Imprime PGG, a yeast β-glucan immunomodulator, has the potential to modulate the subtypes of immunosuppressive M2 macrophages

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Imprime PGG – An Innate Immunomodulator for Cancer Therapy

- Imprime PGG (Imprime) is a yeast-derived, soluble β-glucan being developed for the treatment of cancer in conjunction with anti-tumor antibodies (Abs).
- In multiple myelogenous and xenograft tumor models, Imprime administered in combination with tumor-targeting monoclonal antibodies (mAbs) has demonstrated anti-tumor activity exceeding that of either agent alone.
- In the clinic, in combination with antibody therapeutics, Imprime has shown promising results in early studies in colorectal cancer (CRC), non-small cell lung cancer (NSCLC), and chronic lymphocytic leukemia (CLL) with additional studies ongoing, including a Phase 3 study in CRC.
- Previous in vitro human studies have demonstrated that Imprime forms an immune complex with endogenous immunoglobulin IgG or IgA anti-β-glucan antibodies (Abs) that are commonly present in patients with chronic inflammation, representing a potential therapeutic target.

Skewed immunostimulatory Versus Immunosuppressive Balance in Tumor Microenvironment

Background/Objectives

- Recent in vitro human mechanistic work revealed that Imprime inhibits the polarization of immunosuppressive M2 macrophages. These studies used macrophage-culture systems to differentiate human peripheral blood monocytes.
- Given that the macrophages undergo specific differentiation depending on the cytokines in the local tissue microenvironment, the overall objective of the current study was to further evaluate the phenotype and functional effects of Imprime on the other subtypes of M2 macrophages.

Specific objectives:

- Evaluate the ability of M2 macrophages derived from Imprime-treated monocytes to enhance the phagocytic activity of tumor cells
- Evaluate the ability of M2a macrophages derived from Imprime-treated monocytes to enhance CD4 T cell proliferation

Experimental Design

In Vitro Culture of Human Macrophages

Whole blood (5 ml) treated with Imprime vs vehicle for 2 hours at 37°C

Isolated peripheral blood mononuclear cells (PBMCs) by Ficoll separation

Enriched CD14+ monocytes by negative selection

Macrophages were cultured in IFN-γ medium for a day

M2a or M1 culture medium with 10 ng/ml of LPS

M2c culture medium with the addition of 1 ng/ml of LPS

M2a and M2c macrophages were prepared as described in the Experimental Design. Macrophages were subsequently evaluated for:

A) phenotype, B) enhancement of CD4+ and CD8+ T cell proliferation, and C) modulation of TNF-α and IL-10 production.

Figure 2. Establishment and characterization of M1/M2a/M2c/macrophages from human peripheral blood monocytes.

Results

Previous Results: M2 Macrophages Derived from Imprime-Treated Monocytes are M1-like

Differential Morphology, Phenotype, and Function of In Vitro Cultured M2 and M2a

M2c Derived from Imprime-Treated Monocytes Have Enhanced Phagocytic Ability Potentially Due to Increase in Surface Expression of Other FcγRs

Figure 3. Evaluation of M1/M2 macrophages derived from Imprime-treated monocytes. M1 and M2 macrophages were prepared as described in the Experimental Design. Macrophages were subsequently evaluated for:

A) phenotype, B) modulation of CD4+ and CD8+ T cell proliferation, and C) production of TNF-α and IL-10.

Shown here are representative results from 4 different experiments.

Figure 4. In vitro differentiation and characterization of M2/M2a macrophages derived from monocytes in human whole blood. M2 and M2a macrophages were prepared and characterized as described in the Experimental Design. Results for:

A) morphology and phenotype of M2a/M2c macrophages, and B) CD4 T cell proliferation and cytokine analysis in the co-cultures of macrophages with CD4 T cells are presented. Shown here are representative results from 4 different experiments.

M2 and M2a Macrophages Derived from Imprime-Treated Monocytes are M1-like

Figure 5. Evaluation of M2a macrophages derived from Imprime-treated monocytes. M2a and M2c macrophages were prepared as described in the Experimental Design. Macrophages were subsequently evaluated for:

A) phenotype, B) enhancement of CD4+ T cell proliferation, and C) modulation of TNF-α and IL-10 production.

Shown here are representative results from 3 different experiments.

Figure 6. Upregulation of potential co-stimulatory mechanisms compensate for PD-L1 expression on M2a-Imprime and enhances T-cell proliferation. M2a macrophages were prepared as described in the Experimental Design. Macrophages were subsequently evaluated for:

A) PD-L1 expression by flow cytometry, B) mRNA levels of PD-L1 by qRT-PCR, and C) CD3- and CD8- T cell proliferation. Shown here are representative results from 3 different experiments.

Figure 7. Evaluation of phagocytic ability of M2c macrophages derived from Imprime-treated monocytes. M2c were prepared as described in the Experimental Design and antibody-dependent cell-mediated phagocytosis (ADCP) of M2c was evaluated with tumor cell lines Raji (Burkitt’s lymphoma) and L1210 (murine cell lymphoma) in the presence of anti-CD20 mAbs, A) Rituximab and B) Obinutuzumab. Viable Raji cells were labeled with cell-trace violet and then incubated with 1 μg/ml of Rituximab or 0.001 μg/ml of Obinutuzumab at 4°C for 20 minutes. Macrophages and treated tumor cells were then mixed in triplicates and incubated at 37°C for 4 hours in the presence of 10% autologous human serum. M2c were stained for CD40 and CD11b, fixed and acquired on LSRII with counting beads. ADCP was interpreted as cells positive for both CD11b and cell trace violet (double positive), divided by the total tumor cells. Data is representative of at least 2 dishes in 5 experiments.

M2c Derived from Imprime-Treated Monocytes Have Enhanced Phagocytic Ability and a Potential Consequence of Engagement of Activating FcγR

• Imprime PGG-ABA immune complex has been shown to bind to FcγRIIA (CD32A) on both neutrophils and monocytes.

- CD32A is an activating FcγR. It lowers the threshold of immune activation.

Figure 8. Blocking of CD32 significantly inhibits Imprime binding to neutrophils and monocytes in whole blood. For FcγR blocking, whole blood was treated with 25 μM of the respective blocking antibodies (clone 3G8 for CD32A, clone AT10 for CD32) one hour prior to CD32 measurement (30 minutes) before incubation with Imprime at 37°C for 30 minutes. Cell surface binding of Imprime was subsequently evaluated by staining with an anti-β-glucan specific mAb. Morphology and functional activity of the M2c derived from Imprime-treated monocytes with and without blocking in neutrophils (left) and monocytes (right) are shown. Anova analysis of neutrophils and monocytes is p<0.001 and 0.0011, respectively with F=12.