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HIV-1 Nef Protein Visits B-Cells via Macrophage Nanotubes: A Mechanism for AIDS-Related Lymphoma Pathogenesis?

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Abstract

This letter refers to the recent demonstration that HIV-1 infected macrophages form specialized conduits that connect to B-cells (1). The conduit selectively transports the HIV-1 nef protein, providing nef with numerous means to interfere with cellular processes. Currently, no consideration of the connection between the conduit and the development of AIDS-related lymphoma (ARL) has been offered. ARL is one of the primary causes of death in the HIV-infected population and is related to B-cell proliferation and activation. In this letter we discuss several studies that link HIV-infected macrophages and specific forms of the nef protein to the development of ARL. The conduits discovered by Xu *et al.* [1] may lead to a better understanding of how HIV infection results in lymphomagenesis.

Keywords

HIV-1; AIDS-related lymphoma; macrophages; HIV-1 nef protein

A recent study by Xu *et al.* presented a series of exciting live-cell confocal images of HIV-1 infected macrophages reaching out to make contact with B-cells [1]. The group defined a major purpose for these macrophage protrusions: the creation of a conduit that selectively transfers HIV-1 nef protein into B-cells. These conduits then exclusively carry nef from macrophages to B-cells in order to evade protective virus-specific immunoglobulin responses (IgG2 and IgA) at the mucosal sites of entry. The transfer of nef to B-cells also decreases CD40-dependent activation, interferes with germinal center factors such as Bcl-6, AID, IRF4 and increases differentiation factors and receptors Blimp-1 and CD138 [1–3]. Swingler *et al.* also described an intricate relationship between nef, macrophages and B-cells, indicating that the HIV-1 nef protein carries a pathogenic determinant that governs B-cell defects in HIV-1 infection [4].

The publication and follow-up commentaries to Xu *et al.*'s work, however, failed to address the important relationship of macrophage-mediated B-cell activation to the development of AIDS-related lymphoma (ARL) [1–3]. Lymphoma is the second most common cause of

death among HIV-infected individuals (60 times more likely to occur in the HIV-infected population than in the uninfected population, highly metastatic, and always fatal). Although HIV-1 does not productively infect B-cells, they are influenced by the presence of HIV-infected macrophages to overproduce stimulatory cytokines that may result in B-cell activation *via* cytidine deaminase-associated DNA modification errors and oncogenic translocations; mechanisms associated to ARL [5]. HIV-infected individuals are frequently co-infected with the Epstein Barr virus (EBV), a known contributor to tumorigenesis through B-cell immortalization and proliferation. However we recently reported that 23% of ARL tumor biopsies were HIV+/EBV-, suggesting that a novel class of ARL tumors exists that is linked to HIV infection alone [5].

Monocyte-derived macrophages are the primary differentiated cell in the mononuclear phagocyte system. They are derived from bone marrow, distributed throughout the body and display great structural and functional heterogeneity [6]. Macrophages are the first immune cells to recognize pathogens and signal other immune cells, primarily T-cells, to mount an immune response. They contribute to many disease processes and usually increase in number during wound healing, inflammation, or malignancy. Consequently, macrophages have great potential to modulate the immune response, which can be either positive or can mediate tissue destruction. In a recent review, Herbein and Varin [7] described three different macrophage activation states: M1 is the IFN- γ classically activated macrophage that displays a pro-inflammatory response, M2 is the activation of macrophages by IL-4 and IL-13 that display an anti-inflammatory response and dM represent macrophages deactivated by IL-10, which leads to immune suppression. Polarization of macrophages into classically activated M1 and alternatively activated M2 macrophages is critical in mediating an effective immune response against invading pathogens. However, during HIV infection, the virus uses these pathways to facilitate viral dissemination and pathogenesis [8]. M1 macrophages that are recruited to sites of infection typically have a short half-life and may cause tissue damage. M2 macrophages produce immunosuppressive cytokines that may promote tumor growth and progression [8–10]. dM macrophages strongly express CD206, CD36 and TGF- β and do not produce inflammatory cytokines [11]. dM macrophages are typically observed only at very late stages of HIV disease [12].

HIV-1 nef is a multi-factorial protein that is required to maintain high viral loads [13]. It is the first HIV protein to accumulate following infection of macrophages, at which time it is estimated to represent three-quarters of the viral load [14]. Additionally, nef promotes the survival of infected cells [15] and migration [16, 17]. In the cell, nef can bind MHC class II receptors, allowing it to interfere with a variety of cellular processes such as triggering rapid CD4 endocytosis, targeting the trans-golgi network (TGN) and activating phosphatidylinositol 3-kinases (PI3K). The MHC class II molecules may also bind nef at the plasma membrane resulting in lysosomal degradation [18, 19].

Nef consists of two primary domains: 1) a myristoylation domain that allows it to bind to lipid membranes and 2) a transmembrane domain including a conserved core that is cleaved from the protein after attachment and inserted into cellular membranes. The two domains have been crystallized independently (PDB# 1EFN, 1QA4, 2NEF). In one of these structures (1EFN), nef is shown binding the SH3 domain of a Fyn tyrosine kinase, thus demonstrating one of the important interactions of the protein [20]. Bentham *et al.* stated that the myristoylation of nef alone is insufficient for lipid binding and suggests that it is an interaction of basic residues at the N-terminus, including electrostatic interactions that allow nef to migrate and bind to target membranes [21]. Giese *et al.* agreed and elaborated further that the association of nef to lipid membranes is governed by a complex pattern of amino acid motifs that contribute differentially to individual nef activities [22]. The association of nef with cellular membranes is believed to be essential for virtually all of its effects in

infected cells [22]; however, a significant amount of nef is cytosolic, unbound to any membrane [21, 23–26]. Fig. (1) summarizes some of the known interactions of nef within a M2 activated macrophage.

The population dynamics of HIV-1 within ARL tumors suggest that different tumor sites within the same individual contain closely related HIV genetic populations. The HIV population within ARL tumors expands at a 100× faster rate than at non-tumor locales [27]. As Salemi *et al.* stated, “The different population dynamics between the viruses found in tumors versus the non-tumor associated viruses suggest that there is a significant relationship between HIV evolution and lymphoma pathogenesis.” Furthermore, at the recent 2010 International AIDS Conference in Austria, we described the finding of specific signatures in nef genomes found in ARL-tissues when we compared ARL viruses to viruses derived from brain tissues of patients with dementia [28]. Dementia is an inflammatory disease where macrophages are classically M1 activated. The ARL signatures mapped to regions of the nef structure involved with SH3 binding, the internal core, and the flexible loop domain. Interestingly, this latter amino acid signature exists in a nef domain analyzed in [1] and was considered by Xu *et al.* to be an important motif for nanotube formation. In another study [29], we used a neural network (details of the approach are described in [30]) to determine if positional hydrophobicity in the nef protein could separate sequences derived from lymphoma tissues from sequences derived from non-lymphoma within ARL patients. The result was a 97% correct identification of HIV sequences derived from ARL tumors. When taken together, these findings support a link between the HIV-1 nef protein and ARL development; however, it remained unclear exactly how nef alone could promote B-cell activation.

The determination that nef is responsible for nanotube formation and transfer of nef proteins from macrophages to B-cells further defines the growing list of ways that nef manipulates host-cell machinery during HIV progression. Since nef proteins can remain liberated, this may explain why it can easily move through the macrophage conduits described by Xu *et al.* [1]. As the number of known cellular interactions associated with nef increases, it is likely that both the myristolic and cytosolic forms of nef will be found to contribute to the biological activity of the protein and will be linked to HIV disease pathogenesis. Due to the notoriously high mutation rate of HIV, further studies should address how specific nef variants, especially those associated with disease pathogenesis, might impact nanotube formation or if an abundance of such structures are found in tumor tissues. Additionally, if nef can move through nanotubes into B-cells, can HIV small RNAs move through as well? A recent study that used a deep sequencing approach identified over 100 non-coding HIV small RNAs, including two previously published HIV microRNAs [31]. One of these microRNAs, HIV1-miR-N367, is found within the nef gene. It will be interesting to further understand the impact of the newly discovered macrophage nanotube connection to B-cells and the associated mechanisms that might allow this structure to contribute to ARL pathogenesis.

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REFERENCES

1. Xu W, Santini PA, Sullivan JS, et al. HIV-1 evades virus-specific IgG2 and IgA responses by targeting systemic and intestinal B cells *via* long-range intercellular conduits. *Nat Immunol.* 2009; 10(9):1008–1017. [PubMed: 19648924]

2. Moir S, Fauci AS. Nef, macrophages and B cells: a highway for evasion. *Immunol Cell Biol.* 2010; 88(1):1–2. [PubMed: 19859083]
3. Rudnicka D, Schwartz O. Intrusive HIV-1-infected cells. *Nat Immunol.* 2009; 10(9):933–934. [PubMed: 19692990]
4. Swingler S, Zhou J, Swingler C, et al. Evidence for a pathogenic determinant in HIV-1 Nef involved in B cell dysfunction in HIV/AIDS. *Cell Host Microbe.* 2008; 4(1):63–76. [PubMed: 18621011]
5. Huysentruyt LC, McGrath MS. The role of macrophages in the development and progression of AIDS-related non-Hodgkin lymphoma. *J Leukoc Biol.* 2009; 87(4):627–632. [PubMed: 20042471]
6. McGrath MS. T-cells and macrophages in HIV disease. *Clin Rev Allergy Immunol.* 1997; 14(4): 359–366. [PubMed: 9040966]
7. Herbein G, Varin A. The macrophage in HIV-1 infection: from activation to deactivation. *Retrovirology.* 2010 Apr 9:7:33. [PubMed: 20380696]
8. Cassol E, Cassetta L, Alfano M, Poli G. Macrophage polarization and HIV-1 infection. *J Leukocyte Biol.* 2010; 87(4):599–608. [PubMed: 20042468]
9. Mantovani A, Sica A, Allavena P, Garlanda C, Locati M. Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. *Hum Immunol.* 2009; 70(5):325–330. [PubMed: 19236898]
10. Sica A, Larghi P, Mancino A, et al. Macrophage polarization in tumour progression. *Semin Cancer Biol.* 2008; 18(5):349–355. [PubMed: 18467122]
11. Umemura N, Saio M, Suwa T, et al. Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. *J Leukoc Biol.* 2008; 83(5):1136–1144. [PubMed: 18285406]
12. Herbein G, Varin A. The macrophage in HIV-1 infection: from activation to deactivation? *Retrovirology.* 2010; 7:33. [PubMed: 20380696]
13. Carl S, Greenough TC, Krumbiegel M, et al. Modulation of different human immunodeficiency virus type 1 Nef functions during progression to AIDS. *J Virol.* 2001; 75(8):3657–3665. [PubMed: 11264355]
14. Klotman ME, Kim S, Buchbinder A, DeRossi A, Baltimore D, Wong-Staal F. Kinetics of expression of multiply spliced RNA in early human immunodeficiency virus type 1 infection of lymphocytes and monocytes. *Proc Natl Acad Sci USA.* 1991; 88(11):5011–5015. [PubMed: 1711215]
15. Choi HJ, Smithgall TE. HIV-1 Nef promotes survival of TF-1 macrophages by inducing Bcl-XL expression in an extracellular signal-regulated kinase-dependent manner. *J Biol Chem.* 2004; 279(49):51688–51696. [PubMed: 15459189]
16. Lehmann MH, Walter S, Ylisastigui L, et al. Extracellular HIV-1 Nef increases migration of monocytes. *Exp Cell Res.* 2006; 312(18):3659–3668. [PubMed: 16978607]
17. Swingler S, Mann A, Jacque J, et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nat Med.* 1999; 5(9):985–986. [PubMed: 10470067]
18. Peterlin BM, Trono D. Hide, shield and strike back: how HIV-infected cells avoid immune eradication. *Nat Rev Immunol.* 2003; 3(2):97–107. [PubMed: 12563294]
19. Chaudhry A, Verghese DA, Das SR, et al. HIV-1 Nef promotes endocytosis of cell surface MHC class II molecules *via* a constitutive pathway. *J Immunol.* 2009; 183(4):2415–2424. [PubMed: 19620308]
20. Lee CH, Saksela K, Mirza UA, Chait BT, Kuriyan J. Crystal structure of the conserved core of HIV-1 Nef complexed with a Src family SH3 domain. *Cell.* 1996; 85:931–942. [PubMed: 8681387]
21. Bentham M, Mazaleyrat S, Harris M. Role of myristoylation and N-terminal basic residues in membrane association of the human immunodeficiency virus type 1 Nef protein. *J Gen Virol.* 2006; 87(3):563–571. [PubMed: 16476977]
22. Giese SI, Woerz I, Homann S, Tibroni N, Geyer M, Fackler OT. Specific and distinct determinants mediate membrane binding and lipid raft incorporation of HIV-1(SF2) Nef. *Virology.* 2006; 355(2):175–191. [PubMed: 16916529]
23. Coates K, Boulton V, Cooke S, Mann D, Harris M. Analysis of the interactions between Nef and cellular proteins. *Res Virol.* 1997; 148(1):68–70. [PubMed: 9017838]

24. Fackler OT, Kienzle N, Kremmer E, et al. Association of human immunodeficiency virus Nef protein with actin is myristoylation dependent and influences its subcellular localization. *Eur J Biochem.* 1997; 247(3):843–851. [PubMed: 9288906]
25. Kaminchik J, Margalit R, Yaish S, et al. Cellular distribution of HIV type 1 Nef protein: identification of domains in Nef required for association with membrane and detergent-insoluble cellular matrix. *AIDS Res Hum Retroviruses.* 1994; 10(8):1003–1010. [PubMed: 7811531]
26. Niederman TM, Hastings WR, Ratner L. Myristoylation-enhanced binding of the HIV-1 Nef protein to T cell skeletal matrix. *Virology.* 1993; 197(1):420–425. [PubMed: 8212577]
27. Salemi M, Lamers SL, Huysentruyt LC, et al. Distinct patterns of HIV-1 evolution within metastatic tissues in patients with non-Hodgkins lymphoma. *PLoS One.* 2009; 4(12):e8153. [PubMed: 19997510]
28. Lamers, S.; Salemi, M.; Fogel, G.; McGrath, M. HIV dynamics, signatures and structures associated with the development of AIDS-related lymphoma; International AIDS Conference; July 18–23, 2010; Vienna, Austria. 2010. (Abstract # 16160).
29. Lamers, S.; Fogel, G.; Huysentruyt, L.; Poon, A.; McGrath, M. The HIV nef protein within ARL is genetically and structurally distinct from those in the brain of patients with HAD; 12th International Conference on Malignancies in AIDS and Other Acquired Immunodeficiencies; 2010.
30. Lamers SL, Salemi M, McGrath MS, Fogel GB. Prediction of R5, X4, and R5X4 HIV-1 coreceptor usage with evolved neural networks. *IEEE/ACM Trans Comput Biol Bioinform.* 2008; 5(2):291–300. [PubMed: 18451438]
31. Yeung ML, Bennasser Y, Watashi K, Le SY, Houzet L, Jeang KT. Pyrosequencing of small non-coding RNAs in HIV-1 infected cells: evidence for the processing of a viral-cellular double-stranded RNA hybrid. *Nucleic Acids Res.* 2009; 37(19):6575–6586. [PubMed: 19729508]

Fig. (1). Nef interactions in an M2 activated macrophage interacting with B-cells
Once HIV integrates into macrophage DNA, an abundance of cytosolic nef accumulates that is capable of binding MHC II class receptors, the transgolgi network and Src homology 3 proteins (SH3). The overall result is the interference with several biological pathways and production of anti-inflammatory cytokines. In addition, nef induces nanotube formation that allows selective transport of the protein into B-cells, thus nef is capable of bypassing immunoglobulin receptors on the surface of B-cells. In B-cells, nef increases differentiation factors and interferes with germinal center factors. Tumor-associated B-cells are larger, abnormally shaped and contain increased cytoplasm. The nef conduit also changes the morphology of a normal macrophage to one with long spindle-like arms.