Elucidating the Effects of Prolonged Leukocytic and Metastatic Cell Exposure to P-selectin and ICAM-1 on Cell Activation and Survival

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Elucidating the Effects of Prolonged Leukocytic and Metastatic Cell Exposure to P-selectin and ICAM-1 on Cell Activation and Survival

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To the students of Georgia Institute of Technology

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LIST OF SYMBOLS AND ABBREVIATIONS

PS	Phosphatidylserine
P-selectin	a protein that in humans is encoded by the SELP gene
ICAM-1	Intercellular Adhesion Molecule-1

SUMMARY

The immune system has been well studied for its importance in protection against pathogens and healing of inflamed human tissues. It is known to intervene in chronic inflammation of diseases including cancer. Although much attention has been given to its role in protection against diseases, emerging research show that the immune system contributes to the development of some cancers like the colon carcinoma. The purpose of this research is to analyze what signaling mechanism cancer uses to manipulate the immune system to survive and metastasize. The study looks at the first process of immune intervention by analyzing the process of monocyte recruitment. Cell adhesion molecules located in the endothelium initiate the rolling and firm adhesion of monocytes, P-selectin and ICAM-1 respectively, near the location of inflammation. Apoptosis, a voluntary cell death, is a mechanism this research analyzes for monocyte activation after interacting with cell adhesion molecules. Recent work from our lab has shown that monocytes have more interaction with P-selectin than colon carcinoma, indicating a potential critical point of avoiding being attacked by the immune system. Results in this study show that P-selectin increases the number of Phosphatidylserine (PS) in formation of apoptosis in both monocytic THP1 cells and colon carcinoma LS174 T cells. In addition, current study shows that co-incubation of P-selectin with intracellular adhesion molecule-1(ICAM-1) decreases the number of PS exposure. These results might be crucial in understanding the underlying mechanisms in which cancer cells survive and thus, suggesting that developing therapeutic drugs that target ICAM-1 might be a possible route in decreasing cancer's ability to metastasize.

CHAPTER 1

INTRODUCTION

The recruitment of leukocytes in the vasculature is critical to the progression and resolution of various physiological and pathophysiological processes ranging from atherosclerosis[1] to infection[2], cancer[3], and injury[4]. Leukocyte recruitment begins when adhesive ligands on a circulating cell engage selectins via fast kinetic interactions, thus facilitating rolling adhesion, which slows a cell down relative to free flow. Rolling adhesion in turn enables slower kinetic interactions between integrins and apposing immunoglobulin cell adhesion molecules for firm adhesion and eventually extravasation into the surrounding tissue[5]. In addition to its well characterized role in initiating leukocyte recruitment[2, 6, 7], P-selectin has been reported to activate leukocytes. Specifically, this effect has been studied in the context of coagulation, where engagement of soluble P-selectin by leukocytic THP-1 cells was shown to induce translocation of phosphatidylserine (PS) to the surface of THP-1 cells in a manner which was dependent on both the concentration and duration of P-selectin exposure[8]. PS translocation to the outer leaflet of the cell membrane typically indicates apoptotic cell death and signals for phagocytic clearance of the dying cells from circulation[9,10].

However, the effect of simultaneous engagement of P-selectin and immunoglobulin cell adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1) which typically facilitates firm adhesion after P-selectin mediated rolling adhesion during cell recruitment, on PS translocation remains unclear. Cancer cells similarly utilize a rolling to firm adhesion cascade to escape the vasculature and form metastatic foci in distant tissues, a process in which P-selectin and ICAM-1 have also

been implicated[11, 12]. Yet, recent work in our lab has suggested that, unlike leukocytes, metastatic cells exhibit reduced rolling adhesion persistence on P-selectin[13], implying that their total exposure to P-selectin is impaired.

While little is known about the apoptotic signaling effects of selectin engagement by cancer cells, if P-selectin engagement by circulating cancer cells has similar effects on PS translocation, it might suggest a mechanism by which reduced rolling adhesion persistence facilitates evasion of apoptosis, a hallmark of cancer[14]. Therefore, understanding how both dose and duration of P-selectin and ICAM-1 exposure affect metastatic cancer cell PS translocation may provide critical insight into mechanisms by which metastatic dissemination maintains efficiency.

CHAPTER 2

LITERATURE REVIEW

Human tissue protection and repair during inflammation is a well-studied function of the immune system, but its inability to fight cancer remains a great area of research. Monocytes normally circulate in the blood and they are recruited short after the inflammation. The process of recruitment to the site of inflammation begins with an interaction of P-selectin, found on the surface of the endothelium, and its ligand, P-selectin Glycoprotein Ligand (PSGL-1) found on the surface of monocytes. This rolling adhesion slows down the monocytes and eventually, a firm adhesion occurs between monocytes' integrin and the cell adhesion molecule called ICAM-1 on the endothelium. Then, monocytes transverse and migrate in damaged tissue[4]. P-selectin and ICAM-1 are increasingly expressed on the surface of endothelium in inflamed tissues in many diseases including atherosclerosis[1,9] and cancer[12].

In addition to its well characterized role in initiating leukocyte recruitment[2, 6,7], P-selectin has been reported to activate leukocytes. Specifically, this effect has been studied in the context of coagulation, where engagement of soluble P-selectin by leukocytic THP-1 cells was shown to induce translocation of phosphatidylserine (PS) to the surface of THP-1 cells in a manner which was dependent on both the concentration and duration of P-selectin exposure[8]. Apoptotic cells have been characterized by the expression of Phosphatidylserine (PS) on their surface[8]. Due to this property, macrophages recognize, engulf , and isolate them from the rest of the environment.

The synergy function of P-selectin and ICAM-1 in monocyte rolling and firm adhesion has also been studied in metastatic colon cancer and monocytic cells. Edwards *et al* treated monocytic THP1 cells with different concentrations of P-selectin with or without the presence of 2.5 μg/mL of ICAM-1 in hemodynamic flow to analyze the effect ICAM-1 has on P-selectin. Results showed that the firm adhesion was increased as the concentrations of P-selectin increase. However, they found that the increase in ICAM-1 had no effect on the firm adhesion. One interesting observation was that the co-presentation of P-selectin and ICAM-1 enhances monocytes' firm adhesion but not the rolling adhesion[3].

Previous research studied the role of immune cells in preventing diseases. However, Richard *et al's* study holds the belief that monocytes promote tumor development and metastasis. Monocytes develop into tumor associated macrophages(TAMS) when recruited to the tumor microenvironment. TAMS have been found to promote the tumor growth and suppress the immune system by enhancing angiogenesis. In addition, TAMs facilitate cancer to relocate from its primary site to the blood vessels where it is transported to its metastatic site[12]. P-selectin's role is not limited to monocyte recruitment; it is also involved in tumor development. The absence of P-selectin resulted in a decrease of tumor growth[11]. Therefore, understanding how both dose and duration of P-selectin exposure on metastatic cancer cells and PS translocation may provide critical insight into mechanisms by which metastatic dissemination maintains efficiency. This study aims to understand how ICAM-1 modulates P-selectin-induced cell activation (measured via PS translocation) in leukocytic THP-1 cell and to

compare the extent of cell activation (PS translocation) by leukocytic THP-1 and metastatic LS174T colon carcinoma cell P-selectin and ICAM-1 engagement.

CHAPTER 3

MATERIALS AND METHODS

Fluorophore-conjugated Annexin V and Propidium Iodide (PI) were purchased from Thermo Fisher Scientific company (catalog number: V13241). A fluorescent microscope and Image J were used for image acquisition and analysis in quantifying apoptosis (PS exposure) and necrosis, respectively. In all experiments, 3% hydrogen peroxide and untreated cells were used as positive and negative controls, respectively. Statistical analysis was performed using GraphPad Prism. All the experiments were performed three times to minimize error.

THP-1 cells were cultured in RPMI 1640 that contains 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 10 mM 4-(2-hydroxyethyl)- 1-piperazineethanesulfonic acid (HEPES), and 1% penicillin– streptomycin. LS174T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic-antimycotic. Both cell lines were incubated at 37ºC and 5% CO2. After 72 hours, cells were passaged to maintain them at an average concentration of 2*10^5 cells/mL to 1.6*10^6 cells/mL. For the experiment, 2*10^5 cells were seeded in a 96 trans well plate. P-selectin and ICAM-1 concentrations were determined based on previous work[6].

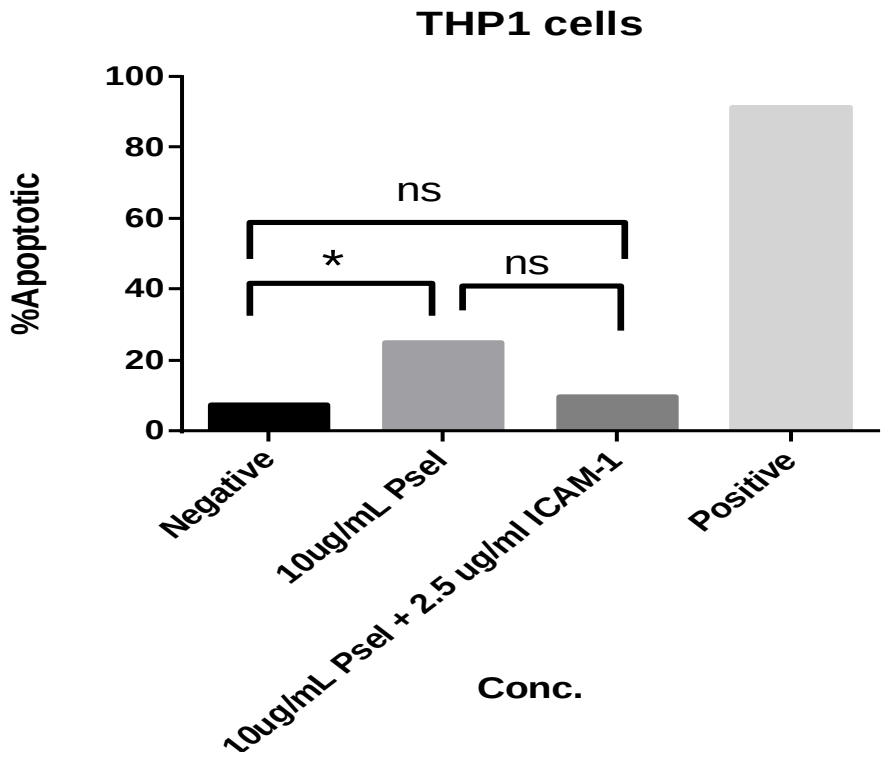
The first phase of this study was to determine the minimum concentration at which THP-1 and LS174T cell lines express a significant number of apoptosis. Each cell line was incubated with 1, 2.5, and 10 µg/mL P-selectin.

The second phase focused on analyzing if ICAM-1 might have an influence on P-selectin's apoptotic effects in both cell lines. This was done by incubating THP1 cells with 10 µg/mL P-selectin or LS174T cells with 25 µg/mL P-selectin. An additional treatment group was added where both 10 µg/mL P-selectin was co-incubated with 2.5 µg/mL ICAM-1 and 25 µg/mL P-selectin was co-incubated with 2.5 µg/mL ICAM-1 for 24 h before staining with Annexin V.

Bright field and green fluorescent images were taken in each well using EVOS fluorescent microscope. Images were then analyzed using Image J to quantify the number of PS exposure on the surface of the cells. To statistically analyze the data using GraphPad PRISM, a one-way ANOVA was done among the experimental group. The data shown in this study are representative of three replicates in both experimental and control groups.

CHAPTER 4

RESULTS



Results indicated that a concentration of 10 µg/ml and 25 µg/ml P-selectin

Figure 1. THP1 cells were treated with 10 µg/ml of P-selectin with or without 2.5 µg/ml ICAM-1.

increased the extent of PS exposure on the surface of THP-1 cells and LS174T cells, respectively, indicating that the concentration-dependence of P-selectin exposure is specific to cell subtype(data not shown). We then sought to understand how addition of ICAM-1 would alter cell fate with respect to PS exposure. We found that, co-incubation of 2.5 ug/ml ICAM-1 with 10 ug/ml P-selectin decreased PS exposure in THP-1 cells relative to P-selectin-only treatment (**Figure 1**).

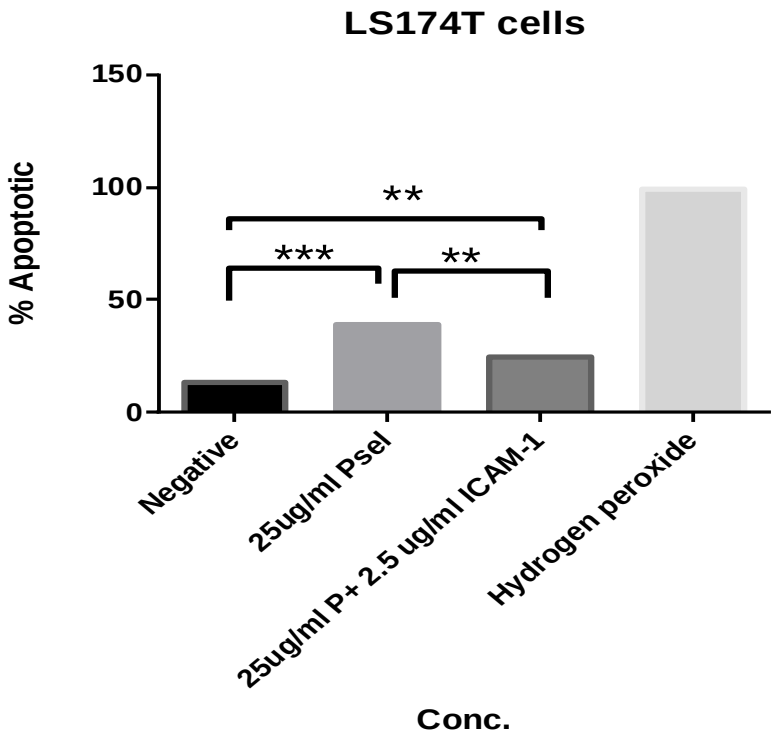


Figure 2. LS174 T cells were treated with 25ug/ml P-selectin with or without 2.5ug/ml ICAM-1.

While 10 µg/ml was the concentration found to induce a significant effect in THP1 cells, this concentration was 25 µg/ml in LS174T cells. Similar to previous results, co-incubation of 2.5 µg/ml ICAM-1 with 25 µg/mL P-selectin decreased PS exposure on the surface of LS174T cells (**Figure 2**). Altogether, these results suggest that while exposure to P-selectin may induce apoptosis in both leukocytic and metastatic cells, co-incubation with ICAM-1 attenuates this effect.[7]

CHAPTER 5

DISCUSSION AND CONCLUSION

An increase in PS exposure on the surface of THP-1 cells due to P-selectin observed in this experiment is similar to del Conde *et al*'s research findings: as the concentration of P-selectin increased, the extent of PS exposure on the surface of THP-1 cells increased. In isolation of other signaling cues, this increase in supposed apoptosis may be a control mechanism by which non-recruited cells are cleared from circulation. Since ICAM-1 is known to facilitate THP-1 firm adhesion when co-presented with P-selectin[6] and thus stipulates that a cell's fate is ultimately recruitment into surrounding tissue, we suspected that ICAM-1 engagement may decrease the extent of THP-1 PS exposure. This trend has been observed in this experiment although the data was not statistically significant (**Figure 1**). Knowing that cancer cells use the same adhesion cascade to invade the tissue in hematogenous metastasis, we expected P-selectin and ICAM-1 would induce similar effects in LS174T cells. This was consistent with the results that show a decrease in apoptosis upon co-incubating ICAM-1 with P-selectin (**Figure 2).**

Some of the challenges met in this study include a limited access to modern techniques of quantifying apoptotic cells like a flow cytometer. This process was done manually by counting each apoptotic cell in ImageJ. While it might be accurate than an automated process, it might not be a time effective method for future studies in case multiple images would have to be analyzed.

The findings of this study indicate that ICAM-1 might modulate metastasis of colon cancer cells. Thus, developing therapies that directly target this molecule might be

a potential solution in preventing circulating cancer cell survival. Howbeit, further studies would need to investigate if a decrease in PS exposure in colon cancer cell depends on both time of exposure and increase in concentration of ICAM-1. Additionally, various types of cancers could be tested to see if this trend holds true.

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