Virus Genes 16:1, 39–46, 1998

Arenavirus Phylogeny: A New Insight

CÉSAR G. ALBARIÑO,[1,2] DIEGO M. POSIK,[1,2] PABLO D. GHIRINGHELLI,[2,1] MARIO E. LOZANO,[2,1] & VÍCTOR ROMANOWSKI[1,2]*

[1]*Instituto de Bioquímica y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata*
[2]*Departamento de Ciencia y Technología, Universidad Nacional de Quilmes*

Abstract. *Arenaviridae* is a worldwide distributed family, of enveloped, single stranded, RNA viruses. The arenaviruses were divided in two major groups (Old World and New World), based on serological properties and genetic data, as well as the geographic distribution. In this study the phylogenetic relationship among the members of the *Arenaviridae* was examined, using the reported genomic sequences. The comparison of the aligned nucleotide sequences of the S RNA and the predicted amino acid sequences of the GPC and N proteins, together with the phylogenetic analysis, strongly suggest a possible kinship of Pichindé and Oliveros viruses, with the Old World arenavirus group. This analysis points at the evolutive relationships between the arenaviruses of the Americas and can be used to evaluate the different hypotheses about their origin.

Key words: arenavirus, S RNA, phylogeny, sequences

Introduction

The *Arenaviridae* family is composed of a growing number of viruses with, at least, 18 recognized members around the world. According to the geographical site of isolation, the serological cross-reactivity and the molecular genetic data, arenaviruses are classified into two different groups. These clades (probably genera), are called the Old World and New World arenavirus groups (1,2).

The prototype of the family, Lymphocytic Choriomeningitis virus (LCM), is the only member with a worldwide distribution, whilst all other described arenaviruses are geographically restricted. LCM is a member of the Old World arenavirus group, which also includes Ippy, Lassa, Mobala and Mopeia viruses. The New World arenavirus group comprises Amapari, Flexal, Guanarito, Junín, Latino, Machupo, Oliveros, Paraná, Pichindé, Sabiá, Tacaribe, Tamiami and Withewater Arroyo viruses (3–6).

All arenaviruses, except Tacaribe, are known to have a rodent host, and some of them (Lassa, Junín, Machupo, Sabiá and Guanarito) are known to be highly pathogenic for humans. In particular, the South American viruses produce hemorrhagic fevers in Argentina, Bolivia, Brazil and Venezuela, respectively.

These diseases have endemo-epidemic characteristics with cardiovascular, renal, immunological and neurological alterations; albeit the low number of documented Sabiá infections in humans does not allow its inclusion in this category.

Arenavirus genome is composed of two single stranded RNA molecules designated L (for large, *ca*. 7 kb) and S (for small, *ca*. 3,5 kb). Both genomic RNAs have two, non overlapping, open reading frames (ORFs), arranged in opposite orientations (7). In addition to the *Arenaviridae*, this ambisense coding strategy was found, only in some genera of *Bunyaviridae*. Arenavirus ORFs are separated by non coding intergenic regions that fold into stable secondary structures, in the form of hairpin-loops (8,9). The L RNA, codes for the RNA polymerase (L) and a zinc-finger-like protein (Z) and the S RNA codes for the viral nucleocapsid protein (N) and the precursor of the envelope glycoproteins (GPC). The N and L proteins are translated from antigenomic (or viral complementary) sense mRNA species that are encoded by the 3′ half of the viral S or L RNA,

*Corresponding author: Dr. V. Romanowski, Instituto de Bioquímica y Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 115 entre 49 y 50, La Plata, (1900). Buenos Aires, Argentina. E.mail: victor@nahuel.biol.unlp.edu.ar

respectively. GPC and Z proteins are translated from a genomic (or viral) sense mRNA corresponding to the 5′ half of the S or L RNA, respectively (9).

The sequence information reported on the L RNA is limited to LCM (10,11), Tacaribe (12,13), Pichindé (D. Harnish, personal communication) and Lassa viruses (14). Conversely, the information about the S RNA is more abundant: the complete nucleotide sequence of this RNA species has been reported for Pichindé (15), LCM (16), Tacaribe (17), Lassa (18,19), Junín (20–22), Mopeia (23), Oliveros (24) and Sabiá (25). In addition, partial sequence data have been reported for some strains of Junín virus, and for Machupo, Amapari, Flexal, Guanarito, Latino, Paraná, Tamiami, Whitewater Arroyo and Pampa viruses (6,26–29).

In the present study, we used the available S RNA sequences and the encoded gene products to examine the phylogenetic relationship in the arenavirus family. The results suggest a possible evolutionary relatedness between Oliveros and Pichindé viruses—isolated in the American continent—with the arenaviruses of Europe and Africa. Alternative hypotheses can be proposed to explain these results, and this could be regarded as a first step in the attempt to elucidate the pathway for the worldwide distribution of the arenaviruses.

Materials and Methods

Sequence Information

The nucleotide sequences data from Junín virus MC2 (Jun-mc2), XJ (Jun-xj), XJ#44 (Jun-xj44) and Candid #1 (Jun-can) strains were obtained in our laboratory. Briefly, these viruses were propagated in cell culture. The viral RNA was purified from virions, isolated from supernatant media and from infected cells. Subsequently, the cDNAs corresponding to different regions of the S RNA were cloned and sequenced (20–22).

The nucleotide sequences of the S RNAs from the following arenaviruses were obtained from the GenBank (National Institutes of Health, Bethesda, Maryland, USA): Machupo (MAC), Tacaribe (TAC), Sabiá (SAB), Pichindé (PIC), Oliveros (OLV), LCM WE and Armstrong strains (LCM-we and LCM-ar); Lassa Nigeria and Josiah strains (LAS-ni y LAS-jo), Mopeia (MOP). The sequence data corresponding to the tospovirus INSV were obtained from the same databank.

The access numbers of the sequences used are: JUN-mc2, D10072; PIC, K02734; TAC, M20304, M65834; MAC, X62616; SAB, U41071; OLV, U34248; LCM-we, M22138; LCM-ar, M20869; LAS-ni, X52400; LAS-jo, J04324; MOP, M33879; INSV, M74904, L20886.

The sequence analyses were done in a MicroVax 3100 computer (Digital, Maynard, USA) using different routines from the program package by GCG (Genetics Computer Group, Sequence Analysis Package, Version 7.1, University of Wisconsin, Madison, USA). The ORFs (*open reading frames*) were located with the MAP program and the sequence translation was obtained with the TRANSLATE program. The pairwise sequence comparisons were done with the GAP program, which generates an alignment and presents the similarity and identity values for the pair of nucleotide or amino acid sequences. The PILEUP program was used for the multiple sequence comparisons.

Initially, a multiple alignment was performed on the complete nucleotide sequences of the S RNA from JUN-xj, JUN-xj 44, JUN-mc2, TAC, SAB, OLV, PIC, LCM-ar, LCM-we, LAS-ni, LAS-jo and MOP. In parallel, deduced amino acid sequences for the N and GPC proteins, and the proteolytic products, G1 and G2, were aligned. The PILEUP program, also, generates a graphic representation (dendrogram), based on the overall similarity, that shows the relationship among the analyzed sequences.

Phylogenetic Analysis

The phylogenetic analysis based on the cladistic approach, was done using different routines from the PHYLIP program package (30) on the sequence alignments. The complete nucleotide sequences of *Impatiens Necrotic Spot* virus (INSV), a tospovirus of the *Bunyaviridae* family was chosen as an outgroup. The *bootstrapping* method was used to sample the alignments with the SEQBOOT program for 100 consecutive cycles.

The parsimony analysis of the amino acid sequence alignments was done with the PROTPARS program. Alternatively, these alignments were analyzed with the distance matrix program PROTDIST, according to the similarity table of Dayhoff-PAM. From the nucleotide sequence of the S RNA, a distance

matrix was constructed using the DNADIST program, calculated according to the substitution model of Kimura. The parsimony analysis of the distance matrices and the generation of the cladograms, were done with the programs FITCH y NEIGHBOR. In all cases, the consensus trees were obtained by the majority rule with the CONSENSE program.

Results

Similarity Relationship between Arenavirus

The study of the relations among the arenaviruses began with the analysis of the dendrograms generated by the PILEUP program. At this point, it should be reminded that dendrograms are graphic representations of relations based on the general similarity, but they do not constitute a phylogenetic analysis. This program calculates the identity values of nucleotide and the identity and similarity values of amino acids from the pairwise comparisons and then, generates a grouping order based upon the figures.

A dendrogram corresponding to the nucleotide sequence analysis of the S RNAs is shown in Fig. 1A. It can be noted that this virus family is divided in two large groups; one of them includes the New World arenaviruses (JUN-xj, JUN-xj 44, JUN-mc2, TAC, SAB, OLV y PIC) and the other encompasses the Old World ones (LCM-ar, LCM-we, LAS-ni, LAS-jo y MOP). The same relations can be appreciated in Table 1, which presents the identity values of the viral sequences.

The dendrogram corresponding to the amino acid sequences of the N proteins, also shows the same distribution in two large groups (Fig. 1B). The sequences of Machupo and Junín Candid #1 viruses were included in this alignment. In contrast with the above mentioned analyses, OLV and PIC viruses appear related with the Old World arenaviruses in the dendrogram derived from the amino acid sequences of the GPC protein (Fig. 1C).

The discrepancy about OLV and PIC location in dendrograms, is coincident with the similarity values obtained in the pairwise comparisons (Table 2). Examining the average similarity of PIC with the rest of the arenaviruses it is possible to simplify the table observation. Considering the N protein, PIC presents a 72% of similarity with the New World arenaviruses, and a lower similarity value (68%) with those of the Old World. Regarding to the GPC protein, the average similarity values of PIC with the New and Old World viruses (64% and 66%, respectively) explain its inclusion in the last group. A very similar situation is observed in OLV virus grouping.

Interestingly, another discrepancy is observed when the similarity values of G1 and G2 polypeptides are examined. Analyzing the G1 region, PIC presents a 55% of similarity with the New World arenavirus and a larger value (60%) with those of the Old World.

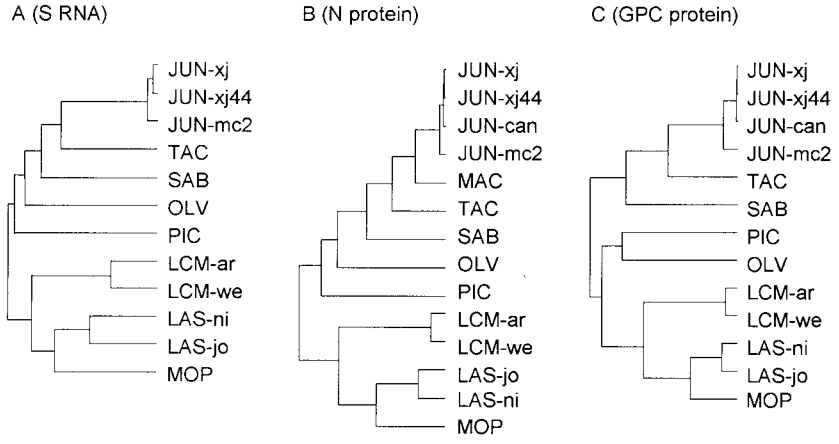

Fig. 1. The overall sequence similarity of arenavirus. The complete nucleotide sequence of the S RNA was considered in A, while the predicted amino acid sequences of N and GPC proteins were considered in B and C, respectively. In dendrograms generated with PILEUP program, the branch length is proportional to the similarity between the analyzed sequences.

	JUN xj	JUN xj44	JUN can	JUN mc2	TAC	SAB	OLV	PIC	LCM ar	LCM we	LAS ni	LAS jo
JUN-xj44	99											
JUN-can	99	99										
JUN-mc2	97	98	98									
TAC	69	69	69	68								
SAB	64	64	63	63	62							
OLV	58	58	58	58	57	58						
PIC	**54**	**54**	**54**	**53**	**56**	**56**	58					
LCM-ar	52	52	52	52	53	54	55	**54**				
LCM-we	53	52	52	52	52	53	55	**54**	84			
LAS-ni	54	54	54	54	55	54	55	**55**	61	61		
LAS-jo	54	55	54	54	54	54	55	**55**	63	62	78	
MOP	52	52	52	52	53	52	55	**56**	62	62	68	69

Table 1. Identity values of the complete S RNA nucleotide sequences, obtained in pairwise comparisons. Data indicated in bold fonts were used to obtain average similarities. OLV was excluded from these average calculation due to the close relationship with PIC. It can be observed that PIC and New World arenaviruses presents an average similarity of 54.5% (bold row); while PIC and Old World arenaviruses presents an average similarity value of 54.8% (bold column)

Although, examining the G2 region, the average similarity of PIC indicates a different relation with the arenavirus of the New and Old World (74% and 72%, respectively).

Phylogenetic Reconstruction

In order to further evaluate the possible relation of OLV and PIC with the Old World arenaviruses, a phylogenetic analysis was done, using the approach of

	JUN xj	JUN xj44	JUN can	JUN mc2	MAC	TAC	SAB	OLV	PIC	LCM ar	LCM we	LAS ni	LAS jo	MOP	
JUN-xj		99	99	98	94	90	85	77	**73**	68	68	68	68	68	
JUN-xj44	100		99	99	94	90	84	77	**72**	68	68	68	68	68	
JUN-can	99	99		99	94	89	84	77	**72**	68	67	68	68	68	
JUN-mc2	97	97	97		93	88	84	77	**72**	67	67	68	67	67	
MAC	*	*	*	*		89	85	77	**73**	69	68	68	68	68	
TAC	83	83	82	81	*		82	76	**73**	68	68	69	68	67	
SAB	73	73	73	72	*	73		74	**72**	69	68	69	68	71	N
OLV	65	65	65	65	*	66	69		75	67	67	69	69	67	
PIC	**64**	**64**	**64**	**64**	*	**64**	**65**	71		**68**	**67**	**69**	**69**	**69**	
LCM-ar	60	60	59	58	*	58	63	68	**66**		97	77	76	79	
LCM-we	59	59	59	58	*	60	64	67	**64**	97		78	77	79	
LAS-ni	63	63	63	62	*	62	64	66	**67**	77	77		94	85	
LAS-jo	62	62	62	62	*	59	63	67	**66**	76	77	96		85	
MOP	64	64	64	63	*	61	62	67	**67**	77	77	89	88		
	GPC														

Table 2. Similarity values obtained in the pairwise comparisons of the N and GPC amino acid sequences. Data indicated in bold fonts were used to obtain averages similarities. OLV was excluded in these average calculation due to the close relationship with PIC. Considering the N protein (right triangle), a 72% average similarity is obtained for PIC and New World arenaviruses (bold column); while a 68% value is obtained for PIC-Old World arenaviruses (bold row). In the GPC protein analysis (left triangle), a 64% average similarity is obtained for PIC and New World arenaviruses (bold row); while a 66% value is obtained for PIC and Old World arenaviruses (bold column).
*The corresponding GPC sequences of Machupo virus have not been reported yet

cladism or systematic phylogenetics. The results of this procedure should yield a classification reflecting the genealogic relationships and to establish an hypothetical phylogenetic reconstruction. To this end, different computational routines from the PHYLIP program package were used; in particular, algoritms that operate over the sequence alignments to construct the phylogeny and others that produces a distance matrix as a previous step.

In this approach, an *outgroup* is included and re-sampling procedures are done to statistically evaluate the tree topology and the consistency of the branching points. The sequence of the tospovirus INSV (*Bunyaviridae*) was selected as the outgroup based on the relative similarity with arenaviruses regarding its genomic structure and coding strategy. The genome of the *Bunyaviridae* family is composed by three single-stranded RNA molecules: S, M and L (small, medium and large). INSV genes are coded in an ambisense way in the S and M RNAs, and in negative sense in the L RNA (31, 32). The re-sampling method of *bootstrapping* generates a set of alignments with random column replacements to be subsequently analyzed by the parsimony criterion. Then, the consensus cladogram is obtained by the majority rule, and the frequency of each monophyletic group (consensus value) is indicated in the figure.

The first cladistic analysis was done with the application of the PROTPARS program (*Protein Sequence Parsimony Method*), which calculates the nucleotide changes associated for each amino acid change. Examining the N protein cladogram (Fig. 2A), it is observed that arenavirus from the New and Old World are clearly separated, as they were in the corresponding dendrogram. However, in the GPC protein cladogram (Fig. 2B), similarly to the previously shown dendrogram (Fig. 1C), OLV and PIC viruses were grouped with the Old World arenaviruses (consensus value $=$ 67%). In further analyses, the sequences of polypeptides G1 and G2 were defined according to the proteolytic signals reported for the GPC precursor protein of LCM virus (33). The cladogram for G1 (without the signal peptide sequence), presents a more significant consensus (82%) for the clustering of OLV and PIC with the Old World arenaviruses (Fig. 2C). When the G2 amino acid sequences were analyzed by the phylogenetic approach, we obtained a similar clustering for OLV and PIC viruses, but with a lower consensus value (61%, Fig. 2D).

In addition, we used distance matrix programs to generate the phylogeny of this group, in order to confirm the previous results. The PROTDIST algorithm makes pairwise comparisons and calculates distance values according to the Dayhoff-PAM amino acid homologies table. These matrices were further analyzed with the FITCH program (Fitch and Margoliash algorithm). The resulting cladograms (not shown) corresponding to the proteins N and GPC, and the polypeptides G1 and G2, present a topology similar to those constructed with the PROTPARS. OLV and PIC relationship with Old World arenavirus was observed in cladograms corresponding to GPC and G1 (without the signal peptide), with a consensus of 76 and 98%, respectively. The G2 cladogram, also, presents the same distribution but with much lower consensus (45%) for the mentioned group. In G1 cladogram, also, it is observed the inclusion of SAB in the mentioned group, albeit with a low consensus (60%). Moreover, we applied the NEIGHBOR program (*Neighbor-Joining* algoritm) on the distance matrices. Again, the resulting consensus cladograms present the general topology like those obtained before.

The analysis of G1 polypeptide, including the signal peptide sequence (data not shown), results in cladograms with a similar clustering for OLV and PIC with the Old World arenaviruses. In fact, this clustering was observed in cladograms generated with PROTPARS and PROTDIST, with a consensus value of 79 and 86%, respectively. These values are lower due to the addition of a more conserved sequence stretch to the G1 sequence, which is the most variable structural polypeptide in the *Arenaviridae* family.

Discussion

This phylogenetic study was done using different approaches and methodologies in order to obtain significant results. Initially, a classical approach was applied, considering only de overall similarity. This simple and widely used analysis was followed by the cladistic method. The latter emphazises the clustering of monophyletic groups, by including a reference outgroup and employing random re-sampling techniques. To this end, programs were employed that deal directly with the sequence alignments and others that generate a distance matrix prior to producing the

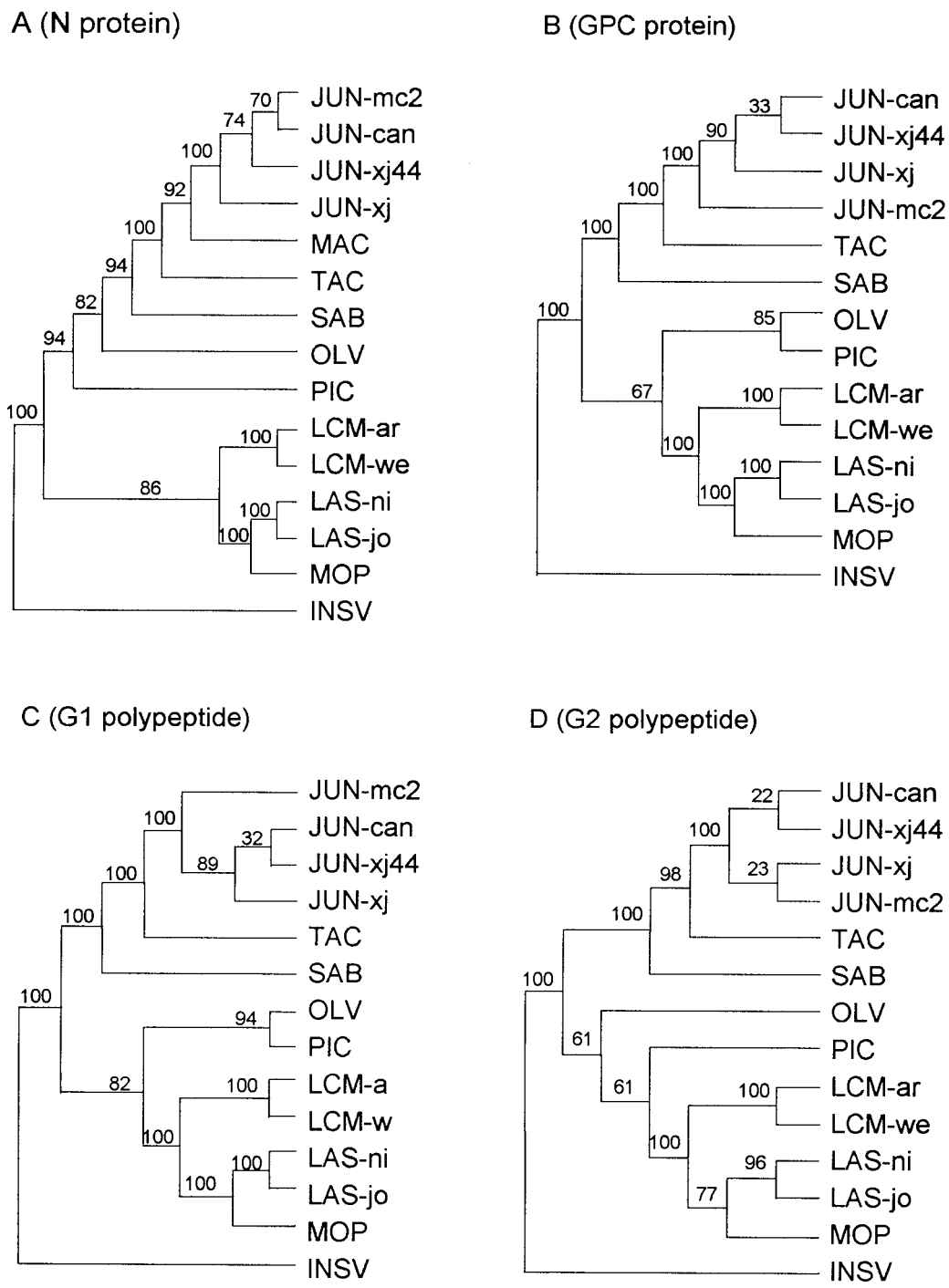

Fig. 2. Arenavirus relationships highlighted in consensus cladogram. The amino acid sequence of the N and GPC proteins were considered in A and B, respectively. Meanwhile, the corresponding sequences of G1 and G2 polypeptides were considered in C and D, respectively. The consensus value over the branches, represents the frequency of each monophyletic group and indicates the nodes consistence; while the branch length lack significance. Cladograms were generated with PROTPARS and CONSENSE of the PHYLIP programs package.

cladograms. Also, different regions and products encoded in the S RNA were examined: the complete nucleotide sequence of the S RNA, the amino acid sequence of the N and GPC proteins, and the proteolytic products, G1 and G2.

This study showed an evident separation between the New and Old World arenaviruses, as was previously reported in the literature (34 and references therein). Nevertheless, the S RNA dendrogram (Fig. 1A) indicates that OLV and PIC are slightly different from the rest of the America's arenaviruses. This separation is also observed examining the identity values generated in the pairwise comparisons (Table 1), i.e., considering the average similarity: PIC-New World $= 54.5\%$; PIC-Old World $= 54.8\%$. These results might suggest that these viruses are more related with the ancestral arenaviruses than others. At this point, it must be mentioned that PIC was considered by Bowen *et al.* (6) an ancestral virus with respect to the New World arenaviruses. Anyway, the support for this proposal has not been reported.

In this study, we decided to examine the complete sequences of the S RNA and its coded products, GPC and N, in order to reduce the putative artifactual results arising from the analysis of partial data. The N protein amino acid sequence analysis yielded similar results to those previously reported by Clegg (34) and Bowen (6). In contrast, the analysis of GPC and its products—G1 and G2—produced different results. Considering GPC and G1, OLV and PIC are clearly related with the Old World arenaviruses, while, examining the G2 region, this relation appears less convincing.

At least, four hypotheses could be proposed to explain these apparent discrepancies (Fig. 3). Firstly, the possibility might be considered that OLV and PIC have a close relation with the ancestral group of the New World arenaviruses (Fig. 3A). This ancester, coming from the Old World, would have originated the lineages in the Americas; and, their closer descendants could retain characteristics reflecting their origin.

A second hypothesis is that these viruses would have a relatively modern common ancestor with genomic characteristics, corresponding to the two large arenaviruses groups. The mixed characteristics could have been originated by a recombination event in the S RNA, during a co-infection by arenavirus of the New and Old World in a wild host (Fig. 3B). Although, presently it is not possible to identify the

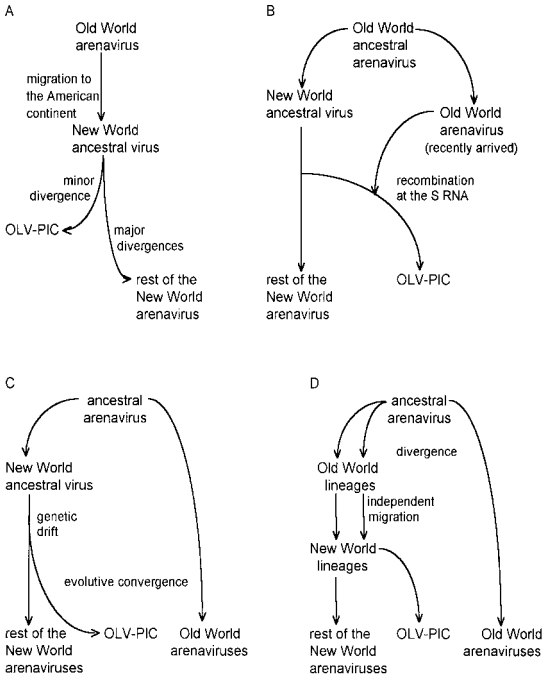

Fig. 3. Graphic representation of four hypotheses that could explain the relationships between OLV and PIC with the rest of the arenaviruses. A close relation to an ancestral virus was considered in A; and a recent S RNA recombination between New a Old World arenaviruses was considered in B. An evolutive convergence was considered in C; while migration of two independent lineages was considered in D. Detailed explanations for each hypothesis are presented in the text.

recombination region, it can be speculated that the event could have taken place in the intergenic region or indeed, within the GPC gene (V. Blinov, personal comunication). In the first case, the GPC gene would have its origin in an Old World arenavirus, while in the second case, only the G1 region would have that origin.

On the other hand, a third possibility could be that OLV and PIC would have a superficial similarity with the Old World arenaviruses. That kind of situation, would have been originated by an accumulation of random mutations in the GPC gene, generating a genic drift in the New World viruses group (Fig. 3C). At this point, it must be remembered that the extensive variability of the GPC gene is a reflection of the mild selective pressure operating in this region and favouring the evolutive phenomena of this kind. Subsequently, the similarity of OLV and PIC with the Old World arenaviruses would have emerged, as a result of an evolutive convergence.

Another hypothesis could be proposed to explain the existence of two arenavirus lineages in the New World. It is known that different subfamilies of the *Muridae* family—the rodent hosts for arenaviruses—migrated to the Americas millions of years ago. Therefore, the ancestors for the OLV-PIC group and the rest of the New World arenaviruses, could have been introduced in the Americas by rodents of different subfamilies, infected with viruses for each of the two lineages (Fig. 3D).

Finally, more complex schemes could be considered superimposing the elements of the hypotheses described before.

References

1. Wulff H., Lange J.V., and Webb P.A., Intervirology *9*, 344–350, 1978.
2. Buchmeier M.J., Clegg J.C.S., Franze-Fernández M.T., Kolakofsky D., Peters C.J., and Southern P.J. in Virus Taxonomy Sixth Report of the International Committee on Taxonomy of Viruses. Murphy F.A., Fauquet C.M., *et al*. (ed.), Springer Verlag, New York, 1995. pp. 319–323.
3. McCormick J.B. in: *Virology* 2nd ed. Fields B.N. and Knipe D.M. (ed). Raven Press, New York, 1990. pp. 1245–1267.
4. Salas R., Manzione N., Tesh R.B., Rico-Hesse R., Shope R., Betancourt A., Godoy O., Bruzual R., Pacheco M., Ramos B., Taibo M.E., García Tamayo J., Jaimes E., Vasquez C., Araoz F., and Querales J., Lancet *338*, 1033–1036, 1991.
5. Coimbra T.L.M., Nassar E.S. Burattini M.N., de Souza L.T.M., Ferreira I.B., Rocco I.M., Travassos da Rosa A.P.A., Vasconcelos P.F.C., Pinheiro F.P., LeDuc J.W., Rico-Hesse R., Gonzalez J.P., Jahrling P.B., and Tesh R.B.; Lancet *343*, 391–392, 1994.
6. Bowen M.D., Peters C.J., and Nichol S.T. Virology *219*, 285–290, 1996.
7. Bishop D.H.L. in: *Virology*, 2nd ed. Fields B.N. and Knipe D.M. (ed.) Raven Press, New York, 1990. pp. 1231–1243.
8. Romanowski, V. in: *The Arenaviridae*. Salvato M.S. (ed). Serie: The Viruses. Wagner, R. and Frankel Conrat, H. (ed.) Plenum Press, New York, 1993. pp. 51–83.
9. Salvato M.S. in: *The Arenaviridae*. Salvato M.S. (ed.), Serie: The Viruses. Wagner, R. and Frankel Conrat, H. (ed.) Plenum Press, New York, 1993. pp. 133–156.
10. Salvato M. and Shimomaye E.M., Virology *173*, 1–10, 1989.
11. Salvato M., Shimomaye E., and Oldstone, M.B.A., Virology *169*, 377–384, 1989.
12. Iapalucci S., Lopez R., Rey O., Lopez N., Franze-Fernandez M.T., Cohen G.N., Lucero M., Ochoa A., and Zakin, M.M., Virology *170*, 40–47, 1989a.
13. Iapalucci S., Lopez N., Rey O., Zakin M.M., Cohen G.N., and Franze-Fernandez M.T., Virology *173*, 357–361, 1989b.
14. Lukashevich I.S., Djavani M., Shapiro K., Sanchez A., Ravkov E., Nichol S.T., and Salvato M.S., J Gen Virol *78*, 547–551, 1997.
15. Auperin D., Romanowski V., Galinski M.S., and Bishop D.H.L., Virology *52*, 897–904, 1984.
16. Romanowski V., Matsuura Y., and Bishop D.H.L., Virus Research *3*, 101–114, 1985.
17. Franze-Fernandez M.T., Zetina C., Iapalucci S., Lucero M.A., Boissou C., Lopez R., Rey O., Daheli M., Cohen G., and Zakin M., Virus Research *7*, 309–324, 1987.
18. Auperin D.D. and McCormick J.B., Virology *168*, 421–425, 1989.
19. Clegg J.C., Wilson S.M., and Oram J.D., Virus Research *18*, 151–164, 1990.
20. Ghiringhelli P.D., Rivera Pomar R.V., Lozano M.E., Grau O., and Romanowski V., J Gen Virol *72*, 2129–2141, 1991.
21. Ghiringhelli P.D., Albariño C.G., Piboul M., and Romanowski V. Am. J Trop Med Hyg *56:2*, 216–225, 1997.
22. Albariño C.G., Ghiringhelli P.D., Posik D.M., Lozano M.E. Ambrosio A.M., Sanchez A., and Romanowski V., J Gen Virol 78, 1605–1610, 1997.
23. Wilson S.M. and Clegg J.C.S., Virology *180*, 543–552, 1991.
24. Bowen M.D., Peters C.J., Mills J.M., and Nichols S.T., Virology *217*, 362–366, 1996.
25. González J.P., Bowen M.D., Nichol S.T., and Rico-Hesse R. Virology *221*, 318–324, 1996.
26. Griffiths C.M., Wilson S.M., and Clegg J.C.S., Archives in Virology *124*, 371–377, 1992.
27. González J.P., Sánchez A., and Rico-Hesse, R., Am J Trop Med Hyg *53(1)*, 1–6, 1995.
28. Fulhorst C.F., Bowen M.D., Ksiazek T.G., Rollin P.E., Nichol S.T., Kosoy M.Y., and Peters C.J., Virology *224*, 114–120, 1996.
29. Lozano M.E., Posik D.M., Albariño C.G. Schujman G., Ghiringhelli P.D., Calderón G., Sabattini M., and Romanowski V., Virus Research, *49* 78–79, 1997.
30. Felsenstein J., Phylogeny Inferencing Package, Version 3.5, University of Washington, USA, 1995.
31. Law M.D., Speck J., and Moyer J.W., Virology *188*, 732–741, 1992.
32. Dewey R., Semorile L.C., Grau O., and Crisci J.V., Virus Genes *13(3)*, 255–262, 1996.
33. Burns J.W. and Buchmeier M.J. (1993). Glycoproteins of Arenaviruses. En: The Arenaviridae. Editado por Salvato M.S., Serie: The Viruses (Wagner, R. & Frankel Conrat, H. Eds.). New York. Plenum Press. pp: 17–35.
34. Clegg J.C.S. in: *The Arenaviridae*. Salvato, M.S. (ed), Serie: The Viruses. Wagner, R. and Frankel Conrat H. (ed.) Plenum Press, New York, 1993. pp: 175–187.