Functions of the HIV-1 matrix protein p17

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SUMMARY

HIV-1 replication is a dynamic process influenced by a combination of viral and host factors. The HIV-1 matrix protein p17 is a structural protein critically involved in most stages of the life cycle of the retrovirus. It participates in the early stages of virus replication as well as in RNA targeting to the plasma membrane, incorporation of the envelope into virions and particle assembly. Besides its well established functions, p17 acts as a viral cytokine that works on preactivated - but not on resting - human T cells promoting proliferation, proinflammatory cytokines release and HIV-1 replication after binding to a cellular receptor (p17R). Thus, p17 might play a key role in the complex network of host- and virus-derived stimulatory factors contributing to create a favourable environment for HIV-1 infection and replication.

Here, we present a brief overview of the functions played by the matrix protein p17 in the HIV-1 life cycle and summarize the current understanding of how p17 could contribute to the pathogenesis of HIV-1 disease.

KEY WORDS: Matrix protein p17; HIV-1 life cycle; immune activation; AIDS pathogenesis

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The human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS). Currently, more than 40 million people worldwide are infected with HIV-1. HIV-1, as member of the retrovirus family (Coffin *et al.,* 1997, Frankel and Joung 1998), possesses a single-stranded RNA genome which is converted to double-stranded DNA shortly after infection of host cells. DNA is then transported into the nucleus where it is integrated into chromosomal DNA and replicated by host factors. The late stages of viral assembly occur on the inner leaflet of the plasma membrane and virus is released by budding, thus obtaining a host-derived lipid envelope. The compositions of the viral envelope and the plasma membrane are not equivalent, suggesting that virus assembly may occur in non random subdomains of cellular membranes.

The HIV-1 genome comprises nine genes. The three major coding regions are 5'-*gag-pol-env*-3' which are synthesized as a single precursor, whilst the other six genes encode the so-called accessory proteins that are multifunctional molecules playing an important role in the pathogenesis of HIV-1 infection. Although all nine viral genes are essential for assembly of an infectious particle, the *gag* gene alone can direct the synthesis, transport to the plasma membrane, and assembly of the structural precursor polyprotein Gag resulting in formation of non infectious virus-like particles in the absence of other viral proteins or packageable RNA (Wills and Craven 1991, Hunter 1994, Swanstrom and Wills 1997, Vogt 1997). The HIV-1 Gag protein is initially synthesized as a polyprotein precursor encoded by the HIV-1 *gag* gene. During or shortly after virus budding the HIV-1 protease cleaves the Gag pre-

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cursor Pr55 (Pr55^{Gag}) into the mature proteins p17 matrix, p24 capsid, p7 nucleocapsid, p6 and two smaller spacer peptides p1 and p2. The matrix domain is the N-terminus of the Pr55^{Gag} polyprotein and, during Pr55^{Gag} synthesis, the N-terminal Gly residue of the matrix is modified by the covalent attachment of a myristic acid moiety. In mature virions, the matrix protein p17 is a 132-amino acid polypeptide that forms a protective shell attached to the inner surface of the plasma membrane of the virus (Gottlinger *et al.,* 1989, Nermut *et al.,* 1994). The three-dimensional structure of p17 has been determined by nuclear magnetic resonance and X-ray crystallography (Massiah *et al.,* 1994, Hill *et al.* 1996). Individual p17 molecules are composed of five major α helices and a highly basic platform consisting of three β strands (Figure 1).

The matrix protein plays a key role in several steps during virus replication, both in the early and late stages of the virus life cycle (Figure 2). Late during infection, p17, as part of Pr55^{Gag}, directs unspliced viral RNA to the site of virus assembly. It is clear that a major function of the matrix protein is to direct binding to and assembly at the plasma membrane both *in vitro* and *in vivo* (Zhou *et al.,* 1994, Erlich *et al.,* 1996, Hermida-Matsumoto and Resh 2000). The highly basic domain between residues 17 and 31 as well as the N-terminal myristyl moiety facilitate interactions between p17 and the plasma membrane. The protein assembles into a trimer and trimerization appears to create a large, bipartite membrane binding surface in which exposed basic residues could cooperate with the N-terminal myristoyl groups to anchor the protein on the acidic inner membrane of the virus (Yuan *et al.,* 1993, Zhou *et al.,* 1994, Massiah *et al.,* 1994, Hill *et al.,* 1996). It was initially reported that the mutation of the N-terminal Gly residue, which serves as the site of myristic acid attachment, blocks virus assembly and impairs binding of Gag to the membrane (Gheysen *et al.,* 1989). Subsequently it was observed that a single amino acid change near the N-terminus, in the vicinity of residues 55 and 85 also causes virus assembly defects (Freed *et al.,* 1994b). More recently, it became clear that any substitutions of residues that prevent the correct folding of p17, as well as mutations at the trimer interface, completely inhibit particle assembly (Cannon *et al.,* 1997). During assembly, the uncleaved Gag polyprotein interacts with the viral RNA and the envelope glycoprotein complex (Env) to coordinate the production of infectious virions. A direct interaction between HIV-1 matrix protein and the cytoplasmic domain of gp41 has been described (Cosson 1996). This evidence is also supported by studies showing that deletions and amino acids substitutions in p17 prevent the incorporation of Env into viral particles (Yu *et al.,* 1992, Dorfman *et al.,* 1994, Freed and Martin 1996). Recently, many investigators have focused their studies on the targeting of p17 to lipid rafts in cells (Campbell *et al.,* 2001). It is noteworthy that one factor responsible for raft localization in other protein systems is the presence of two saturated acyl chains, such as myristoyl. However, studies using model systems indicate that the p17 membrane targeting signal, i.e. the basic residues and myristylation, will not result in raft-targeting (McCabe and Berthiaume 2001) and thus for HIV-1 to localize to rafts, some other factors such as interaction of p17 and envelope proteins must be responsible (Zheng *et al.,* 2001).

New findings have demonstrated that membrane targeting is cell-type dependent and in some cells (i.e. macrophages) HIV-1 virus budding occurs

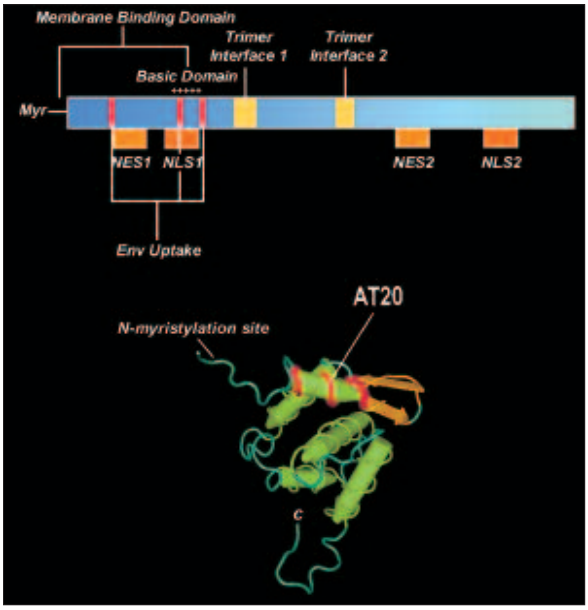

FIGURE 1 - *(A) Linear representation of the HIV-1 p17. Major functional domains are indicated.* (B) *Ribbon diagram of the matrix protein. The receptor binding region (AT20) is shown.*

directly in multivescicular bodies (MVB) rather than from the plasma membrane (Nguyen *et al.,* 2003, Ono and Freed 2004, Pelchen-Matthews *et al.,* 2003). It is likely that specific trafficking machineries are responsible for the Gag transportation within the cellular compartments. Dong *et al.,* (2005) have shown that the amino-terminal α-helical segment of p17 interacts directly with the δ subunit of the Adaptor Protein-3 complex, which belongs to a family of protein complexes involved in sorting of cargo proteins throughout membrane compartments of the late secretory and endocytic pathways. Disruption of this interaction prevents Gag from reaching the MVB compartment and inhibits particle formation. These results show that the trafficking of Gag to MVB is part of the normal productive pathway of HIV-1 assembly and represents an essential step in HIV-1 virus particle production in macrophages where virion release may follow an exosomal pathway.

HIV-1 p17 is also essential in early postentry events of the virus life cycle (Figure 2). An important aspect of the pathophysiology of HIV-1 infection is the ability of the virus to enter and replicate in non-dividing cells. Three HIV proteins, p17, integrase and viral protein R, have been proposed as karyophilic agents that recruit the cellular nuclear import machinery to the viral preintegration complex (PIC). While most of p17 in the virions localizes outside the core and forms the layer between the viral capsid and the envelope, some p17 molecules are found in tight association with the HIV-1 core and the PIC (Miller *et al.,* 1997). Historically, matrix protein p17 was the first implicated in HIV-1 nuclear import (Bukrinsky *et al.,* 1993, von Schwedler *et al.,* 1994, Freed *et al.,* 1994a) and some studies have suggested that it is essential for PIC transport to the nucleus (Bukrinsky *et al.,* 1993, Haffar *et al.,* 2000). The HIV-1 p17 carries two functional, yet rather weak, nuclear localization signals (NLS) (Nadler *et al.,* 1997), but whether they are able by themselves to regulate the HIV-1 nuclear import, enabling the virus to replicate in non dividing cells, remains controversial (Freed and Martin 1994, Freed *et al.,* 1995, Fouchier *et al.,* 1997). In fact, while p17 is clearly required for efficient nuclear import of the PIC, it appears to be nonessential and is likely only one of several factors regulating this process (Reil *et al.,* 1998). Interestingly, one region of the NLS lies in the basic region of p17 that is also involved in membrane binding. It has been hypothesized that protein modifications such as phosphorylation weaken p17 membrane binding thus revealing its karyophilic properties within the PIC. It was initially proposed that the matrix protein is phosphorylated at Tyr-132 by a virion-associated cellular protein kinase (Gallay *et al.,* 1995a) and this modification induces the binding of p17 to integrase (Gallay *et al.,* 1995b), thus allowing its incorporation into the uncoated viral nucleoprotein complexes. However, other groups have been unable to confirm this finding and have suggested that a greater complexity in the phosphate acceptor sites that regulate p17 localization in the target cell and that influence HIV-1 infectivity must occur. In fact, they have provided evidence that p17 is phosphorylated predominantly on tyrosine prior to and during virus assembly, but in native virions and in PIC isolated from target cells p17 is phosphorylated exclusively on serine. It is possible that tyrosine and serine phosphorylation of p17 operate independently in virus infectivity (Bukrinskaya *et al.,* 1996, Freed *et al.,* 1997, Kaushik and Ratner 2004).

Therefore p17, during the entire virus life cycle, mediates two opposing targeting functions. Dupont *et al.,* (1999) have shown that, although p17 lacks the canonical leucine-rich nuclear export signal (NES), it possesses a nuclear export activity. A mutation that disrupts the p17 NES mislocalizes $Pr55^{Gag}$ and genomic viral RNA to the nucleus, thereby severely impairing viral replication. Hence, the p17 NES is required to counteract the p17 NLS, thus ensuring the cytoplasmic availability of the components required for virion assembly (Dupont *et al.,* 1999) (Figure 1). Myristoylation of the Gag matrix domain functions as the regulator of intracellular localization, targeting the $Pr55^{Gag}$ to the plasma membrane during virus assembly and dissociating from the membrane during infectivity for nuclear targeting of PIC (Figure 2). Membrane release is triggered by proteolytic cleavage of $Pr55^{Gag}$. Despite the fact that both Gag and p17 are efficiently myristoylated, their membrane-binding properties are quite different: the Gag precursor binds well to membranes, whereas p17 binds poorly (Zhou and Resh 1996). To explain the differential membrane binding of p17 and Gag, it has been

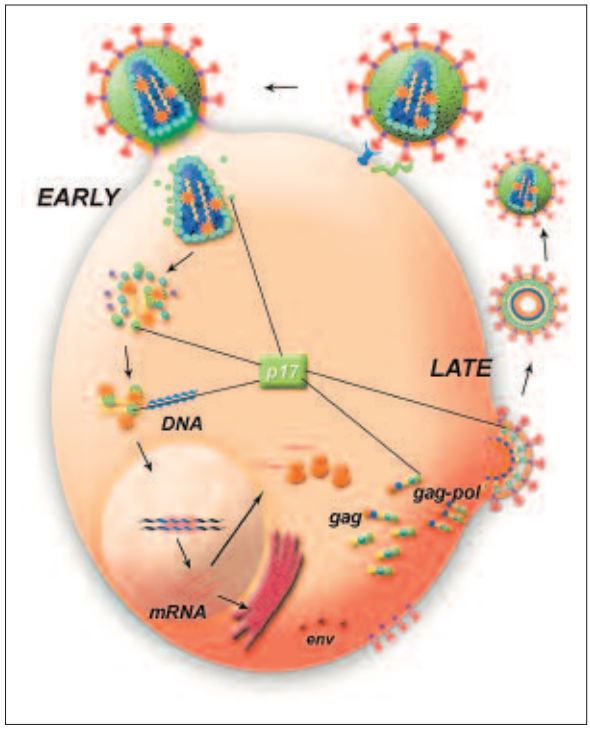

FIGURE 2 - *The HIV-1 life cycle. Major steps in virus replication that involves the p17 matrix protein are schematically represented.*

proposed that accessibility of the myristate moiety is regulated by a "myristoyl switch" mechanism: myristate would be exposed in Gag but sequestered in the context of p17 (Zhou and Resh 1996). A recent article by Tang *et al.,* (2004) provided structural information confirming that the multimerization state of Gag regulates the orientation of the myristate moiety and Gag membrane binding during the virus life cycle, so providing direct support for the myristoyl switch model. It is noteworthy that, unlike other myristoyl-protein structures, p17 does not undergo a dramatic conformational change when switching between states (Tang *et al.,* 2004, Wu *et al.,* 2004).

A pivotal study performed in 1994 (Matthews *et al.,*) showed that the helical topology of the matrix protein is unusual, sharing a strict similarity with the immune modulator interferon (IFN)-γ. The structural similarity is intriguing since IFN-γ is functional as a homodimer (Fountolakis *et al.,* 1990) and p17 possesses the capacity to oligomerize (Scarlata *et al.,* 1998). In agreement with the results obtained by Matthews and co-workers, our group described the specific interaction between p17 and IFN-γ proteins. This interaction was found to be dose-dependent and to involve conformational epitopes on both sides. However, p17 did not displace the binding of IFN-γ to its cellular receptor, nor did it interfere with the capability of the lymphokine to exert its immunomodulatory activity (Caruso *et al.,* 1989, Flamminio *et al.,* 1995). It cannot be ruled out that p17 can form heterodimers with IFN-γ still allowing the cytokine to perform its biological activities. However, p17 *per se* does not bind IFN-γ receptors nor is it able to mimic IFN-γ biological properties (Flamminio *et al.,* 1995). It is well established that viruses encode cytokine homologues that bind to specific receptors and either trigger signal transduction and biological responses (agonist) or occupy receptor binding sites and prevent binding of host cytokines (antagonist) (reviewed by Alcami *et al.,* 2002). Viral cytokines may therefore have immunomodulatory activity or they may promote viral replication by inducing the proliferation of immune cells that are good targets for the virus (reviewed by Alcami, 2003), thus contributing directly to the pathology that is associated with viral infection. Among viruses, HIV-1 is highly successful in modifying the immune system despite its small genome as most of the HIV-1 proteins have been shown to disregulate immunological responses. Because of the strict similarity of the viral protein with IFN-γ, it has been investigated whether p17 could act as a viral cytokine. Recent studies have shown that p17 specifically binds to a cell surface receptor (p17R) which is costitutively expressed on freshly collected peripheral B cells but not on peripheral $CD4^+$ and $CD8^+$ T cells (De Francesco *et al.,* 2002). The stability of the p17R positive phenotype by B cells and the acquisition of p17R expression by T cells has been demonstrated in PBMC cultures stimulated with IL-2. After 72 h of IL-2 stimulation, a large percentage of $CD4^+$ and $CD8^+$ T cells acquire the surface expression of p17R. Time course experiments, performed to study the kinetics of the *de novo* p17R expression on purified $CD4^+$ and $CD8^+$ T cell subsets following IL-2 stimulation, demonstrated that the appearance of p17Rs occurs at the very early stages of T cell activation, reaching a peak at 48 h of culture. HIV-1 p17 binds to its receptor with an apparent K_d of 2.5 x 10^{-8}

M as determined by Scatchard analysis. Binding of p17 to mitogen-activated T cells is more pronounced on a proportion of cells or absent on cells within the $CD4^+$ and $CD8^+$ subset, suggesting that T cells can be more or less responsive, or even not responsive, to a potential p17 biological activity.

Exogenous p17 does not induce any biologically relevant effect on resting T cells but - at a concentration as low as 5 ng/ml - it acts synergistically with a signal provided by IL-2 to promote cell proliferation and a Th-1 cytokine profile. PBMCs obtained from various donors respond to p17 stimulation in the presence of varying doses of IL-2 and even the weakest activation achieved with IL-2 has shown to be sufficient for p17 to increase the T-cell proliferative response to the lymphokine (De Francesco *et al.,* 1998). At the same time, addition of p17 to PBMC cultures positively influences the production of the proinflammatory molecules IFN-γ and tumor necrosis factor (TNF)-α induced by IL-2 treatment (Figure 3). The potential of p17 to sustain

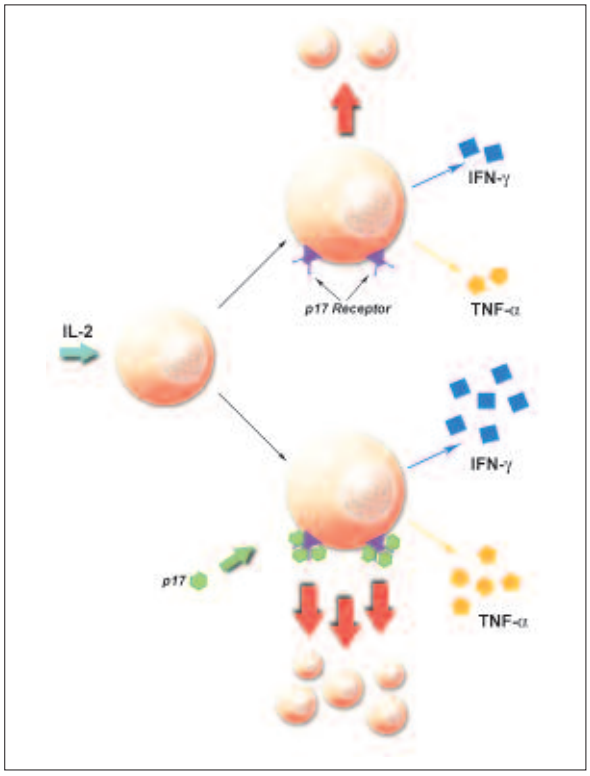

FIGURE 3 - *A model for the role of p17 in inducing T lymphocytes activation and proliferation. For details refer to the text.*

an inflammatory microenvironment is further confirmed by its capability to counteract the anti-inflammatory properties of IL-4. Addition of IL-4 to IL-2 stimulated PBMC cultures suppresses both TNF-α and IFN-γ production, whereas the presence of p17 completely restores the capability of PBMCs to respond to IL-2 stimulation and produce TNF-α and IFN-γ (De Francesco *et al.,* 2002). It is well known that IL-12 synergizes with IL-2 in driving proliferation and differentiation of T cells towards a Th1 effector type (Chan *et al.,* 1991) and that IL-15 has overlapping biological effects with IL-2 that are partly due to the shared use of the IL-2 receptor subunit gamma chain (Tagaya *et al.,* 1996). For these reasons it was analysed whether p17 contributes to lymphocyte stimulation induced by the IL-12 and IL-15 (De Francesco *et al.,* 2002). PBMCs treated with IL-12 or IL-15 are induced toward a proinflammatory phenotype and release both TNF-α and IFN-γ. The addition of p17 to IL-12 and IL-15 stimulated cultures does not increase the production of either cytokine, confirming that p17 is mainly able to sustain the biological effects driven by the IL-2 dependent pathway. This is true not only for T cells but also for natural killer (NK) cells. In fact, HIV-1 p17 synergizes with IL-2 in inducing NK cell proliferation and production of proinflammatory cytokines (Vitale *et al.,* 2003). On the other hand, p17 does not affect NK cytotoxicity or target-induced apoptosis, in agreement with the previous observations that NK cell proliferation and cytotoxicity can occur independently (Biron *et al.,* 1999).

Replication of HIV-1 is regulated by virus-encoded regulatory proteins as well as by a variety of cellular factors including cytokines. In fact, cellular activation is believed to be necessary for an optimal viral spreading. Proinflammatory cytokines can profoundly enhance the ability of HIV-1 to replicate in $CD4^+$ target cells, such as T lymphocytes and macrophages (Vicenzi *et al.,* 1997). It is well established that HIV-1 replication in IL-2 stimulated PBMCs is driven in an autocrine/paracrine manner by endogenously produced cytokines (Kinter *et al.,* 1995). By modulating cellular proliferation and cytokine milieau, p17 has been shown able to favour HIV-1 replication *in vitro*. PBMCs infected with HIV-1 and then cultured in the presence of IL-2 and p17 release higher amounts of virus than cells

cultured in the presence of IL-2 alone (De Francesco *et al.,* 1998). All these findings emphasize the capability of p17 to exert its biological activity on the IL-2-dependent activation/proliferation pathway and suggest a role of the viral protein in creating a suitable environment for HIV-1 replication.

Demonstration of extracellular presence of p17 in abundant amounts in lymph nodes has established that this structural viral protein *in vivo* most likely exerts biological activities (Popovic *et al.,* 2005). Popovic *et al.,* (2005) highlighted the long-term persistence of the p17 in tissue specimens during the 13 months of the study follow-up, providing evidence that the matrix protein p17 is accumulated in the germinal center light zone of lymph nodes in HIV-1-infected patients who received anti-viral treatment (HAART) in the absence of any – even in situ – HIV-1 replication. Moreover, p17 detection did not noticeably differ in tissue specimens taken from patients either before initiation of therapy or during treatment where the virus burden significantly decreased. This finding strongly suggests that the HIV-1 structural protein persists in patients under HAART for a long time in the absence of detectable virus replication, thus strengthening the recent *in vitro* evidence that HIV-1 infected cells are able to release significant amounts of p17 (Figure 4). The mechanisms underlying these events are not yet understood but it can be hypothesized that HIV-1 p17 can reach consistent concentrations in the HIV-1 infected microenvironment through mechanisms of virus disintegration or by immune lysis of infected cells or even exocytotic pathways (Dong *et al,* 2005).

All these data therefore support the possibility that exogenous p17 may act as a virokine especially in organs such as lymph nodes, where it can interact with target cells.

The addition of a neutralizing antibody able to inhibit the interaction between the viral protein and its cellular receptor during functional assays completely blocks all the p17 activities, suggesting that the p17 cytokine-like functions occur through its binding to the cellular receptor (De Francesco *et al.,* 2002). The receptor binding region (RBR) of p17 was identified by epitope mapping analysis. RBR is located in the N-terminus of the p17 molecule encompassing 20 amino acids (AT-20) (Figure 1). The identified RBR has been confirmed to be strategical in the fulfilment of its cytokine-like activities since the use of AT-20 peptide - coupled with KLH - as immunogen generated mouse and rabbit neutralizing anti-p17 antibodies. Sera obtained from immunized animals blocked the interaction

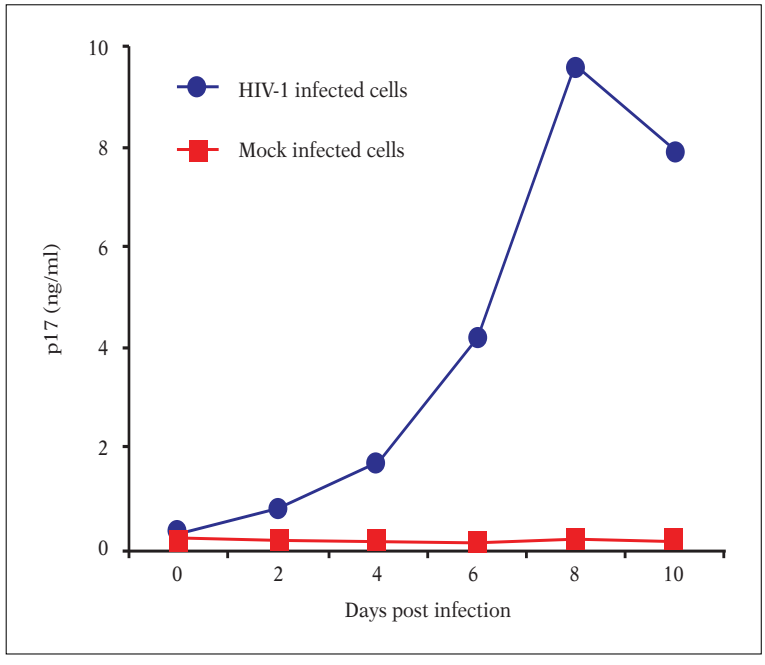

FIGURE 4 - *Quantification of HIV-1 p17 in the supernatant of infected cell cultures. H9 cells were acutely infected with an HIV-1-BH10 inoculum and the presence of p17 in the cell culture supernatant was evaluated at different time points after infection using a capture ELISA. Mock-infection indicates the supernatants obtained from H9 cultured in the absence of HIV-1. During the entire period of culture both HIV-1-infected and mock-infected cells did not show any cytopathic effect.*

of the p17 with its receptor, as defined by flow cytometric p17 binding assays (Fiorentini *et al.,* 2004, Becker *et al.,* in press). However, it cannot be ruled out that other epitopes contribute to the binding and/or stabilization of the p17/p17R interaction.

Recent findings have shown that p17Rs are expressed also on murine immune cells, which behave strikingly like their human counterpart in responding to p17 stimulation. The development of a mouse model, where T cell activation was achieved through an abortive HSV-1 infection, demonstrated that *in vivo* pre-activated murine T cells do express p17Rs and are highly susceptible to p17 biological activities. Indeed, p17 treatment triggers proinflammatory cytokines release and strongly promotes $CD4^+$ T cell survival and expansion. Furthermore, coculture of *in vivo*-activated splenocytes with macrophages induces the release of IFN-γ, which is strongly enhanced by p17. The immune modulatory properties of p17 have also been highlighted at the mucosal site where the viral protein was found to enhance immune responses against a coadministered model antigen (Fiorentini *et al.,* in press). The mucosa plays a fundamental role in HIV-1 disease by serving as a site for virus entry, replication and amplification, and constitutes the initial site of $CD4^+$ T cell depletion. Thus, AIDS is now considered a disease of the mucosal immune system (Belyakov and Berzofsky 2004). The demonstration that p17 exerts its biological activity also at mucosal level highlights the possibility that matrix protein may play an important role at the sites of HIV-1 infection.

A large body of evidence has highlighted how the matrix protein p17 functions during HIV-1 replication both at the early and late stages of the virus life cycle. Nevertheless, studies on the mechanisms that finely tune interactions between p17 and other viral proteins and specific host components are still ongoing.

In addition, p17 has all the characteristics of a viral cytokine and is likely to be considered an additional member of the group of viral regulatory proteins that use immune system activation to the advantage of HIV-1 infection and replication. The future characterization of the p17 receptor and knowledge on its role in promoting p17 activity will enhance our understanding of the mechanisms used by HIV-1 to better replicate into different target cells. Studies on the capacity of molecules representative of the AT20-p17 domain to induce an immune response able to neutralize the p17/p17R interaction will suggest new ways to approach anti-HIV-1 therapeutic strategies.

REFERENCES

ALCAMI, A. (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nat Rev Immunol*, **3**, 36-50.

ALCAMI, A., GHAZAL, P., AND YEWDELL, J.W. (2002). Viruses in control of the immune system. Workshop on molecular mechanisms of immune modulation: lessons from viruses. *EMBO Rep*, **3**, 927-932.

BECKER, P.D., FIORENTINI, S., LINK, C., TOSTI, G., EBENSEN, T., CARUSO, A., AND GUZMÁN C.A. The HIV-1 matrix protein p17 can be efficiently delivered by intranasal route in mice using the TLR 2/6 agonist MALP-2 as mucosal adjuvant. *Vaccine*, in press.

BELYAKOV, I.M., AND BERZOFSKY, J.A. (2004). Immunobiology of mucosal HIV infection and the basis for development of a new generation of mucosal AIDS vaccines. *Immunity* **20**, 247-253.

BIRON, C.A., NGUYEN, K.B., PIEN, G.C., COUSENS, L.P., AND SALAZAR-MATHER, T.P. (1999). Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev of Immunol,* **17**, 189-220.

BUKRINSKAYA, A.G., GHORPADE, A., HEINZINGER, N.K., SMITHGALL, T.E., LEWIS R.E., AND STEVENSON, M. (1996). Phosphorylation-dependent human immunodeficiency virus type 1 infection and nuclear targeting of viral DNA. *Proc Natl Acad Sci USA,* **93**, 367-371.

BUKRINSKY, M.I., HAGGERTY, S., DEMPSEY, M.P., SHAROVA, N., ADZHUBEL, A., SPITZ, L., LEWIS, P., GOLDFARB, D., EMERMAN, M., AND STEVENSON, M. (1993). A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. *Nature*, **365**, 666-669.

CAMPBELL, S.M., CROWE, S.M., AND MAK, J. (2001). Lipid rafts and HIV-1: from viral entry to assembly of progeny virions. *J Clin Virol*, **22**, 217-227.

CANNON, P.M., MATTHEWS, S., CLARK, N., BYLES, E.D., IOURIN, O., HOCKLEY, D.J., KINGSMAN, S.M., AND KINGSMAN, A.J. (1997). Structure-function studies of the human immunodeficiency virus type 1 matrix protein, p17. *J Virol*, **71**, 3474-3483.

CARUSO, A., POLLARA, P., FORESTI, I., BONFANTI, C., DE FRANCESCO, M., GELMI, M., AND TURANO, A. (1989). Interferon-gamma is associated with the surface

of the human immunodeficiency virus and binds to the gag gene product p17. *AIDS Res Hum Retroviruses*, **5,** 605-612.

CHAN, S.H. , PERUSSIA, B., GUPTA, J.W., KOBAYASHY, M., POSPISIL, M., YOUNG, H.A., WOLF, S.W., YOUNG, D., CLARK, S.C., AND TRINCHIERI, G. (1991). Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J Exp Med,* **173**, 869-879.

COFFIN, J.M., HUGHES, S.H., AND VARMUS, H.E. (1997). *Retroviruses*, Cold Spring Harbor Laboratory Press, Woodbury, NY.

COSSON, P. (1996). Direct interaction between the envelope and matrix proteins of HIV-1. *EMBO J*, **15**, 5783-5788.

DE FRANCESCO, M.A., BARONIO, M., FIORENTINI, S., SIGNORINI, C., BONFANTI, C., POIESI, C., POPOVIC, M., GRASSI, M., GARRAFA, E., BOZZO, L., LEWIS, G.K., LICENZIATI, S., GALLO, R.C., AND CARUSO, A. (2002). HIV-1 matrix protein p17 increases the production of proinflammatory cytokines and counteracts IL-4 activity by binding to a cellular receptor. *Proc Natl Acad Sci USA*, **99**, 9972-9977.

DE FRANCESCO, M.A., CARUSO, A., FALLACARA, F., CANARIS, A.D., DIMA, F., POIESI, C., LICENZIATI, S., CORULLI, M., MARTINELLI, F., FIORENTINI, S., AND TURANO, A. (1998). HIV p17 enhances lymphocyte proliferation and HIV-1 replication after binding to a human serum factor. *AIDS*, **12**, 245-252.

DONG, X., LI, H., DERDOWSKI, A., DING, L., BURNETT, A., CHEN, X., PETERS, T.R., DERMODY, T.S., WOODRUFF, E., WANG, J.J., AND SPEARMAN, P. (2005). AP-3 directs the intracellular trafficking of HIV-1 Gag and plays a key role in particle assembly. *Cell*, **120**, 663-674.

DORFMAN, T., MAMMANO, F., HASELTINE, W.A., AND GOTTLINGER, H.G. (1994). Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein. *J Virol*, **68**, 1689-1696.

DUPONT, S., SHAROVA, N., DEHORATIUS, C., VIRBASIUS, C.M., ZHU, X., BUKRINSKAYA, A.G., STEVENSON, M., AND GREEN, M.R. (1999). A novel nuclear export activity in HIV-1 matrix protein required for viral replication. *Nature*, **402**, 681-685.

EHRLICH, L.S., FONG, S., SCARLATA, S., ZYBARTH, G., AND CARTER, C. (1996). Partitioning of HIV-1 Gag and Gag-related proteins to membranes. *Biochemistry,* **35,** 3933-3943.

FIORENTINI, S., BECKER, P.D., MARINI, E., MARCONI, P., AVOLIO, M., TOSTI, G., LINK, C., MANSERVIGI, R., GUZMAN, C.A. AND CARUSO, A. HIV-1 matrix protein p17 modulates in vivo preactivated murine T cell response and enhances the induction of systemic and mucosal immunity against intranasally coadministered antigens. *Viral Immunol*, in press

FIORENTINI, S., MARINI, E., BOZZO, L., TRAININI, L., SAADOUNE, L., AVOLIO, M., PONTILLO, A., BONFANTI, C., SARMIENTOS, P., AND CARUSO, A. (2004) Preclinical studies on immunogenicity of the HIV-1 p17-based synthetic peptide AT20-KLH. *Biopolymers*, **76**, 334-343.

FLAMMINIO, G., CARUSO, A., POIESI, C., BONFANTI, C., TERLENGHI, L., CANARIS, A.D., VARINACCI, C., MARTINELLI, F., GAROTTA, G., ALBERTINI, A., AND TURANO, A. (1995). Aspects of molecular interaction between HIV p17 and human gamma interferon. *AIDS Res Hum Retroviruses*, **11**, 1441-1447.

FOUCHIER, R.A., MEYER B.E., SIMON J.H., FISCHER U., AND MALIM, M.H. (1997). HIV-1 infection of non-dividing cells: evidence that the amino-terminal basic region of the viral matrix protein is important for Gag processing but not for post-entry nuclear import. *EMBO J*, **16**, 4531-4539.

FOUNTOULAKIS, M., JURANVILLE, J.F., MARIS, A., OZMEN, L. AND GAROTTA, G. (1990). One interferon gamma receptor binds one interferon gamma dimer. *J Biol Chem,* **265**, 19758-19767.

FRANKEL, A.D., AND YOUNG, J.A. (1998). HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem*, **67**, 1-25.

FREED, E.O., ENGLUND, G., AND MARTIN, M.A. (1995). Role of the basic domain of human immunodeficiency virus type 1 matrix in macrophage infection. *J Virol*, **69**, 3949-3954.

FREED, E.O., ENGLUND, G., MALDARELLI, F., AND MARTIN, M.A. (1997). Phosphorylation of residue 131 of HIV-1 matrix is not required for macrophage infection. *Cell*, **88**, 171-173.

FREED, E.O., AND MARTIN, M.A. (1994). HIV-1 infection of nondividing cells. *Nature*, **369**, 107-108.

FREED, E.O., AND MARTIN, M.A. (1996). Domains of the human immunodeficiency virus type 1 matrix and gp41 cytoplasmic tail required for envelope incorporation into virions. *J Virol,* **70**, 341-351.

FREED, E.O., ORENSTEIN, J.M., BUCKLER-WHITE, A.J., AND MARTIN, M.A. (1994). Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production. *J Virol*, **68**, 5311-5320.

GALLAY, P., SWINGLER, S., AIKEN, C., AND TRONO, D. (1995). C-terminal tyrosine phosphorylation of the viral matrix protein is a key regulator. *Cell,* **80**, 379-388.

GALLAY, P., SWINGLER, S., SONG, J., BUSHMAN, F., AND TRONO, D. (1995). HIV nuclear import is governed by the phosphotyrosine-mediated binding of matrix to the core domain of integrase. *Cell*, **83**, 569-576.

GHEYSEN, D., JACOBS, E., DE FORESTA, F., THIRIART, C., FRANCOTTE, M., THINES, D., AND DE WILDE, M. (1989). Assembly and release of HIV-1 precursor Pr55gag virus-like particles from recombinant baculovirus infected insect cells. *Cell*, **59**, 103–112.

GOTTLINGER, H.G., SODROSKI, J.G., AND HASELTINE, W.A. (1989). Role of capsid precursor processing and

myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. *Proc Natl Acad Sci USA,* **86**, 5781–5785.

Haffar, O.K., Popov, S., Dubrovsky, L., Agostini, I., Tang, H., Pushkarsky, T., Nadler, S.G., and Bukrinsky, M. (2000). Two nuclear localization signals in the HIV-1 matrix protein regulate nuclear import of the HIV-1 pre-integration complex. *J Mol Biol*, **299**, 359-368.

Hermida-Matsumoto, L., AND Resh, M.D. (2000). Localization of human immunodeficiency virus type 1 Gag and Env at the plasma membrane by confocal imaging. *J Virol,* **74**, 8670-8679.

Hill, C.P., Worthylake, D., Bancroft, D.P., Christensen, A.M., and Sundquist, W.I. (1996). Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. *Proc Natl Acad Sci USA,* **93**, 3099-3104.

Hunter, E. (1994). Macromolecular interactions in the assembly of HIV and other retroviruses. *Semin Virol,* **5**, 71–83.

Kaushik, R., and Ratner, L. (2004). Role of Human Immunodeficiency Virus Type 1 Matrix Phosphorylation in an Early Postentry Step of Virus Replication. *J Virol*, **78**, 2319-2326.

Kiernan, R.E., Ono, A., Englund, G., and Freed, E.O. (1998). Role of matrix in an early postentry step in the human immunodeficiency virus type 1 life cycle. *J Virol,* **72**, 4116-4126.

Kinter, A.L., Poli, G., Fox, L., Hardy, E., Fauci, A.S. (1995). HIV replication in IL-2-stimulated peripheral blood mononuclear cells is driven in an autocrine/paracrine manner by endogenous cytokines. *J Immunol*, **154**, 2448-59.

Massiah, M.A., Starich, M.R., Paschall, C., Summers, M.F., Christensen, A.M., and Sundquist, W.I. (1994). Three-dimensional structure of the human immunodeficiency virus type 1 matrix protein. *J Mol Biol*, **244**, 198-223.

Matthews, S., Barlow, P., Boyd, J., Barton, G., Russell, R., Mills, H., Cunningham, M., Meyers, N., Burns, N., Clark, N., Kingsman, s., Kingsman, A., and Campbell, I. (1994). Structural similarity between the p17 matrix protein of HIV-1 and interferon-gamma. *Nature*, **370**, 666-668.

McCabe, J.B., and Berthiaume, L.G. (2001). N-terminal protein acylation confers localization to cholesterol, sphingolipid-enriched membranes but not to lipid rafts/caveolae. *Mol Biol Cell*, **12**, 3601-3617.

Miller, M.D., Farnet, C.M., and Bushman, F.D. (1997). Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. *J Virol,* **71**, 5382-5390.

Nadler, S.G., Tritschler, D., Haffar, O.K., Blake, J., Bruce, A.G., and Cleaveland, J.S. (1997). Differential expression and sequence-specific interaction of karyopherin alpha with nuclear localization sequences. *J Biol Chem,* **272**, 4310-4315.

Nermut, M.V., Hockley, D.J., Jowett, J.B., Jones, I.M., Garreau, M., and Thomas, D. (1994). Fullerene-like organization of HIV gag-protein shell in virus-like particles produced by recombinant baculovirus. *Virology*, **198**, 288-296.

Nguyen, D.G., Booth, A., Gould, S.J., and Hildreth, J.E. (2003). Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. *J Biol Chem,* **278**, 52347–52354.

Ono, A., and Freed, E.O. (2004). Cell-type-dependent targeting of human immunodeficiency virus type 1 assembly to the plasma membrane and the multivesicular body. *J Virol,* **78**, 1552–1563.

Pelchen-Matthews, A., Kramer, B., and Marsh, M. (2003). Infectious HIV-1 assembles in late endosomes in primary macrophages. *J Cell Biol,* **162**, 443-545.

Popovic, M., Tenner-Racz, K., Pelser, C., Stellbrink, H.J., van Lunzen, J., Lewis, G., Kalyanaraman, VS., Gallo, R.C., and Racz, P. (2005). Persistence of HIV-1 structural proteins and glycoproteins in lymph nodes of patients under highly active antiretroviral therapy. *Proc Natl Acad Sci USA,* **102**, 14807-14812.

Reil, H., Bukovsky, A.A., Gelderblom, H.R., and Gottlinger, H.G. (1998). Efficient HIV-1 replication can occur in the absence of the viral matrix protein. *EMBO J,* **17**, 2699-2708.

Scarlata, S., Ehrlich, L.S., and Carter, C.A. (1998). Membrane-induced alterations in HIV-1 Gag and matrix protein-protein interactions. *J Mol Biol,* **277**, 161-169.

Swanstrom, R., and Wills, J.W. (1997). Synthesis, assembly, and processing of viral proteins. In *"Retroviruses"* (Coffin, J.M., Hughes, S.H., and Varmus, H.E. Eds.), 263–334. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Tagaya, Y., Bamford, R.N., DeFilippis, A.P., and Waldmann, T.A. (1996). IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity,* **4**, 329-336.

Tang, C., Loeliger, E., Luncsford, P., Kinde, I., Beckett, D., and Summers, M.F. (2004). Entropic switch regulates myristate exposure in the HIV-1 matrix protein. *Proc Natl Acad Sci USA,* **101**, 517-522.

Vicenzi, E., Biswas, P., Mengozzi, M., and Poli, G. (1997). Role of pro-inflammatory cytokines and beta-chemokines in controlling HIV replication. *J Leukoc Biol*, **62**, 34-40.

Vitale, M., Caruso, A., De Francesco, M.A., Rodella, L., Bozzo, L., Garrafa, E., Grassi, M., Gobbi, G., Cacchioli, A., and Fiorentini, S. (2003). HIV-1 matrix protein p17 enhances the proliferative activity of natural killer cells and increases their abil-

ity to secrete proinflammatory cytokines. *Br J Haematol,* **120**, 337-343.

VOGT, V.M. (1997). Retroviral virions and genomes. In *"Retroviruses"*(COFFIN, J.M., HUGHES, S.H., AND VARMUS, H.E. Eds.), 27–70. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

VON SCHWEDLER, U., KORNBLUTH, R.S., AND TRONO, D. (1994). The nuclear localization signal of the matrix protein of human immunodeficiency virus type 1 allows the establishment of infection in macrophages and quiescent T lymphocytes. *Proc Natl Acad Sci USA*, **91**, 6992-6996.

WILLS, J.W., AND CRAVEN, R.C. (1991). Form, function, and use of retroviral Gag proteins. *AIDS,* **5**, 639–654.

WU, Z., ALEXANDRATOS, J., ERICKSEN, B., LUBKOWSKI, J., GALLO, R.C., AND LU, W. (2004). Total chemical synthesis of N-myristoylated HIV-1 matrix protein p17: structural and mechanistic implications of p17 myristoylation. *Proc Natl Acad Sci USA,* **101**, 11587-11592.

YU, X., YUAN X., MATSUDA, Z., LEE, T.H., AND ESSEX, M. (1992). The matrix protein of human immunodeficiency virus type 1 is required for incorporation of viral envelope protein into mature virions. *J Virol*, **66**, 4966-4971.

YUAN, X., YU, X., LEE, T.H., AND ESSEX, M. (1993). Mutations in the N-terminal region of human immunodeficiency virus type 1 matrix protein block intracellular transport of the Gag precursor. *J Virol*, **67**, 6387–6394.

ZHENG, Y.H., PLEMENITAS, A., LINNEMANN, T., FACKLER, O.T., AND PETERLIN, B.M. (2001). Nef increases infectivity of HIV via lipid rafts. *Curr Biol,* **11**, 875-879.

ZHOU, W., PARENT, L.J., WILLS, J.W., AND RESH, M.D. (1994). Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. *J Virol,* **68**, 2556–2569.

ZHOU, W., AND RESH, M.D. (1996). Differential membrane binding of the human immunodeficiency virus type 1 matrix protein. *J Virol,* **70**, 8540-8548.