African Swine Fever: genomic

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The effect of passage in pigs and pig bone marrow cultures on the genome of the Cameroon isolate (CAM/82) of ASF virus

Investigation of the effect of serial passage of the CAM/82 ASFV isolate in pigs and in primary pig bone marrow cultures by restriction enzyme analysis and restriction enzyme site mapping

Introduction

Many reports have been written describing the major rearrangements that occur during adaptation of ASF virus to tissue culture (2, 13, 15). Comparison of restriction enzyme site maps of genomes of tissue culture adapted virus with those of unadapted virus showed that most changes resulted from deletions or additions of sequences from regions close to both termini. In two different experiments (2, 13) it was observed that large deletions of 7 kb and 15 kb of sequences occurred between about 7 and 23 Kb from the left terminus during adaptation to MS and Vero monkey cell lines. In addition, small increases in the size of both terminal fragments and a fragment about 25 kb from the right end were observed (13). Viral DNA sub-populations were also produced when the ASFV E70 isolate from Spain was grown in monkey kidney MS cells (10). Mapping the genomes of four of these virus variants showed that the restriction enzyme fragments located at both termini, one fragment about 25 kb from the right end and one fragment in the center of the genome varied in length when virus variants were compared to the parent viruses (10). Virus genome heterogeneity has also been observed in plaque-purified virus isolates obtained after serial passage in Vero cells (15).

Most biochemical studies have been made with the virus adapted to grow in monkey kidney cells and the DNA of such tissue culture- adapted virus varies in size (160 kbp) when compared to the wild type virus (170 kbp) (1, 13). This study was carried out to investigate the effect of serial passage of the CAM/82 ASFV isolate in pigs and in primary pig bone marrow cultures by restriction enzyme analysis and restriction enzyme site mapping.

Materials and methods

Crossbred Large White x Landrace pigs of 20-30 kg live weight were used in the study. Two ml of spleen suspension containing the original CAM/82 ASF virus at a titre of 104.1 HAD50/gm of spleen was first inoculated intravenously into a pig after taking a pre-inoculation blood sample. When the pig developed a temperature of 40°C or more, blood was collected and virus DNA was prepared from the infected packed red blood cells as described by Wesley and Tuthill (16). The same procedure was carried out for 20 pig passages of the virus and each time a suspension prepared from the spleen of a previously infected pig was used to inoculate a new pig.

The restriction enzymes BamHI, Asp718 and EcoRI obtained from Boehringer Mannheim were used according to manufacturer?s recommendations to digest the virus DNA prepared from the infected red blood cells obtained from the different pig passages. The end-labeling with 32PdATP using the Klenow fragment of DNA polymerase I was performed using standard procedures (5, 6) and electrophoresis of the digested products in 0.6% agarose gel. A Southern blot prepared after digesting the various DNA samples with BamHI was used to hybridize with plasmid DNA clones, RK?, RA/SC, RB and RD? of the Vero cell-adapted Spanish isolate of ASF virus DNA (7). These

clones hybridize with fragments which map to the left terminus, the region 35-40 kb from the left terminus, the central region and the right terminus which contain the variable regions of the ASF genome (2, 3, 12, 14).

The PBM cultures were prepared as described by Plowright et al (9) and established in 75 ml plastic tissue culture flasks (FalconR). The monolayers were infected with 2 ml of the CAM/82 ASF virus at a titre of 104.1 HAD50/gm of spleen after 3 days of incubation at 37°C. When the cytopathic effect (CPE) was 80-90% complete, the cells were pooled and an aliquot of 20 ml was stored at - 70°C for the next virus passage while the rest was centrifuged at 1000 G for 15 min at 4°C. The preparation of virus DNA from the infected cells was carried out essentially as described by Wesley and Pan (15). The process was repeated until the virus had been passed 17 times in PBM cultures and at each passage the virus, stored at - 70°C from the previous passage, was used to infect new PBM cultures.

The restriction enzymes BamHI and EcoRI were used to digest the virus DNA from the different PBM passages and the digests were end-labeled and electrophoressed in 0.6% agarose gel following standard procedures (5, 6). A Southern blot was prepared from fragments produced after digesting the DNA samples with BamHI (11). The blot was used to hybridize with plasmid clones RA/SC, RB RD?, SH and RK?. This was to determine whether any differences had occurred in the variable region as a result of the PBM culture passage.

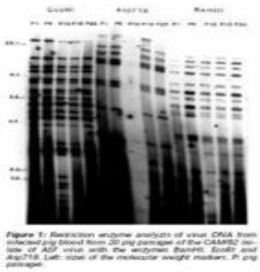
Results

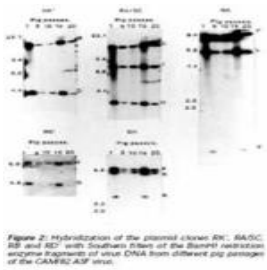
Virus DNA prepared from infected red blood cells obtained from the pig passages 1, 5, 10, 15 and 20 of the CAM/82 virus isolate was digested with the restriction enzyme BamHI, EcoRI and Asp718. The digested products were analyzed by electrophoresis on 0.6% agarose gel. No variations in the electrophoretic mobility of the restriction enzyme fragments were observed between the genomes with the three enzymes (figure 1). Cross-hybridizations of the BamHI fragments of the virus genome from the various pig passages on a Southern blot were carried out using the plasmid DNA clones RK?, RA/SC, RB, SH and RD? as probes to determine whether any variations had occurred in the fragments in the variable regions of the genome. No size differences were observed using these probes (figure 2).

Virus DNA of the CAM/82 isolate prepared from 5 passages of the virus in PBM cultures was analyzed by restriction enzyme analysis on 0.6% agarose gel with the enzymes BamHI and EcoRI. No differences in restriction enzyme fragment pattern were observed between the DNA from the PBM passages 1, 5, 8, 10 and 17 with BamHI and EcoRI (figure 3) with the parent virus isolate.

The virus became non-haemadsorbing after the 10th passage and remained non haemadsorbing in the subsequent passages. No differences between restriction enzyme fragments of the genome of the CAM/82 virus from the different PBM passages was observed when a Southern blot of these fragments was crosshybridized with the plasmid DNA clones RK?, RA/SC, RB, SH and RD?, which hybridize to fragments mapping to the variable regions of the genome (figure 4).

Discussion

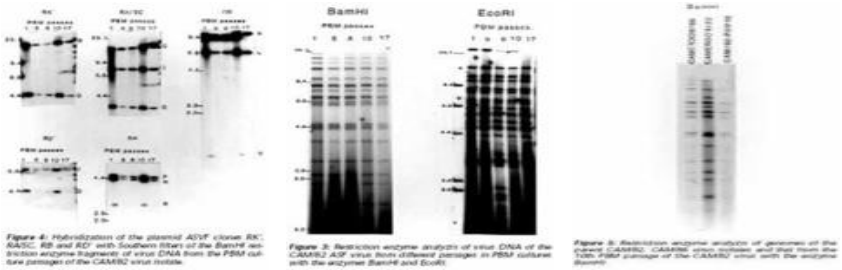




The results of the restriction enzyme analysis and cross-hybridizations of the restriction enzyme fragments with plasmid DNA clones of ASF virus, have shown that no changes were detected in the genome of the CAM/82 isolate of ASFV when the virus was passed either in pigs or PBM cultures. This stability of the ASFV genome has also been observed with other isolates during natural infection in domestic pigs. The Dominican Republic and Haiti isolates were obtained three years apart and from different geographical areas, yet they showed identical restriction enzyme fragment profiles which were also very closely related to those of the genome of the CAM/82 isolate of ASFV (16). The CAM/82 isolate was indistinguishable from the CAM/88 isolate by restriction enzyme analysis (4) and these isolates were obtained six years apart from different parts of the enzootic area. Also seven 1986 Portuguese ASFV isolates from outbreaks in domestic pigs in different regions of the country were shown to be identical by restriction enzyme analysis while other European isolates of ASFV from outbreaks in domestic pigs were observed to be very closely related (Wilkinson et al., personal communications). The results presented in this study and those of other workers mentioned do suggest that the ASFV genome is quite stable during pig-to-pig transmission of the virus both in natural and in experimental conditions.

No differences in restriction enzyme fragment pattern were observed with the virus DNA from the different PBM culture passages (figure 3). Cross-hybridizations of the BamHI restriction enzyme fragments on a Southern blot with plasmid clones RK?, RA/SC, RB, SH and RD? failed to detect any differences within the variable regions of the genome between virus DNA from the different PBM passages. A number of different workers have described major genome rearrangements that occur during adaptation of ASFV to grow in other tissue culture cell lines (2, 13, 15). Most changes resulted from deletions or additions of sequences from regions close to both termini. Large deletions of 7-15 kbp of sequences between about 7 and 23 kb from the left terminus occurred during adaptation of the virus to MS and Vero monkey cell lines (2, 13). In addition, small increases in the size of both terminal fragments and of a fragment about 25 kb from the right terminus were also observed (13). This latter fragment also varied in length in virus variants obtained after sequential passage in MS tissue culture cells (13). However, the Badajoz isolate of ASFV (BA71 clone 1) did not change in its SalI restriction enzyme fragment pattern after 20 passages in pig macrophage cultures (2). Also, the virus BA71H which was derived from BA71 by more than 100 passages in pig macrophage cultures, showed no change in the SalI restriction enzyme pattern with respect to the predominant virus in the original population (2). Their results were very similar to those obtained in the present study with the CAM/82 virus isolate.

It was observed that the virus (CAM/82) became non-haemadsorbing after the 10th PBM culture passage. It is quite possible that the passaging process had selected a new predominant virus population which did not possess this biological marker and had a similar genome to the earlier population as shown by the similar restriction enzyme fragment patterns (figure 5). This multiclonal concept of ASF virus was first proposed by Pan and Hess (8). They stated that ASFV isolates are composed of heterogeneous virus populations that can be separated by cloning and that they may differ in their biological characteristics with respect to haemadsorption, virulence, plaque size, antigenic composition and genomic structure. Therefore, the characteristics of any particular virus population can change if the proportion of the different clones is altered by passage in tissue culture (or pig) and this is probably the situation that was generated from the 10th PBM passage in relation to the haemadsorption characteristic. The results presented in this study provide further epidemiological evidence for the persistence of ASF in Cameroon by continuous circulation of viruses in pig populations.



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References

1. ALMENDRAL J.M., BLASCO R., LEY V., BELOSO A., TALAVER A., VINUELA E., 1984. Restriction site map of African swine fever virus DNA. Virol., 133: 258-270.
2. BLASCO R., AGUERO M., ALMENDRAL J.M. AND VINUELA E., 1989. Variable and constant regions in African swine fever virus DNA. Virol., 168: 330-338.
3. DIXON L.K., WILKINSON W.P., 1988. Genetic diversity of African swine fever virus isolates from soft ticks (Ornithodoros moubata) inhabiting burrows in Zambia. J. Gen. Vir., 69: 2981-2993.
4. EKUE N.F., 1989. The epidemiology of African swine fever in Cameroon. Ph.D. Thesis, University of Surrey, Guildfort, U.K., 231 p.
4. FEINBERG A.P., VOGELSTEIN B., 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 132: 6-13.
5. FEINBERG A.P., VOGELSTEIN B., 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 137: 266 -267.
6. LEY V., ALMENDRAL J.M., CARBONERO P., BELOSO A., VINUELA E., TALAVERA A., 1984. Molecular cloning of African swine fever virus DNA. Virol., 133: 249-257.
7. PAN I.C., HESS W.R., 1985. Diversity of African swine fever virus. Am. J. Vet. Res., 66: 314 - 320.
8. PLOWRIGHT W., PARKER J., STAPLE R.F., 1968. The growth of a virulent strain of ASF virus in domestic pigs. J. Hyg., 66: 117-134.
9. SANTURDE G., GONZALVO RUIZ F.,CARNERO M.E., TABARES E., 1988. Genetic stability of African swine fever virus grown in monkey kidney cells. Arch. Virol., 98: 117-122.
10. SOUTHERN E.M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol., 98: 503- 518.
11. SUMPTION K.J., HUTCHINGS G.H., WILKINSON P.J., DIXON L.K., 1990. Variable regions on the genome of the Malawi isolates of ASF virus. J. Gen. Virol., 71: 2331-2340.
12. TABARES E., OLIVARES I., SANTURDE C., GARCIA M.J., MARTIN E., CARNERO M.E., 1987. African swine fever virus DNA: deletions and additions during adaptation to growth in monkey kidney cells. Arch. Virol., 97: 333-346.
13. VINUELA E., 1985. African swine fever. Curr. Top. Microbiol. Immunol., 116: 151-170.
14. WESLEY R.D., PAN I.C., 1982. African swine fever virus DNA: Restriction endonuclease cleavage patterns of wild type, Vero celladapted and plaque purified virus. J. Gen. Virol., 63: 383-391.
15. WESLEY R.D., TUTHILL A.E., 1984. Genome relatedness among African swine fever field isolates by restriction endonuclease analysis. Prev. Vet. Med., 2: 53-62.

Yes