Analysis of Theiler's Virus Isolates from Persistently Infected Mouse Nervous Tissue

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SUMMARY

The DA strain of Theiler's virus causes a chronic progressive demyelination in mice following intracerebral inoculation. Virus was isolated from chronically infected mice, and then grown in cell culture, and the isolates were compared with the parent virus used for inoculation. No defective interfering particles or temperature-sensitive virus were recovered, and capsid proteins appeared identical by SDS-PAGE. One of three isolates had evidence of genomic mutation by T1 ribonuclease oligonucleotide fingerprinting. The significance of these findings with regard to the generation and maintenance of persistence and to adaptation to cell culture is discussed. Also of interest was the marked difference between the DA fingerprint and that of GD VII, a serologically related strain with different biological activity.

Theiler's murine encephalomyelitis viruses (TMEV) are a group of picornaviruses that produce enteric and neurological disease in mice. There are two subgroups of Theiler's viruses which differ in their biological activity (Lipton, 1980). The group typified by the GD VII strain is highly virulent, produces large plaques in vitro, causes acute encephalitis in vivo, and does not persist in mice (Lipton, 1980). The group typified by the DA strain is less virulent, produces small plaques in cell culture (Lipton, 1980), and causes a chronic, progressive demyelinating disease in weanling mice (Daniels et al., 1951; Lehrich et al., 1976; Lipton, 1975). Small amounts of virus can be isolated from the central nervous system of demyelinated mice for months in the face of a vigorous immune response manifest by large amounts of antibody in the blood as well as in the central nervous system (Lipton & Gonzalez-Scarano, 1978). It is unclear whether viral titres are low because the levels of virus are low or because the virus is in a non-productive form.

Picornaviruses are generally regarded as acutely lytic viruses with little tendency to persist in vivo in the immunocompetent host; there are examples, however, of persistence by picornaviruses reported for foot-and-mouth disease virus (Fellowes & Sutmoller, 1970) and poliovirus (Miller, 1981). The factors enabling DA, a potentially acutely lytic virus, to persist in vivo are presently unknown. In the case of some RNA viruses, the evolution of virus populations by means of mutation enables the virus to persist for prolonged periods (Youngner & Preble, 1981). In visna virus infection, for example, the immune response is bypassed by repeated mutations of the virus which change its antigenicity (Narayan et al., 1978). In subacute sclerosing panencephalitis, a mutation involving measles virus M protein, important in virus assembly, presumably leads to a more chronic infection (Hall & Choppin, 1981). Defective interfering (DI) particles and temperature-sensitive (ts) mutations of several viruses have been associated with persistent infections in vitro and experimental chronic infections in vivo (Rabinowitz et al., 1976; Spandidos & Graham, 1976), but there is only suggestive evidence that these mutations evolve during the course of a naturally occurring chronic infection in vivo (Buchmeier et al., 1980; Fellowes & Sutmoller, 1970; Straver & Van Bekkum, 1972).

We sought to determine whether DA virus develops mutations during the chronic disease, thereby producing a more indolent infection and avoiding host immune clearance. We have begun an investigation to characterize the virus populations which are used to produce DA chronic disease, which exist in the persistently infected animal, and those which emerge from the persistently infected animal when the isolates are again passed in cell cultures. Direct analysis of virus present in mouse brain and spinal cord is difficult, however, and will require the development of new methodology, which is in progress. In addition, virus isolated from central...
nervous tissue of persistently infected mice grows poorly in cell cultures in the laboratory, and in
general must be passed several times before reasonable yields can be obtained; the changes
occurring during this ‘adaptation’ period are unknown. If virus mutations exist in persistently
infected mice, the mutations may become fixed in the viral genome and be retained by virus
which is subsequently passaged in culture. In this report, we describe the analysis of several
isolates of virus from persistently infected nervous tissue which have been subsequently cultured
in the laboratory and compared with the parent virus used to establish the persistent infections.

The DA strain, designated DAwt, was inoculated intracerebrally (0.03 ml) into 3-week-old
SJL/J mice (Jackson Laboratory, Bar Harbor, Me., U.S.A.). Progressive demyelination with
weakness began approximately 4 weeks after inoculation. Three months after inoculation, a
10% homogenate in Hanks' buffered saline solution was prepared from spinal cords from each
of three mice, and was inoculated separately onto BHK-21 (baby hamster kidney) cells. In order
to build up sufficient titre, the isolates were passed an additional six times in BHK-21 cells.
These three isolates are designated DA1, DA2 and DA3. In the case of DA1, the initial BHK-21
monolayer was overlaid with minimum essential medium containing 0.5% agarose (Seakem
Marine Colloids Inc., Springfield, N.J., U.S.A.) and 2% calf serum; after 3 days a
microscopically visible, isolated plaque was picked and passed onto further BHK-21 cells.

Initial experiments were conducted to search for the presence of mutants or DI particles in the
virus preparations grown from persistently infected mice. When plaque-assayed on BHK-21
cells (Lipton, 1980) the DAwt and isolates demonstrated small plaques in neutral red-stained
agarose in contrast to the larger plaques seen with GD VII, as previously described (Lipton,
1980). The titre of wild-type DA virus was $10^{6.3}$ plaque-forming units (p.f.u.) per ml at 32.5 °C
and at 39.5 °C with an efficiency of plating (e.o.p.) at 39.5 °C/32.5 °C of 1. The titre of DA1 was 10^6.8 p.f.u. at 32.5 °C and 10^6.2 p.f.u. per ml at 39.5 °C with an e.o.p. at 39.5 °C/32.5 °C of 0.3, indicating no significant temperature sensitivity of this isolate.

All virus isolates were purified as previously described (Roos et al., 1982) and banded at a density of approximately 1.36 g/ml in CsCl. No evidence of DI particles banding at a lighter buoyant density was apparent in any virus preparation. Since several described picornavirus DI particles have been shown to contain RNA genomes with small deletions, yielding particles not readily resolvable from complete virus in CsCl, RNA extracted from purified preparations of DAwt and the three isolates was analysed in denaturing gels containing methylmercury hydroxide according to previously published methods (Batt-Humphries et al., 1979). The viruses studied contain one predominant RNA species, corresponding to a size of approximately 7400 nucleotides (data not shown). The GD VII strain of TMEV contains RNA of an identical size. No other homogeneous RNA species was detectable, indicating that virus preparations from chronically infected animal isolates subsequently grown in cell cultures are not enriched for DI particles, compared with preparations from the parent strain. One would have expected that if significant numbers of DI particles had been present then they would have been amplified during several rapid growth cycles in vitro rather than be selected against during the subsequent passages in cell cultures. However, the role of host factors in the generation and/or amplification of DI particles is still unknown and the specific cell type in which the virus persists may be important.

Viral capsid proteins from DA1 and DA2 were compared with those of the wild-type DA parent by one-dimensional SDS–PAGE (Laemmli, 1970) on 12.5% polyacrylamide gels of virions purified from cells infected in the presence of [35S]methionine. The two isolates contained a polypeptide profile identical to that of DAwt (data not shown). Three major polypeptides corresponding to VP1, VP2, and VP3 (molecular weights 25000 to 35000) were visible, as well as a smaller polypeptide of less than 10000 corresponding to VP4. This pattern is typical of picornavirus capsids and is similar to that previously described for DA virus (Lipton & Friedman, 1980).

Despite the similarity in the mobilities of the capsid proteins of DA virus grown from the isolates from persistently infected mice and from the parent virus, RNase T1 oligonucleotide maps of the genomes of each virus isolate were prepared according to previously described techniques (Roos et al., 1982) in order to evaluate the possibility of mutations not detectable by SDS–PAGE or located at sites coding for non-structural proteins. Fig. 1 shows a comparison of two-dimensional fingerprints of DAwt RNA (Fig. 1b) and RNA from GD VII (Fig. 1a), a serologically related strain of Theiler's virus which has marked biological differences from DA, and which does not produce persistent infection. The RNA fingerprints of these two strains are substantially different from one another. In particular, the largest T1 oligonucleotides from GD VII RNA migrate more rapidly than the xylene cyanol dye marker in the second dimension, whereas DA RNA contains two prominent very large T1-resistant oligonucleotides which migrate more slowly than the dye. A fingerprint of DAwt material was also prepared after eight lytic cell culture passages (DAwt + 8) to compare with those which were isolated from the persistently infected mice and then similarly passaged (Fig. 2a). No changes in the fingerprint of DAwt were detected. Fig. 2 also shows fingerprints of virus grown from the three animal isolates (Fig. 2b, c, d). Animal isolates DA2 (Fig. 2c) and DA3 (Fig. 2d) had fingerprints indistinguishable from the parent wild-type. DA1 (Fig 2b), however, lacks one prominent oligonucleotide and contains at least one oligonucleotide not present in the fingerprint of DAwt. Since the large identifiable oligonucleotides represent only about 10% of the total genome, this is a minimum estimate of mutation. Similarly, the absence of detectable change in the fingerprints of the other two isolates does not guarantee that mutation has not occurred. Thus, mutations in the viral genome do arise which can be detected by this method of analysis, but the virus which emerged after growth in cell culture following isolation from persistently infected mice was very little changed from the original parent. In addition, no single consistent change appears to have been found in the genome as a result of the persistent virus infection.

The present study represents an initial effort to determine whether changes in virus
Fig. 2. Two-dimensional oligonucleotide maps of RNase T1-digested DA viral RNAs. (a) DAwt + 8; (b) DA1; (c) DA2; (d) DA3. The circle indicates the position of an oligonucleotide present in DAwt and DAwt + 8 but not in DA1. The arrow indicates the position of an oligonucleotide present in the isolates, but not in DAwt. The first dimension is from left to right; the second dimension is from bottom to top.
populations occur during replication and establishment of persistence in vivo and during subsequent adaptation of isolates from the persistently infected animals to growth in cell culture. We have characterized the DA strain of TMEV, which consistently produces a chronic demyelinating disease in weanling mice, and shown that its RNA fingerprint is stable during lytic passage in cell culture; and we have also shown that its fingerprint is distinctly different from the GD VII strain of TMEV, a serologically related strain which produces only acute disease and no persistent infection. [While this manuscript was in preparation, the marked difference in oligonucleotide maps between DA and GD VII was reported by Lorch et al. (1981).] In addition, we have shown that virus which is recovered from virus populations isolated from persistently infected tissue and then grown in cell cultures is very little changed from the original parent. If replication and persistence in vivo were accompanied by significant mutation of the viral genome, it is possible that isolates derived from the persistently infected animals would retain some of their genome alterations even after passage in cell culture. For example, analyses of poliovirus type 1 isolated from clinical infections and subsequently grown in culture showed clear evidence of evolution of the viral genome following replication in vivo, but stability of the genome in vitro (Nottay et al., 1981). Alternatively, growth in cell culture might be a sufficiently selective pressure so that the adaptation procedure would produce very similar viruses, regardless of the starting population. The results presented in this report emphasize the need for direct analysis of this virus population present in mouse nervous tissue, without subsequent passage in vitro. It is also possible that no significant changes in the viral genome occur during persistent infection, but that host cell permissiveness and/or host defence mechanisms produce the persistent infection without virus alteration.

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