Platelet Factor 4 Oligomeric State Correlates With Antiviral Activity

Zahra F. Parker, Ann H. Rux, Ph.D., Amber M. Riblett, Fang-Hua Lee, Ph.D.,
Mortimer Poncz, M.D., Bruce Sachais, M.D., Ph. D., Robert W. Doms, M.D., Ph.D.
Department of Microbiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104
Division of Hematology, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104

Introduction

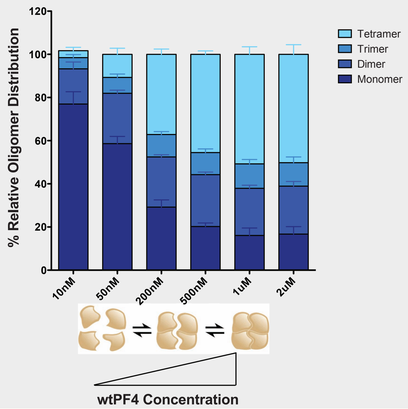
The platelet-derived chemokine platelet factor 4 (PF4) is a cationic chemokine released by alpha granules in activated platelets during platelet aggregation. PF4 promotes blood coagulation by moderating the effects of heparin-like molecules and also serves as a chemoattractant for monocytes and neutrophils.

Recently PF4 has been shown to inhibit HIV-1 infection *in vitro* at concentrations less than 0.5μM. However, despite plasma and local tissue concentrations of PF4 ranging from 0.25nM to 10μM, HIV is able to successfully replicate and escape the inhibitory effects of PF4 *in vivo*. The mechanism of this discordance remains unclear.

Using wildtype and mutant PF4s, we demonstrate that the inhibitory effects of PF4 are limited to a narrow concentration range, corresponding to the predominance of a monomeric state. At concentrations of greater than 1μM, PF4 multimerizes and viral infection *in vitro* is enhanced rather than inhibited.

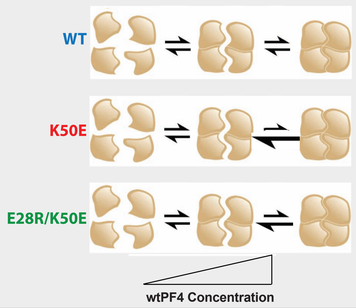
PF4 exists in dynamic equilibrium in solution

PF4 exists in solution in a dynamic state where at low concentrations, it is largely monomeric while at high concentrations it forms tetramers. We confirmed this equilibrium using the synthetic PF4 used in our studies.



Mutant PF4

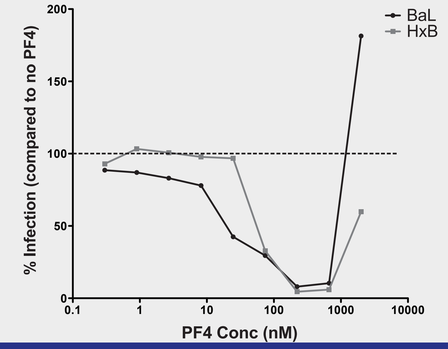
The oligomeric state of PF4 can be modulated independent of concentration through the use of specific mutants.



PF4 exhibits dual activity on HIV-1 entry

- MAGI-R5 cells were pretreated with increasing concentrations of PF4 for 1 hour, and subsequently infected with CCR5 (R5)- and CXCR4 (X4)-using HIV-1 (BaL and HxB, respectively) pseudoviruses.

- PF4 inhibited infection of R5- and X4-using HIV-1 at concentrations below 666nM, with the nadir at approximately 250nM. However, at concentrations above 666nM PF4 significantly enhanced viral infection of HIV-1 BaL and inhibited HxB less efficiently. Additionally, PF4 inhibited and enhanced infection of 24 primary HIV-1 Envs comprising subtypes B and C over a similar concentration range.



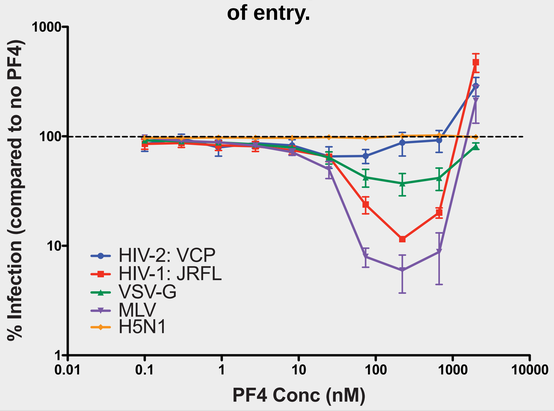
Representative graph

PF4 activity is not restricted to HIV-1

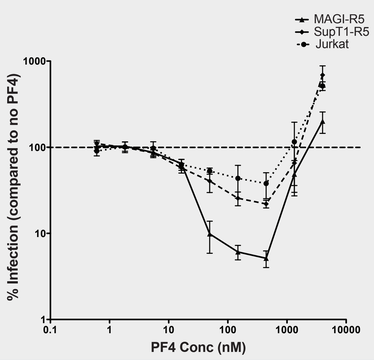
- To assess the breadth of PF4 activity against other viruses, MAGI-R5 cells were pretreated with PF4 and infected with HIV-1 pseudoviruses comprising a common HIV-1 core (NL4.3) and studded with the viral glycoproteins of different viruses.

- HIV-1 and MLV pseudoviruses were robustly inhibited and then enhanced by PF4 as concentrations increased. VSV-G and HIV-2 were affected to a lesser degree, and influenza (H5N1) pseudotype infection was unaffected by PF4.

These data suggest envelope specificity, and are consistent with PF4 impacting infection at the level of entry.



PF4 activity is not cell-type specific

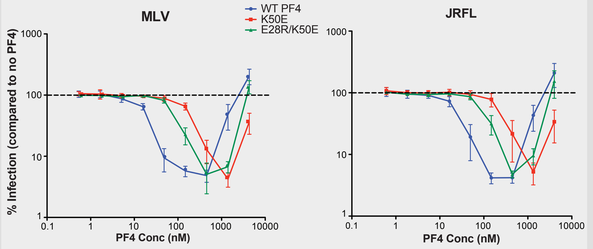


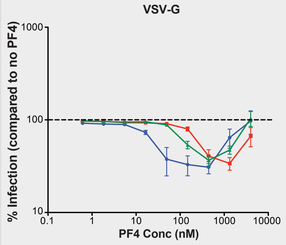
Immortalized cell lines were infected with HIV pseudotypes (HIV_{MLV} shown) in the presence of increasing concentrations of PF4.

The dual activity of PF4 was observed in all cell types.

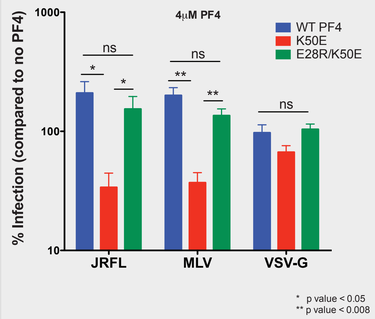
Complex-formation is associated with PF4 dual action on viral entry

Tetrameric PF4 has been shown to interact with cell surface glycosaminoglycans (GAGs), forming large complexes. PF4 mutants were used to explore the effect of complex-formation on PF4 activity.





At 1μM WT PF4, viral infection was enhanced. The K50E mutant, which exists mainly as monomers and dimers, inhibited infection at 1μM, while the complementary mutant (E28R/K50E) exhibited an intermediate IC_{50}.



* p value < 0.05
** p value < 0.008

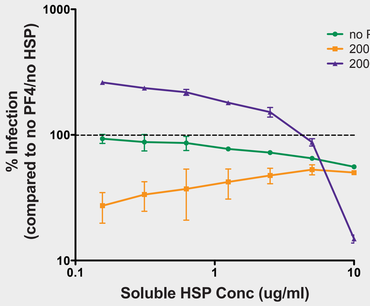
The bar graph depicts the activity of the three forms of PF4 on viral infection at the highest concentration tested (4μM).

These results suggest that PF4 oligomeric state (and ability to form large complexes) may be critical for its anti- and pro-viral effects.

Soluble GAGs neutralize PF4 activity

To address the role of cell surface GAGs on PF4 activity, competition assays using soluble GAGs were performed.

MAGI-R5 cells were infected with HIV pseudotypes (HIV_{JRFL} shown) in the presence of increasing concentrations of soluble heparan sulfate proteoglycan and no, low (200nM), or high (2000nM) PF4.



As soluble heparan sulfate concentrations were increased, the ability of the low concentration of PF4 to inhibit infection were attenuated, and the ability of the high concentration of PF4 to enhance infection were ablated.

Future Directions

- To evaluate whether PF4 interacts with the viral glycoprotein, co-immunoprecipitation will be performed using soluble HIV-1 gp120.

- Virion capture assay will be performed to assess the interaction of PF4 with gp120 in the context of the virus.

- To further explore the role of cell surface GAGs, cells will be treated with heparitinase (to cleave GAGs), and infected with virus in the presence of PF4.

Conclusions/Implications

- PF4 inhibits diverse HIV-1 strains at low concentrations, when it exists mainly in a monomeric state, and enhances infection when it exists predominantly as a tetramer at high concentrations .

- The dual action of PF4 on viral infection involves binding of PF4 to cell surface GAGs.

- Since PF4 exists in a dynamic equilibrium, with tetramers predominating at physiologic levels, we conclude that the *in vitro* inhibitory effects of PF4 are less likely to predominate at physiologic PF4 concentrations.

Acknowledgements

Research funded by:
-NIH RO1 AI45378
-ZFP is supported by the National Science Foundation Graduate Research Fellowship under grant number 2012140562

We would like to thank Lubica Rauova and Douglas Cines for insightful discussion and reagents.