Tetherin
Getting to grips with cross-species transmission

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Cross-species transmission

Many of the most challenging infectious diseases of the last century are the result of cross-species transmission events. This includes both quickly appearing and disappearing pathogens such as Ebola (from fruit bats Leroy et al. (2005)), the SARS outbreak of 2003 (also from bats Li et al. (2005)), and new influenza strains, such as the 2009 swine flu outbreak. However, not all pathogens that transmit to humans come in the guise of a 'scare' that, although causing much concern due to high mortality rates, quickly disappears again. The most well known cross-species pathogen that has firmly established itself in the human population is the Human Immunodeficiency Virus (HIV). Since the initial HIV infection in Central Africa near the beginning of the twentieth century, HIV has spread to virtually every country on the globe. The latest estimate is that 35 million people are currently infected with HIV, with over 2 million new infections per year world wide (www.unaids.org). Clearly, transmission of pathogens between different species poses a risk to human health. Many factors influence the probability of zoonosis between two species. Population based factors play an important role, such as the geographical overlap between different hosts, and the amount of interaction between the species. For example, most new flu variants arise in Asia, where contact between humans and live poultry is more common than elsewhere. Cultural factors, such as urbanisation, intravenous drug use or the reuse of needles in medical settings can increase the chances of human-to-human transmission of new pathogens. Differences in cell types, cellular receptors and the immune response can act as a barrier to cross-species transmission, although to what extend depends on the evolutionary distance between the hosts. For closely related hosts, many of these factors can be conserved, and will not prevent the transmission of pathogens across species boundaries.

One protein that has the potential to affect cross-species transmission is tetherin, a recently discovered restriction factor of enveloped viruses.

Tetherin

Tetherin is a broad acting restriction factor that tethers newly produced virions to infected cells, preventing the spread of the infection. The goal of this paper is to determine the effect of tetherin on cross-species transmission of enveloped viruses. Tetherin, also called bst2, CD317, and HM1.24, is an 20 kD protein that is part of the innate immune response against enveloped viruses (Douglas et al., 2010). Tetherin inhibits the release of newly formed virions from infected cells by physically tethering budding virions to the cell surface, as is illustrated in Figure 1.

The role of tetherin in immunity was first discovered in 2008 when it was shown that tetherin is responsible for the well known, but little understood phenomenon that Vpu-deletion inhibits HIV replication in some cell lines, but has no effect in others. Neil et al. (2008) analysed the gene expression patterns of both restrictive and permissive cell lines, and was able to demonstrate that

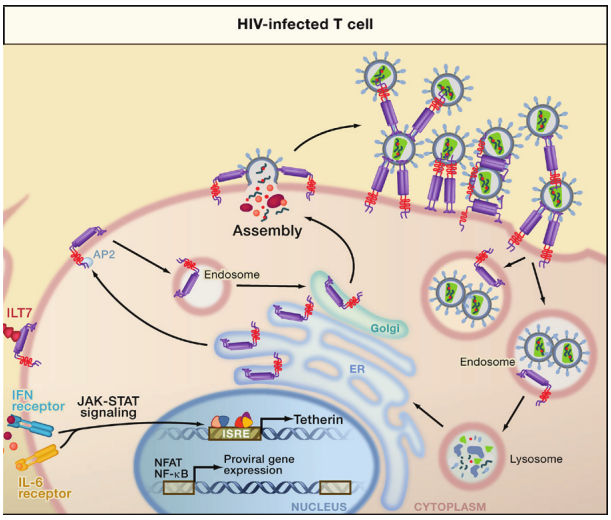

Figure 1: Tetherin mediated restriction of HIV virion release, tetherin in shown as the purple dimers at the cell surface. Taken from Sauter et al. (2010).

bst2/tetherin expression was the only factor that differentiated between the two.

Tetherin is a protein with a unique architecture among eukaryotic proteins, with the exception of an isoform of the prion protein (Kupzig et al., 2003; Moore et al., 1999). It has a short N-terminal cytoplasmic tail, followed by a trans membrane region, a coiled-coil extracellular domain, and a GPI anchor, giving it two separate membrane attachments (see Figure 2). Tetherin can form parallel homodimers via three conserved cystine residues that are located near the N-terminal part of the coiled-coil of the protein. Tetherin undergoes several post-translational modifications: it has two N-linked glycosylation sites, and the C-terminus is cleaved at position 160 to add a GPI anchor, as shown in Figure 3 (Kupzig et al., 2003). Surprisingly, a completely artificial tetherin-like protein, constructed by taking the N-terminal and trans membrane part of the transferrin receptor, a coiled-coil from Myotonin protein kinase and the GPI anchor from the Urokinase receptor, also localises to the plasma membrane, and is also able to restrict virus release of HIV Perez-Caballero et al. (2009).

The bst2 gene is present in a single copy on human chromosome 19, and is not expressed on most cells, unless in response to interferon-I or II treatment (Blasius et al., 2006). Tetherin is conserved among all mammals, and appears to be under positive selection, at least within primates (Lim et al., 2010). Figure 3 shows an alignment of a diverse set of mammalian tetherins, which shows considerable divergence in amino acid sequence, but not in domain organisation. Notice how the cystine residues that are critical for dimerisation are conserved in all species.

Lim et al. (2010), who used a dataset of 20 primate tetherin proteins, found that only three residues in tetherin are under positive selection in primates. One

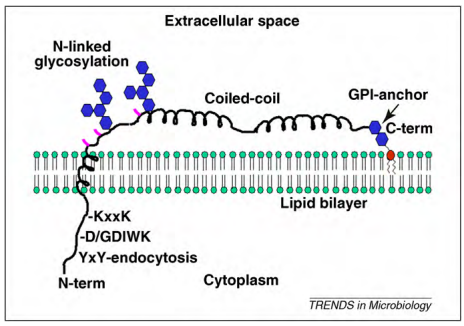

Figure 2: Cartoon representation of tetherin. The three conserved cystine residues are indicated in red. Taken from Evans et al. (2010).

of these residues, at position 17 in the cytoplasmic tail, is the site where SIV Nef interacts with tetherin. This shows that primates have faced selection pressure from a virus that encodes Nef or a protein with Nef-like specificity for a long time. Interestingly, human tetherin has a 5 amino acid deletion that includes position 17, rendering it completely resistant against antagonism by SIV Nef (see Figure 3).

Mature tetherin proteins accumulate in the Trans-Golgi Network (TGN) and as dimers at the plasma membrane, where they associate with lipid rafts (Kupzig et al., 2003). Lipid rafts act as a scaffold for protein complexes on the plasma membrane, and are also involved in the budding of viruses such as HIV and Ebola (Lopez et al., 2012). During viral budding, one of tetherin's two membrane anchors (either the GPI anchor or the N-terminal trans membrane domain) gets inserted into the budding virion, which tethers the virion to the host cell, preventing its release. Tethered virions are then internalised via clatherin mediated endocytosis Rollason et al. (2007) and subsequently degraded in the late endosome (Kueck and Neil, 2012). Dimer formation of tetherin (via the three conserved cystine residues) is required for efficient virion retention of HIV, though not of Lassa or Marburg virus (Andrew et al., 2009; Watanabe et al., 2011).

Tetherin functions by physically tethering the virus envelope, which is derived from the host cell, to the plasma membrane. Because of this tetherin able to restrict the release of any virus that buds from a membrane that is accessible to tetherin. In most cases, this will be the plasma membrane of the cell, which is the case for HIV/SIV and other retroviruses, Ebola Virus and Marburg Virus. All these viruses are restricted by tetherin. Ebola and Marburg viruses represent a family of filamentous viruses that bud from the plasma membrane. This family of viruses is restricted by tetherin, and uses the conserved Glycoprotein GP to antagonise this restriction. Retroviruses such as HIV and SIV are also restricted by tetherin, but unlike filoviruses, the protein used to antagonise tetherin is not conserved.

However, tetherin also restricts the release of at least two herpes viruses,

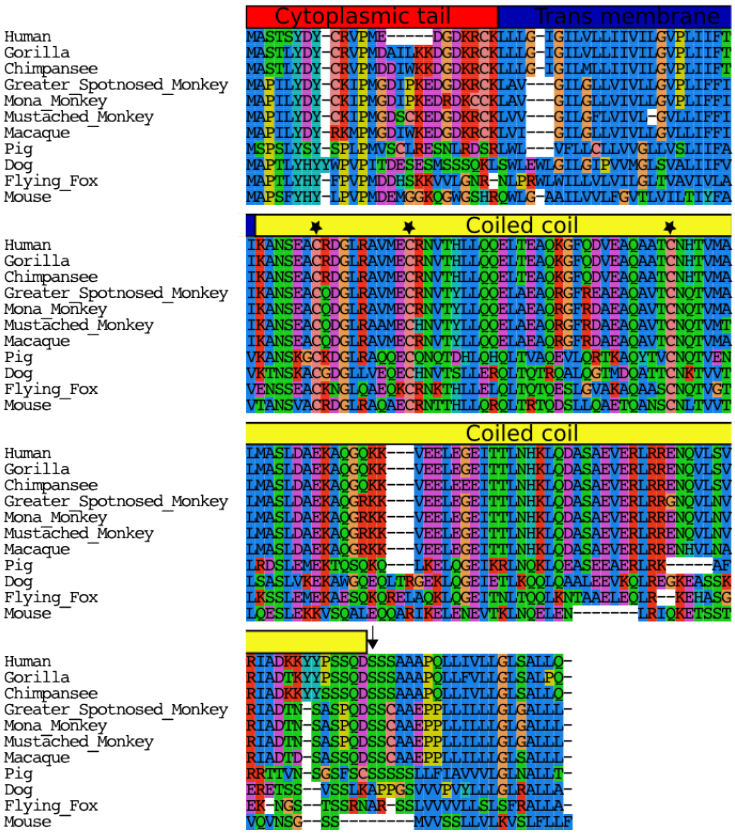

Figure 3: Alignment of tetherin proteins of several mamalian species. Stars indicate the three conserved cystine residues, the arrow bordering the coiled coil domain is the site that is cleaved to add the GPI anchor. Note the 5 amino acid deletion in the cytoplasmic tail of human tetherin that makes it resistant to SIV Nef.

which do not bud from the plasma membrane (Mansouri et al., 2009; Mettenleiter et al., 2006). Herpes viruses have a complex assembly process (reviewed by Mettenleiter et al. (2006)), which includes assembly of the capsid with DNA within the nucleus, followed by exit from the nucleus by fusion of the pro-virion with the nuclear membrane. Secondary envelopment occurs at the TGN, which is presumably where tetherin interacts with the herpes virions. Secondary envelopment in the TGN results in a vesicle containing the mature virion, which is released when the vesicle fuses with the plasma membrane. Nonetheless, both Kaposi's sarcoma-associated herpesvirus and Herpes simplex virus-1 have been shown to be restricted by tetherin (Mansouri et al., 2009; Blondeau et al.,

2013). Surprisingly, tetherin expression actually increases infection rates for another herpes virus, Human cytomegalovirus, which is able to use tetherin as an entry co-factor (Viswanathan et al., 2011). Tetherin restriction by herpes viruses will not be discussed in detail in this paper, since herpes viruses tend to co-evolve with their host (McGeoch et al., 1995), and only two herpesviruses have been characterised in terms of tetherin antagonism.

Tetherin antagonism

Since tetherin interacts with the virus envelope, rather than specific viral proteins, it is not possible for viruses to escape tetherin by mutating certain key proteins. The only option for viruses to escape tetherin restriction is to disrupt tetherin function, either by disrupting upstream regulatory elements for tetherin, or by directly inhibiting the protein itself from functioning.

As demonstrated by the artificial tetherin-like protein constructed by Perez-Caballero et al. (2009), the amino acid composition of tetherin is hardly relevant for its function. This means that tetherin proteins of different species can diverge significantly, without effecting the function of tetherin. The differences in tetherin between different mammals are shown in Figure 3, and present a significant challenge to cross-species transmission. As a result, different enveloped viruses have developed different approaches to antagonise tetherin. This paper focuses on the effect of tetherin on the cross-species transmission of viruses. I therefore only discuss viruses restricted by tetherin that show signs of recent or ongoing cross-species transmission. Influenza virus is not included for several reasons. First, the evidence on the effect of tetherin on influenza virus release is conflicted, with some papers claiming tetherin restricts influenza budding (Yondola et al., 2011; Mangeat et al., 2012), while others cannot find an effect (Watanabe et al., 2011; Winkler et al., 2012). However, the papers that see an effect of tetherin seem to have somewhat higher tetherin expression. Second, human influenza viruses are often derived from influenza viruses that infect birds, which lack a tetherin homolog. Third, the influenza protein NSP1 inhibits the upstream regulatory pathway of tetherin, the interferon response. This means that it is not clear if cells infected by influenza virus are able to express tetherin.

Tetherin antagonism in retroviruses

All known retroviruses are restricted by tetherin (Neil, 2013). This includes both extant retroviruses such as HIV/SIV, but also endogenous retroviruses such as HERV-K (Jouvenet et al., 2009).

Here, I will focus on the most extensively studied group of retroviruses, primate lentiviruses, which includes HIV and SIV. Since all retroviruses are restricted by tetherin, it is not surprising that primate lentiviruses have evolved proteins to counter restriction by the tetherin proteins of their respective hosts. Surprisingly, different lentiviruses use different proteins to antagonise tetherin. HIV-1 uses Vpu, while HIV-2 uses Env, while most SIV use Nef.

To date, over 50 species of SIV that infect different primate hosts have been identified (Greenwood et al., 2013). SIV tends to co-evolve with its host, as is evident by the fact that the phylogeny of most SIV mimics the phylogeny of the host species, rather than the geographical distribution of the different host species (Hahn et al., 2000), see Figure 4. The infection rate in primate populations naturally infected with SIV usually lies around 50% (e.g Santiago et al. (2005)), while complications due to SIV infections are rare in the natural host (Compton et al., 2013).

Most primate lentiviruses use the conserved Nef protein (see Figure 4) to antagonise tetherin (Zhang et al., 2009).

The effect of this protein on the evolution of tetherin is demonstrated by the fact that the site of Nef-tetherin interaction is under the strongest positive selection in Old World Primates (Lim et al., 2010). This indicates that Nef, or another protein with the exact specificity of Nef, has been evolving with Old World Monkeys for a long time.

Nonetheless, not all primate lentiviruses use Nef to antagonise tetherin. Some, including HIV, use Vpu to antagonise tetherin. Unlike Nef, which is conserved across primate lentiviruses, Vpu is found in two related lineages. Vpu arose first in the lineage marked with SIV_{gsn} in Figure 4, which also includes viruses that infect Mona monkeys, Dent's mona monkeys and Mustached monkeys (SIV_{mon}, SIV_{den} and SIV_{mus}, respectively). Each of which uses Vpu to antagonise the tetherin molecules of their respective hosts (Yang et al., 2010).

One of the clinically most important cross-species SIV infections was the formation of SIV_{cpz}, precursor to both SIV_{gor} and HIV-1. SIV_{cpz}, which is part of the group of primate lentiviruses that encode a Vpu protein, arose as a recombinant virus between $\text{SIV}_{gsn/mon/mus}$ (which provided Vpu, tat, rev and Env) and SIV_{rcm} (5' LTR, Nef and 3' LTR) (Sharp and Hahn, 2011). SIV_{cpz} appears to be a stable recombinant virus, with over 30 strains so far that show the same mosaic organisation (Sharp and Hahn, 2011).

Because of its recombinant origin, SIV_{cpz} contains two potential tetherin antagonists: The Vpu from $\text{SIV}_{gsn/mon/mus}$ that could antagonise tetherin from the original host, and SIV_{rcm} Nef, which antagonises tetherin from Red-Capped Mangabey. However, it is unlikely that either SIV_{gsn} Vpu or SIV_{rcm} Nef would be a potent antagonist of Chimpanzee tetherin, due to the fact that most tetherin antagonism by SIV is species specific, and the fact that Chimpanzee tetherin only shares around 78% sequence identity with the tetherin from these hosts.

Eventually, SIV_{cpz} Nef evolved the ability to efficiently counteract tetherin, while SIV_{cpz} Vpu did not gain the ability to antagonise Chimpanzee tetherin (Sauter et al., 2009). The reason why SIV_{cpz} evolved to use Nef instead of Vpu is not clear, although Sauter et al. (2009) speculates it might be due to the fact that Nef interacts with the cytoplasmic tail of tetherin, which is more conserved between Chimpanzee and Red-Capped Mangabeys than the trans membrane region of tetherin, which is where Vpu interacts with tetherin.

Dating the zoonosis event that gave rise to SIV_{cpz} is difficult. Molecular data place the root of SIV_{cpz} around 1500, which is very recent (Wertheim and Worobey, 2009). On the other hand, only two of the four Chimpanzee subspecies

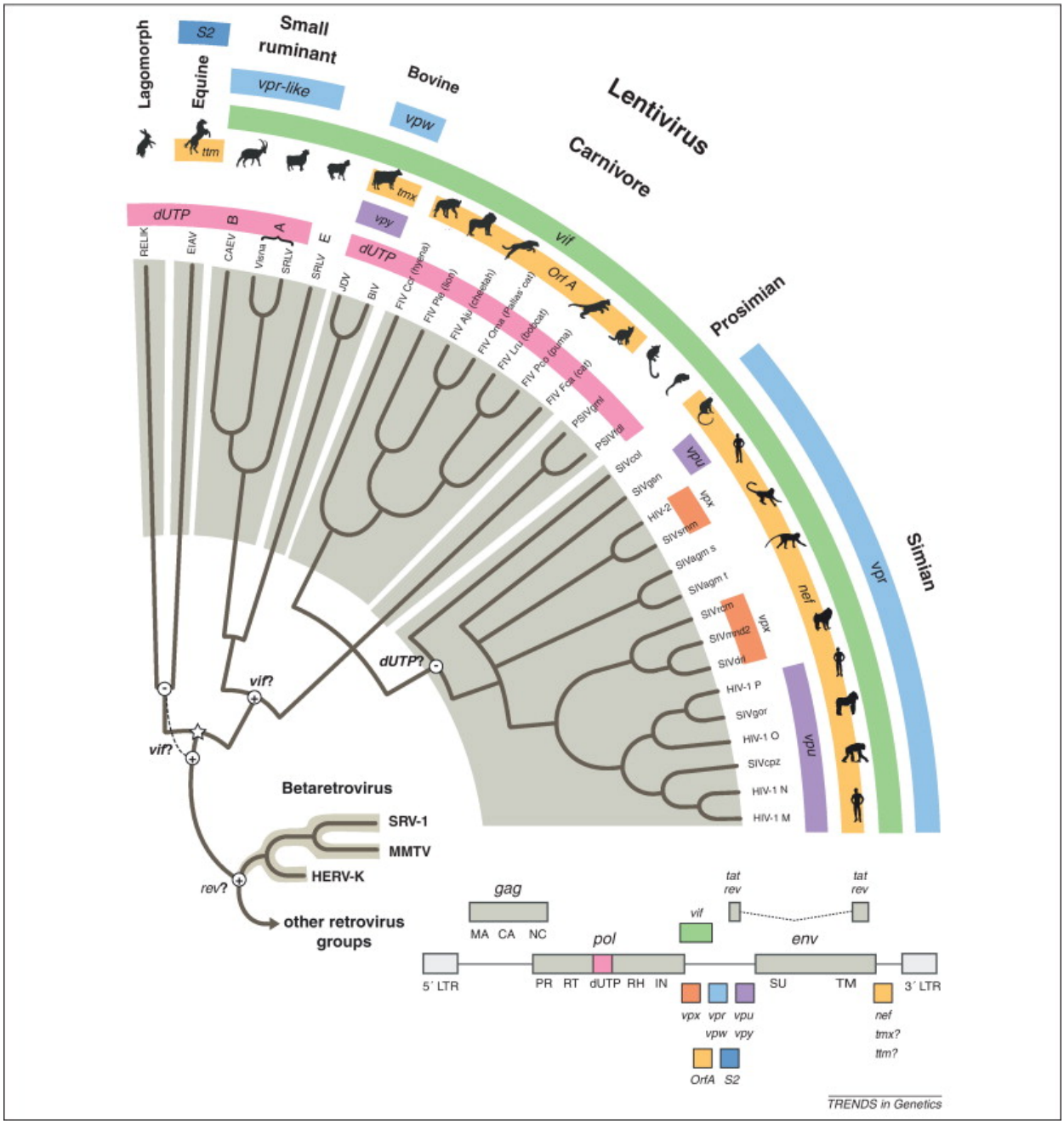

Figure 4: Tree of lentiviruses, taken from Gifford (2012)

(*P.t. troglodytes* and *P.t. schweinfurthii*, Central and Eastern Chimpanzee) are naturally infected with SIV_{cpz}. SIV_{cpz} has not been found in either *P.t. verus*, *P.t. elioti* or bonobo's (*Pan paniscus*). However, the fact that the SIV_{cpz} strains form *P.t. troglodytes* and *P.t. schweinfurthii* cluster independently from each other suggests that SIV_{cpz} is much older (Sharp and Hahn, 2011).

Most of the restriction factors that could potentially prevent SIV_{cpz} from replicating in human hosts, such as APOBEC and TRIM5α, are conserved between Chimpanzee and humans, and do not restrict SIV_{cpz}/HIV (Sauter et al., 2009). One exception is tetherin, which is resistant to SIV_{cpz} Nef due to the deletion discussed before. In the following, I will review different HIV-1 groups and their adaptation to human tetherin.

HIV-1 subgroups

Besides the pandemic HIV-1 group M, three other HIV-1 groups have been identified, each of which is believed to be the result of an independent cross-species transmission event. HIV-1 group M and HIV-1 group N are derived from SIV_{cpz}, while HIV-1 group O and HIV-1 group P are more similar to SIV_{gor} (Mourez et al., 2013). SIV_{gor} is closely related to SIV_{cpz}, with the zoonosis estimated around 100-200 years ago (Takehisa et al., 2009). Even if HIV-1 group O and HIV-1 group P passed through Gorilla first, all four viruses represent very recent cross-species transmission of SIV_{cpz} to humans. The first HIV-1 group to be discovered after the main HIV-1 group M is HIV-1 group O, for 'outlier'. HIV-1 group O was discovered in two individuals of Cameroonian origin who were living in Belgium at the time (De Leys et al., 1990). A third HIV-1 Group was discovered in Cameroon in 1995 by Simon et al. (1998), in a 40 year old Cameroonian woman with AIDS, who died later that same year. Because the virus isolated by Simon et al. (1998) was equally distant from HIV-1 group M and SIV_{cpz}, it was termed HIV-1 group N, for 'Non-M, Non-O'. A fourth group, named HIV-1 group P, was first reported by Plantier et al. (2009), and only two cases have been identified to date.

Although four HIV-1 groups have been identified, only HIV-1 group M is responsible for the world-wide AIDS pandemic. By studying the characteristics and differences of these four HIV-1 groups, we can identify which factors are responsible for the fact that only one of the four viruses caused the global AIDS pandemic. Here, I would like to hypothesise that tetherin is one of the important factors in determining the spread of these viruses in the human population.

HIV-1 group M

Even though SIV_{cpz} uses Nef to antagonise tetherin, this has not been conserved in any of the existing HIV-1 viruses. The reason is a 5 amino acid deletion in the cytoplasmic tail of human tetherin (see Figure 3), which completely abolishes the ability of Nef to antagonise human tetherin (Lim et al., 2010). Instead, the pandemic HIV-1 group M has evolved the ability to antagonise tetherin with its Vpu protein. Vpu binds to the tetherin transmembrane domain (Lopez et al.,

2010), leading to poly-ubiquination (Tokarev et al., 2011) and endolysosomal degradation of tetherin (Agromayor et al., 2012). Surprisingly, blocking the ability of Vpu to induce the ubiquitination of tetherin, by preventing its association with the SCF-ubiquitin ligase complex, did not result in an increased virion production. This suggests that the degradation of tetherin is not required for effective tetherin antagonism by Vpu (Tervo et al., 2011; Neil, 2013). In contrast, blocking the cellular protein UBAP1, part of the endosomal degradation pathway, completely abolished Vpu-mediated tetherin antagonism (Agromayor et al., 2012). This shows that Vpu act by both subverting the normal cycling of tetherin between the plasma membrane and the TGN, while also preventing newly formed tetherin from reaching the plasma membrane.

It is currently not known why Vpu induces tetherin degradation, even though this is not required for efficient tetherin antagonism. Interestingly, the only other known tetherin antagonist that leads to tetherin degradation is the K5 protein of Kaposi's sarcoma associated herpesvirus. Human tetherin is unique among all mammalian tetherins for its ability to induce NFκB signalling, especially upon binding viral particles on the plasma membrane (Galão et al., 2012; Tokarev et al., 2013). This might be the reason why only human tetherin antagonists induce tetherin degradation (Neil, 2013).

HIV-1 group O

It is believed HIV-1 group O crossed from Chimpanzee to humans around the same time as HIV-1 group M, early in the twentieth century (Lemey et al., 2003). The earliest confirmed infection with HIV-1 group O was in the 1960's in Europe, as documented by Jonassen et al. (1997), which shows HIV-1 group O was already in wide circulation long before it was first described. While HIV-1 group O spreads less efficiently than HIV-1 group M, it does cause AIDS in infected individuals (Jonassen et al., 1997). If HIV-1 group O is similar to HIV-1 group M in many respects, including the time of transmission to the human population and ability to cause AIDS, what caused the difference in global spread between these viruses?

HIV-1 group O Nef has been shown to be unable to restrict human tetherin, as was expected. Surprisingly, neither HIV-1 group O Vpu nor any other protein encoded by HIV-1 group O has evolved the ability to antagonise human tetherin (Yang et al., 2011). Despite the lack of activity against tetherin, HIV-1 group O Vpu retains the ability to down-regulate CD4 via its conserved βTrCP motif (Yang et al., 2011).

HIV-1 group O Vpu's lack of tetherin antagonism can be traced to two key factors that differ between HIV-1 group M and HIV-1 group O Vpu. First, HIV-1 group O Vpu localises to the ER, while HIV-1 group M Vpu localises to the TGN (Vigan and Neil, 2011). HIV-1 group M Vpu mutants that localise to the ER loose the ability to antagonise tetherin (Vigan and Neil, 2011), indicating that co-localisation of Vpu and tetherin within the cell is essential for tetherin antagonism. The second difference between HIV-1 group O and HIV-1 group M Vpu is that HIV-1 group O Vpu fails to interact with the trans membrane

region of tetherin. Again, mutating HIV-1 group M Vpu such that it is unable to interact with tetherin abolishes its anti-tetherin activity (Yang et al., 2011). The interaction between HIV-1 group M Vpu and tetherin is mediated by multiple amino acids in their respective trans membrane regions, while TGN localisation is determined by a single residue in the membrane proximal region of the Vpu cytoplasmic domain. Only when both tetherin binding and localisation to the TGN were conferred to HIV-1 group O Vpu was this hybrid protein able to antagonise restriction by tetherin (Yang et al., 2011).

This shows that unlike HIV-1 group M Vpu, which only needed to gain the ability to bind human tetherin, HIV-1 group O Vpu needs mutations in two distinct parts of the protein to become effective against human tetherin. In the case of HIV-1 group M Vpu, it was shown that hybrids between HIV-1 group M Vpu and its most closely related SIV_{cpz} Vpu had intermediate tetherin binding affinity (Lim et al., 2010).

It is surprising that HIV-1 group O Vpu failed to evolve the ability to counteract tetherin, considering the fact that the progenitor virus to SIV_{cpz}, $SIV_{gsn/mon/mus}$, uses Vpu to antagonise the tetherin of their respective hosts. In fact SIV_{gsn} Vpu even has limited activity against human tetherin (Yang et al., 2010).

HIV-1 group N

To date, only 15 infections with HIV-1 group N have been identified, all but one in Cameroon Mourez et al. (2013). Nonetheless, there is evidence for circulation of HIV-1 group N viruses outside of Cameroon: a 57 year old French man has been identified who contracted HIV-1 group N in Togo (Delaugerre et al., 2011). The fact that this virus was contracted in Togo, which does not share a border with Cameroon, makes it likely that HIV-1 group N is in wider circulation in that region.

Despite its limited spread outside of Cameroon and the small number of HIV-1 group N strains that have been identified so far, there is evidence of ongoing adaptation of HIV-1 group N to its human host. For example, HIV-1 group N Vpu's ability to counteracts human tetherin differs markedly between different strains of HIV-1 group N Sauter et al. (2009).While HIV-1 group N strain YBF30 is able to moderately enhance virus release, strains 2693 and CK162 only show very limited anti-tetherin activity. A fourth HIV-1 group N strain, identified in France, showed even stronger tetherin antagonism, comparable with that of HIV-1 group M Vpus Sauter et al. (2012). Surprisingly, however, all HIV-1 group N Vpus have lost the ability to down-regulate CD4 from the cell surface, despite the fact that this function of Vpu is conserved. Unlike the anti-tetherin activity of Vpu, CD4 antagonism is not species specific, so this cannot explain the lack of activity of HIV-1 group N Vpu against human CD4 Sauter et al. (2009).

It is not clear why HIV-1 group N Vpu has lost the ability to down-regulate CD4, since even HIV-1 group N strains that are unable to antagonise tetherin have lost the ability to down-regulate CD4. Furthermore, Sauter et al. (2012)

were able to show that the closest SIV_{cpz} relative of HIV-1 group N, EK505, localises to the TGN, and only requires 4 amino acid changes to become a potent tetherin antagonist, which is still able to down-regulate CD4.Why this has not happened in HIV-1 group N is not clear, but it could be related to the fact that the region coding for these amino acids overlaps with regulatory sequences for Env, which could be under more stringent selection (Sauter et al., 2012).

Whatever the reason for the apparent trade-off between CD4 down-regulation and tetherin antagonism, it is clear that HIV-1 group N is still evolving to escape tetherin based restriction. In that light, it is interesting to note that the first HIV-1 group N to be identified outside of Cameroon is also the most potent tetherin antagonist (Delaugerre et al., 2011; Sauter et al., 2012). This supports the hypothesis that tetherin is an important determinant of the spread of HIV in the human population.

HIV-1 group P

HIV-1 group P is the rarest of the four known HIV-1 groups, with only two infections identified to date. Based on the sequence of these two isolates, Sauter et al. (2011b) estimates that the last common ancestor of these two isolates arose between 1845 and 1989. Like HIV-1 group O, HIV-1 group P is unable to antagonise tetherin via either Nef, Env or Vpu (Sauter et al., 2011a). Surface CD4 down-regulation by both Nef and Vpu is conserved, unlike HIV-1 group N where Vpu lost the ability to down-regulate CD4 (Sauter et al., 2011a). Whether the rarity of P is due to it's recent transmission to humans, a lack of adaptation or other factors is not known, and will remain unclear until more strains of this virus can be isolated.

HIV-2

HIV-1, which is derived from SIV_{cpz}, is not the only primate lentivirus that has crossed from primates to humans in the last 150 years. HIV-2 was first isolated in 1986 in West Africa (Clavel et al., 1986). HIV-2 is derived from SIV_{sm}, a primate lentivirus that infects the Sooty Mangabey. So far, there have been 8 independent cross-species transmission events of SIV_{sm} to humans, which are classified as groups A to H. Of these groups, C-H are believed to be dead end infections, that have only been identified in one or two patients. Zoonosis most likely occurs through the consumption or preparation of bush meat (Chen et al., 1997). The peak of HIV-2 infections occurred approximately 20 year ago, with prevalence in Guinea Bissau of 8% in adults. Since then, the prevalence of HIV-2 has steadily decreased due to replacement by HIV-1 (Sharp and Hahn, 2011).

Unlike HIV-1, most patients infected with HIV-2 do not progress to AIDS, but are able to control the infection (Poulsen et al., 1997). If patients do progress to AIDS, the disease is indistinguishable from AIDS caused by HIV-1. Set point viral load is around 30 fold lower in HIV-2 when compared to HIV-1 (de Silva et al., 2008), and as a result both horizontal and vertical transmission is slow.

SIV_{sm}, the progenitor of HIV-2, uses Nef to antagonise tetherin, which is not active against human tetherin (Jia et al., 2009). Surprisingly, SIV_{sm} Env appears to have a very limited activity against both Sooty Mangabey and human tetherin, although this effect is negligible when compared to the activity of SIV_{sm} Nef against Sooty Mangabey tetherin.

HIV-2 group A uses Env instead of Nef to antagonise human tetherin. The way in which HIV-2 group A Env antagonises tetherin is remarkably similar to the way SIV Nef antagonises tetherin. HIV-2 group A Env binds to tetherin, and removes it from the plasma membrane by sequestering it in the TG N(Le Tortorec and Neil, 2009). Unlike HIV-1 group M Vpu, HIV-2 group A Env does not reduce the total cellular levels of tetherin Sauter et al. (2010), i.e. it does not induce degradation of tetherin. So far, only HIV-2 group A has been characterised in terms of its ability to antagonise tetherin. The ability of the other HIV-2 groups to antagonise tetherin has not been determined. Without information about the other groups of HIV-2, it is unclear how difficult it is to adapt Env, or another protein, to antagonise human tetherin. Based on evidence from HIV-1, one would hypothesise that HIV-2 group A, and possibly HIV-2 group B, have evolved the ability to antagonise tetherin, since these groups have spread considerably within and outside Africa. Due to their lack of spread in the human population, groups C-H are expected to be poor tetherin antagonists, which would explain why these viruses have failed to spread in the human population, despite the fact that they occur in the same geographic region where other primate lentiviral zoonosis have proven to be successful.

Tetherin antagonism in filoviruses

Not all viruses that can infect humans are able to permanently establish themselves in the human population. One such example is the group of related filoviruses that include Marburg and Ebola viruses. Ebola viruses were first discovered in 1976, with two concurrent outbreaks of haemorrhagic fever in Zaire and Sudan (Organization et al., 1978; Cox et al., 1983), which were later shown to be due to the Zaire and Sudan Ebola virus, respectively. While the case fatality rate of Ebola virus infection is extremely high, up to 90% (Feldmann and Geisbert, 2011), outbreaks tend to be self-limiting. Most infections occur in rural hospitals, due to the lack of isolation of infected patients or reusing unsterilised needles (Peters and LeDuc, 1999). Since 1976, there have been regular outbreaks of Ebola, which typically infect no more than 100 people before dying out again. Despite repeated outbreaks, ebola viruses have not established themselves in the human population. The identity of the reservoir species for Ebola has long been a mystery, but has recently been identified to be fruit bats (Leroy et al., 2005).

Infected fruit bats shed Ebola virus in faeces, which provides a plausible route of transmission to other species. Ebola virus outbreaks are unique in that multiple species become infected during each outbreak of the disease, apparently without the need of the virus to adapt to the different hosts (Leroy et al., 2004). In fact, most Ebola outbreaks do not begin in humans, but in other species such

as Gorilla, Chimpanzee or Duiker, a type of deer (Leroy et al., 2004). Ebola virus infection has a high mortality rate in these species, and Ebola outbreaks in humans are often preceded by increased mortality in local wildlife (Leroy et al., 2011; Bermejo et al., 2006). The most common route of Ebola into the human population is by bush-meat hunters that find carcasses of animals that were infected by Ebola (Leroy et al., 2004).

Unlike most viruses, filoviruses are filamentous rather the spherical, and encode a single strand negative sense RNA genome of approximately 19kb bases. Ebola virus particles are enveloped, and are thus potentially restricted by tetherin. The first evidence for tetherin restriction of filoviruses came when it was shown that Ebola virus like particles were restricted by tetherin (Radoshitzky et al., 2010). The same study showed that full virions were not restricted by tetherin, suggesting that Ebola viruses encode a tetherin antagonist.

Ebola GP can antagonise restriction by human tetherin (Kaletsky et al., 2009). Unlike other tetherin antagonists, Ebola GP does not reduce the total cellular levels of tetherin, nor does it remove tetherin from the cell surface (Kühl et al., 2011). Lopez et al. (2010) was able to show that Ebola GP also does not change the localisation of tetherin within the plasma membrane, where it stays associated with lipid rafts, which are the site of viral budding for many viruses, including HIV and Ebola (Lopez et al., 2010). At this point, it is not known by which mechanism Ebola GP is able to antagonise tetherin. The importance of tetherin restriction is shown by the fact that this function of GP is conserved in all four Ebola viruses and in the GP of the related Marburg virus, despite the fact that Ebola and Marburg GP only share 28% amino acid sequence identity.

Not only is the tetherin antagonism of GP conserved, it is also the most broadly acting tetherin antagonist that has been identified to date. Ebola GP restrict the activity of human tetherin, but it is also active against tetherin from other primates, and even murine tetherin (Lopez et al., 2010). Even more surprising is the finding by Lopez et al. (2010) that Ebola GP can antagonise the completely artificial tetherin-like molecule constructed by Perez-Caballero et al. (2009). The activity of Ebola GP against bat tetherin has not yet been characterised, but the broad activity of GP suggests that it should be active against the tetherin homolog encoded by the reservoir species.

The broad tetherin antagonism of Ebola viruses is accompanied by a very broad host range. Ebola viruses have been shown to infect bats, the proposed reservoir species, but also humans, chimpanzees, Gorillas and other primates, as well as pigs and dogs (Olson et al., 2012).

Discussion

It is now known that many enveloped viruses can be restricted by tetherin, and that most of these have evolved proteins to counteract this restriction. This applies both to short-lived infections such as influenza and Ebola, as well as to viruses that establish chronic infection, such as HIV/SIV, or herpesviruses such as KSHV and Herpes simplex virus 1. The fact that these diverse viruses have

independently evolved the ability to antagonise tetherin shows the importance of this restriction factor on viral fitness.

Tetherin is an important restriction factor that inhibits the release of many retroviruses, including lentiviruses. To counteract this restriction, primate lentiviruses encode proteins to antagonise tetherin. However, these tetherin antagonists tend to be host-species specific. As a result, tetherin acts as a barrier against the transmission of these viruses between different host-species.

One of the most important cross-species transmission events in the last century was the transmission of SIV_{cpz} to humans, giving rise to HIV-1. SIV_{cpz} uses Nef to counteract Chimpanzee tetherin, but is ineffective against human tetherin. Of the four zoonosis events from $SIV_{cpz/gor}$ to human, only the pandemic HIV-1 group M has evolved the ability to potently antagonise tetherin. HIV-1 group O and HIV-1 group P are unable to antagonise tetherin, despite the fact that HIV-1 group O entered the human population around the same time as HIV-1 group M. The activity of HIV-1 group N Vpu against tetherin differs between strains, and HIV-1 group N Vpu has lost the ability to antagonise CD4 with Vpu altogether. It is, however, interesting to note that the only HIV-1 group N strain identified so far that has spread outside Cameroon encodes a Vpu that is a potent tetherin antagonist. These data confirm the hypothesis that tetherin is an important factor in determining the successful spread of HIV. The importance of tetherin is underscored by the fact that the non-pandemic HIV-1 groups are not otherwise poorly adapted to humans. Both HIV-1 group N and HIV-1 group O replicate efficiently in human cells, and cause AIDS in infected individuals. The clinical outcome of HIV-1 group P infection is not known, since only two HIV-1 group P viruses have been identified to date.

The case of HIV-2 is less clear, since only HIV-2 group A, the most successful group, has been tested for tetherin antagonism. Nonetheless, it is telling that HIV-2 group A evolved the ability to counteract tetherin, while remaining susceptible to other restriction factors such as TRIM5α(Takeuchi et al., 2013). The fact that the most widespread HIV-2 group has evolved the ability to counteract tetherin but not other important restriction factors, shows the importance of restriction by tetherin. Further research on the anti-tetherin capabilities of other HIV-2 groups is needed to draw stronger conclusions on the effect of tetherin on the spread of HIV-2.

Unlike HIV, Ebola viruses have not established themselves in the human population. Rather, each Ebola outbreak is believed to be the result of a cross-species transmission, based on the fact that most Ebola outbreaks are preceded by an increase in wildlife mortality (Leroy et al., 2004). Ebola and Marburg viruses encode a conserved, extremely broad acting tetherin antagonist. The Ebola/Marburg Glycoprotein (GP) can counteract human, primate and murine tetherin, despite both considerable differences between Ebola and Marburg GP (28% amino acid identity) and differences between human and murine tetherin (45% amino acid identity). Consistent with its broad-acting tetherin antagonist, Ebola viruses can infect a broad range of hosts, including bats, humans, apes and monkeys, but also pigs and dogs.

In the case of HIV/SIV, successful cross species transmissions are rare, and

only viruses that are able to restrict the tetherin proteins of their new host are able to spread successfully. In the case of Ebola, which can restrict the tetherin proteins of a wide range of hosts, cross species transmission to many diverse species is frequent. I therefore conclude that tetherin is an important factor limiting the success of cross-species transmission of enveloped viruses.

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