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The genetic properties accompanied of "quasi-species" crossing of Small Ruminant LentiViruses (SRLVs)

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ABSTRACT

The env gene of Small Ruminant LentiViruses (SRLV) encodes a polypeptide precursor which after glycosylation is cleaved to give the two glycoproteins of the viral envelope: the transmembrane protein (TM) and the surface protein (SU). The passage and adaptation of SRLVs in new hosts is always accompanied by genetic mutations in the SU region of the env gene that determines the tropism of the virus by recognition of the cellular receptor. We studied the genetic properties of SRLVs accompanied of the "quasi-species" in Capra ibex. 16 Blood samples of Capra ibex were tested for presence of specific antibodies against SRLVs using a commercially available ELISA. Peripheral blood mononuclear cells (PBMC) isolated on Ficoll gradient were cultured in macrophage differentiation medium to obtain monocyte-derived macrophage (MDM) monolayers for virus isolation. DNAs from non cultured PBMC were used as templates for PCR and Nested PCR amplification of proviral genome. Nested PCR products (108 nt) were cloned and sequenced. Sequences were analysed using ClustalW software. The alignments of the SU region sequences show three important types of genetic mutations: deletion, addition and replacement nucleotides. We present data showing that the sequences of C. ibex SRLV isolates are closer to the prototypic CAEV-Co isolate. This might indicate a recent "quasi-species" infection of SRLV in C. ibex.

الخصائص الجينية المصاحبة لـ "العبور بين الأجناس" للفيروسات البطيئة عند المجترات الصغيرة SRLV

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الكلمات المفتاحية:
الفيروسات البطيئة
جين *env*
SRLV
موضع *SU*
Nested PCR
عبور الاجناس.

الملخص

يشفر الجين env لفيروسات الـ SRLV لعديد الببتيد، الذي ينقسم إلى نوعين من السكريات البروتينية المكونة للغلاف الفيروسي: البروتين الغشائي(TM) ، والبروتين السطحي .(SU) العبور الفيروسي، والتأقلم، لفيروسات الـ SRLVداخل العوائل الجديدة يكون دائما مصحوبا بطفرات وراثية في الجزء SU من الجينenv ، الذي بواسطته يمكن التعرف على المستقبل الخلوي لخلية العائل، والاندماج معه. في هذه الورقة قمنا بدراسة الخصائص الوراثية لفيروساتSRLV ، المصاحبة لعملية "العبور بين الأجناس" في الوعول البرية.

ثم اخذ 16 عينة دم من الوعول البرية، وتحليلها مصليا، بالبحث عن الأجسام المضادة باستخدام تقنية الـ ELISA.بعدها تمت زراعة الخلايا الدموية المحيطية (PBMC) المعزولة على الوسطFicoll ، للحصول على الخلايا الليمفاوية البعلمية أحادية النواة (MDM) بغية عزل الفيروس .

اختبر الحمض النووي DNA للفيروس المعزول، وتم تحليله باستخدام تقنيتي الPCR ، والNestedPCR ، للحصول على نسخة مضاعفة من الحمض النووي الفيروسي. خضع الحمض النووي المتحصل عليه (108

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نيوكليوتيد) لعملية استنساخ، و"كولنة" للحصول على السلاسل الوراثية النقية، والتي تم تحليلها فيما بعد باستخدام برنامج.ClustalW تحليل السلاسل الوراثية للجزء SU المتحصل عليها بين ثلاث أنواع من الطفرات الوراثية: حذف، إضافة واستبدال. كما بينت النتائج المتحصل عليها أن السلاسل الوراثية قريبة جينيا من الفيروس CAEV-Co، الأمر الذي يؤكد حدوث عملية "اختراق للأجناس" لفيروسات SRLV في الوعول البرية.

Introduction

The genome of SRLV is composed of repeated non-coding terminal sequences, LTR (Long Terminal Repeat), flanking the internal sequences encoding viral proteins. The main genes are distinguished by genes encoding viral enzymes (*pol*) and structural proteins (*gag* and *env*).

The *env* gene encodes a polypeptide precursor which after glycosylation is cleaved to yield the two viral envelope glycoproteins, the transmembrane protein (TM) that binds to the infected cell membrane and the virion and surface protein (SU). which will form "spicules" on the outside of the envelope and determine the tropism of the virus by the recognition of the cellular receptor. The SU protein consists of constant zones and variable zones [1], these last form 5 sites (V1 - V5) in CAEV (Caprine Arthritis Encephalitis Virus) [2].

All lentiviruses have restricted replication in pro-monocytes and monocytes present in bone marrow and blood. A stage of maturation of these cells carrying integrated macrophage proviruses is essential to obtain a productive viral replication [3]. Cell tropism is the ability of a retrovirus to infect a given cell type in which it will be able to multiply. The penetration of the virus is related to the presence of membrane receptors on the surface of the target cells. Cellular receptors remain unknown for SRLV, but it seems that goat lentivirus uses a receptor that restricts its ability to infect ruminant cells while K1514 virus uses a different receptor that allows it to infect cells of several species mammals [4,5].

When the virus is inside the host cell, innate immunity can interfere with SRLV replication, but the virus develops neutralization mechanisms to escape, multiply, and survive, creating a quasispecies [6].

The SRLV include viruses with diverse genotypes that frequently cross the species barrier between domesticated animals and wild small ruminants and that display a great genetic variability [7]. The infection of new cells and the passage of SRLVs in new hosts is accompanied by genetic mutations in the *env* gene [1].

The objective of our work was to study the tropism of SRLV, and the risk of interspecific passage leading to the emergence of new pathogens. In this paper, we have studied the genetic characteristics (SU region) that accompany natural infection and SRLV adaptation in *Capra ibex*.

Materials and methods

Blood samples were taken from ibex captured at three sites in the French Alps (Bonneval sur Arc, Entraigues and Modane) and from small domestic ruminants likely to interact with wild ungulates at these sites.

In total, 16 Blood samples of *Capra ibex* were tested for presence of specific antibodies against SRLVs using a commercially available ELISA based on detection of the recombinant p28-gag protein. Peripheral blood mononuclear cells (PBMC) isolated on Ficoll gradient were cultured in macrophage differentiation medium to obtain monocyte-derived macrophage (MDM) monolayers for virus isolation. DNAs from non cultured PBMC were used as templates for PCR amplification of proviral genome with primer sets chosen in a conserved region of *env* gene sequences from published CAEV genomes (Genbank accession number M33677) [8]. The primers ECE5' (5'- GGC CTC TCT GGC AAA TGG AG -3') and ECE3' (5'- AGC TTC CAC TCG AGC CAC TCG-3') were used for the first amplification (PCR). Primers ECI5' (5'- GTG GTG CTG GTA TAC AGC GGC C-3') and ECI3' (5'- CCC AGA GAA GCC CCT GCG GCA GC-3') were used for the second amplification (Nested PCR). N-PCR products (180 nt) were cloned and sequenced. Sequences were analysed using ClustalW software [9]. The nature of these variations is shown in phylogenetic trees constructed using the program NEIGHBOR [10] method Neighbor-Joining (NJ) [11], with bootstrap values determined over 1000 iterations [12].

Results:

Seven clones were obtained from independent cloning reactions realized from the independent Nested PCRs and sequenced to assess the genetic characteristics of the SU region-*env* gene. The different sequences obtained were aligned using ClustalW with CAEV-Co reference sequence (Figure 1). The sequences alignments corresponding to the surface protein SU (5' end of *env* gene) shows three types of genetic mutations: the first is a deletion of one nucleotide: deletion of A at position 599 (sequence S3ENV) and G at position 663 (sequence S2ENV). The second type of genetic mutations is an addition of one nucleotide: addition of C at position 512 (sequence S4ENV) and G at position 512 (sequence S5ENV).

The third type of genetic mutations is a simple replacement of one nucleotide by another: (Table 1).

A phylogenetic tree illustrating the relationships between these different SU sequences was constructed using the neighbor joining method, but very similar results were obtained using other tree-building algorithms. It can be seen that proviruses from the S1ENV and S2ENV form a closely related group, quite distinct from the CAEV-Co reference sequence. The third group shows the sequences S4ENV, S5ENV, S3ENV, S6ENV and S7ENV (Figure 2).

Table 1: Replacement of nucleotides: Po.=Position, Nt= Nucleotide, Rep. by= Replacement by.

Po.	Nt	Rep. by:	Sequences
402	G	C	S4ENV, S6ENV
405	T	C	All sequences
408	A	C	S4ENV
511	A	C	S4ENV, S5ENV
514	A	C	S4ENV, S5ENV, S3ENV, S6ENV, S7ENV
518	A	T	S4ENV
527	T	C	S4ENV, S5ENV, S6ENV, S7ENV
532	T	C	All sequences
535	G	A	S4ENV, S5ENV, S6ENV, S7ENV
544	C	A	All sequences
561	T	C	S4ENV
573	G	A	All sequences
574	A	T	S4ENV
578	T	C	All sequences
588	A	G	All sequences
590	A	C	S4ENV, S5ENV, S6ENV, S7ENV
593	C	T	All sequences
595	A	C	S4ENV, S5ENV, S1ENV, S2ENV, S3ENV
596	A	C	S5ENV

597	A	G	S1ENV, S2ENV, S3ENV
604	G	C	S4ENV
608	T	C	S1ENV, S2ENV, S3ENV
610	A	C	S3ENV
611	A	G	S1ENV, S2ENV, S3ENV
614	A	C	S3ENV, S5ENV, S6ENV, S7ENV
624	G	T	All sequences
627	C	T	S6ENV, S7ENV
633	T	A	S4ENV, S5ENV
638	A	G	S4ENV, S5ENV, S1ENV, S2ENV, S6ENV, S7ENV
642	G	C	S5ENV
645	T	C	S6ENV
662	G	A	S4ENV, S5ENV, S6ENV, S7ENV
663	G	C	S3ENV
666	G	A	All sequences
671	G	A	S4ENV, S5ENV
672	A	G	S1ENV, S2ENV
674	A	T	S4ENV, S5ENV
680	A	G	S4ENV, S5ENV
680	A	T	S3ENV

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                                  510       520       530       540       550       560
CAEV-Co GGATTACATAA-CACTAATATCAGATCCCTATGGGTTCTCACCCATAAAAAATGTGTCTG
S4ENV   CGACTACCTCTCCCCTATTATCAGACCCCTACGGATTCTCACCAGTAAAAAATGTGTCCG
S5ENV   CGACTACATCTGCCCTAATATCAGACCCCTACGGATTCTCACCAGTAAAAAATGTGTCAG
S1ENV   GGACTACATAA-CACTAATATCAGATCCCTACGGGTTCTCACCAGTAAAAAATGTGTCAG
S2ENV   GGACTACATAA-CACTAATATCAGATCCCTACGGGTTCTCACCAGTAAAAAATGTGTCAG
S3ENV   GGACTACATCA-CCCTAATATCAGATCCCTACGGGTTCTCACCAGTAAAAAATGTGTCAG
S6ENV   CGACTACATCA-CCCTAATATCAGACCCCTACGGATTCTCACCAGTAAAAAATGTGTCAG
S7ENV   CGACTACATCA-CCCTAATATCAGACCCCTACGGATTCTCACCAGTAAAAAATGTGTCAG
Consen  ** ***.*.: *.***:******* ***** **.********..********** *
                                  570       580       590       600       610       620
CAEV-Co GGGTACCAGTGACTTGTGTAACAAAAGAATTCGCAAAATGGGGATGTCAACCACTAGGAG
S4ENV   GGGTACCAGTATCCTGCGTAACAAAGGACTTTGCCAAATGGGCATGTCAACCCCTAGGAG
S5ENV   GGGTACCAGTAACCTGCGTAACAAAGGACTTTGCCCAATGGGGATGTCAACCCCTAGGAG
S1ENV   GGGTACCAGTAACCTGCGTAACAAAGGAATTTGCCAGGTGGGGATGCCAGCCACTAGGAG
S2ENV   GGGTACCAGTAACCTGCGTAACAAAGGAATTTGCCAGGTGGGGATGCCAGCCACTAGGAG
S3ENV   GGGTACCAGTAACCTGCGTAACAAAGGAATTTGCCAG-TGGGGATGCCCGCCACTAGGAG
S6ENV   GGGTACCAGTAACCTGCGTAACAAAGGACTTTGCAAAATGGGGATGTCAACCCCTAGGAG
S7ENV   GGGTACCAGTAACCTGCGTAACAAAGGACTTTGCAAAATGGGGATGTCAACCCCTAGGAG
Consen  **********.:* ** ********.**.** **... **** *** *..**.*******
                                  630       640       650       660       670       680
CAEV-Co CGTACCCTGATCCAGAAATAGAATACAGAAATGTGAGTCAGGAAGTAGTGAAAGAAGTAT
S4ENV   CTTACCCTGAACCAGAGATAGAATATAGAAATGTGAGTCAAGAAATAGTAAATGAAGTGT
S5ENV   CTTACCCTGAACCAGAGATACAATATAGAAATGTGAGTCAAGAAATAGTAAATGAAGTGT
S1ENV   CTTACCCTGATCCAGAGATAGAATATAGAAATGTGAGTCAAGAAATAGTGGAAGAAGTAT
S2ENV   CTTACCCTGATCCAGAGATAGAATATAGAAATGTGAGTCAG-AAATAGTGGAAGAAGTAT
S3ENV   CTTACCCTGATCCAGAAATAGAATATAGAAATGTGAGTCAGCAAATAGTGAAAGAAGTTT
S6ENV   CTTATCCTGATCCAGAGATAGAACACAGAAATGTGAGTCAAGAAATAGTGAAAGAAGTAT
S7ENV   CTTATCCTGATCCAGAGATAGAACACAGAAATGTGAGTCAAGAAATAGTGAAAGAAGTAT
Consen  * ** *****:*****.*** ** * **************. **.****..*:****** *
```

Figure 1: Comparison of the *env* nucleotide sequence (SU region) of seven clones with the sequences of prototype CAEV-Co (Genbank accession number M33677) [8]. Sequences were aligned versus the CAEV-Co sequence (ClustalW program). Consen= Consensus.

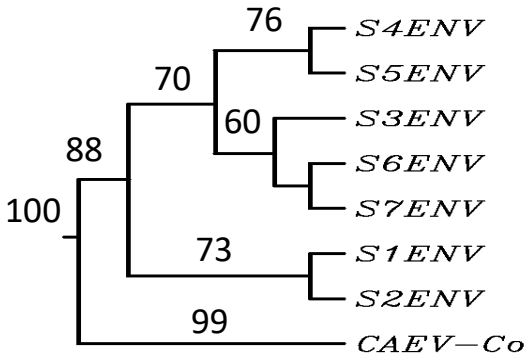

Figure 2: *Neighbour-joining* tree of a 180nt fragment of the SU region(*env* gene) from the 7 clones of the present study and Genbank sequence representing the CAEV-Co prototypic strain. The tree was constructed with the distance matrix calculated with the Neighbor-Joining (NJ) method and gamma distribution. Bootstrap testing of phylogeny was performed with 1000 replications.

Discussion:

The objective of this work was to study the genetic properties of SRLV present in Capra ibex, in order to determine the genetic modifications that this retrovirus accumulated essentially in the part corresponding to the SU region of the envelope of its genome for adapt to this new species. The experiments yielded results that led us to conclude that the adaptation of SRLV in *Capra ibex* was accompanied by genetic modifications that accumulated preferentially in the SU part of the *env* gene of its genome.

These modifications in the gene encoding envelope glycoproteins would correspond to a selection of genotypes that have a selective advantage of replication in the cells. Indeed, to enter the cells the SRLV must interact with membrane receptors present on the surface of the target cells. This interaction is followed by a fusion between the viral and cellular membranes to allow internalization of the viral particle. The receptors used by the SRLV for its entry into the target cells are not yet known, whereas those used by the primate lentiviruses are well identified.

The primary receptor of the HIV1 virus has been identified, it is the CD4 molecule [13], as well as galactosyl ceramide (GalCer) which has been identified as a potential receptor of the HIV-1 virus in the cells of the nervous system. Recently, chemokine co-receptors (CXCR4, CCR5) have been described as a co-receptor of the HIV1 virus [14], and the chemokine CXCR6 as a co-receptor of the SIV virus [15].

One of the hypotheses that can be advanced is that cell surface receptor molecules of *C. ibex* are divergent from those on the cell surface of other ruminants; therefore only genotypes that have accumulated a large number of mutations can interact with these receptors and are positively selected. It is interesting to note that the 5' end of the viral sequence, corresponding to the surface glycoprotein SU, have much more genetic changes than the 3' corresponding the transmembrane glycoprotein TM. Indeed, the interaction with the cellular receptors is essentially carried out with the SU and the merger is ensured by the TM. As a result, to enter the target cells of a new species (*Capra ibex*), it is necessary for the SRLV to vary the part of the envelope glycoproteins (SU) which interacts specifically with the receptors [1].

In addition, highly conserved areas were observed in analyzed alignments, which was observed at the SRLV LTR region [16]. In contrast, the amino acid sequences of immuno-dominant epitopes in the *gag* region prove to be highly conserved [17].

Conclusion:

The fine characterization of the mutations that accumulate in *C. ibex* during the adaptation of SRLV in this new host species will allow a better understanding of the mechanisms involved in the *"quasi-species*" passage and the emergence of new pathogens and new pathologies.

References:

[1]- Erhouma E., Guiguen F., Gauthier D.and Chebloune Y. 2017. Risk of lentiviral genetic mutations in *env* gene. 1th Conference of Applied Science CAS. Zintan – Libya 19-20 September.

[2]- Valas S., Benoit C., Baudry C., Perrin G., Mamoun R.Z. 2000. Variability and immunogenicity of caprine arthritis-encephalitis virus surface glycoprotein. J. Virology. 13: 6178-85.

[3]- Gendelman H. E., Narayan O., Kennedy-Stokopf S., Kennedy P. G., Ghotbi Z., Clements J. E., Stanley J., and Pezeshkpour G. 1986. Tropism of sheep lentiviruses for monocytes: susceptibility to infection and virus gene expression increase during maturation of monocytes to macropohages. J. Virology. 58: 67-74.

[4]- Hötzel I. and Cheevers W.P. 2003. Caprine arthritis-encephalitis virus envelope surface glycoprotein regions interacting with the transmembrane glycoprotein: structural and functional parallels with the human immunodeficiency virus type 1 gp120. J. Virology. **77**: 11578–11587.

[5]- Hötzel I., William P. and Cheevers W.P. 2005. Mutations increasing exposure of a receptor binding site epitope in the soluble and oligomeric forms of the caprine arthritis-encephalitis lentivirus envelope glycoprotein. Virology. 339: 261–272.

[6]- Ramírez H., Reina R., Amorena B., De Andrés D. and Martínez H.A. 2013. Small ruminant lentiviruses: genetic variability, tropism and diagnosis. Viruses. 23: 5(4):1175-1207.

[7]- Erhouma E., Guiguen F., Chebloune Y., Gauthier D., Mselli Lakhal L., Greenland T., Mornex J.F., Leroux C., and Alogninouwa T. 2008. Small ruminant lentiviruses (SRLV) proviral sequences from wild ibex in contact with domestic goats. Journal of General Virology, 89: 1478-1484.

[8]- Saltarelli M., Querat G., Konings D. A., Vigne R. and Clements J. E. 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology. 179 : 1893-1903.

[9]- Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., and Higgins D.G. 1997. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignement aided by quality analysis tools. Nucleic Acids Research. 25: 4876-4882.

[10]- Perriere G. and Gouy M. 1996. WWW-query: an on-line retrieval system for biological sequence banks. Biochimie 78: 364-369.

[11]- Saitou N. and Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4: 406-425.

[12]- Felsenstein J. 2002. PHYLIP: phylogeny inference package, version 3.6 (alpha3), University of Washington, Seattle.

[13]- Dalgleish A. G., Beverly P., Clapham P. R., Crawford D. H., Greaves M. F,. and Weiss R. A. 1984. The CD4 (T4) antigen is an essential component of the receptor for AIDS retrovirus. Nature.312: 763-767.

[14]- Doranz B. J., Rucker J., Hi Y., Smyth R. J., Samson M., Peiper S. C., Parmentier M. , Collman R. G. and Doms R. W . 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 85: 1149-1158.

[15]- Wetzel K. S., Yi Y., Elliott S. T., Romero D., Jacquelin B., Hahn B. H., Muller-Trutwin M., Apetre C., Pandrea I. and Collman R. G. 2017. CXCR6-Mediated Simian Immunodeficiency Virus SIVagmSab Entry into Sabaeus African Green Monkey Lymphocytes Implicates Widespread Use of Non-CCR5 Pathways in Natural Host Infections. J Virology. 91(4): 1616-1626.

[16]- Mendiola W. P. S., Tórtora J. L., Martínez H. A., García M .M., Cuevas-Romero S., Cerriteño J. L. and Ramírez H. 2019. Genotyping Based on the LTR Region of Small Ruminant Lentiviruses from Naturally Infected Sheep and Goats from Mexico. Biomed Research International. 12:573-579.

[17]- Gayo E., Cuteri V, Polledo L., Rossi G., García Marín J.F. and Preziuso S. 2018. Genetic Characterization and Phylogenetic Analysis of Small Ruminant Lentiviruses Detected in Spanish Assaf Sheep with Different Mammary Lesions. Viruses.10(6): 315-330.