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ORIGINAL PAPER

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Polymorphism of human and primate *RANTES*, *CX$_3$CR1*, *CCR2* and *CXCR4* genes with regard to HIV/SIV infection

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Abstract Among genes that influence human susceptibility to HIV (human immunodeficiency virus) infection or AIDS (acquired immunodeficiency syndrome) progression, chemokine-receptor and chemokine genes were extensively studied because of their role as HIV co-receptors or co-receptor competitors, respectively. We have studied in non-human primates (chimpanzee, gorilla, gibbon, orang-utan, crab-eating and rhesus macaque, baboon and marmoset) the *RANTES*, *CCR2* and *CX$_3$CR1* gene sequences in regions surrounding human mutations that were associated with susceptibility to HIV or AIDS progression: *RANTES* G–403A and C–28G, *CCR2* V64I, *CX$_3$CR1* V249I and *CX$_3$CR1* T280M. Among these five dimorphisms, only *RANTES* G–403A is observed in one of the eight primate species studied here (gibbon). This suggests that these mutations appeared recently in humans and probably do not account for variable HIV/SIV disease progression in primates. It is noteworthy that chimpanzees, which are naturally resistant to HIV-1- and HIV-2-induced AIDS, do not have the human mutations associated with delayed disease progression. Inter-species and intra-species polymorphic positions are observed in primates and we discuss the potential impact of these mutations on HIV/SIV disease progression. Particularly, we identified polymorphisms in old-world monkey (OWM) genes, and it could be of great importance to analyse the possible association between these polymorphisms and disease progression in OWM species that are currently used in research for HIV vaccine and therapy.

Keywords Co-receptors · Chemokines · HIV · Primates · Polymorphism

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Introduction

African primates such as chimpanzee, sooty mangabey, African green monkey (AGM), Sykes mangabey and l'Hoest monkey are natural hosts for simian immunodeficiency viruses (SIV) (de Beer et al. 1999; Hirsch et al. 1995). Virus sequence comparison and phylogenetic studies have demonstrated that each of these primate species is infected by a monophyletic virus lineage (SIV_{cpz}, SIV_{sm}, SIV_{agm}, SIV_{syk} and SIV_{lhoest}). Co-evolution of animal species and their respective virus lineage leads in each case to a non-pathogenic persistence of the infection (de Beer et al. 1999). Some African species, such as baboons, lacked their own strain of SIV but have become naturally infected from AGM and remain healthy when infected with SIV_{agm} (de Beer et al. 1999). As for Asian species of primates, such as macaques and gibbons, and new-world monkeys (NWMs), the presence of species-specific SIV was not apparent (de Beer et al. 1999).

By contrast to the non-pathogenicity of primate lentiviruses for their natural host, cross-species infections, either natural or experimental, lead to pathogenic infection evolving toward AIDS (acquired immunodeficiency syndrome). In humans, HIV-1 (human immunodeficiency virus) and HIV-2 epidemics arose through cross-species transmission of SIV from chimpanzee (HIV-1) and sooty mangabey (HIV-2) (Gao et al. 1999; Korber et al. 2000). The origin of the main HIV-1 group in the human population was estimated to have occurred during the first half of the 20th century (around 1930), and cross-species transmission from chimpanzee to man probably occurred

earlier (Foley 2000; Korber et al. 2000), because from sequence comparison the common ancestor of the HIV-1 group and the SIV_{cpz} dates to the late 17th century (Foley 2000).

The transfer of HIV-1 and HIV-2 viral ancestors from chimpanzees and mangabeys to humans resulted in a critical change of virulence, as the natural simian hosts do not develop AIDS (Chakrabarti et al. 2000; Gougeon et al. 1997; Kaur et al. 1998; Weiss 2001). In the same way, experimental transfer of virus strains from one naturally infected species to another free of the strain resulted in a change of virulence (Barnett et al. 1994; de Beer et al. 1999; Kaur et al. 1998). Experimentally HIV-2-infected baboons and SIV_{sm}-infected macaques develop AIDS-like symptoms (Barnett et al. 1994; Kaur et al. 1998). As in the course of human HIV infection, symptoms and rate of disease progression can vary from one animal to another (Holterman et al. 2000; Locher et al. 1998). Recent studies reported that a very small number of chimpanzees experimentally infected by a recombinant HIV strain could progress toward AIDS (O'Neil et al. 2000; Villinger et al. 1997). Recently, marmosets were shown to be resistant to experimental HIV-1 infection (LaBonte et al. 2002). The follow-up of experimentally infected gibbons was not long enough to detect AIDS-like symptoms (Lusso et al. 1988).

Therefore, there is both inter-species and intra-species variability in primate sensitivity to lentivirus-induced AIDS (Barnett et al. 1994; Benveniste et al. 1996; Chakrabarti et al. 2000; Gougeon et al. 1997; Kaur et al. 1998; Lusso et al. 1988). Molecular mechanisms of resistance to AIDS progression in humans and non-human primates have not yet been fully elucidated. In humans, a small fraction (<5%) of HIV-infected people do not develop AIDS in the absence of treatment and are called long-term non-progressors (LTNP). Comparison of human LTNP and progressors revealed that multiple genetic factors such as MHC (major histocompatibility complex), α-defensin genes, chemokine and chemokine-receptor genes influence the rate of disease progression (Rowland-Jones et al. 2001; Zhang et al. 2002). In this study, we focused our interest on chemokine and chemokine-receptor polymorphisms in primates.

Indeed, infection of human and non-human primate cells by HIV and SIV requires not only the presence of the main virus receptor CD4 but also a co-receptor which corresponds to one of various chemokine receptors (Chen et al. 1997; Dragic et al. 1996; Feng et al. 1996; Kristiansen et al. 1998; Moore et al. 1997; Owen et al. 2000). Natural ligands of these chemokine receptors compete, at least in vitro, with HIV/SIV in binding to the receptor. Clinical studies in humans have demonstrated that mutations involving chemokine-receptors (CCR5, CCR2, CX_3CR1) or their natural ligands [SDF1 (stromal cell-derived factor-1 and RANTES (regulated on activation, normal T-cell expressed and secreted)] influence the susceptibility to HIV infection and/or the rate of progression toward AIDS (Faure et al. 2000; McDermott et al. 2000a; Samson et al. 1996; Smith et al. 1997; Winkler et al. 1998).

We report here a sequence comparison of chemokine and chemokine-receptor genes in primates, in species representative of African and Asian apes, old-world monkeys (OWMs) and NWMs.

Materials and methods

Animals

Blood samples from ten chimpanzees (*Pan troglodytes troglodytes),* ten crab-eating macaques (*Macaca fascicularis)*, five rhesus monkeys (*Macaca mulatta*), three baboons (*Papio papio)* and eight marmosets (*Calithrix jaccus*) were obtained from the Laboratory for Experimental Medicine and Surgery In Primates (LEMSIP, New York University, New York, USA). Ten gorilla (*Gorilla gorilla)* blood samples were obtained from the CIRMF (Centre Internationnal de Recherche Médicale de Franceville, Franceville, Gabon), one orang-utan (*Pongo pygmaeus)* blood sample from the zoo of Saint Martin La Plaine, France, and two gibbon (*Hylobates lar)* blood samples from the zoo of Nay, France.

For comparison, various sequences were obtained from databanks. Access numbers are given in figure legends.

PCR and sequencing

Animal genomic DNA samples, purified from peripheral blood, were amplified by polymerase chain reaction (PCR) using pairs of primers described previously (Faure et al. 2000; Liu et al. 1999; Smith et al. 1997). Forward and backward primers were as follows (5′→3′ orientation):

- TTGCACATTGCATTCCCAAAGACCC and GGATTGAACAAGGACGCATTTCCCC for *CCR2*,
- GAGGTCCTCCAGGAAATCTGGCCCGTG and AGACACAAGGCTTTGGGATTC for *CX_3CR1*,
- GACCCTCCTCAATAAAAC and GCGCAGAGGGCAGTAGCAA for *RANTES*,
- ACTACACCGAGGAAATGGGCTC and TGTCCACCTCGCTTTCCTTTG for *CXCR4*.

PCR products were purified by agarose-gel electrophoresis and sequenced on a 373 Applied Biosystems sequencer. When the analysis of sequences revealed possible heterozygosity, amplicons were cloned (Topo TA cloning kit; Invitrogen, Cergy Pontoise, France) and at least 20 clones were sequenced to determine both allelic sequences.

Comparison of sequences

Sequences were aligned using Cluster W (Mac Vector package).

For designation of human mutations in the text, the base or amino acid observed in the more frequent allele is given first, followed by the position number and then the base or amino acid observed in the less frequent allele.

Results

Inter-species polymorphism

RANTES

As two mutations (G–403A and C–28G) in the promoter region of *RANTES* were shown to influence HIV disease progression in humans, we analysed the homologous

Fig. 1 Comparison of sequences of the *RANTES* promoter region (from −443 to +104). *Man*: human sequence of *RANTES*; numbers indicate nucleotide positions relative to the major transcription start site (Liu et al., 1999); *Gogo*: *Gorilla gorilla*; *Patr*: *Pan troglodytes*; *Popy*: *Pongo pygmaeus*; *Hyla*: *Hylobates lar*; *Mamu*: *Macaca mulatta*; *Mafa*: *Macaca fascicularis*; *Papa*: *Papio papio*; *Caja*: *Callithrix jacchus*. Names of species are followed by a number when more than one allele was characterised. *Minus* or *plus* above position numbers corresponds to position before and after the major transcription site, respectively

region (547 bp) in eight non-human primate species. Primers based on the human sequence allowed the amplification of the *RANTES* promoter in all primates but marmosets (Fig. 1). All non-human primates studied presented an A at position −403, as in the less frequent human allele. At position −28, apes have a C, similar to the more frequent human allele, while OWMs (*Macaca mulatta, Macaca fascicularis and Papio papio*) have a T, which is different from the two human alleles. Consequently, the haplotype [*A−403; C−28*] is shared by all apes (gorilla, chimpanzee, orang-utan, gibbon) but is absent from OWMs, which displayed a [*A−403; T−28*] haplotype.

Other mutations distributed widely along the analysed region were found in the seven species, some of them leading to length polymorphisms (Fig. 1): gibbons had a duplication of 13 bp between positions 25 and 26; OWMs exhibited a 2-bp insertion between positions 28 and 29; rhesus monkeys had a 7-bp deletion between positions −424 and −430.

OWMs present numerous mutations in the *RANTES* promoter region compared with humans. Some of these mutations occur on transcription factor binding sites: C−339T, C−330A on AP-1 binding sites; T−220G, T−221G and A−222G on NF-AT binding sites; G−11A on NF-IL-6 binding sites and A−36G, G−34A on NF-kb binding sites. By contrast, these transcription-factor binding sites are highly conserved in apes, except NF-kb binding sites in gorilla, gibbon and orang-utan (A−36G). The positions of the CAAT and TATA boxes were conserved among all studied species.

CX_3CR1

In man, two mutations in the *CX_3CR1* coding sequence, referred to as V249I and T280M, have been recently described and their association with HIV disease progression still remains controversial (Faure et al. 2000; McDermott et al. 2000b). At position 280, all primates displayed a threonine codon, identical to the most frequent human allele (ACG for Thr_{280}) (Fig. 2).

At codon 249, ape and OWM genes are identical to the most frequent human allele (GTT for V_{249}). Only marmoset exhibited an isoleucine codon (ATT), as for the least frequent human allele (Fig. 2c).

Other primate species displayed non-conservative amino-acid changes located in the carboxy-terminal domain of the protein (positions 326 and 334), in the second and the third extracellular loops (positions 195 and 270, respectively) (Fig. 5).

CCR2

The *CCR2*-V64I mutation is associated with delayed HIV disease progression in humans. All primate species studied harboured a valine at position 64, as in the more frequent human allele.

By reference to humans, OWMs have ten mutations in common, one in the promoter region and nine in the coding region (seven synonymous and two non-synonymous) (Fig. 3). One non-synonymous mutation leads to a conservative amino-acid change at position 75, in the first intracellular loop of CCR2. The other one leads to amino-acid changes in OWMs (E→G) and also in gibbons (E→D), in the amino-terminal domain of CCR2 (position 15). It can be noted that gibbons exhibited four amino-acid changes compared with humans (positions 15, 54, 104 and 105); only the mutation at position 105 is non-conservative.

CXCR4

As numerous *CXCR4* coding sequences, except crab-eating macaque sequences, were available in the databanks, we just sequenced *CXCR4* of ten crab-eating

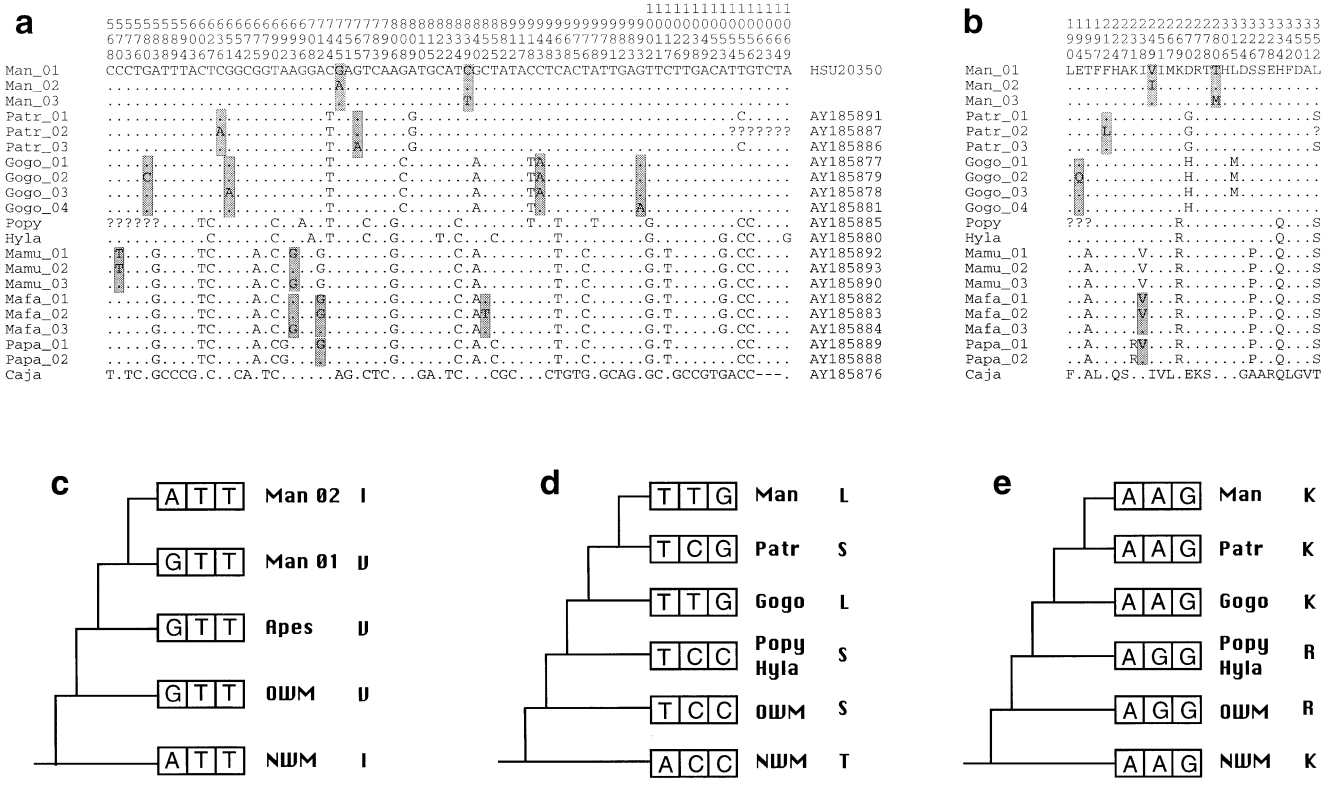
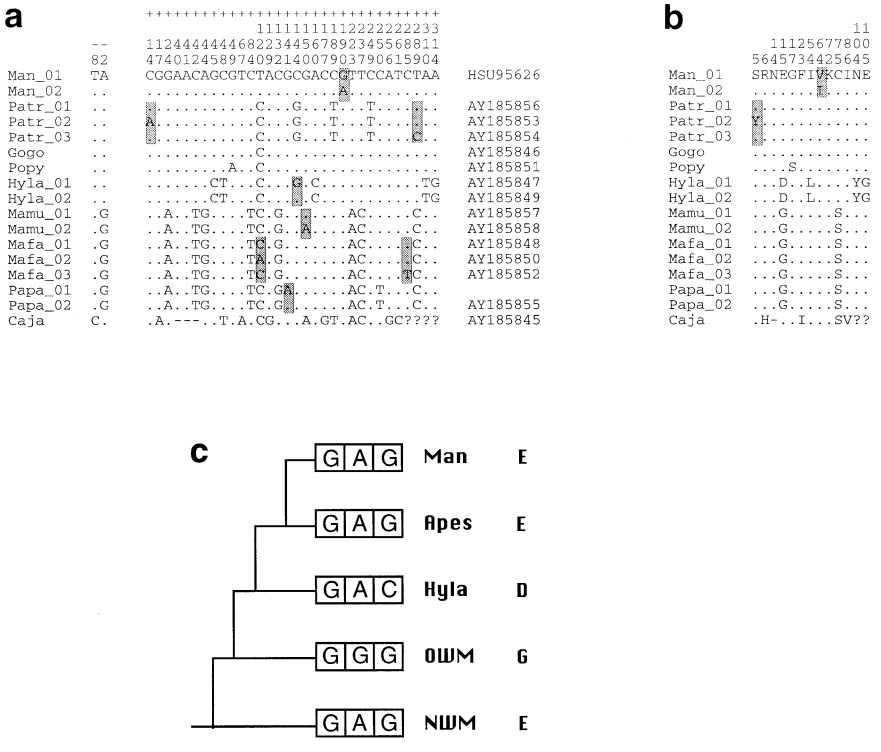


Fig. 2a–e Sequence analysis of the *CX₃CR1* gene. **a** Comparison of gene sequences from nucleotides 568 to 1,071, counting from the ATG initiation codon. **b** Comparison of protein sequences. **c–e** Evolution of the *CX₃CR1* gene at codons 249, 269 and 352, respectively

Fig. 3a–c Sequence analysis of the *CCR2* gene. **a** Comparison of gene sequences from nucleotides −14 to +316, counting from the A of ATG initiation codon. *Minus* or *plus* above position numbers corresponds to position before and after the initiation codon, respectively. **b** Comparison of protein sequences. **c** Evolution of *CCR2* gene at codon 15



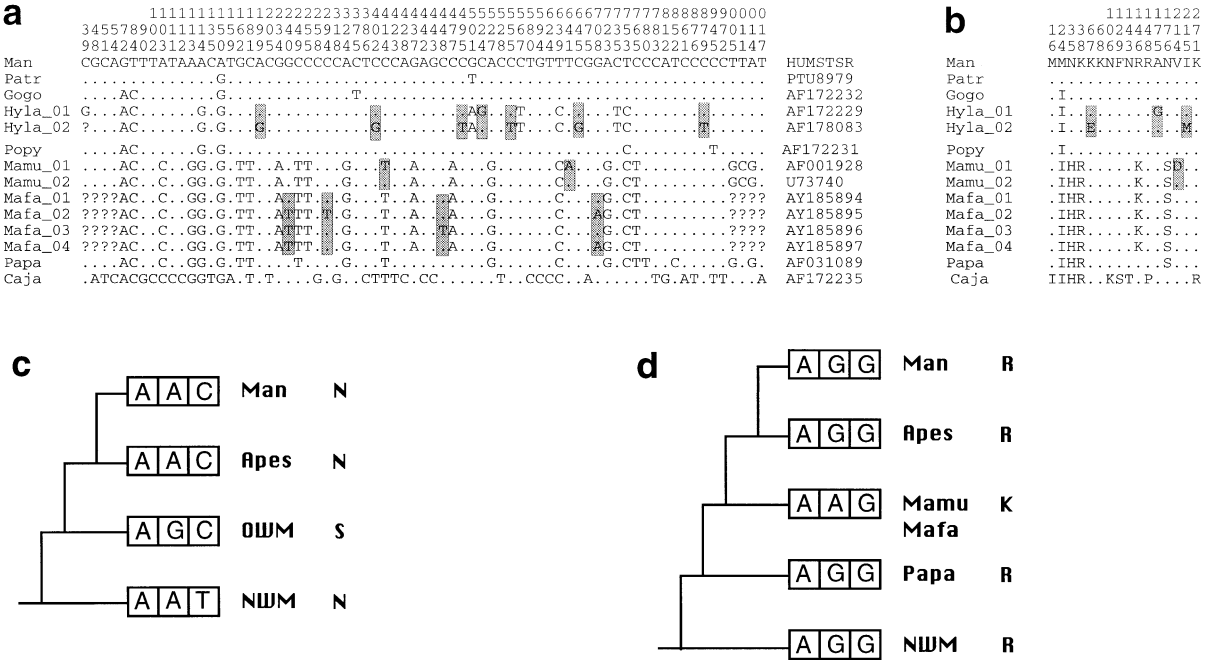


Fig. 4a–d Sequence analysis of the *CXCR4* gene. Sequences were obtained from the literature (see accession numbers) except *Macaca fascicularis* sequences (nucleotides 69–989). **a** Comparison of gene sequences from nucleotides 1 to 1,058, counting from ATG initiation codon. **b** Comparison of protein sequences. **c, d** Evolution of *CXCR4* gene at codons 146 and 176, respectively

macaques (920 bp) and aligned these sequences with those from the literature corresponding to species involved in our study (1,059 bp, see Fig. 4). At position 24 on the human protein, chimpanzee harboured a methionine, while all other primate species had an isoleucine (Fig. 4b). OWMs shared three amino-acid changes in comparison with humans and apes (positions 35, 38 and 176, Fig. 4b), one of them being non-conservative (position 35). Marmoset also displayed the two mutations at positions 35 and 38. The amino-acid changes at positions 24, 35 and 38 are located in the amino-terminal domain of the receptor, and the mutation at position 176 is located in the second extracellular loop of CXCR4 (Fig. 5).

Interestingly, some inter-species polymorphic positions are discordant with species phylogenesis and several reversions can be observed.

At *CX_3CR1* codon 249, human, ape and OWM alleles possess a valine (Val=GTT), while marmosets have an isoleucine (I=ATT), as for the least frequent human allele (Fig. 2c). Consequently, there are two hypotheses: either the common ancestral codon is GTT and two independent mutations, G to A, occur in marmoset and human, or the ancestral codon common to all primates studied here is ATT. In that case, one human allele presented a reversion from the ancestral codon (GTT to ATT).

At position 352, on the carboxy-terminal domain of the CX_3CR1 receptor, three amino acids were observed: humans and gorillas both exhibited a leucine; chimpanzees, apes from Asia (gibbon and orang-utan) and OWMs displayed a serine. Only marmosets had a threonine. It is noteworthy that the serine codon of chimpanzee differs from the one shared by OWMs, gibbons and orang-utans (Fig. 2d). The chimpanzee gene most probably presents a back mutation in the second base of the 352 codon (T to C).

At position 269, on the third extracellular loop, human, chimpanzee, gorilla and marmoset displayed the same codon, which differs from the OWM, gibbon and orang-utan one (Fig. 2e). One hypothesis is that the common ancestral codon is AAG and one point mutation occurs in OWMs, gibbon and orang-utan.

The same phenomenon is observed at position 15 of CCR2, where the common ancestral codon is GAG, and OWMs and gibbons presented point mutations (A→G and G→C, respectively).

At position 176 of the CXCR4 protein, ape and human reverted to the marmoset amino acid but did not present the same codon (Fig. 4c).

At position 146, rhesus and cynomolgus macaques harboured a conservative amino-acid change compared with humans (Fig. 4b). At that position, apes and humans reverted to the marmoset and baboon amino acid and codon (Fig. 4d).

Intra-species polymorphism

We did not find any polymorphism in the *RANTES*, *CX_3CR1*, *CCR2* and *CXCR4* genes of orang-utan (one animal) and marmoset (eight animals). Thus, only six species (gorilla, chimpanzee, gibbon, rhesus macaque,

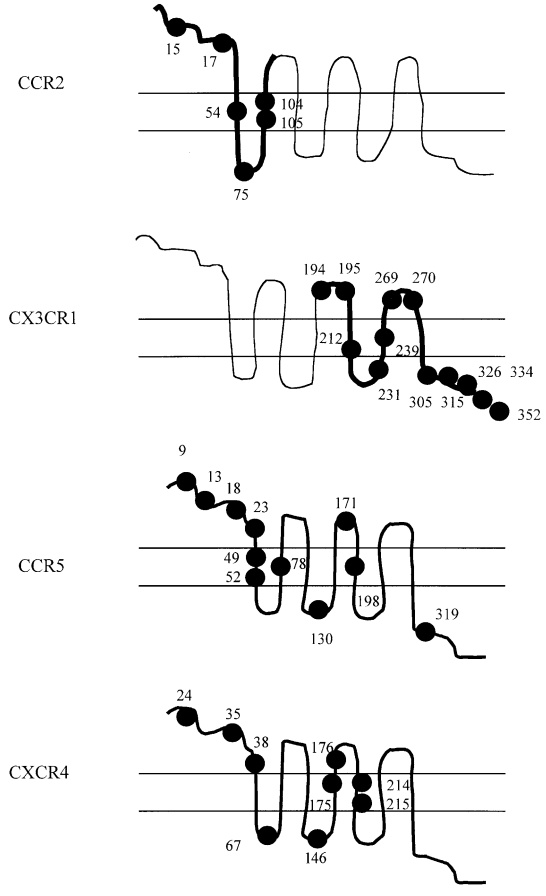


Fig. 5 Variable positions (compared with humans) in apes and OWMs. For CCR2 and CX_3CR1, the *dark line* shows the part of the protein that was sequenced in our study

crab-eating macaque and baboon) are considered hereafter.

RANTES

Polymorphisms consisted of either point mutations or deletions (Fig. 1). None of these intra-species polymorphic positions are located on known transcription factor binding sites (AP-1, STAT, NF-AT, NF-IL6, NF-kB). The gibbon was the only primate species harbouring the human G–403A polymorphism. Gorillas (ten animals) were not polymorphic.

CX_3CR1

At the nucleotide level, we observed four alleles in gorillas, and three alleles in chimpanzees, rhesus macaques and crab-eating macaques (Fig. 2). Baboons displayed two alleles. Most of these mutations are synonymous. Gibbons are not polymorphic.

At the amino-acid level, gorilla and chimpanzee present one point mutation at positions 194 (E→Q) and 212 (F→L), respectively. These two mutations are located on the second extracellular loop and the fifth trans-membrane domain of the receptor, respectively. Crab-eating macaques and baboons displayed polymorphism at position 238. At this position, an isoleucine is replaced by a valine. It is interesting to note that this replacement involves the same amino acids as the mutation at position 249, and also occurs in the sixth trans-membrane domain of the receptor (Fig. 5).

CCR2

We observed three alleles in chimpanzees and crab-eating macaques, and two alleles in gibbons, rhesus macaques and baboons (Fig. 3).

Only the chimpanzee displayed amino-acid polymorphism, in the amino terminal domain of CCR2 protein (position 5, S to Y). Gorilla was not polymorphic.

CXCR4

At the nucleotide level, from databanks, we found only one *CXCR4* allele in chimpanzee, gorilla, orang-utan, baboon and marmoset. Gibbon and rhesus macaques harboured two alleles. From our study, we identified four alleles in crab-eating macaques (Fig. 4).

At the protein level, gibbons presented three polymorphic positions that are intra-species specific. Rhesus macaques also displayed a polymorphic position at 214.

Discussion

Because of their phylogenetic closeness to man, non-human primates are of utmost importance for the study of lentivirus infection physiopathology and for vaccine development. In the present study, we compared primate chemokine and chemokine-receptor genes to their human counterparts. We restricted our study to genes whose polymorphisms were associated with susceptibility to HIV infection or AIDS progression in humans. Likewise, genetic variation within these genes could influence non-human primate susceptibility to HIV- or SIV- induced AIDS.

Chemokine and chemokine-receptor genes in primates were studied in a few species. The *CCR5* gene was studied in several primate species and a *CCR5* deletion was reported in Sooty Mangabey (Palacios et al. 1998; Zhang et al. 1999). The comparison of chimpanzee and baboon *CXCR4* sequences was also analysed (Benton et al. 1998). In a former study, we characterised the 3′-untranslated region of *SDF1* in primates (Puissant and Blancher 2001). SDF1 is the natural ligand of CXCR4 and homozygosity for a transition in the promoter of *SDF1*, referred to as SDF1-3′A, was first associated with a delay in disease progression (Winkler et al. 1998). However, subsequent observations demonstrated that

SDF1-3′A homozygous individuals showed an accelerated progression of HIV disease (Brambilla et al. 2000; van Rij et al. 1998). In our previous study, we found the presence of the SDF1-3′A mutation in primate species that are the most susceptible to HIV- or SIV-induced AIDS (*M. mulatta, M. fascicularis* and *P. anubis*) (Barnett et al. 1994; Benveniste et al. 1996; Chakrabarti et al. 2000). In contrast, species which are significantly more resistant than humans to lentivirus-induced AIDS (*Pan troglodytes* and *Hylobates lar*) do not have the SDF1-3′A mutation (Gougeon et al. 1997; Lusso et al. 1988). These results tend to associate the SDF1-3′A mutation with high sensitivity to SIV-induced AIDS in non-human primates and were concordant with those observed in humans.

In the present study, we analysed the sequence of four other genes in primates. Comparison of human polymorphic positions with non-human primate sequences allows the reconstruction of human allele phylogenesis. Among the genes studied here, three amino-acid positions are polymorphic in humans (*CX_3CR1* V249I, *CX_3CR1* T280M, *CCR2* V64I). At homologous positions in primates, in all cases we found the amino acid corresponding to the most frequent human allele. In only one case, CX_3CR1 −249, was the amino acid present in the rare human allele found in marmoset (isoleucine). Obviously, all three amino-acid mutations arose recently in human species. The existence of these human-specific alleles cannot be the consequence of the recent HIV epidemic in the human species but could result from selection by another pathogen in the past. In the same way, the presence of the *CCR5Δ32* allele in some human ethnic groups has been proposed to be the scar of the exposure of European populations to an unknown pathogen centuries ago. On the other hand, because *CCR2* is in close proximity to *CCR5* on human chromosome 3, the *CCR2*-V64I mutation could have been selected by a "hitchhiking" effect due to selective pressure exerted on the *CCR5* gene. Indeed, the mechanism by which the *CCR2*-V64I mutation favours delayed disease progression in humans is not fully elucidated. As it was shown that *CCR5* and *CCR2* were in linkage disequilibrium, *CCR2* V64I could be linked to another mutation in the *CCR5* and/or *CCR2* genes. This uncharacterised mutation would then be responsible for delayed disease progression toward AIDS in humans (Smith et al. 1997).

Two nucleotide mutations (G−403A and C−28G) have been described in the human *RANTES* promoter (Gonzalez et al. 2001). Although four haplotypes were theoretically possible, only three have been described (GC, AC and AG in order of frequency) (Gonzalez et al. 2001). Previous analysis of *RANTES* promoter sequences in chimpanzees defined an ancestral haplotype (−403A and −28C) (Gonzalez et al. 2001). Our results confirm that all apes, including gibbon, share that *AC* ancestral haplotype. It is noteworthy that in humans, the more frequently found haplotype is *GC* (Gonzalez et al. 2001; Liu et al. 1999). OWM species exhibited a third *RANTES* promoter allele, *AT*. Humans homozygous for the *RANTES AC* allele (−403A and −28C) exhibited an increased susceptibility to HIV infection and disease progression (Gonzalez et al. 2001). It is remarkable that chimpanzees, which are resistant to HIV-induced AIDS, displayed the *AC* haplotype. Therefore, the poor prognosis associated with the *RANTES AC* haplotype in man could be an indirect consequence of the linkage of this mutation to another one located in the gene cluster surrounding *RANTES* (Gonzalez et al. 2001). Either this (these) unknown mutation(s) is (are) absent in chimpanzees or chimpanzees display this (these) mutation(s) while other mutations counteract its (their) effect. Interestingly, OWM species are susceptible to SIV-induced AIDS and the *RANTES* promoter allele *AT* could be related to their sensitivity to SIV-induced AIDS.

In total, the five human genetic markers known to be associated with the LTNP phenotype are absent in most primates studied here. It is noteworthy that chimpanzees, which are naturally resistant to HIV-1- and HIV-2-induced AIDS, do not have the human mutations associated with the LTNP phenotype. Although these five human genetic markers are mostly absent from non-human primates, our results revealed inter-species polymorphisms among primates. Compared with man, primates exhibit numerous mutations in transcription factor binding sites on the *RANTES* gene. Primates also display other mutations in the promoter region that could influence the level of RANTES expression. Because RANTES could compete with HIV/SIV for binding to CCR5, different levels of RANTES expression may have an impact on HIV/SIV disease progression in primates. Plasma levels of RANTES were studied in macaques at the early stage of infection (1 month post-infection) (Kwofie et al. 2000). RANTES levels are not significantly different according to the pathogenicity of the virus. However, RANTES polymorphism of macaques was not studied in this paper.

OWMs exhibited one mutation (at position 15) in the amino-terminal domain of CCR2. This domain was previously shown to be the co-receptor binding site for HIV (Frade et al. 1997). So, this mutation could have an impact on HIV/SIV binding to CCR2 in primates and facilitate SIV infection in OWMs.

Few allelic variations have been identified in the human *CXCR4* gene, the receptor for T-tropic HIV strains, but as the mutated variants were found at very low frequency, their effect on HIV disease progression could not be studied (Alvarez et al. 1998; Cohen et al. 1998). OWMs shared mutations on the amino-terminal domain (positions 35 and 38) and on the second extracellular loop (position 176) of CXCR4. The amino-terminal domain of CXCR4, in association with extracellular loop 2, was previously shown to play an important role in CXCR4 co-receptor activity (Brelot et al. 2000). Thus, the three mutations at amino acids 35, 38 and 176 identified in OWM sequences could modify HIV/SIV binding to CXCR4 and contribute to the relative sensitivity of OWMs to SIV-induced AIDS. These positions are not intra-species polymorphic and are probably not responsible for variable rates of disease progression in OWMs.

Interestingly, three other amino acids, known to be associated with the binding of T-tropic HIV strains on human CXCR4 (Asp_{97}, Asp_{193}, Glu_{288}), are not polymorphic in primate CXCR4 (Brelot et al. 2000).

In total, interspecies polymorphisms occurred in the *RANTES* promoter and in chemokine-receptor sites involved in HIV/SIV binding. Thus, these polymorphisms could be related to differences of susceptibility to AIDS in primates.

We also found synonymous and non-synonymous nucleotide intra-species polymorphism in non-human primates. Most of the polymorphic positions in the coding region of CX_3CR1, CCR2 and CXCR4 are synonymous, and therefore cannot influence HIV/SIV susceptibility. However, some of these single-base polymorphisms could serve as genetic markers to detect mutations located in other members of the same gene cluster.

Until now, almost all chimpanzees infected by HIV, either naturally or experimentally, remain healthy. Therefore, the polymorphisms we observed in the *RANTES* promoter and in the CX_3CR1 and CCR2 proteins of chimpanzees cannot be associated with variations in susceptibility to HIV infection or disease progression. Likewise, chimpanzees displayed a CXCR4 protein identical to the human one and CXCR4 is probably not involved in the resistance of chimpanzees to AIDS.

By contrast with chimpanzees, OWMs can be infected with HIV or SIV and present inter-individual variation of the rate of progression toward AIDS, as do humans. We demonstrate here that OWMs present intra-species polymorphism in the *RANTES* promoter, in CX_3CR1 protein (crab-eating macaques and baboons) and in CXCR4 protein (rhesus macaques). The recent transmission to man of HIV strains infeodated and adapted to various African primates revealed the influence of human chemokine and chemokine-receptor gene polymorphism in sensitivity to HIV infection and/or disease progression. In the same way, experimental infection of OWMs by SIV from other primate species could parallel the situation observed in man. Obviously, the OWM chemokine and chemokine-receptor polymorphism could have an impact on sensitivity to infection and/or disease progression. Thus, we estimate that it is of great importance to analyse the possible association of the polymorphisms described in this study with disease progression in primates currently used in research for HIV vaccines and therapy.

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