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Selective downmodulation of HLA-A and -B by Nef alleles from different groups of primate lentiviruses

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Abstract

It has been demonstrated that the HIV-1 NL4-3 and IIIB Nef alleles downregulate HLA-A and -B but not -C or -E from the cell surface. It remained elusive, however, whether selective modulation of specific HLA molecules is conserved between different groups of human and simian immunodeficiency viruses, respectively. To address this, we analyzed a large panel of primate lentiviral Nef proteins and we found that this property is conserved among *nef* alleles from the M, N and O groups of HIV-1, as well as those from SIVcpz, the precursor of HIV-1, and a variety of other highly divergent primate lentiviruses. In conclusion, our data indicate that Nef's ability to selectively downregulate HLA-A and -B alleles to prevent CTL lysis and NK killing of virally infected cells is conserved among different primate lentiviral lineages and preceded the zoonotic transmission of SIVcpz from chimpanzees to humans.

Keywords: HIV; SIV; HLA; Alleles; Nef; CTL lysis; NK killing

Introduction

Downmodulation of class I major histocompatibility complex molecules (MHC-I) is one of the best established functions of the accessory Nef protein of primate lentivirures (Akari et al., 2000; Le Gall et al., 1997, 2000; Schwartz et al., 1996). It has been demonstrated that Nef-mediated removal of MHC-I from the cell surface protects HIV-1-infected cells against killing by cytotoxic T lymphocytes (CTLs) (Collins et al., 1998; Yang et al., 2002). Nef alleles isolated from HIV-1-infected individuals early in infection are frequently more effective in MHC-I downmodulation than those obtained from late stage AIDS patients (Carl et al., 2001), supporting an important role of Nef-mediated immune evasion in the progression to AIDS (reviewed in Collins and Baltimore, 1999; Desrosiers, 1999; Doms and Trono, 2000). Experiments with specific simian immunodeficiency virus (SIV) Nef mutants demonstrated that efficient MHC-I downmodulation is associated with a strong selective advantage in infected rhesus macaques (Münch et al., 2001; Swigut et al., 2004), confirming that this function is important for viral immune evasion and effective persistence *in vivo*.

Multiple studies have analyzed the mechanism(s) of Nef-mediated downmodulation of MHC-I. Altogether, they suggest that Nef binds to the cytoplasmic tail of MHC-I (Williams et al., 2002) and utilizes at least two pathways to reduce its expression on the cell surface: (i) recruitment of AP-1 to the MHC-I cytoplasmic tail to re-route MHC-I from the trans-Golgi network (TGN) to lysosomes and (ii) endocytosis of MHC-I from the plasma membrane to the TGN in a PACS-1, AP-1 and clathrin-dependent manner (Blagoveshchenskaya et al., 2002; Kasper and Collins, 2003; Kasper et al., 2005; Larsen et al., 2004; Le Gall et al., 1998; Lubben et al., 2007; Mangasarian et al., 1999; Piguet et al., 2000; Roeth et al., 2004). However, the exact

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mechanisms and the relative contribution of these pathways to MHC-I downmodulation are controversial (reviewed in Roeth and Collins, 2006). Moreover, the magnitude of the effects may be cell type dependent, possibly because Nef inhibits export and increases turnover of MHC-I in HIV-1-infected T cells but mainly affects endocytosis in other cell types commonly used to study Nef function, such as HeLa-derived cell lines (Kasper and Collins, 2003; Kasper et al., 2005).

Reduced cell surface levels of MHC-I are beneficial for HIV-1 in avoiding CTL recognition but could increase the susceptibility of virally infected cells to lysis by natural killer (NK) cells. An elegant study by Cohen et al. (1999), however, has demonstrated that the HIV-1 Nef protein protects infected cells against both CTLs and NK cells by affecting MHC-I cell surface expression in a selective manner, i.e. it specifically downmodulates HLA-A and -B but not HLA-C or -E alleles. This selectivity most likely allows HIV-1 to balance escape from CTL lysis with protection from NK attack and is presumably important for effective viral immune evasion and persistence. It has been established that a large variety of HIV-1, HIV-2 and SIV Nef alleles downmodulate human MHC-I from the surface of infected T cells (Kirchhoff et al., 2004; Münch et al., 2005; Schindler et al., 2006). Selective downmodulation of specific HLA molecules, however, has only been demonstrated for *nef* alleles of the T cell line adapted HIV-1 group M subtype B NL4-3 and IIIB strains (Adnan et al., 2006; Cohen et al., 1999; Williams et al., 2002).

Results and discussion

To assess whether selective downmodulation of HLA-A and HLA-B is conserved between different groups of HIV-1 and other primate lentiviruses, we generated Jurkat T cell lines stably expressing the ectodomain of the CD8α chain fused to the cytoplasmic domains of HLA-A, -B, -C or E. The predicted amino acid sequences for the cytoplasmic domains of these HLA alleles and the position of the Y320, A323 and D327 residues in HLA-A and -B, known to be critical for Nef interaction (Cohen et al., 1999; Williams et al., 2002), are indicated in Fig. 1. A total of 23 HIV-1 and SIV *nef* alleles was selected for functional analysis based on their species-origin and phylogenetic relationship (Schindler et al., 2006). This set encompassed the HIV-1 M and N groups, which arose from independent transmissions of SIVcpz from the *Pan troglodytes troglodytes* (*P.t.t.*) subspecies of chimpanzees to humans (reviewed in Hahn et al., 2000); HIV-1 group O, that is most closely related to SIVs found in gorillas

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          310        320        330        340
          |          |          |          |
Cons      RRKSSGGKGGSYSQAASSDSAQGSDVSLTACKV
HLA-A     .....DR.....................
HLA-B     ..............................
HLA-C     ...........C......N......E..I...A
HLA-E     .K..........K.EW.......ESHSL
Patr-E    .K.......R..K.EW....
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Fig. 1. Alignment of MHC-I cytoplasmic domain sequences. Dots indicate amino acid identity and the positions critical for Nef interaction are shaded (6, 32). The numbering corresponds to the amino acid position in HLA-Cw4 (6).

(Van Heuverswyn et al., 2006); SIVcpz from both *P.t.t.* and the *Pan troglodytes schweinfurthii* (*P.t.s.*) subspecies that has not been found in humans (Hahn et al., 2000), and a variety of SIVs from different primate lentiviral lineages. Notably, the great majority of SIVcpz *nef* alleles were obtained directly from uncultured chimpanzee material, such as the spleen (US), PBMC (GAB2 and Ch-Ni), plasma (Ch-No) and feces (TAN1, TAN2 and TAN3) (Kirchhoff et al., 2004; Takehisa et al., 2007). Thus, they do not contain changes representing adaptation to human cells.

To examine whether residues proposed to be critical for MHC-I downmodulation are conserved, we aligned the amino acid sequences of the Nef alleles selected for detailed analysis. The alignment revealed substantial variation in the M20 residue, the acidic region and the proline-rich motif, which have been implicated in MHC-I downmodulation by HIV-1 Nef (Blagoveshchenskaya et al., 2002; Greenberg et al., 1998; Mangasarian et al., 1999). For example, M20 (numbering corresponds to the position in the NL4-3 Nef) in the amino-terminal-proximal α-helix is conserved in HIV-1 Nefs but not in those of SIV (Fig. 2). The acidic region (EEEE65), proposed to represent a PACS-1 binding site (Piguet et al., 2000), is conserved in HIV-1 group M and N Nefs but variable in all remaining Nef sequences, although a minimum of two negatively charged residues is always preserved at this location (Fig. 2). It is controversial whether PACS-1 is required for Nef-mediated rerouting of MHC-I to the trans-Golgi network (Blagoveshchenskaya et al., 2002; Lubben et al., 2007; Piguet et al., 2000). The proline-rich motif $(PXXP)_3$ implicated in binding of cellular kinases and MHC-I modulation (Greenberg et al., 1998; Yamada et al., 2003) is preserved in HIV-1 and SIVcpz Nefs. However, the last proline is changed to aspartic acid or glutamine in many SIV Nefs (Fig. 2). Similarly, a thioesterase binding site (FPD123), known to be critical for multiple HIV-1 Nef functions including MHC-I downmodulation (reviewed in Ref. Roeth and Collins, 2006), is conserved in SIVcpz but altered in the remaining SIV Nef sequences. Thus, the domains previously implicated in MHC-I downmodulation and binding by HIV-1 Nef show substantial variation among the HIV-1 and SIV Nef sequences investigated.

In agreement with the results of previous studies (Kirchhoff et al., 2004; Münch et al., 2005; Schindler et al., 2006), the great majority of HIV-1 and SIVcpz Nef alleles efficiently downregulated MHC-I (examples shown in Fig. 3A, upper panel). Our analysis demonstrated that all HIV and SIVcpz *nef* alleles specifically downmodulate CD8-HLA-A and -B, but not CD8-HLA-C or -E fusions (Figs. 3A, B). This effect was Nef-specific because infection with otherwise isogenic reporter viruses containing disrupted *nef* genes did not affect MHC-I or CD8-HLA fusion expression levels (Fig. 3A, lane 2). Unexpectedly, the HIV-1 O 8161 Nef allele moderately upregulated CD8-HLA-C surface expression (Figs. 3A, lane 5 and B). To quantify the efficiency of receptor modulation, the red mean fluorescence intensity (MFI) of cells infected with the *nef* defective control virus was divided by the MFI of cells infected with reporter viruses coexpressing Nef and eGFP. In agreement with the results of Cohen et al. (1999), the NL4-3 Nef downmodulated CD8