Association of Plasminogen with Dipeptidyl Peptidase IV and Na⁺/H⁺ Exchanger Isoform NHE3 Regulates Invasion of Human 1-LN Prostate Tumor Cells*

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Binding of plasminogen type II (Pg 2) to dipeptidyl peptidase IV (DPP IV) on the surface of the highly invasive 1-LN human prostate tumor cell line induces an intracellular Ca²⁺ ([Ca²⁺]i) signaling cascade accompanied by a rise in intracellular pH (pHi). In endothelial cells, Pg 2 regulates intracellular pH via Na⁺/H⁺ exchange (NHE) antiporters; however, this mechanism has not been demonstrated in any other cell type including prostate cancer cells. Because the Pg 2 receptor DPP IV is associated with NHE3 in kidney cell plasma membranes, we investigated a similar association in 1-LN human prostate cancer cells and a mechanistic explanation for changes in [Ca²⁺]i or pHi induced by Pg 2 in these cells. Our results suggest that the signaling cascade initiated by Pg 2 and its receptor proceeds via activation of phospholipase C, which promotes formation of inositol 3,4,5-trisphosphate, an inducer of Ca²⁺ release from endoplasmic reticulum stores. Furthermore, our results suggest that Pg 2 may regulate pHi via an association with NHE3 linked to DPP IV in these cells. These associations suggest that Pg has the potential to simultaneously regulate calcium signaling pathways and Na⁺/H⁺ exchanges necessary for tumor cell proliferation and invasiveness.

The growth of many tumors, including invasive breast cancer, non-small cell lung cancer, and prostate carcinoma, is associated with changes in the tumor microenvironment leading to hypoxia and decreases in extracellular pH (1–3). Typically, the pH in the extracellular environment is 0.5 pH units or lower than in normal tissues, whereas intracellular pH (pHi) in tumors is either similar or more alkaline than normal tissues (4, 5). A key factor in prostate tumors is the rapid fluctuations in cytosolic free Ca²⁺ concentrations ([Ca²⁺]i) that result from regulated signal transduction events associated with cell proliferation (6). One of these events occurs when plasminogen type II (Pg 2) interacts with dipeptidyl peptidase IV (DPP IV) on the surface of human prostate tumor 1-LN cells (7), producing a rapid increase in [Ca²⁺], associated with expression of metalloproteinase-9, which in an in vitro model enhances the invasiveness of these cells (7). Using this model, we recently demonstrated that a Pg fragment containing kringles 1–3 (angiotatin) is able to inhibit the Ca²⁺ signaling cascade induced by Pg via competition for binding to DPP IV (8).

In renal brush-border membranes, DPP IV exists in multimeric complexes with NHE3, a member of the NHE antiporter family (9, 10). Both appear to follow a common apical secretion pathway (11, 12). In humans there are nine NHE isoforms that are members of a gene family called SLC9A (13). The NHE family can be divided into plasma membrane and intracellular organellar isoforms. The established plasma membrane isoforms include NHE 1–5 (13). The plasma membrane isoforms are further divided into those that cycle to and from the recycling endosomes/plasma membrane including NHE3 (14) and NHE5 (15) and those that permanently reside on the plasma membrane and include NHE1, -2, and -4 (16, 17). The organellar isoforms include NHE6, NHE7, NHE8, and NHE9 (13). NHE1–3 are present in all gastrointestinal organs, and NHE6, although predicted to be ubiquitous, is present only in pancreas and liver. NHE4 together with NHE8 and NHE9 is present only in liver (13). NHE5 is present only in brain (18). NHE2 is also expressed in prostate tissue (19).

We investigated a possible association of DPP IV with NHEs and a mechanistic explanation for changes in [Ca²⁺], or pHi induced by Pg 2 in 1-LN cells. We found that interaction between Pg 2 and its receptor induces activation of phospholipase C (PLC), promoting the formation of inositol 3,4,5-triphosphate (IP₃), which in turn stimulates release of Ca²⁺ from endoplasmic reticulum stores. We also found that Pg 2 may regulate pH, via an association with NHE3 linked to DPP IV in these cells. These associations have the potential to regulate simultaneously calcium signaling pathways and Na⁺/H⁺ exchanges necessary for tumor cell invasiveness.

EXPERIMENTAL PROCEDURES

Materials—Culture media were from Invitrogen. Porcine pancreatic elastase was from Sigma. The 16-amino acid peptide NH₂-VTRFTKH-VRIIEPGFV (Val²⁷⁵–Val²⁹⁰) of NHE3 was purchased from Genemed Synthesis, Inc. (San Francisco, CA). Fura-2/AM and bis(carboxyethyl)carbonyl fluorescein and 1,2-bis (2-amino-5-fluorophenoxylethane-N,N,N',N'-tetraacetic acid tetrak(isoacetyl) ester (BAPTA-AM) were purchased from Molecular Probes, Inc. (Eugene, OR). The compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and the electron-coupling reagent phenazine ethosulfate were purchased from Promega Corp. (Madison, WI) as one solution cell proliferation assay kit, CellTiter 96AQueous 1–[6]-[17-β-3-Methoxyestra-1,3,5(10)-trien-17-yl][aminohexyl]-1H-pyrrole-2,5-dione (U-73122), 1-[6]-[17-β-3-Methoxyestra-1,3,5(10)-trien-17-yl][aminohexyl]-2,5-pyrrolidine-dione (U-73343), and amiloride-HCl were purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA).

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1 The abbreviations used are: Pg, plasminogen; DPP IV, dipeptidyl peptidase IV; NHE, Na⁺/H⁺ exchange antiporter; IP₃, inositol 3,4,5-trisphosphate; PLC, phospholipase C; Val²⁷⁵–Val²⁹⁰; the NHE3 peptide sequence NH₂-VTRFTKH-VRIIEPGFV; HBSS, Hanks’ balanced salt solution; CELISA, cellular enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; BAPTA-AM, 1,2-bis (2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrak(isoacetyl) ester; 6-AHA, 6-aminohexanoic acid.
Proteins—Human Pg was purified from human plasma by affinity chromatography on 1-lgase-Sepharose (20) and separated into its two major classes of glycoforms, Pg 1 and Pg 2, by affinity chromatography on concanavalin A-Sepharose (20). Antibodies—Rabbit IgG against DPP IV was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against the 16-amino acid sequence (Val275–Val290) of NHE3 conjugated to keyhole limpet hemocyanin (15) were prepared in rabbits by Covance (Denver, PA). The IgG fraction specific to NHE3 was purified by immunoaffinity on a resin containing the NHE3 peptide conjugated to carboxyethyl-Sepharose. Affinity-purified anti-NHE1 IgG raised in rabbits against a 22-amino acid domain of human NHE1 (23), affinity-purified anti-NHE2 IgG raised in rabbits against a 20-amino acid domain common to both human and rat NHE2 (24), and affinity-purified anti-NHE3 IgG raised in rabbits against a 22-amino acid domain of human NHE3 (25) were all purchased from Chemicon International Inc. (Temecula, CA).

Cell Cultures—The LL/LN cell line L1-N was grown in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum on glass coverslips and allowed to adhere overnight. Cells were incubated for 4°C in PBS containing 10% bovine serum albumin with anti-DPP IV, anti-NHE1, anti-NHE2 (negative control), or preimmune IgG. Cells were washed and incubated at 4°C with goat anti-rabbit IgG conjugated to Alexa Fluor® 488 dye purchased from Molecular Probes-Invitrogen before washing and fixing in 4% paraformaldehyde. Immunofluorescence microscopy was performed by using an Olympus BX-60 microscope (Olympus, Lake Success, NY).

Immunoprecipitation of DPP IV and NHE1, NHE2, and NHE3 from Plasma Membranes—1-LN cell monolayers from 5 × 150 cm² were gently detached and washed twice with HBSS. After centrifugation at 5,000 rpm for 5 min, the cell pellet was suspended in HBSS buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 μM benzamide, and 10 μM leupeptin. The cell suspension was transferred to chilled homogenizing tubes, and plasma membranes were isolated as described previously (31). Cell plasma membrane lysates were prepared by suspending the membranes in the above buffer containing 0.5% Nonidet P-40 and passing the suspension through a syringe (22-gauge needle) six times followed by centrifugation at 5,000 rpm for 5 min at 4°C to remove particles in suspension. The supernatant was divided in five aliquots (200 μl), and each one was incubated with 50 μl of protein A-Sepharose beads for 1 h at 22°C containing 0.1 M sodium bicarbonate, pH 7.1. The cell suspension was then incubated with 10 μg of IgG anti-DPP IV, anti-NHE3 (Val275–Val290), or specific anti-NHE1, -NHE2, or -NHE3 in the presence of 30 μl of protein A-Sepharose beads overnight at 4°C with shaking. Then the mixtures were centrifuged at 5,000 rpm for 5 min at 4°C. The beads were washed three times in solubilization buffer and then prepared for SDS-PAGE and immunoblotting.

Western Blotting with Cytosolic Membranes—Protein samples were solubilized in SDS sample buffer, and proteins were separated by SDS-PAGE using 7.5% polyacrylamide gels according to Laemmli (32). The dye-conjugated molecular weight markers (Bio-Rad) used were myosin (Mr ~220,000), β-galactosidase (Mr ~116,000), phosphorylase b (Mr ~97,000), bovine serum albumin (Mr ~68,000), and ovalbumin (Mr ~45,000). For immunoblottings, proteins were transferred to nitrocellulose membranes and blocked in 10% nonfat dry milk in PBS-Tween. The membranes were incubated overnight at 4°C with the mixture of nitrocellulose membranes containing transferrin proteins were incubated first in a solution containing 5% nonfat dry milk and 0.1% Tween 80 in PBS for 1 h at 22°C to block nonspecific binding of antibody followed by incubation overnight at 4°C with specific rabbit anti-DPP IV or anti-NHE3 IgGs (1 μg/ml) in PBS containing 0.1% Tween 80 (PBS-Tween). The membranes were then washed three times in PBS-Tween and incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit IgG. Bound antibody was detected by incubating the membranes in PBS containing the horseradish peroxidase substrate 4-chloro-1-naphtol (1 mg/ml) and 0.01% (v/v) H2O2.

Plasminogen Binding to Immobilized NHE3—Solubilized membrane aliquots (100 μl) were incubated with 10 μg anti-NHE3 IgG raised in rabbit (26) in 10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.5% (w/v) Triton X-100, and 10 μM leupeptin. Immediately after, the presence of 30 μl of protein A-Sepharose beads as described above. After separation of the proteins by SDS-PAGE and blocking of nonspecific sites, nitrocellulose membranes containing individual lanes were incubated with human Pg 2 (1 μM) in the absence or presence of 100 mM 6-aminohexanoic acid (6-AHA) in PBS-Tween at 4°C overnight. After extensive rinsing in PBS-Tween, the membranes were incubated with an affinity-purified goat anti-human Pg IgG (1 μg/ml) at 22°C for 3 h. A membrane containing a control lane was reacted with an anti-NHE3 (Val275–Val290) IgG. The membranes were then washed three times in PBS-Tween and incubated for 1 h with horseradish peroxidase-conjugated anti-goat IgG. Bound antibody was detected as described above.

RESULTS

Changes in [Ca2+]i in Response to Pg 2 Binding to the Surface of 1-LN Cells—Pg 2 (0.1 μM) added to 1-LN cells induces a transient rise in [Ca2+]i lasting about 90 s before returning to the base line (Fig. 1). Cells preincubated with the calcium chelator EGTA (1 mM) (Fig. 1) do not significantly influence the [Ca2+]i, induced by Pg 2 (0.1 μM); however, the rise in [Ca2+]i, was almost completely abolished when cells were preincubated for 20 min with the intracellular calcium chelator BAPTA-AM (10 μM), which permeates the cell (34), prior to the addition of Pg 2 (0.1 μM) (Fig. 1A). These experiments suggest that Pg 2 induces calcium release from intracellular calcium stores, which are activated by signaling pathways initiated by cell-
we hypothesized that their effects on pH$_i$ may be mediated by the same NHE receptor. Preincubation of 1-LN cells with amiloride (100 nM) prior to addition of Pg 2 (0.1 μM) inhibits the rise in pH$_i$ (Fig. 2), suggesting that Pg 2 may regulate pH$_i$ in 1-LN cells via mechanisms involving NHE receptors. The association between DPP IV and NHE3 exists in kidneys (11–12); however, a similar association has not been reported in other cell types. Our goal then was to determine whether NHE3 in association with DPP IV also serves as an additional receptor for Pg 2 on the 1-LN cell surface.

Synthesis of an Antibody against a Cell-surface Epitope on NHE3—A hidden Markov model was used to search for the transmembrane topology corresponding to NHE3 (37, 38). The membrane topology model predicts three outside loops in NHE3 (39), including the amino acid segments Asn$^{133}$–Thr$^{142}$, Thr$^{275}$–Phe$^{281}$, and Asp$^{277}$–Leu$^{281}$. The sequence including Val$^{275}$–Val$^{280}$ (Fig. 3B) in the second outside loop of NHE3 contains one L-lysine residue (Lys$^{280}$) flanked by two hydrophilic amino acids (Thr$^{277}$ and His$^{281}$) suggested a candidate binding site for Pg 2. A comparison of this primary amino acid sequence in NHE3 with similar sequences in other members of the NHE family (Fig. 3A), shows segments in NHE1, -2, and -5 with primary structural homologies greater than 60%, whereas NHE4 and NHE6 show only 50 and 35% homologies, respectively.

A topology model comparing the second outside loop of NHE3 with homologous structures in NHE1, -2, -4, and -5 (Fig. 3B), predicts outside loops only in NHE2 and -5. However, only the outside loop in NHE5 contains a candidate binding site for Pg 2, L-Lys$^{280}$. These comparisons suggest NHE3 as a unique receptor for Pg 2 in prostate cells because NHE5 is expressed solely in the brain (18).

Identification of NHE3 on 1-LN Cell Surface by CELISA and Immunofluorescence Microscopy—The NHE3 peptide Val$^{275}$–Val$^{280}$ was synthesized and antibodies were prepared and purified as described under “Experimental Procedures.” The anti-NHE3 IgG was then used as a reagent to assess the presence of NHE3 on the surface of 1-LN cells by CELISA and its possible association with DPP IV using immunoprecipitation techniques. Confluent cultures of 1-LN cells in 96-well culture plates were titrated with anti-NHE3 IgG and a control preimmune rabbit IgG (Fig. 4). The anti-NHE3 IgG binds to these cells in a dose-dependent manner, whereas very little specific binding was observed with the nonimmune IgG, suggesting the presence of NHE3 in the 1-LN cell membrane. This experiment was validated by immunofluorescence microscopy studies in non-permeabilized 1-LN cells (Fig. 5). The cells were incubated first with anti-DPP IV (Fig. 5A), anti-NHE3 (Va$^{275}$–Va$^{280}$) (Fig. 5B), anti-actin (negative control) (Fig. 5C), and anti-Ca$^{2+}$ (negative control) (Fig. 5D) followed by a secondary goat anti-rabbit IgG conjugated to Alexa Fluor® 488 dye, showing surface expression of both DPP IV and NHE3 on 1-LN cells.

Specificity of the Association between NHE3 and DPP IV on 1-LN Cell Surface—To examine the presence of an NHE3-DPP IV complex we performed immunoprecipitation of solubilized
plasma membranes from 1-LN cells with anti-NHE3 IgG and analyzed the precipitate for the presence of DPP IV by Western blotting and reaction with an anti-DPP IV IgG. The co-precipitation of a protein with a molecular mass of 120 kDa corresponding to a monomer of NHE3 (Fig. 6, lane 2), again demonstrating an association between DPP IV and NHE3.

To assess the specificity of the anti-NHE3 (Val\textsuperscript{275–290}) IgG we performed immunoprecipitations of solubilized 1-LN cell plasma membranes with monospecific anti-NHE1, -2, or -3 and reacted the proteins immunoprecipitated with anti-DPP IV or anti-NHE3 (Val\textsuperscript{275–290}) IgGs. The results (Fig. 7, lanes 1–3) show co-precipitation of DPP IV with each single NHE1, -2, or -3. Similarly, each single precipitated NHE1, -2, or -3 appears to cross-react with the anti-NHE3 (Val\textsuperscript{275–290}) IgG (Fig. 7, lanes 4 – 6) as suggested by the analyses shown in Fig. 3A. These results suggest that DPP IV is associated not only with NHE3 but also with NHE1 and -2 on the surface of 1-LN cells.

Association between Pg 2 and NHEs on 1-LN Cell Surface—We examined the effect of anti-NHE3 IgG on the pH\textsubscript{i} changes induced by Pg 2 on 1-LN cells. Incubation of cells with anti-NHE3 IgG (10 μg/ml) in serum-free RPMI 1640 followed by addition of Pg 2 (0.1 μM) shows almost no changes in pH\textsubscript{i} (Fig. 8), suggesting that the antibody blocks Pg 2 access to NHE3 on the cell surface. To assess the interaction of Pg 2 with NHEs we monitored binding of Pg 2 directly to NHEs precipitated with anti-NHE3 (Val\textsuperscript{275–290}) IgG separated by electrophoresis and electroblotted to nitrocellulose membranes. We show that NHE3 (Fig. 9, lane 1) reacts efficiently with Pg 2 (Fig. 9, lane 2), whereas a membrane incubated with Pg 2 in the presence of 6-AHA (100 μM) shows no specific binding, thereby suggesting an L-lysine-dependent binding of Pg 2 to NHE3. This interaction was confirmed when we monitored the binding of Pg 2 directly to the Val\textsuperscript{275–290} peptide conjugated to carboxyhexyl-Sepharose. When Pg 2 (2.5 mg) in PBS was filtered through this resin, almost 90% of the Pg 2 is bound (Fig. 10), thereby confirming that Pg 2 may bind to NHE3.

Effect of Anti-NHE3 IgG on the Pg 2-induced Stimulation of 1-LN Cell Invasiveness—We determined previously that Pg 2 enhances the ability of 1-LN cells to penetrate the synthetic basement membrane Matrigel® (7). To determine whether anti-NHE3 IgG influences Pg-induced cell invasiveness, we incubated cells with increasing concentrations of anti-NHE3 IgG and a single concentration of a control nonimmune rabbit IgG in serum-free RPMI 1640 followed by activation of PLC, which forms IP\textsubscript{3}, a direct inducer of 

\[ \text{Ca}^{2+} \text{ release from endoplasmic reticulum stores.} \]

A similar signaling pathway has been demonstrated for the epidermal growth factor receptor in DU-145 prostate tumor cells (43). Cells transfected with a dominant-negative fragment of PLC-γ, reaction of the precipitate with anti-NHE3 IgG shows proteins of molecular mass of 84 kDa corresponding to a monomer of NHE3 (Fig. 6, lane 2), again demonstrating an association between DPP IV and NHE3.

DISCUSSION

In a previous report (7), we demonstrated that Pg 2 in association with DPP IV is pivotal in the invasive capacity of 1-LN prostate cancer cells. This association induces a Ca\textsuperscript{2+} signaling cascade, which regulates the expression of matrix metalloproteinase-9, essential for invasiveness of these cells in an in vitro model (7). Pg 2 binds to DPP IV via its carbohydrate chains (7, 40), and this interaction is easily inhibited by angiostatin, which competes with Pg for the DPP IV binding site on 1-LN cells (8). On endothelial cells, angiostatin induces a decrease in pH\textsubscript{i} (36), whereas Pg 2 induces a sustained increase in pH\textsubscript{i} (42). Conversely, angiostatin does not induce any changes on 1-LN cells (7, 8). In the present study, we investigated the mechanisms involved in the changes of Ca\textsuperscript{2+} concentration and pH\textsubscript{i} induced by Pg 2 on 1-LN cells.

Our results suggest that Pg 2 induces a rise in [Ca\textsuperscript{2+}]\textsubscript{i}, as a result of activation of PLC, which forms IP\textsubscript{3}, a direct inducer of Ca\textsuperscript{2+} release from endoplasmic reticulum stores. A similar signaling pathway has been demonstrated for the epidermal growth factor receptor in DU-145 prostate tumor cells (43). Cells transfected with a dominant-negative fragment of PLC-γ,
which induced a decrease in IP3 generation, were significantly less invasive than control cells (43).

Intracellular pH plays a central role in the regulation of many aspects of cell physiology, and protons may function as a second messenger in a manner similar to that of Ca2+ (44). pH is also one of the factors thought to control the rate of cell proliferation and transformation (45). In many tumor cell lines pH is more alkaline than in normal cells (46). Because cell alkalization precedes cell proliferation, a correlation between pH and cell cycle has been proposed (46). Intracellular alka-

![Fig. 5. Immunofluorescence microscopy of DPP IV and NHE3 on 1-LN cell surface. Non-permeabilized cells were incubated with specific rabbit polyclonal IgGs as described under “Experimental Procedures.” A, 1-LN cells showing immunofluorescence surface staining for DPP IV. B, 1-LN cells showing immunofluorescence staining for NHE3. C, 1-LN cells stained with an anti-actin IgG (negative control). D, 1-LN cells stained with preimmune rabbit serum.](image)

![Fig. 6. Blot binding assay of solubilized 1-LN membrane proteins immunoprecipitated with specific anti-DPP IV or anti-NHE3 IgGs. Immunoprecipitation of solubilized proteins, separation by SDS-PAGE (reducing conditions), and transference to nitrocellulose membranes were performed as described under “Experimental Procedures.” Lane 1, reaction of proteins immunoprecipitated by an anti-NHE3 IgG with an anti-DPP IV IgG. Lane 2, reaction of proteins immunoprecipitated by an anti-DPP IV IgG with an anti-NHE3 IgG.](image)

![Fig. 7. Blot binding assay of solubilized 1-LN membrane proteins immunoprecipitated with specific anti-NHE1, -NHE2, or -NHE3 IgGs. Immunoprecipitation, SDS-PAGE, and transference to nitrocellulose membranes were performed as described under “Experimental Procedures.” Lanes 1–3, reaction of proteins precipitated by anti-NHE1–3 IgGs with an anti-DPP IV IgG, respectively. Lanes 4–6, reaction of proteins precipitated by anti-NHE 1–3 IgG with an anti-NHE3 (Val275–Val279) IgG.](image)

![Fig. 8. Changes in pH induced by Pg 2 in 1-LN cells exposed to an anti-NHE3 IgG. Cells preloaded with Fura-2/AM were exposed to anti-NHE3 (peptide Val275–Val279) IgG for 20 min prior to addition of Pg 2 (0.1 μM). The arrow indicates the time of addition of Pg 2. ●, effect of Pg 2 on pH; ○, effect of Pg 2 on pH, in cells preincubated with anti-NHE3 IgG (10 μg/ml).](image)

![Fig. 9. Pg 2 binding to immobilized NHE3. Immunoprecipitation, SDS-PAGE, transference to nitrocellulose membranes, and detection of bound proteins were performed as described under “Experimental Procedures.” Lanes 1, reaction of proteins precipitated by an affinity-purified IgG against a 22-amino acid C-terminal domain of human NHE3 by an anti-NHE3 (Val275–Val279) IgG (control). Lane 2, reaction of proteins precipitated by a specific anti-NHE3 IgG with Pg 2 (1 μM). Lane 3, reaction of proteins precipitated by a specific anti-NHE3 IgG with Pg 2 (1 μM) in the presence of 100 mM 6-AHA.](image)
linization is an early event in malignant transformation (47), and the acid extrusion via NHE1 plays a key role in this process (48). Our present data are consistent with these observations. Pg 2 binding to 1-LN cells induces a rise in pH and promotes cell invasiveness. Hypothetically, Pg 2 binds to DPP IV via its carbohydrate chain and to NHE3 via its Lysy-binding sites. This ability gives Pg 2 the capacity to act simultaneously on mechanisms involving changes in [Ca\(^{2+}\)]\(_{i}\) or pH, as suggested by inhibition of the Pg 2-induced changes in [Ca\(^{2+}\)], by inhibitors of PLC or changes in pH by an anti-NHE3 IgG against the epitope including the amino acid sequence V\(^{277}\)TRFTKHKVRIIEPGFV\(^{290}\). This epitope is unique because it targets to limit tumor progression.

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### TABLE I

| Ligand relative invasion | Number of cells/field |
|--------------------------|-----------------------|
| Pg 2                     | 5.5 ± 2.7             |
| Pg 2 + anti-NHE3 IgG (2 μg/ml) | 47.3 ± 3.8          |
| Pg 2 + anti-NHE3 IgG (4 μg/ml) | 31.6 ± 5.1          |
| Pg 2 + anti-NHE3 IgG (10 μg/ml) | 12.4 ± 2.3          |
| Pg 2 + nonimmune IgG (10 μg/ml) | 68.3 ± 5.7          |

### FIG. 10

**Binding of Pg2 to the NHE3 peptide Val\(^{277}\)–Val\(^{279}\)**

The NHE3 peptide Val\(^{277}\)–Val\(^{279}\) was covalently attached to Sepharose 4B as described under “Experimental Procedures.” Pg 2 (2.5 mg) in PBS was adsorbed to the resin and then eluted with 100 mM 6-AHA.
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