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MOLECULAR VARIABILITY AND IDENTITIES AMONG EGYPTIAN RVFV ISOLATES

(With 2 Tables and 1 Figure)

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التباينات والتشابهات بين بعض العترات المصرية لفيروس حمى الوادي المتصدع

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أوددت العترات المصرية لفيروس حمى الوادي المتصدع اختلافات من حيث القدرة على عدوى الفئران الرضيعة واليافعة المحقونة بالمخ وبالغشاء البريتونى على الترتيب. وأثبت العترة ZH-501 أنها مميتة لكلا النوعين من الفئران ولكن العترة ZH-548 M12 والعترة فليو فيروس مميتة فقط للفئران الرضيعة. ولذا كان هذا التناقض هو الباعث على عمل بعض الاستكشافات الجزيئية التي تستهدف المقطع "M" من جينوم تلك الفيروس بغرض تحديد أوجه التشابه و الاختلاف بينهم والتي قد تفسر ذلك التضارب في القدرة على إحداث العدوى بين تلك العترات. وقد دلت النتائج على أن درجة تشابه بين العترة ZH-501 والعترة ZH-548 M12 كان ٩٩,٤% ، ٩٩,٧٤% وكان التشابه أيضا بين العترة ZH-501 والعترة فليو ٩٩,٨٨% ، ٩٩,١% وذلك على المستوى النيوكليوتيدى والأميني على الترتيب. ووجد أن ستة مواقع عل التسلسل النيوكليوتيدى بين العترات الثلاثة متطابقة ، أربعة منها تقع على الجين ج٢ واثنتان فقط على الجين ج او هذا التطابق والتماثل بين العترات الثلاث يمكن أن يكون راجعا لنشوء هذه العترات من العترة ZH-501أما التباينات فقد تركزت على الجين ج٢ وهو بدوره المسبب التي يحتمل أن يؤدى إلى اختلافات هذه الفيروسات في القدرة على الإصابة وإحداث المرض بينما التطابق الذي تركز على الجين ج٢ يمكن أن يكون المصدر في تشابه الصفات الأنتيجينية لهذه العترات ولذا ينبغي عمل المزيد من الأبحاث لدراسة العترات المستضعفة (ZH-548 M12 + Phlebving) و إمكانية استخدامها في عمليات التحصين وخاصة في أماكن ظهور المرض وأيضا التعرف على دور الأنتجينات المتماثلة في إحداث المناعة ودراسة الاختلافات التي تبعث على ظهور المرض.

SUMMARY

The Egyptian isolates of RVF viruses exhibited variable pathogenicity to intracerebrally and intrapretonially inoculated baby and adult mice respectively. The ZH-501 proved to be lethal for both animals whereas

ZH-548M12 and phlebovirus are only mortals for intracerebrally inoculated mice. Such discrepancy in the infectivity was the motif for molecular speculations targeting the M. segment of these viruses in order to figure out the degree of resemblance and divergence in their genotypic constitution that might elucidate their peculiar phenotypic criteria. These investigations have proved that the degree of homology between ZH-501 and ZH-548M12 was 99.4% and 99.74%, while the identity between ZH-501 and phlebovirus was 99.88% and 99.1% either on the nucleotide and amino acid levels respectively. Six consensus sequences were also detected along, four of them were carried on G_2 gene and two are harboured on G1 gene. This curious highly conserved sequence might be responsible for the epitopes that elicit the RVF specific antibodies (especially the neutralizing ones). Also, the high degree of homology among the Egyptian isolates may give a clue for their unique source being derived most probably from ZH-501. The area of divergence are located mainly on the G1 sequence denoting variable domains that might have a role concerning their virulence phenotypes. Further investigations should be devoted to studying the attenuated strains as immunogens particularly in the enzootic areas. Moreover, for speculating the role and the topology of the common antigenic determinants.

Key words: RPF virus

INTRODUCTION

Rift Valley Fever (RVF) is an African arthropod-born viral disease that primarily affects ruminants (Peters and Meegan, 1984).

Rift Valley Fever virus was first isolated in 1930 during an epidemic among ewes and lambs on a farm in the Rift Valley in East Africa (Daubney and Hudson, 1931).

An epizootic epidemic of unprecedented size swept Egypt for the first time in 1977 to 1978 during which 25% to 50% of sheep and cattle were infected. The scourge extended to involve humans with severe sorts of illness including haemorrhagic hepatitis, meningioencephalitis and retinitis (Meegan, 1979). The disease resurgence was documented in 1993. Several Egyptian RVFV isolates were identified since that time (Arthur et al., 1993).

Judging by the morphological and basic molecular features, Rift Valley Fever virus (RVFV) was classified as member of Bunyaviridae Family (Murphy et al., 1973). Whereas, its antigenic characteristics postulated that it belongs to genus Phlebovirus (Yanagihara et al., 1985).

RVF virus contains a single stranded RNA genome of mostly a negative polarity that has tripartite nature, the large (L) segment codes for L-protein (large polymerase), the medium (M) segment codes for the viral glycoproteins G1 and G2 and the small (S) segment codes for the nucleocapsid protein (N) (Figure1) (Field's et al., 1996). Another two nonstructural proteins, one encoded by the M-segment (NS_M) and the other by the S-segment (NS_S) both proteins have undefined role (Collier et al., 1998). G1 is supposed to be the major virulence determinant that mediates virus attachment and fusion to the receptors of erythrocytes and host cells together with G2 (Ludwig and Israel, 1991).

The main objective of this study has been directed to finding out the degree of resemblance and differences amongst the Egyptian RVFV isolates in order to elucidate their genotypic and phenotypic characteristics that could be devoted for devising a safe and potent RVFV vaccine.

MATERIAL and METHODS

1. Viruses:

The M segment RNA of the Egyptian RVFV isolates was aligned using PC-Gene and Align computer programs. The strains were:

a. RVF G PNSA:

Rift Valley Fever virus (Egyptian isolate ZH-501). The strain was isolated from the serum of human being with RVF in 1977. The fall sequence of the M-segment was published by Takahera et al. (1989).

b. RVF MRNA:

Rift valley fever virus Egyptian isolate, the complete nucleotide sequence M-RNA segment was published by Collett et al. (1985).

c. RVF MPRVAC:

Rift valley fever virus Egyptian isolate, vaccine strain ZH-548M12, its full MRNA sequence was published by Takehara et al. (1989).

2. PC/Gene Computer Program:

PC / gene release 6.6 (February, 1991) from Intalligeneyics contains over 70 programs for the analysis of protein and nucleic acids and management of sequence data.

3. Alignment Computer Program:

This program allows to perform multiple alignment of DNA or protein sequencing using the method developed by Higgins et al. (1992).

4. Translation Computer Program:

This program allows the translation of a nucleic acid sequence to a polypeptide sequence using one of the three open reading frames.

5. Reform Computer Program:

It includes converting a sequence file from intelligenetics suite format or other commercial and data bank formats into PC/gene format. It can also convert a PC/gene sequence file into intelligenetics suite format, GeneBank format, and protein or nucleic acid format.

6. Internet, EMBL and GenBank:

Internet (the World's largest computer network) has many facilities concerning the use of database and transfer files. EMBL is the European Molecular Biology Laboratory in Heidelberg, Germany. It has an important collections of molecular biology computer databases including the Gen Bank nucleotide and protein sequences.

RESULTS

RVFV Egyptian isolates infectivity to baby and adult mice:

RVFV ZH-501 that was isolated from human case during the epidemic of 1977 was intracerebrally and intrapretonially inoculate into baby and adult mice respectively. The virus was lethal for both as all animals died after a week of inoculation, whereas phlebovirus and ZH-548M12 RVF viruses are only lethal for baby mice (Table 1).

Homology percent among RVFV Egyptian isolates:

RVFV ZH-501 exhibited 99.40% degree of nucleotide homology with RVFV ZH-548M12 while on the amino acid level the degree of homology between them was 99.74%. The identity between ZH-501 and phlebovirus on the nucleotide and amino acid levels were 99.88% and 99.1% (Table 2).

Determination of the consenous antigenic determinants:

Six unifying sequences were located between the residues 80-400, 410-705, 1010-1700, 2320-2700, 2750-2980 and 3040-3360, whereas the intervening sequences among the previous ones showed some degree of variability. The trailer showed the hypervariable sequence that might give a clue for virulence difference on G_1.

DISCUSSION

Rift valley fever virus produces severe disease in domestic animals, sheep being more susceptible than cattle, whereas goats are least susceptible. Lambs experience over 90% mortality, adult sheep about 25% and pregnant ewes usually abort (Field's et al., 1996). Virulent RVFV kills baby as well as adult mice but non-pathogenic strains do not (Fenner et al., 1993).

The Egyptian RVF isolates manifested variable infectivity to intracerebrally and intraperitonially baby and adult mice respectively, ZH-501 proved to be lethal for both, but ZH-548M12 and phlebovirus are only mortals to baby mice. The loss of infectivity for the later two viruses might be due to their exposure to some sort of attenuation that led to losing their infectivity to adult mice (Morril and Carpenter, 1991 and Taha et al., 1994). The above mentioned results are completely coincident with those given by Battles and Dalrymple (1988) who ascribed such discrepancy to the variation in the genetic constitution of RVFV isolates.

In terms of the genetic characterization of the Egyptian isolates, our herein studies figured out that the degree of homology between ZH-501 and ZH-548M12 on both the nucleotide and deduced amino levels were 99.4% and 99.74%, while the corresponding parameters between ZH-501 and phlebovirus were 99.88% and 99.1%. Takahera and his colleagues (1989) came out to the same results. They attributed the high degree of resemblance amongest the genomes of the RVF Egyptian isolates to the source that they might have been derived that could be most probably from ZH-501. The genotypic difference of the isolates might be due to the chemical mutation by using the 5-fluorouracil as in ZH-548M12 (Caplen et al., 1985) or to the natural reassortment that spoilt some of the virulence determinants that are responsible for the disease performance (Saluzzo and Smith, 1990).

However, in spite of such mild divergence, consensus sequences were detected between positions 80 and 400, 410 and 705, 1010 and 1700, 2320 and 2700, 2750 and 2980 then 3040 and 3360. These unifying sequences might have a role concerning the neutralizing, haemagglutinating or precipitating antibody production as they may carry the epitopes relevant to such antibodies. These motions were previously ascertained by Battles and Dalrymble (1988). Also, Keegan and Collett (1986) detected hydrophilic areas along the M-segment of

various isolates of RVF virus. They emphasized on the role played by such areas in eliciting similar RVF antibodies in animal body.

Hydrophobic regions were also recognized mostly on G1 glycoprotein that might act as virulence determinants that have major importance in the process of viral infection and pathogenesis (Besselaar and Blackburn, 1991).

It could be concluded that the attenuated isolates such as ZH-548M12 and phlebovirus could be utilized as vaccines against the virulent RVF isolates e.g. ZH-501 in condition that they must be subjected to further investigations so as to prove their safety as well as their immunogenicity.

REFERENCES

Arthur, R.; El-Sharkawy, M.S. and Cope, S.E. (1993): Recurrence of Rift Valley Fever in Egypt. Lancet 1993, 342 : 1149 - 1150.

Battles, J.K. and Dalrymple, J.M. (1988): Genetic variation geographic isolates of Rift Valley Fever virus. Am. J. Med. Hyg., 39, 617 - 631.

Besselaar, T.G. and Blackburn, N.K. (1991): Topological mapping of antigenic sites on the Rift Valley Fever virus envelope glycoproteins using monoclonal antibodies. Arch. Virol., 121 (1-4) : 111 - 124.

Caplen, H.; Peters, C.J. and Bishop, D.L. (1985): Mutagen-directed attenuation of Rift Valley Fever virus as a method for vaccine development". J. Gen. Virol., 66 : 2271-2277.

Collett, M.S.; Purchio, A.F.; Keegan, K.; Frazier, S.; Hays, W.; Anderson, D.K.; Parker, M.D.; Schmaljohn, C.; Schmidt, J. and Dalrymple, J.M. (1985): Complete nucleotide sequence of the M-segment of Rift Valley Fever virus. Virology 144, 228 - 245. MEDLINE identifier 86045943.

Collier, L.; Balows, A. and Sussman, M. (1998): Toply & Wilson's "Microbiology and Microbial Infections" Ninth Edition. Vol. I Virology, Vol Edit. (Brion, W.J., Leslie Collier). A member of the Hodder Headline Group, London. Sydney. Auckland Co. Published in the USA by Oxford University Press, Inc. New York.

Daubney, R.; Hudson, J.R. (1931): Enzootic hepatitis or Rift Valley Fever. An undescribed virus disease of sheep, cattle and man from East Africa. J. Pathol., 34 : 545 - 557.

Fenner, F.J.; Gibbs, E.P.J.; Murphy, F.A.; Rott, R.; Studdert, M.J. and White, D.O. (1993): "Veterinary Virology". Second Edition, Academic Press, 369: 389.

Fields, B.N.; Knipe, D.M.; Howley, P.M.; Chanock, R.M.; Melnick, J.L.; Monath, T.P.; Roizman, B. and Straus, S.E. (1996): Fields "Virology" Third Edition. Lippincott-Roven Publishers, Philadelphia, New York.

Higgins, D.G.; Bleasby, A.J. and Fuchs, R. (1992): Clustal V: Improved software for multiple sequence alignment computer applications in the Biosciences, 8 : 189-191.

Keegan, K. and Collett, M.S. (1986): Use of bacterial expression cloning to define the amino acid sequences of antigenic determinants on the G_2 glycoprotein of Rift Valley Fever virus. J. Virol., 58 : 263-270.

Ludwig, G.V. and Israel, B.A. (1991): Role of La Crosse virus glycoproteins in attachment of virus to host cell. Virology, 181: 504 - 71.

Meegan, J. (1979): The Rift Valley Fever epizootic in Egypt 1977 - 78. 1. Description of the epizootic and virological studies. Trans. R. Soc. Trop. Med. Hyg., 73 : 618 - 623.

Morril, J.C. and Carpenter, L. (1991): Further evaluation of a mutagen attenuated Rift Valley Fever vaccine in sheep. Vaccine, 9: 35 - 41.

Murphy, F.; Horrison, A.K. and Whitfield, S.G. (1973): Bunyaviridae morphologic and morphogenetic similarities of Bunyamwera serologic supergroup viruses and several other anthropod borne viruses. Intervirology, 1 : 297 - 316.

Peters, C.J. and Meegan, J.M. (1984): "Handbook Series in Zoonoses". 1, 403-420.

Saluzzo, J.F. and Smith, J.F. (1990): Use of reassortment viruses to map attenuating and temperature sensitive mutations of the Rift Valley Fever virus MP-12 vaccine. Vaccine, 8(4): 369 - 375.

Taha, M.M.; Ellian, K.; Mohamed, G.K.; Soliman, S.M.; Mohsen, A.Y.A. and El-Debagy, A. (1994): Studies on Rift Valley Fever virus trials for production of attenuated Rift Valley Fever

variant from original (ZH-501) Egyptian strain". J. Egypt Vet. Med. Ass., 54 (4): 348-357.

Takehara, K.; Min, M.K.; Battles, K.; Sugiyama, V.C.; Dalrymple, J.M. and Bishop, D.H.L. (1989): Identification of mutations in the M RNA of a candidate vaccine strain of Rift Valley Fever virus. Virology, 169, 452 - 457. MEDLINE identifier, 89204917.

Yanagihara, R.; Goldgaber, D. and Gajduesk, D.C. (1985): Propagation of nephrophathia epidemica virus in Mongolian gerbils. J. Virol., 53 : 973 - 975.

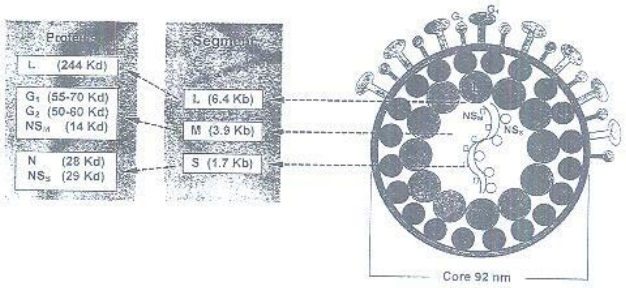

Fig (1): STRUCTURE AND CODING ASSIGNMENTS OF RVF VIRUS.

Table 1: Pathogenicity of ZH-501 RVF virus in baby and adult mice.

Animals ⇨	Baby Mice		Adult Mice	
DPI ⇩	Living	Dead	Living	Dead
1	20	0	15	0
2	16	4	14	1
3	11	9	12	3
4	7	13	8	7
5	2	18	3	12
6	1	19	1	14
7	0	20	0	15

DPI: Days Post Inoculation.

Table (2): Nucleotide and amino acid divergencies among ZH-501, ZH-548 and phlebovirus of RVF Egyptian isoltes.

Serial number	Gene order	Nucleotide			Amino acid			Nucleotide			Amino acid		
		No.	ZH 501	ZH 548	No.	ZH 501	ZH 548	No.	ZH 501	ZH 548	No.	ZH 501	ZH 548
1		10	C	T	9	I	T	847	A	G	276	E	G
2		45	T	C	17	V	I	2318	T	A	767	F	I
3		69	G	A	129	E	K	2748	G	A	910	G	S
4		404	A	T	232	Q	L	3633	C	A	1159	T	L
5		714	T	C	259	Y	H				1160	I	Y
6		795	G	A	329	H	L				1161	L	S
7		857	A	T	566	G	D				1162	L	S
8		1005	T	C	602	V	I				1163	I	F
9		1697	G	A	641	H	D				1164	C	A
10		1706	G	A	747	I	L				1165	L	C
11		1824	C	G	1182	R	G				1166	Y	M
12		1941	A	T							1167	V	L
13		2258	C	T							1168	A	H
14		2711	A	C							1169	L	Y
15		2981	A	T							1170	S	Q
16		3564	T	C							1171	I	L
17		3621	C	G							1173	L	S
18		3632	A	C							1175	F	S
19		3633	C	A							1176	L	S
20		3644	A	G							1178	I	Y
21		3655	C	T							1179	V	I
22		3660	A	T							1181	G	E
23		3678	G	A							1182	R	E
24											1183	T	Q
25											1184	G	A
26											1185	L	S
27											1186	S	L
28											1188	M	C
29											1189	W	Q
30											1191	A	L
31											1192	A	P
32											1193	T	L