, a cancer immunotherapeutic agent

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Abstract

Imprime complex isolation and characterization is a challenging area of pharmaceutical research. Here, we disclose our efforts to better understand an immune complex formed between Imprime PGG® (Imprime), antibodies, and complement proteins. Imprime is a soluble yeast-derived beta-glucan immunomodulator that is currently being developed as a cancer immunotherapeutic drug. Imprime is in phase 3 and multiple phase 2 clinical trials in combination with anti-tumor monoclonal antibodies (e.g., bevacizumab and cetuximab). Previous research demonstrated that Imprime forms an immune complex with anti-beta-glucan antibodies (ABA), specifically IgG and IgM, present in the sera of healthy donors and oncology patients. This immune complex activates the classical complement pathway, leading to opsonization of Imprime by IgG to form a three-part complex that subsequently binds to the receptors CR3 and Fcy on neutrophils and monocytes. The goal of this study was to isolate or assemble this three-part complex in pure form free of contaminating components, i.e., albumin, other antibodies, etc. ABA was isolated from serum or commercial sources of immunoglobulin. These antibodies were then recombined with pure Imprime and the complexes were characterized. The biological activity of the immune complexes in terms of their ability to bind immune cells in various conditions will be discussed. In summary, the information learned while isolating and purifying this immune complex will provide a better understanding of Imprime’s mechanism of action and aid in clinical development.

Objective

To purify ABA from serum and commercial IgG and show that purified ABA can enhance Imprime binding to immune cells.
To isolate and characterize the immune complex formed between Imprime and ABA.
To show that the Imprime immune complex gets opsonized with complement in serum at normothermia.

Results

Imprime is isolated from the cell wall of a proprietary strain of Saccharomyces cerevisiae. It is a pharmaceutical grade, water soluble beta 1,3 linked glucose polymer with beta 1,3 linked side chains of Imprime is isolated from the cell wall of a proprietary strain of Saccharomyces cerevisiae. It is a pharmaceutical grade, water soluble beta 1,3 linked glucose polymer with beta 1,3 linked side chains of the Saccharomyces cerevisiae. The beta-D-glucosylated dimer (PGG) is the active moiety of Imprime. The structure is a molecular weight disperse aggregate of triple helices with a Mw of ~150 kD.

Supplementing Imprime with Purified ABA Enhances Binding to Immune Cells

Figure 2. ABA is purified using an immobilized Imprime resin. ABA concentration is determined by an ABA ELISA developed at Biothera.

Mechanism of Action

1. Natural anti-beta-glucan antibodies (IgG and IgM) bind to Imprime.
2. The complex is opsonized via the classical pathway of complement activation.
3. The opsonized complex binds to CR3 receptors on neutrophils and monocytes.
4. Activated cells are primed for functional activity.

Biomarker

A correlation exists between the presence of natural ABA in serum and the in vivo binding of Imprime to neutrophils and other functional activities.

In non small cell lung cancer (NSCLC) there is a correlation between the presence of ABA (Biomarker Positive) and clinical response to Imprime.

NSCLC Imprime + Erlotinib® + Carboplatin/Paclitaxel

Tumor Response (LCAn832)

Objective

All Histologies

Control

Imprime PGG®

Imprime Positive Imprime PGG®

Imprime Negative Imprime PGG®

(6/26)

(22/46)

(10/15)

(12/31)

23%

48%

67%

39%

vs. Control

P=0.0478

P=0.0088

P=0.2592

Results

Figure 3. Neutrophil (left), monocyte (middle), and B cell (right) binding to Imprime or immune complex in unwashed and washed human whole blood. Binding in human whole blood was performed by incubating whole blood with Imprime at 10 µg/ml, alone or with ABA at 30 µg/ml, for 30 minutes at 37°C. The surface bound beta glucan was then determined by BD IV (an IgM ABA) staining and flow cytometry.

SEC Analysis of Imprime Complexed with ABA

Figure 4. UV280 overlay of ABA alone and immune complex formation with leftover ABA. Imprime was added to a solution of ABA then immediately injected on an analytical SEC instrument.

Immune Complex purification

Figure 5. Scheme of Imprime PGG and ABA immune complex purification. Imprime and ABA are combined resulting in a mixture of immune complex, Imprime, and ABA. This mixture is passed over a thiophilic resin to capture immune complex. The immune complex and leftover ABA are eluted and then separated using preparative size exclusion chromatography. The larger immune complex is isolated.

Conclusions

ABA was purified from commercial IgG and serum. Addition of ABA to Imprime enhanced binding to neutrophils, monocytes, and B cells.

The immune complex between Imprime and ABA was isolated using a thiophilic resin and preparative SEC and then characterized by analytical SEC. It bound better than Imprime to neutrophils and monocytes in washed and unwashed whole blood.

The immune complex is opsonized with complement when incubated at 37°C in human serum.