October 2002

Effect of Shear Stress on Platelet Activation via the Glycoprotein VI Receptor

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Publisher URL: http://ieeexplore.ieee.org/xpl/tocresult.jsp?isNumber=25190&page=22

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Abstract
Cardiovascular diseases are the nation's leading cause of death. Such diseases are caused by platelet response to collagen especially in the event of vascular injury leading to thrombosis. One of the platelet receptors known to bind to the collagen ligand is glycoprotein VI (GPVI) with co-receptor Fc receptor γ chain (FcRγ). By stably expressing the GPVI receptor in rat basophilic leukemia cells (RBL-2H3), which abundantly express FcRγ, but endogenously lack GPVI, studies have shown that GPVI-FcRγ is sufficient to confer adhesion as well as signaling responses to collagen as long as the receptor density is equivalent to that found on human platelets. While those investigations confirm that the GPVI receptor mediate binding to collagen under static conditions, they do not provide information on how the GPVI receptor interacts with collagen under dynamic conditions. In the present study we have used the GPVI-expressing RBL-2H3 cells to observe the kinetics of adhesion to collagen under hydrodynamic flow conditions *in vitro* using a parallel plate flow chamber coupled with video microscopy. We demonstrate that these cells do adhere to the surface at a low shear rate and do so at a greater adherent cell density than wild-type RBL-2H3 (WT-RBL) cells.

Keywords
GPVI-expressing RBL-2H3 cells, GPVI-173, collagen, cell adhesion, flow chamber

Proceedings of the Second Joint EMBS/BMES Conference
Houston, TX, USA • October 23-26, 2002

EFFECT OF SHEAR STRESS ON PLATELET ACTIVATION VIA THE GLYCOPROTEIN VI RECEPTOR

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Abstract-**Cardiovascular diseases are the nation's leading cause of death. Such diseases are caused by platelet response to collagen especially in the event of vascular injury leading to thrombosis. One of the platelet receptors known to bind to the collagen ligand is glycoprotein VI (GPVI) with co-receptor Fc receptor gamma chain (FcRγ). By stably expressing the GPVI receptor in rat basophilic leukemia cells (RBL-2H3), which abundantly express FcRγ, but endogenously lack GPVI, studies have shown that GPVI-FcRγ is sufficient to confer adhesion as well as signaling responses to collagen as long as the receptor density is equivalent to that found on human platelets. While those investigations confirm that the GPVI receptor mediate binding to collagen under static conditions, they do not provide information on how the GPVI receptor interacts with collagen under dynamic conditions. In the present study we have used the GPVI-expressing RBL-2H3 cells to observe the kinetics of adhesion to collagen under hydrodynamic flow conditions *in vitro* using a parallel plate flow chamber coupled with video microscopy. We demonstrate that these cells do adhere to the surface at a low shear rate and do so at a greater adherent cell density than wild-type RBL-2H3 (WT-RBL) cells.**

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I. INTRODUCTION

One of the primary and critical reactions that can lead to cardiovascular disorders is the adherence and aggregation of platelets at sites of vascular injury in flowing blood [1,2]. Platelet response is generally brought about through interactions between specific receptors on the platelet surface and exposed ligands of the subendothelium, with collagen being a major agonist. The initial interaction between circulating platelets and the subendothelium is believed to occur indirectly through the von Willebrand factor (vWF) bound to collagen and the GPIb platelet receptor [3]. Other platelet surface proteins implicated as being direct platelet/collagen receptors include integrin $\alpha_2\beta_1$, CD36, glycoprotein VI (GPVI), and P65 [4-8].

Although the indirect interaction has been clearly identified, the direct interaction between platelet receptors and collagen remain poorly understood, especially in the case of the GPVI receptor [9-11]. This receptor has been found to be important, since human platelets deficient in it show impaired levels of adhesion and aggregation compared to whole blood [1]. To address the functional role of GPVI in isolation of other receptors, GPVI was expressed in rat basophilic leukemia (RBL-2H3) cells, a cell line that expresses the Fc receptor gamma chain (FcRγ), but does not express endogenous GPVI or integrin $\alpha_2\beta_1$ [12]. Studies with these cells under static conditions demonstrate that "GPVI expression is sufficient to confer both adhesive and signaling responses to collagen independent of other collagen receptors but that GPVI-collagen responses are strictly dependent on receptor density" [11]. Of the three clones studied, GPVI-expressing RBL clone 173 (GPVI-173), expressing the highest level of GPVI, adhered to collagen at a higher optical density than the other two clones [11]. Using the GPVI-173 cells, this study quantifies the adhesion of the cells to collagen type I under flow conditions. The preliminary results show that the GPVI receptor adheres to the collagen surface at a low shear rate.

II. METHODOLOGY

Slide preparation. Microscope slides (3"x1"; Sigma Diagnostics, St. Louis, MO) were coated with fibrillar collagen (100 ug/mL) derived from equine tendons (Chronolog; Havertown, PA). The slide was incubated overnight at 4°C. On the day of experimentation the slide was rinsed with phosphate buffered saline with 0.05% polyoxyethylene-sorbitan monolaurate to remove unbound collagen. The slide was then incubated with 2% bovine serum albumin (BSA) for 1 hour at room temperature on a rotator.

Cell preparation. GPVI-173 cells were generated as previously described. [11,12]. The cells were cultured in Dulbecco Modified Eagle Medium with 10% fetal bovine serum and 10μg/mL gentamicin. On the day of experimentation, the cells were suspended in PBS with 1% BSA (PBS^+) to achieve a cell concentration of $1x10^6$ cells/mL.

Parallel plate flow chamber assembly and video microscopy. The parallel plate flow chamber was assembled in water with a straight channel template and collagen coated glass slide. The flow chamber assembly was mounted onto the stage of a phase contrast inverted microscope (Diaphot-TMD; Nikon, Tokyo, Japan). A CCD camera (Cohu Inc.; San Diego, CA) and Sony VHS recorder (Sony Medical Systems; Montvale, NJ) were used to obtain and record images. After the chamber was flushed with PBS^+, wild-type RBL-2H3 (WT-RBL) and GPVI-173 cells were passed through the flow chamber at a controlled flow rate for 20 minutes using a syringe pump (Model 55-1143, Harvard Apparatus; South Natic, MA). The actual shear stress was calculated by the following equation

$$\tau_w = 6\mu Q/wh^2 \quad (1)$$

where τ_w is the wall shear stress, μ is the fluid viscosity, Q is the volumetric flow rate, w is the chamber width, and h is the gap thickness [13,14].

Adherent cell densities were determined by counting the number of adherent cells and dividing them by the field of view, 0.32 mm^2. Based on three experiments, one-way analysis of variance with a probability value of 0.05 (95% confidence) was performed to test for significance.

III. Results

Visually analyzing the video, the adhesion of GPVI-173 to collagen was studied at a wall shear rate of $63s^{-1}$. The number of cells per 0.32 mm^2 was counted and averaged over 3 experiments. We have presented the average adherent cell densities of GPVI-173 and WT-RBL cells at 5, 10, 15, and 20 minutes (see Table I). It was found that there was significant adhesion of GPVI-173 cells to collagen, with the cells remaining adherent. Over the twenty-minute segment, the adherent cell density gradually increased for both cell types. Control experiments performed with WT-RBL cells showed significantly lower adherent cell densities compared to that of GPVI-173 cells.

IV. Discussion

Studies using static assays show that GPVI-173 is sufficient to mediate adhesion to collagen. While this may be true, the results do not account for the fact that adhesion of platelets, *in vivo*, occur under physiologically shear conditions. We demonstrate that at a low shear rate of $63s^{-1}$, GPVI-173 cells adhere to collagen with a significantly higher adherent cell density than WT-RBL cells. This result suggests that GPVI alone is sufficient to confer adhesion to collagen under hydrodynamic conditions. However the shear rate range over which the receptor is reactive has yet to be determined. Thus, more experiments must be done to test for nonspecific adhesion.

V. Conclusion

This study provides further evidence that GPVI, a collagen receptor, mediates collagen adhesion. In doing the adhesion assay under dynamic conditions, we hope to gain a better understanding of the mechanistic details of the GPVI receptor's involvement in collagen activation of platelets. By determining its functionality, we plan to elucidate the pathomechanism GPVI plays in cardiovascular disorders such as myocardial infarction, angina, stroke, and arteriosclerosis. From there future investigations can provide novel targets for anti-platelet strategies to treat cardiovascular diseases.

Table I. Average adherent cell densities (cells/mm^2) ± SE

	Cell Type	
	GPVI-173	WT-RBL
5 min	87.47±20.56	45.32±15.44
10 min	160.72±33.10	69.03±15.70
15 min	182.33±33.94	72.98±15.33
20 min	192.08±32.85	75.09±17.73

Acknowledgments

We gratefully acknowledge the support of NIH HI62250 and HL64388 to Dr. Daniel Hammer.

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