The effects of plasmin on regulating immune cell-mediated phagocytosis

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Introduction

Phagocytosis is fundamental to the body's resistance to infection and for the elimination of cancer cells.[1]
Study focus: To investigate the role of plasmin in macrophage and dendritic-cell mediated phagocytosis

Plasmin is a serine protease derived from the zymogen, plasminogen (plg), and is regulated by plg activators, tissue-type plg activator (tPA), urokinase-type plg activator (uPA), their inhibitors and various plg receptors[2]

- Plasmin is a primary regulator of thrombolysis[3], infection, immunity and wound healing and its proteolytic activity has been linked to growth factor and cytokine regulation and the activation of various signal transduction pathways[4]
- Plasmin has also been shown to play various roles in cancer progression[5]
- Previous studies have shown that plasmin upregulates macrophage[6], dendritic cell[7] and keratocyte-mediated phagocytosis[8], but the mechanism is unknown.

Cancer represents the second largest burden of disease globally with 10 million cancer deaths in 2020[9]

- Chemotherapeutics remain the most effective and widely used cancer modality[10]
  - Of emerging importance is the role of chemotherapy in eliciting an immune response against cancer cells through immunogenic cell death (ICD)[11]

Objective & Hypothesis

Objectives: To investigate the function of plasmin during the phagocytosis of *E. coli* bioparticles and chemotherapy-treated cancer cells

Hypotheses:

1. Plasmin will enhance the phagocytosis of *E. coli* bioparticles by macrophage cells
2. Plasmin activity will be affected in cancer cells treated with chemotherapeutic drugs
3. Plasmin will enhance the phagocytosis of cancer cells following chemotherapy

Methods

Cell culture: murine fibrocarcinoma, MCA205 cells and marine macrophage, J774A.1 cells were cultured, and bone marrow-derived dendritic cells (BMDCs) were isolated post-mortem from plg-wild-type (Plg-WT) and plg-knock-out (Plg-KO) mice

1. **Phagocytic capacity of macrophage cells:** J774A.1 cells were incubated with fluorescent *E. coli* bioparticles for 2 hours, quenched with trypan blue and *E. coli* bioparticle uptake was measured via fluorescence measurements.
   - Cells were pre-treated for 24 hours or treated concurrently with fluorescent bioparticles for two hours with or without plg and tPA or plg and uPA, +/- inhibitors, epsilon-aminocaproic acid (eAPA) or tranexamic acid (TXA) or aprotinin (AP) and compared to the no-treatment control (Figure 1).

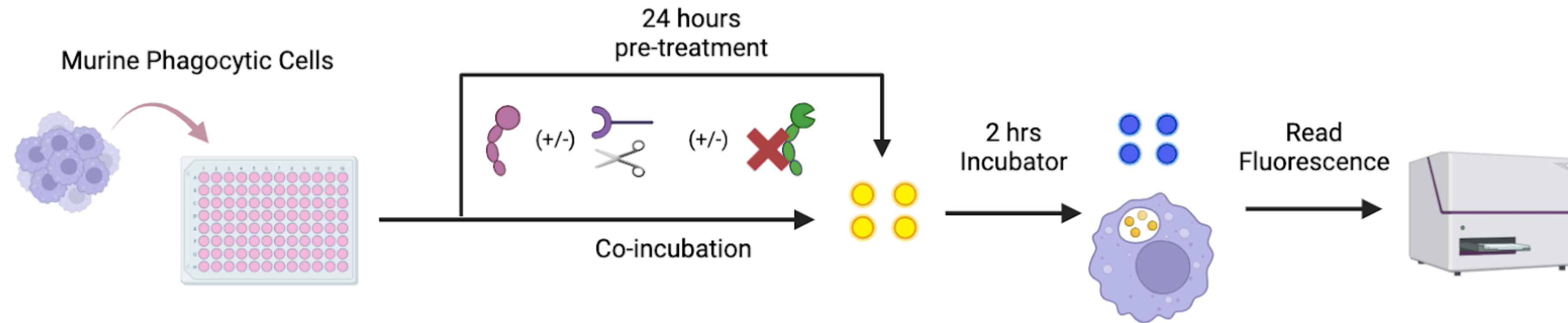

Figure 1 Visual representation of the phagocytic assay where J774A.1 cells were treated concurrently with or 24 hours prior to *E. coli* fluorescent bioparticle incubation with or without plg and tPA or uPA +/- plasmin inhibitors

2. **Plasminogen activation:** MCA205 cells were treated with serial dilutions of chemotherapeutics doxorubicin, mitoxantrone (100nM, 500nM, 1000nM) or oxaliplatin (50 µM, 250 µM, 500 µM) or DMEM for 20 hours.
   - Conversion of inactive plg to active plasmin was assessed in the presence of plg and tPA using a chromogenic substrate and absorbance was measured at A405 nm

3. **Phagocytosis of chemotherapy-treated cancer cells:** MCA205 cells were treated with or without 300µM oxaliplatin for 20 hours, stained with cell trace violet and co-cultured with J774A.1 cells, Plg-WT or Plg-KO BMDCs for 4 hours in the presence or absence plg and plg activators +/- plg inhibitors. Phagocytic cells were stained with macrophage and dendritic cell marker Cd11b/Cd11c tagged with PerCP and data was collected via flow cytometry.
   - Phagocytosis was determined by assessing the percent total cells which stain positively for both dyes.

4. **Statistical Analysis:** significance was assessed via an unpaired t-test or a one-way ANOVA ($p < 0.05$)

Results

1. The phagocytic uptake of fluorescent *E. coli* bioparticles by J774A.1 cells was not significantly affected by 24-hour pre-treatment of plg and tPA (Fig 2A) +/- inhibitors, AP or eACA (Fig 2B). Concurrent treatment plg and tPA or plg and uPA with *E. coli* bioparticles led to marginal but significant increase in phagocytosis (Fig 2C & 2D), but we did not observe a suppression of phagocytosis with the inhibitors, eACA and TXA (Fig 2E).

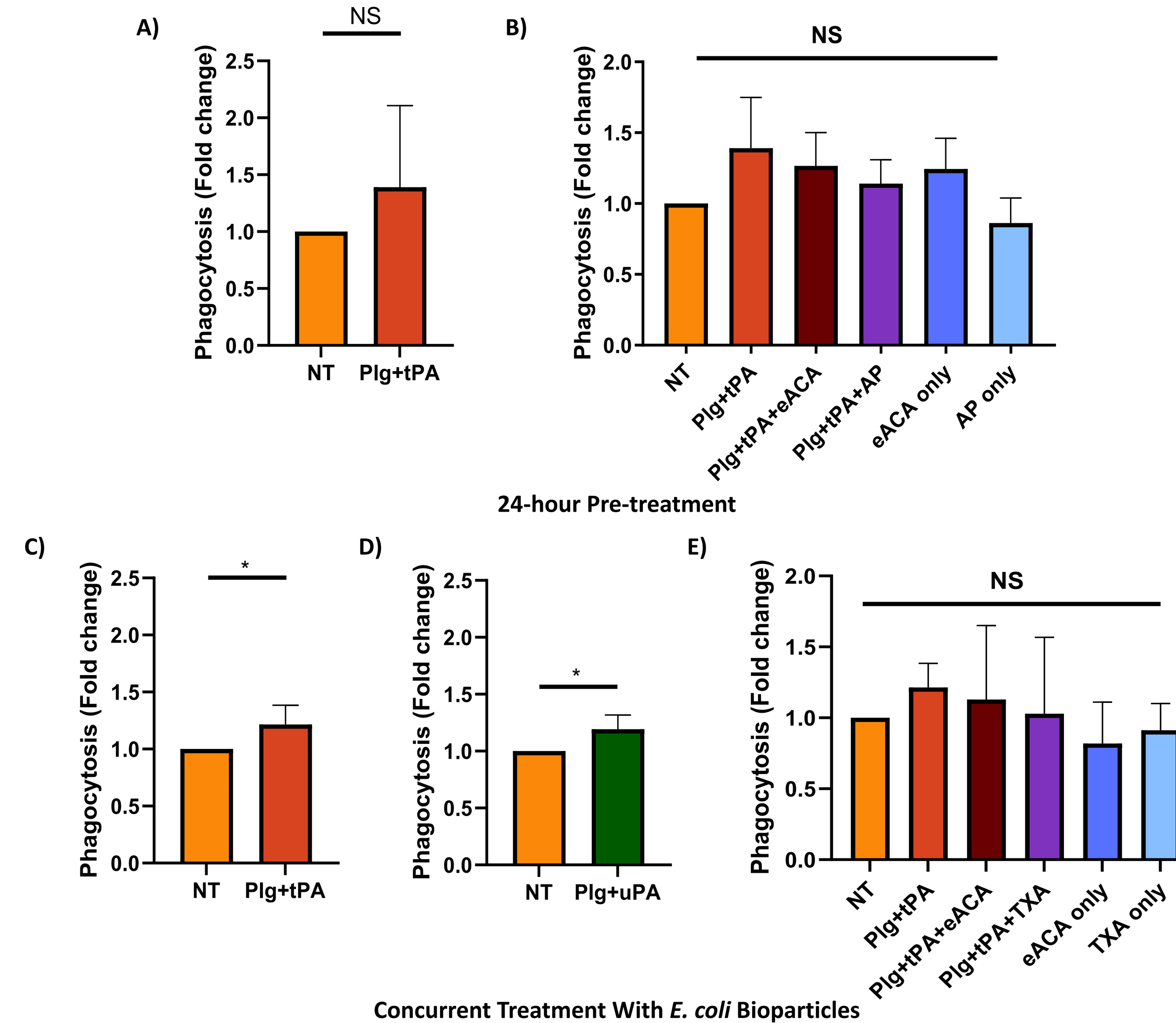

Figure 2 Phagocytic uptake of fluorescent *E. coli* bioparticles by J774A.1 A) pre-treated with plg and tPA for 24 hours (p>0.05, unpaired t-test) B) in the presence or absence of inhibitors, eACA or AP was not increased relative to the no-treatment (NT) control (p>0.05; One-way ANOVA with Brown-Forsythe's multiple comparison). Concurrent treatment of bioparticles in the presence or absence of C) plg and tPA or D) plg and uPA yielded a significantly increased bioparticle uptake (p<0.05, unpaired t-test). These changes were not seen across treatments in the presence or absence of plg and tPA +/- plg inhibitors, eACA or TXA (p>0.05; One-way ANOVA with Brown-Forsythe's multiple comparison). Fold changes were compiled from three independent experiments and values are shown as mean + SD.

2. Chemotherapeutic drug treatments of MCA205 cells led to an increase in plg activation in a dose-dependent manner compared to the no-treatment control (NT) (Figure 3)

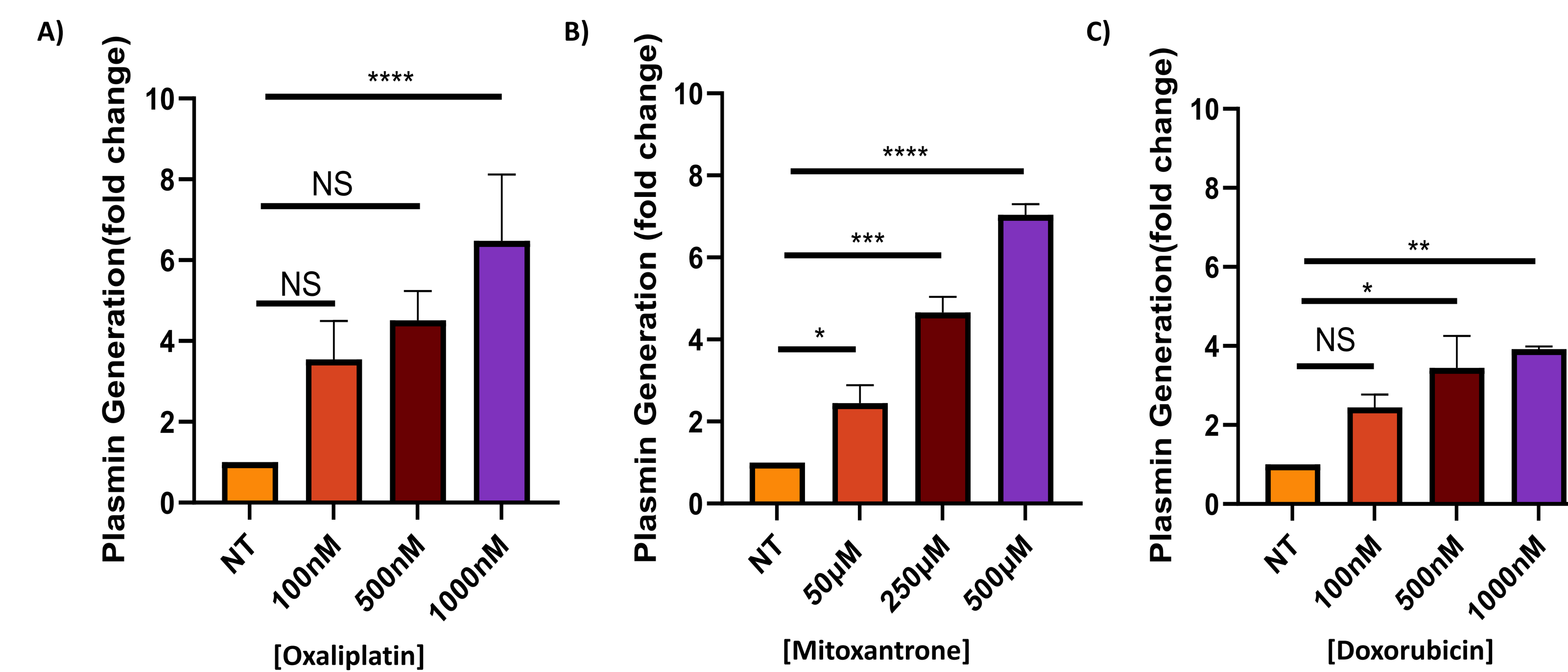

Figure 3 Plasminogen activation enhanced by treatment of chemotherapeutics A) oxaliplatin B) mitoxantrone C) doxorubicin in a dose-dependent manner as compared to a no-treatment (NT) control (p<0.0001; One-way ANOVA with Brown-Forsythe's multiple comparison). The fold change was compiled from three independent experiments and values are shown as mean + SD.

3. Low percent phagocytosis did not allow for meaningful comparisons of MCA205 phagocytosis by J774A.1 cells (Fig 4A), Plg-WT or Plg-KO BMDCs (Fig 4B) as measured by flow cytometry.

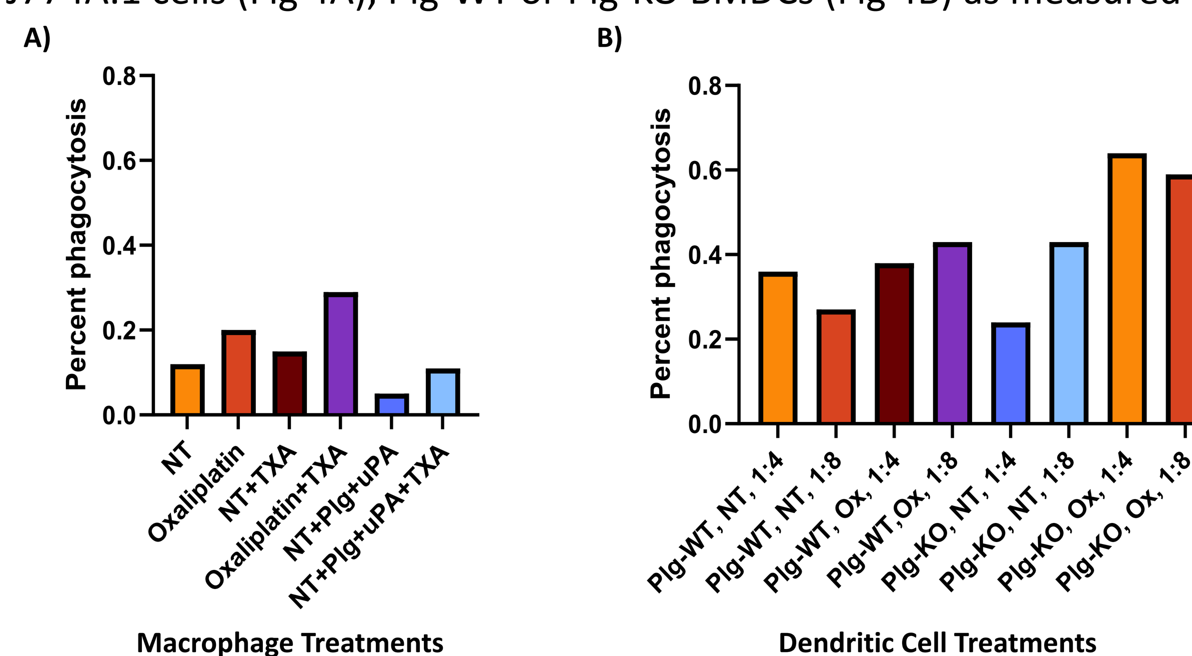

Figure 4 Percent phagocytosis of MCA205 cells, treated with or without (NT) oxaliplatin or plg and uPA +/- plg activation inhibitors, by A) J774A.1 cells or B) Plg-Wild-Type (Plg-WT) or Plg-Knock-out (Plg-KO) BMDCs. The percent phagocytosis is indicated by the cell populations double stained for both Cell Trace Violet (MCA 205) and Cd11b/Cd11c Per-CP. Data was collected from one experiment and plotted using Graph Pad prism version 10 software.

Discussion

- While we did see some degree of phagocytic enhancement in macrophage cells in the presence of plasminogen and plasminogen activator, which did not change in the presence of inhibitors, contrary to previous findings[8,, 9, 10].
  - Suggest that further optimization and replicates may be required to yield significant results
- Our findings that plasminogen activation is upregulated *in cancer cells* following treatment with chemotherapeutic agents has not been previously reported and indicate that plasmin may serve a role in cancer progression following chemotherapy.
  - The mechanism for increased plasmin activity remains unclear
- Unlike Cerrato et al. (2020)[12] who found the phagocytosis of MCA205 cells treated with oxaliplatin by BMDCs was in the magnitude of >20%, we did not see a significant population of phagocytosed cells in our experiment, indicating a further need for assay optimization.

Conclusion

Overall, this study will provide a mechanistic understanding of the role of plasmin in phagocytosis of cancer cells following chemotherapy which will build the foundation for further studies of the role of plasmin in ICD. Therefore, this report may provide important insight in the understanding and development of cancer cure strategies.

References

Canadian Cancer Society