High Molecular Weight Kininogen Regulates Platelet-Leukocyte Interactions by Bridging Mac-1 and Glycoprotein Ib*

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Leukocyte–platelet interaction is important in mediating leukocyte adhesion to a thrombus and leukocyte recruitment to a site of vascular injury. This interaction is mediated at least in part by the β2-integrin Mac-1 (CD11b/CD18) and its counter-receptor on platelets, glycoprotein Ibα (GPIbα). High molecular weight kininogen (HK) was previously shown to interact with both GPIbα and Mac-1 through its domains 3 and 5, respectively. In this study we investigated the ability of HK to interfere with the leukocyte-platelet interaction. In a purified system, HK binding to GPIbα was inhibited by HK domain 3 and the monoclonal antibody (mAb) SZ2, directed against the epitope 269–282 of GPIbα, and its counter-receptor on platelets, glycoprotein Ibα (CD11b/CD18) and its counter-receptor on platelets, glycoprotein ligand-1 is followed by Mac-1-dependent firm adhesion and diapedesis (1). Leukocytes express members of the β2-integrin family including LFA-1 (αLβ2, CD11a/CD18), Mac-1 (αMβ2, CD11b/CD18), and p150,95 (αXβ2, CD11c/CD18), as well as β2-integrins that interact with endothelial counterligands such as ICAM-1, surface-associated fibrinogen, or vascular cell adhesion molecule 1 (VCAM-1) (2–4). After denudation of the endothelial cell lining at sites of vascular injury, leukocyte infiltration occurs through interactions with deposited platelets and fibrin (5). In vivo studies demonstrated an association of both cell types within atherosclerotic and restenotic lesions, or in areas of ischemia-reperfusion injury (6, 7). Reminiscent of the leukocyte-endothelium interactions, a sequential adhesion process including leukocyte attachment to and transmigration across surface-adherent platelets has been proposed. Platelet P-selectin-mediated initial tethering and rolling of leukocytes through their P-selectin glycoprotein ligand-1 is followed by Mac-1-dependent firm adhesion and transplatelet migration (8). Recent work indicated functional adhesion molecule-3 and glycoprotein (GP) Ibα on platelets as potential counter-receptors for Mac-1 (9, 10). Furthermore, ICAM-2 and αMβ2-associated fibrinogen has also been proposed to mediate Mac-1-dependent platelet-leukocyte interactions (11, 12); however, the exact contribution of each system remains to be elucidated.

Multicellular interactions between leukocytes and the vessel wall are important in inflammatory processes or vascular remodeling. Leukocyte recruitment to vascular endothelium requires multistep adhesive and signaling events including selectin-mediated rolling, leukocyte activation, and integrin-mediated firm adhesion and diapedesis (1). Leukocytes express members of the β2-integrin family including LFA-1 (αLβ2, CD11a/CD18), Mac-1 (αMβ2, CD11b/CD18), and p150,95 (αXβ2, CD11c/CD18), as well as β2-integrins that interact with endothelial counterligands such as ICAM-1, surface-associated fibrinogen, or vascular cell adhesion molecule 1 (VCAM-1) (2–4). After denudation of the endothelial cell lining at sites of vascular injury, leukocyte infiltration occurs through interactions with deposited platelets and fibrin (5). In vivo studies demonstrated an association of both cell types within atherosclerotic and restenotic lesions, or in areas of ischemia-reperfusion injury (6, 7). Reminiscent of the leukocyte-endothelium interactions, a sequential adhesion process including leukocyte attachment to and transmigration across surface-adherent platelets has been proposed. Platelet P-selectin-mediated initial tethering and rolling of leukocytes through their P-selectin glycoprotein ligand-1 is followed by Mac-1-dependent firm adhesion and transplatelet migration (8). Recent work indicated functional adhesion molecule-3 and glycoprotein (GP) Ibα on platelets as potential counter-receptors for Mac-1 (9, 10). Furthermore, ICAM-2 and αMβ2-associated fibrinogen has also been proposed to mediate Mac-1-dependent platelet-leukocyte interactions (11, 12); however, the exact contribution of each system remains to be elucidated.

High molecular weight kininogen (HK) and especially two-chain kinin-free kininogen (HKα) were previously reported to regulate adhesive events (13, 14). Initially identified as a non-enzymatic cofactor in the initiation of the contact phase (15), HK appears to be associated with vascular injury, inflammation, or activation of complement in humoral immune defense. In particular, kallikrein can liberate the short lived vasodilator X2-integrin family including LFA-1 (αLβ2, CD11a/CD18), Mac-1 (αMβ2, CD11b/CD18), and p150,95 (αXβ2, CD11c/CD18), as well as β2-integrins that interact with endothelial counterligands such as ICAM-1, surface-associated fibrinogen, or vascular cell adhesion molecule 1 (VCAM-1) (2–4). After denudation of the endothelial cell lining at sites of vascular injury, leukocyte infiltration occurs through interactions with deposited platelets and fibrin (5). In vivo studies demonstrated an association of both cell types within atherosclerotic and restenotic lesions, or in areas of ischemia-reperfusion injury (6, 7). Reminiscent of the leukocyte-endothelium interactions, a sequential adhesion process including leukocyte attachment to and transmigration across surface-adherent platelets has been proposed. Platelet P-selectin-mediated initial tethering and rolling of leukocytes through their P-selectin glycoprotein ligand-1 is followed by Mac-1-dependent firm adhesion and transplatelet migration (8). Recent work indicated functional adhesion molecule-3 and glycoprotein (GP) Ibα on platelets as potential counter-receptors for Mac-1 (9, 10). Furthermore, ICAM-2 and αMβ2-associated fibrinogen has also been proposed to mediate Mac-1-dependent platelet-leukocyte interactions (11, 12); however, the exact contribution of each system remains to be elucidated.

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1 The abbreviations used are: ICAM-1, intercellular adhesion molecule 1; BSA, bovine serum albumin; GPIbα, glycoprotein Ibα; HK, single chain high molecular weight kininogen; HKα, two-chain (kinin-free) high molecular weight kininogen; mAb, monoclonal antibody; CHO, Chinese hamster ovary.

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main 5 of HK binds to the β3-integrin Mac-1 on granulocytes and competes for fibrinogen and ICAM-1 binding in vitro and serves to regulate the Mac-1-dependent leukocyte recruitment in vivo (21). On platelets, cell-associated thrombospondin as well as GP Ibα mediate the binding of HK (22–24). Whereas binding to GP Ibα has been mapped to the region Lys275-Gln292 of HK domain 3, multiple binding sites for HK have been proposed within the GP Ibα molecule, among others the region 269–282 that contains three sulfated tyrosine residues, which is recognized by the mAb SZ2 (25). In contrast, the region 201–236 of GP Ibα recognized by mAb API mediates the Mac-1-GP Ibα interaction (9, 26).

These diverse observations prompted us to define the role and influence of HK on the Mac-1-GP Ibα interaction. Our results demonstrate that HK enhances the binding between leukocytes and platelets by bridging Mac-1 and GP Ibα and thereby provide new insights into the regulatory role of HK for leukocyte-platelet interactions.

EXPERIMENTAL PROCEDURES

Reagents—Single and two-chain HK and HKα were purchased from Enzyme Research Laboratories (South Bend, IN). The purified HK and HKα (>95%) appeared as a major band of 140 and 110 kDa, respectively, on non-reducing SDS gels. HK was digested with plasma kallikrein (HK to kallikrein = 100:1, mol/mol) for 20 min at 37°C. The resulting HKα was composed of two bands of 62 and 46 kDa when analyzed by reduced SDS-gel electrophoresis. Biotin-labeled HK and HKα were produced as previously described (21). Glutathione S-transferase fused to domains 3, 5, and 6 of HK or to sequences derived from domain 5 were produced as previously described (24). Glutathione S-transferase was amino terminally attached to the following sequences of HK: Gly235-Met357 (domain 3), Lys420-Ser513 (domain 5), Thr498-Ser520 (domain 6) as well as Lys420-Asp473 and His475-Lys502 (amino- and carboxyl-terminal domain 5 sequences). The mutants were purified on a glutathione column reaching more than 90% purity (25). Peptide synthesis and high performance liquid chromatography purification to a final concentration of 100 μg/ml was performed by Dr. J. Lambris (University of Boston, MA). Purified Mac-1, LFA-1, and Mac-1 domain 5 (21) while binding of HKα to GPIbα is predominantly mediated by the 268–282 region of GPIbα (6). These diverse observations prompted us to define the role and influence of HK on the Mac-1-GP Ibα interaction. Our results demonstrate that HK enhances the binding between leukocytes and platelets by bridging Mac-1 and GP Ibα and thereby provide new insights into the regulatory role of HK for leukocyte-platelet interactions.

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Enzyme-linked Immunoassay for Ligand-Receptor Interactions—A previously outlined procedure was used (9, 21, 31). Briefly, Maxisorp plates (Greiner, Frickenhausen, Germany) were coated with 1 μg/ml purified glycolicin in PBS overnight at 4°C and then blocked with 5% BSA. Binding of biotin-labeled HK or HKα (10 ng each) to immobilized Mac-1 or glycolicin was tested (same buffers as above but supplemented with 0.05% Tween 20, 0.1% BSA, 10 μg/ml ZnCl2) in the absence or presence of competitors. After incubation for 2 h at 22°C and a washing step, bound biotin-labeled HK or HKα was detected by 1:2000 diluted peroxidase-conjugated streptavidin.

Binding of biotin-glycolicin (0–500 nM) to immobilized Mac-1 was performed in 20 μl HEPES, 150 mM NaCl, 1 mM MnCl2, pH 7.2, with glycolicin (5 μM) in PBS, pH 7.4, containing 1 mM CaCl2, 1 mM MgCl2 overnight at 4°C and then blocked with 3% BSA. Binding of biotin-labeled HK or HKα (10 ng each) to immobilized Mac-1 or glycolicin was tested (same buffers as above but supplemented with 0.05% Tween 20, 0.1% BSA, 10 μg/ml ZnCl2) in the absence or presence of competitors. After incubation for 2 h at 22°C and a washing step, bound biotin-labeled HK or HKα was detected by 1:2000 diluted peroxidase-conjugated streptavidin.

RESULTS

Characterization of the Interactions between HK and Mac-1 or HK and GP Ibα—We have previously demonstrated that binding of HK/HKα to Mac-1 is predominantly mediated by domain 5 (21) while binding of HK/HKα to GP Ibα is mediated by domain 3 of HK (23). In the presence of ZnCl2, HK and HKα bound to both immobilized glycolicin and Mac-1 (1). Binding of HK/HKα to glycolicin was inhibited by HK domain 3 but not by HK domains 5 or 6. Moreover, mAb S22, directed against the 269–282 region within GP Ibα that contains three sulfated tyrosine residues, prevented the binding of HK/HKα to glycolicin, whereas the antibody API recognizing an epitope within the 201–268 region of GP Ibα, previously reported to mediate the binding of GP Ibα to Mac-1 (10) was not effective in this respect (Fig. 1A). In contrast, the interaction between HK/HKα and immobilized Mac-1 was blocked by domain 5 but not domains 3 or 6 of HK. In addition, peptides from the 475–497 region (HK475, HK493) of HK domain 5 inhibited
The peptides derived from domain 5, HK475 and HK483, as well as or presence of domain 3 (\(D_3\)), domain 6 (\(D_6\)) (each 250 nM), mAb AP1 directed to the
A
B
mAb LPM19c against Mac-1 and mAb AP1 (each 200 nM) is shown. B, the binding of biotin-HK (\(D_5\)) or biotin-HKa (\(D_6\)) to immobilized Mac-1 in the absence (\(\text{absorbance at } 405 \text{ nm}\)) or presence of domain 3 (\(D_3\)), domain 5 (\(D_5\)), domain 6 (\(D_6\)) (each 250 nM), the peptides derived from domain 5, HK475 and HK483, as well as or presence of domain 3 (\(D_3\)), domain 6 (\(D_6\)) (each 250 nM), mAb LPM19c (200 nM) is shown. Specific binding is presented as absorbance at 405 nm, and data represent mean ± S.D. (\(n = 3\)) of a typical experiment; similar results were obtained in three separate experiments.

**Effect of HK on the Interaction between Mac-1 and GPIbα—**Because Mac-1 directly interacts with GPIbα and HK binds to both adhesion receptors, we tested the effect of HK on the interaction between both molecules. As previously shown, an epitope within the 201–268 region in GPIbα mediates binding to Mac-1 (10). Indeed, binding of GPIbα to Mac-1 was inhibited by mAb LPM19c against Mac-1 and mAb AP1 directed to the 201–268 region of GPIbα but not by mAb S22 directed to the 269–282 region of GPIbα. In the presence of HK or HKa (data with HKa not shown) binding of glycopcalcin to immobilized Mac-1 was significantly increased about 2-fold. Whereas mAb LPM19c completely abolished the Mac-1-GPIbα interaction both in the absence or presence of HK, mAb AP1 provided a complete inhibition of the Mac-1-GPIbα interaction in the absence of HK but only a 50% inhibition in the presence of HK. In contrast, the effect of HK on the Mac-1-GPIbα interaction was prevented in the presence of mAb S22 (Fig. 2A). The enhancement of the Mac-1-GPIbα interaction by HK was prevented by both domains 3 and 5 but not by domain 6. Moreover, the peptides HK475 and HK483 also reversed the effect of HK on

**HK Enhances the Neutrophil-Platelet Binding via Mac-1-GPIbα Interactions—**Adhesion of myelomonocytic U937 cells to immobilized glycopcalcin is mediated by Mac-1, as it was abolished in the presence of mAb LPM19c (not shown). U937 cell adhesion to glycopcalcin was stimulated about 2-fold in the presence of HK or HKa. The isolated domains 3 and 5 of HK but not domain 6 reversed the pro-adhesive effect of HK, whereas they did not affect adhesion in the absence of HK (Fig. 3).

To corroborate these results further, we investigated the adhesion of U937 cells to non-transfected CHO cells, to CHO cells transfected with GPIbβ/GPIX, or to GPIbβ/GPIX-transfected CHO cells. The expression level of GPIb was comparable in the CHO cells stably expressing GPIbβ/I/X, or to GPIbβ/GPIX-transfected CHO cells. The expression level of GPIb was comparable with the level of GPIb in the co-transfected cells (Fig. 4A). No appreciable adhesion of U937 cells to untransfected CHO cells or CHO cells expressing GPIbβ/I/X was noted both in the absence or presence of HK. In contrast, Mac-1-dependent adhesion to the CHO cells expressing GPIbβ/I/X was detected. Here, cell adhesion was doubled in the presence of HK. Thus, HK enhances Mac-1-dependent adhesion to cells expressing the GPIbβ/I/X complex only through its interaction with GPIbα (Fig. 4B). Moreover, HK and HKa dose dependently stimulated the adhesion of phorbol myristate-acetate-stimulated U937 cells to GPIbα-transfected CHO cells (Fig. 5A). Analogous to the results obtained with purified components, U937 cell adhesion to GPIbα-transfected CHO cells was completely abolished by a blocking mAb against Mac-1 independent of the presence of HK/HKa. Moreover, anti-GPIbα mAb AP1 blocked U937 cell adhesion to GPIbα-transfected CHO cells completely in the absence of HK but only partially (about 50–60% inhibition) in the presence of HK, whereas anti-GPIbα mAb S22 only inhibited the stimulatory effect of HK on U937 cell adhesion. Strikingly, HK-stimulated adhesion of U937 cells to GPIbα-transfected CHO cells was almost completely abolished by combining the inhibitory action of both mAbs, AP1 and S22 (Fig. 5B). The isolated domains 3 and 5 of HK but not domain 6 reversed the pro-adhesive effect of HK, whereas they did not affect cell adhesion in the absence of HK (Fig. 5C).

Similar results were obtained when we investigated the adhesion of Mac-1-transfected K562 (stimulated with the \(\beta_2\)-integrin activating mAb Kim185) to

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**FIG. 1.** Binding of HK to immobilized glycopcalcin (A) and Mac-1 (B). A, the binding of biotin-HK (\(\text{filled bars}\)) or biotin-HKa (\(\text{gray bars}\)) to immobilized glycopcalcin in the absence (\(\text{absorbance at } 405 \text{ nm}\)) or presence of domain 3 (\(D_3\)), domain 5 (\(D_5\)), domain 6 (\(D_6\)) (each 250 nM), mAb AP1 or mAb S22 (each 200 nM) is shown. B, the binding of biotin-HK (\(\text{filled bars}\)) or biotin-HKa (\(\text{gray bars}\)) to immobilized Mac-1 in the absence (\(\text{absorbance at } 405 \text{ nm}\)) or presence of domain 3 (\(D_3\)), domain 5 (\(D_5\)), domain 6 (\(D_6\)) (each 250 nM), the peptides derived from domain 5, HK475 and HK483, as well as or presence of domain 3 (\(D_3\)), domain 6 (\(D_6\)) (each 250 nM), mAb LPM19c (200 nM) is shown. Specific binding is presented as absorbance at 405 nm, and data represent mean ± S.D. (\(n = 3\)) of a typical experiment; similar results were obtained in three separate experiments.

binding of HK/HKa to Mac-1, whereas scrambled peptides from the same region (HK475M, HK483M) or peptides from other regions of domain 5 (HK406, HK440; not shown) had no effect (Fig. 1B). Thus, binding of HK to Mac-1 is mediated by the 475–497 region of domain 5, whereas binding of HK to GPIbα is mediated by domain 3. In GPIbα the 269–282 region participates in the binding to HK, whereas the epitope recognized by the mAb AP1 does not.

To corroborate these results further, we investigated the adhesion of U937 cells to non-transfected CHO cells, to CHO cells transfected with GPIbβ/GPIX, or to GPIbβ/GPIX-transfected CHO cells. The expression level of GPIb was comparable in the CHO cells stably expressing GPIbβ/I/X, or to GPIbβ/GPIX-transfected CHO cells. The expression level of GPIb was comparable with the level of GPIb in the co-transfected cells (Fig. 4A). No appreciable adhesion of U937 cells to untransfected CHO cells or CHO cells expressing GPIbβ/I/X was noted both in the absence or presence of HK. In contrast, Mac-1-dependent adhesion to the CHO cells expressing GPIbβ/I/X was detected. Here, cell adhesion was doubled in the presence of HK. Thus, HK enhances Mac-1-dependent adhesion to cells expressing the GPIbβ/I/X complex only through its interaction with GPIbα (Fig. 4B). Moreover, HK and HKa dose dependently stimulated the adhesion of phorbol myristate-acetate-stimulated U937 cells to GPIbα-transfected CHO cells (Fig. 5A). Analogous to the results obtained with purified components, U937 cell adhesion to GPIbα-transfected CHO cells was completely abolished by a blocking mAb against Mac-1 independent of the presence of HK/HKa. Moreover, anti-GPIbα mAb AP1 blocked U937 cell adhesion to GPIbα-transfected CHO cells completely in the absence of HK but only partially (about 50–60% inhibition) in the presence of HK or HKa, whereas anti-GPIbα mAb S22 only inhibited the stimulatory effect of HK on U937 cell adhesion. Strikingly, HK-stimulated adhesion of U937 cells to GPIbα-transfected CHO cells was almost completely abolished by combining the inhibitory action of both mAbs, AP1 and S22 (Fig. 5B). The isolated domains 3 and 5 of HK but not domain 6 reversed the pro-adhesive effect of HK, whereas they did not affect cell adhesion in the absence of HK (Fig. 5C).
HK and Platelet-Leukocyte Interactions

**DISCUSSION**

Leukocyte binding to platelets enables the recruitment of leukocytes to sites of vascular injury after denudation of the endothelial cell lining. Leukocyte-platelet interactions are largely controlled by the degree and duration of adhesive contacts between both cell types. A sequential adhesion model of leukocyte attachment to and transmigration across surface-adherent platelets has been proposed, including P-selectin-mediated initial rolling (8) and the subsequent Mac-1-dependent firm adhesion and transplatelet migration (32–34). Among others, GPIbα is an important platelet counter-receptor for Mac-1. Through its propensity to interact with both, Mac-1 and GPIbα, HK was shown in the present study to augment leukocyte-platelet interactions by bridging the two adhesion receptors. These data provide new insights into the regulatory role of HK on adhesive events between vascular cells with potential implications in atherothrombotic disease.

The following features are consistent with a specific modulatory role of HK on the Mac-1-GPIb interaction. (i) HK domain 5, and particularly the region 475–497, interacts with Mac-1, whereas HK domain 3 directly binds to GPIbα. The binding site for GPIbα in HK was shown to be within the region 270–292 of HK domain 3 (23). The binding sites for Mac-1 and HK in GPIbα are distinct, as binding GPIbα to Mac-1 and HK is inhibited by mAb AP1 (recognizing an epitope within the region 201–268 of GPIbα) and mAb SZ2 (recognizing the region 269–282 of GPIbα), respectively (10, 23). (ii) Consequently, HK promotes the interaction between isolated Mac-1 and GPIbα in a purified system, the Mac-1-dependent adhesion of myelomonocytic U937 cells to immobilized glycocalcin, and provides an
intercellular bridge between Mac-1 on neutrophils and GPIbα on platelets. These results were corroborated using Mac-1- and GPIbα-transfected cells. Whereas no significant difference in the affinity of the Mac-1-GPIbα interaction was observed in the absence or presence of HK, maximal binding of glycocalcin to immobilized Mac-1 doubles in the presence of HK. Thus, the number of GPIbα binding sites in the Mac-1-GPIbα interaction doubles in the presence of HK. Therefore, we conclude that Mac-1 simultaneously binds to HK and GPIbα, with a bound HK molecule binding another molecule of GPIbα. (iii) The stimulatory effect of HK on the Mac-1-GPIbα interaction was blocked in the presence of isolated domains 3 or 5 that compete for HK binding to GPIbα and Mac-1, respectively. In contrast, neither isolated domains of HK affected the Mac-1-GPIbα interaction in the absence of HK. (iv) Whereas mAb AP1 completely abolished the Mac-1-GPIbα interaction in the absence of HK, it only provided a partial inhibition in the presence of HK. In contrast, mAb SZ2 inhibited the stimulatory effect of HK on the Mac-1-GPIbα interaction, although it did not affect this interaction in the absence of HK. Strikingly, the combination of both antibodies completely abolished Mac-1-GPIbα interaction in the presence of HK. The results with the mAbs strengthen the hypothesis that HK doubles the binding sites of the Mac-1-GPIbα interaction. Thus, in the presence of HK additional binding sites on Mac-1 and GPIbα are engaged, augmenting cell-cell adhesion between leukocytes and platelets. Although it was previously shown that HK also binds to GPIX (23), this interaction is not sufficient to enhance the adhesion between Mac-1-bearing cells and platelets or cells that express GPIX. Thus, HK enhances Mac-1-dependent adhesion to platelets or cells expressing the GPIbα-Ibβ-IX complex only through its interaction with GPIbα. The hypothesis that HK dimers or oligomers may be formed that promote the bridging between the two adhesion receptors needs further experimental proof.

Besides HK, which regulates the Mac-1-GPIbα interaction and thereby leukocyte-platelet interactions, additional Mac-1-dependent and -independent binding sites on platelets have been described previously. Other potential Mac-1 ligands present on the platelet membrane include junctional adhesion molecule-3 (9), fibrinogen (bound to αIIbβ3-integrin) (11), ICAM-2 (35), or glycosaminoglycans (36). Further detailed functional analysis needs to be performed to clarify the contribution of each platelet-associated Mac-1 ligand in leukocyte-platelet interactions. Also Mac-1-independent interactions contribute to leukocyte-platelet aggregate formation including thrombospondin bridging between GPIV receptors on platelets and monocytes (37) or binding of P-selectin on activated platelets to leukocyte P-selectin glycoprotein ligand-1 (38, 39).
similar results were obtained in three separate experiments. The number of adherent cells is expressed as percent of total

Apart from its role as precursor of bradykinin, which serves

properties (14), which is not necessarily contradictory to the

pro-adhesive effect of HK and HKa, described here. In fact, the
data presented here help to explain the anti-adhesive and

anti-inflammatory role of the isolated domain 5 observed in an
acute peritonitis model in vivo (21). Namely, isolated domain 5
may be anti-inflammatory not only by interfering with the

Mac-1-ICAM-1 interaction but also by abrogating the HK-stim-

ulated Mac-1-dependent leukocyte binding to platelets. Fur-

thermore, HK was previously described to regulate platelet
aggregation. Domains 3 and 5, as well as fragments thereof
inhibit thrombin-induced platelet aggregation and interfere
with ligand binding to α5β1-integrin, respectively (24, 40).
However, the present work indicates that the antithrombotic
properties of these domains might also be attributable to their
ability to interfere with Mac-1-GPIb-dependent leukocyte-
platelet interactions.

In the present work the whole molecule, HK or HKa, has
proadhesive functions, whereas isolated domains 3 and 5 are
anti-adhesive. This is in accordance with previous reports com-
paring the actions of the whole HK versus an isolated fragment
or domain. In particular, isolated domain 5 inhibited platelet
aggregation and prolonged bleeding time in vivo, whereas HK
or HKa failed to do so (40). Moreover, HK is proangiogenic as
opposed to the anti-angiogenic actions of the isolated domain
5 (26).

Taken together, HK interacts with platelet GPIbα and leu-
kocyte Mac-1 via its domains 3 and 5, respectively, providing
an intercellular bridge between both adhesion receptors. HK
thereby potentially contributes to the accumulation of leuko-
cytes to surface-adherent platelets at sites of vascular injury
after denudation of the endothelial cell lining, which is relevant
within atherosclerotic and restenotic lesions, or in areas of
ischemia-reperfusion injury. HK may provide a novel molecu-
lar target for reducing inflammatory cell recruitment in ather-
othydromic vascular pathologies.

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