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Stealth adaptation of viruses: review and updated molecular analysis on a stealth adapted african green monkey simian cytomegalovirus (SCMV)

Abstract

The available DNA sequence data on an African green monkey simian cytomegalovirus virus (SCMV)-derived stealth adapted virus are summarized. The data provide important insight into a generic mechanism by which viruses avoid effective immunological recognition by the cellular immune system. This process is termed stealth adaptation and comprises the deletion or mutation of the relatively few virus components, which are normally directly targeted on virus infected cells by T lymphocytes. The sequence data also reveal the potential complexity of stealth adapted virus genomes resulting from genetic instability and also from the apparent involvement with replicating cellular and bacterial genes. Stealth adapted viruses, including those which presumptively originated from SCMV contaminated poliovirus vaccines, pose a serious threat to public health and can readily explain the increasing prevalence of neuropsychiatric illnesses, including autism, mental diseases and the chronic fatigue syndrome (CFS).

Keywords: Herpes virus, Warts, Zoster, Shingles, Papillomavirus, Post-herpetic neuralgia, Phototherapy, Alternative cellular energy, ACE pathway, ACE pigments, KELEA

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Abbreviations: HSV, Herpes Simplex Virus; HZV, Herpes Zoster Virus; HPV, Human Papillomavirus; ACE, Alternative Cellular Energy; KELEA, Kinetic Energy Limiting Electrostatic Attraction; UV, Ultraviolet; CPE, Cytopathic Effect; MHC, Major Histocompatibility Complex; HCMV, Human Cytomegalovirus; CTL, Cytotoxic T Cells; PCR, Polymerase Chain Reaction; CSF, Cerebrospinal Fluid; CFS, Chronic Fatigue Syndrome; IE, Immediate Early; EBV, Epstein Barr Virus; VZV, Varicella-Zoster Virus; HTLV, Human T Lymphotrophic Viruses

Introduction

While inflammation is the expected hallmark of infectious diseases, situations have been described in which the body fails to mount an inflammatory response to an ongoing virus infection. One example is infection occurring in an individual without a functioning cellular immune system, as can be seen with JC virus infections in AIDS patients.[1,2] Prenatal virus infections can potentially induce immunological tolerance to virus antigens, as occurs with congenitally acquired hepatitis B virus.[3,4] Changes in a virus can also explain a lack of an accompanying inflammatory reaction. Although still not widely appreciated among virologists, relatively few components of most viruses are displayed on the surface of virus-infected cells in a manner that allows for effective immunological recognition by T lymphocytes. The restricted diversity of virus antigen presentation is a corollary of the Clonal Section Theory of Acquired Immunity.[5] Each lymphocyte can only engage with cells expressing multiple copies of the actual antigenic specificity for which that particular lymphocyte is genetically preprogrammed to recognize. Virus antigen presentation is a complex process requiring partial degradation of selected virus proteins to small peptides, which then bind to a specific region of newly synthesized major histocompatibility complex (MHC) proteins, prior to these proteins being transported to and lodging within the cell membrane.[6]

The restriction on virus antigenic recognition is particularly striking for human cytomegalovirus (HCMV). Although, this virus codes for more than 200 proteins, the majority of anti-HCMV cytotoxic T cells (CTL) are directed against the protein coded by virus gene UL83, where UL refers to the unique long segment of the virus genome and US refers to an adjoining unique short segment .[7-10] Additional smaller antigenic contributions are made by UL55 and UL123 coded proteins, such that in aggregate these three components comprise over 90% of targeted antigens for the CTL response against HCMV.[11-15] Deletion or mutation in the relatively few genes encoding critical immunogenic virus antigens provides a relatively simple explanation for how active virus infections may persist without evoking an inflammatory reaction.

The successful clinical use of neutral red dye plus light treatment of HSV skin lesions was originally reported in 1971 by Felber,[13] along with colleagues, he published additional findings in 1973.[14] Although, many clinics began utilizing this approach, the results were seemingly too variable to be accepted into mainstream medicine. The issue also arose as to whether neutral red dye plus light could mutate the virus DNA in such a manner that the damaged virus could potentially cause cancer.[15-17] In a major clinical trial published in 1975 in the New England Journal of Medicine, Dr. Meyer's and his colleagues from Harvard Medical School reported that neutral red dye applied to HSV skin lesions followed by light illumination was ineffective.[18] Additional publications supported this negative conclusion and the neutral red dye/light procedure was largely abandoned.

The possibility of this novel immune evasion mechanism was supported by an examination of a brain biopsy obtained in 1990 from an immunocompetent patient with an unexplained neurological illness.[16] She had been experiencing social difficulties as a primary school teacher and sought psychological counseling in an unsuccessful effort to avoid being dismissed. Upon reemployment as a kindergarten

teacher, she found it difficult to express herself, either verbally or in writing. Near confluent, bilateral periventricular opacities were seen on MRI (magnetic resonance imaging), justifying a stereotactic needle biopsy of this region. The biopsy showed no inflammation, yet was weakly positive when tested using the polymerase chain reaction (PCR) method of virus detection.

The PCR assay uses small synthetic oligonucleotides as primers for recycling DNA replication. Ideally the DNA sequences of the synthetic primers match exactly to relatively closely spaced regions on opposing strands of the targeted DNA virus, allowing for the assay to be performed under high stringency conditions and to be uniquely specific for the targeted virus. In the PCR study employed on the brain biopsy, however, the primers used were designed to be more broadly reactive with multiple human herpesviruses when tested using low stringency conditions. It was not possible at the time to further characterize the actual virus presumptively responsible for the weak, but decidedly positive PCR. Cellular damage comprising vacuolization with intracellular inclusions was clearly present on histological examination of the brain tissue and confirmed by electron microscopy.[16,17] The indications of cellular damage, together with the positive PCR, were highly suggestive of a virus infection, in spite of the lack of an inflammatory response. The putative virus causing the cellular brain damage was characterized as "stealth" in it apparent ability to bypass the cellular immune system.

Earlier support for this basic premise was provided by positive PCR assays performed on blood and/or cerebrospinal fluid (CSF) obtained from several patients with unexplained or atypical neurological illnesses. A memorable strikingly positive PCR assay was repeatedly obtained on an infant born with hepatomegaly and thrombocytopenia. The infant remained in neonatal intensive care because of choroid plexus hemorrhage and an overall failure to thrive. While he was suspected of having a virus illness, routine commercial laboratory attempts at virus cultures were negative as was IgM serology for common viruses. A ventriculovenous shunt allowed for repeated sampling of CSF. Consistently positive PCR were obtained on CSF samples using the herpesvirus-reactive PCR primers. While not indicative of regular HCMV, the results strongly supported a viral cause for the infant's illness. Another example was an adolescent with residual brain damage for which his mother was seeking legal compensation. She alleged his clinician had been negligent in not starting Acyclovir therapy soon after her son's hospital admission for headache and cognitive confusion. The early possibility of herpes simplex virus (HSV) encephalitis was considered but not supported by the absence of a cellular reaction in the patient's CSF and the rather slight lowering of mental status (consciousness). Only after the patient clinically deteriorated and developed diplopia, did the physician arrive at the diagnosis of HSV encephalitis and begin to prescribe Acyclovir. While this therapy appeared to improve his medical condition, he remained cognitively impaired with a severe learning and visual disorders. A blood sample yielded clearly positive PCR findings with the broadly herpesvirus-reactive primer set, but was negative when tested using a primer set specific for HSV or primer sets specific for other known human herpesviruses.

PCR Studies in Patients with the Chronic Fatigue Syndrome (CFS).

The capacity to perform PCR assays on the brain biopsy and on patients with complex neurological illnesses, along with the high level of suspicion that atypical viruses were able to inflict brain damage without an accompanying inflammatory reaction, were outcomes of ongoing efforts to find the cause of the chronic fatigue syndrome (CFS). The major impetus to these studies was the report in 1986 of a possible epidemic virus illness at Lake Tahoe, Nevada.[18] Affected patients were experiencing persisting fatigue and were tentatively being diagnosed as having CFS. A new herpesvirus, initially called HBLV and later HHV-6, had been described in the same year.[19] as had the PCR assay.[20] An obvious line of inquiry was to use the PCR assay to search for HHV-6 in CFS patients.

Zaki Salahuddin, working in Dr. Robert Gallo's laboratory at the National Institutes of Health, kindly provided sufficient DNA sequence data on HHV-6 to prepare sets of primers specifically reactive with the newly described herpesvirus. Experience using the PCR assay was gained in other studies aimed at identifying mutant ras gene in some human cancers.[21] human papillomaviruses in cervical.[22] and ocular tissues.[23] and HCMV in HIV infected patients.[24] kidney transplant recipients and tissue samples of salivary gland tumors. Two sets of HCMV primers were generally used, one of which was directed against the gene coding the immediate early (IE) gene (coded by UL123) and the other against the UL83 coded gene. Interestingly, in a small minority of patients, there was a lack of concordance using the two sets of primers, suggesting that variant forms of HCMV may exist.[24] When applied to patients with complex neurological diseases, weak positive PCR responses were not uncommonly observed using the UL83 directed primers. Unlike in the positive HCMV assays, however, quite low amounts of multiple products were being generated, none of which corresponded to the expected size based on the HCMV genome. By reducing the stringency conditions of the PCR assay and employing a sensitive dot blot hybridization detection method for the PCR generated DNA products, positive PCR assays were obtainable using these primers with the other human herpesviruses; HSV-1, HSV-2, Epstein Barr virus (EBV), varicella-zoster virus (VZV) and HHV-6. The assays were conducted such that no discernable hybridizable products were generated in PCR assays performed on DNA samples from laboratory personnel and other normal volunteers. As noted above, however, positive results were obtainable using this assay in several patients with severe neurological illnesses.

Dr. Jay Goldstein, an Orange County physician specializing in CFS, kindly provided blood samples from many of his patients for both virus culture and PCR assays. While the cultures were not definitive, the PCR assays using the broadly reactive herpesvirus primers under low stringency conditions were clearly yielding weak positive reactions in about a third of the CFS patients.[16] Even more efficient was a primer set originally designed to screen for possible retrovirus sequences. The primers corresponded to regions of the tax gene of human T lymphotrophic viruses (HTLV) I and II, respectively.[25] but such that they should not specifically amplify either of the conventional forms of these two retroviruses. Using these SK43 (HTLV-I) and SK44 (HTLV-II) primers, unmistakable, clearly positive findings were obtainable in various patients with complex neurological illnesses, as well as in more than a third of Dr. Goldstein's CFS patients.

The varying patterns of PCR reactivity argued against their being a single, molecularly stable virus common to different patients. The PCR data also distinguished the provisional viruses from known human herpesviruses and from HTLV. Goldstein.[26] provided the clinical insight that his patients were manifesting clinical symptoms more consistent with limbic encephalopathy than encephalitis. Similar, therefore, to the PCR positive patients with more severe neurological illnesses, the CFS patients were apparently not responding with an inflammatory reaction; as would be expected from overt activation of their cellular immune system. This conclusion further justified the term stealth adapted in referring to the putative viruses causing CFS

and potentially also causing a wide spectrum of non-inflammatory neurological, psychiatric and other illnesses.[27]

Culture of stealth adapted viruses

Earlier attempts at culturing viruses from CFS patients were yielding equivocal and essentially unconvincing evidence for a progressive cytopathic effect (CPE). A determined effort was made in 1990 to closely follow the blood cultures of a PCR positive 43-year-old healthcare provider. She had been in her usual state of good health before an acute onset illness in August of that year. Her illness was characterized by intense headaches, generalized myalgia, and fever, developing one week after a sore throat. She was hospitalized with a provisional diagnosis of encephalitis/meningitis. Her CSF had normal protein and glucose levels with only a single white blood cell per cu millimeter. She improved somewhat without therapy and was discharged 7 days after admission. A blood sample was subsequently obtained because of her persisting fatigue and cognitive impairment. It clearly tested positive with both the retrovirus and herpesvirus-based PCR primer sets. Additional blood samples were, therefore, requested for virus cultures, which were performed on human foreskin fibroblasts. After 4-6 weeks, the cultures began showing subtle cellular changes, which were initially thought to possibly reflect a nutritional deficiency. Following several re-feedings of the cultures with fresh medium, the fibroblast cells began to exhibit a CPE with signs of cellular damage somewhat comparable to that seen previously in the brain biopsy. Re-culturing additional blood samples from the patient on MRC-5 human fibroblasts and on rhesus monkey kidney cells also yielded a CPE, which occurred more rapidly than previously because of early and frequent re-feeding of the cultures.[28,29] The striking CPE was characterized by the formation of foamy vacuolated cells that formed large syncytia. The cultures were negative in specific antibody and/or PCR testing for HCMV, HHV-6, HSV, EBV, HTLV and enteroviruses. Yet the cultures reacted strongly with the HTLV-related primers, which had previously and were subsequently shown to test positive on the patient's blood. Using the culture generated PCR products as a probe, the size of the viral DNA isolated from the culture supernatant and from cell pellets was noted to be significantly smaller than that of an intact herpesvirus based on agarose gel electrophosis.[29] The foamy appearance of the CPE, along with reactivity using HTLV-based primers, led to an early consideration of a possible spumavirus (spuma being the Latin word for foamy). Yet, numerous herpesvirus-like virus particles were seen by electron microscopy.[29] A single gene cluster, referred to as bel (between the envelope gene and the long terminal repeat) distinguishes spumaviruses from simple retroviruses and can cause illness independent of the other retrovirus genes.[30,31] A reasonable suggestion, therefore, was that a bel-like gene might have been acquired by an atypical herpesviruses, essentially allowing it to create the observed foamy CPE.

The distinctive syncytial foamy cell CPE was easily transferable to secondary and subsequent cultures of many long-term human cell lines of epithelial, glial and lymphoid origin. Even more striking was the wide species host range of the virus. Thus, CPE was demonstrable on murine, feline, rabbit, hamster, duck and chicken fibroblast cell lines.[29] The virus, designated stealth adapted virus-1, was subsequently noted to be also cytopathic for an insect cell line.

Fifteen of 18 additional blood samples from the patient obtained over a three-year period produced the same characteristic CPE within several days of culturing.[29] Moreover, a stored CSF sample collected at the time of her initial illness also yielded a positive culture. The primary development and expression of CPE by the patient's blood samples was shown to be improved by using frozen thawed extracts of the patient's mononuclear cells cultured in a serum free medium.

A male non-intimate social contact of the patient had concurrent symptoms of fatigue and cognitive impairments. His blood was cultured and yielded very similar CPE to that of the female patient. He was shown to be HIV positive and his clinical condition deteriorated rather rapidly leading to his death within 6 months. The female patient has been unable to work and has remained disabled for more than 20 years with a diagnosis of CFS.

Recovery from the cpe in cultures of stealth adapted viruses

Under routine virus culture conditions, the early CPE caused by cultured stealth adapted viruses can be mistaken for non-specific toxicity, especially since it tends not to progress and commonly disappears in infrequently fed cultures. Reversal of the CPE can even occur in well established, strongly positive cultures and is associated with the production of materials that can seemingly supply a non-mitochondria-based source of energy to the virus infected cells. These materials are referred to as alternative cellular energy (ACE) pigments and have been described elsewhere.[32]

The unappreciated need for repeated re-feeding of the cultures as a means of reducing levels of ACE pigments explained the many suggestive, but never definitive, virus culture results, which were previously obtained in many of Dr. Goldstein's patients. Once the stealth adapted virus culture methodology was standardized, virtually all of the blood samples subsequently received from Dr. Goldstein and other clinicians specializing in CFS yielded positive cultures. Moreover, positive cultures were regularly being obtained on blood samples on autistic children[33] and their mothers and on the majority of tested patients in a psychiatric facility. Other illnesses yielding consistently positive cultures included multiple myeloma, amyotrophic lateral sclerosis, Gulf War syndrome and so-called chronic Lyme disease. In blinded control studies established and read independently by different technologists, patients with severe CFS, multiple myeloma and autism showed consistently positive cultures in contrast to the 10% of selected healthy controls and up to 20% of randomly selected blood samples. Strongly positive cultures of rapid onset, were not seen in control cultures, but were not infrequently observed in the cultures from patients, especially those with more severe illnesses. It should be noted that not all culture positive patients report fatigue as their primary symptom or even as a major component of their present illness. Moreover, some individuals will report being healthy, but on closer questioning reveal either prior or ongoing episodes of sub-optimal cognitive functioning, with accompanying mood and/or sleep disorders. These considerations limit the usefulness of trying to validate a CFS-specific diagnostic assay and basically challenge the many efforts at defining CFS as a discrete illness.

Animal inoculation studies

The functional lack of immunogenic antigens recognizable by the cellular immune system was confirmed by intravenously inoculating cats with a frozen-thawed extract of cultured stealth adapted virus-1 infected cells.[34] Cats were chosen because several CFS patients had reported behavioral illnesses and even unexplained deaths within their household pets. The animal studies were done in a university setting with full institutional approval. The inoculated animals remained asymptomatic for 48 hrs, but then developed a severe neurological illness, which peaked at between 2-4 weeks. During this period, the

animals lost the playfulness that was present prior to injection. Rather, they became reclusive and irritable; yet the altered behavior was not accompanied by a marked reduction in consciousness. The body temperature of the animals dropped 0.6o – 0.8o F below normal, which is characteristic of ill cats. They had dilated pupils and were clearly bothered by the light. Several animals had balding areas on their head from repeated rubbing against the cage. Another animal had torn part of his face from scratching; while two others had bloody ocular and nasal discharges, also probably from scratching. During this initial period, the animals' gums were swollen. Peripheral enlarged lymph nodes could also be easily palpated and many muscle groups were clearly painful when squeezed. The cats were euthanized at 1, 2, 4, 6 and 15 weeks after virus inoculation. Although only a few animals were observed, the severity of the illness began to wane after 4 weeks with definite improvement noted in the cats at 6 weeks. Indeed, the cat on which the necropsy occurred at 15 weeks had seemingly resumed normal activities by week 10.

Histological examination of brain tissue at each of the time points showed foci of cells with cytoplasmic vacuolization, which occasionally formed syncytia. These changes occurred in the complete absence of any inflammatory reaction. Marked intracellular inclusions and extracellular deposited materials were noted upon staining with periodic acid Schiff (PAS) and Stains-all dyes. Electron microscopy confirmed the presence of foamy vacuolated cells with structured intracellular inclusions. Cellular damage was still apparent in the cat on whom the necropsy was performed at week 15. Vacuolating cytopathology without discernable inflammation (except for a suggested slight increase of tissue eosinophils), was observed within other organs, indicating a widespread systemic infection.

Illness did not occur in a cat inoculated with heat inactivated virus infected cells. Interestingly, two follow up inoculation of non-inactivated virus failed to induce any apparent disease in this animal. The protection provided by the heat-inactivated virus may well have been mediated by antibody, since the failure to generate a cellular immune response does not necessarily preclude antibody-mediated protective immunity. Consistent with this interpretation and with the differences among stealth adapted viruses isolated from different patients, the previously protected cat developed a severe, but recoverable illness, when inoculated with a different stealth adapted virus isolate/.[34] The other isolate was obtained from a patient with systemic lupus erythematosus. Interestingly, this patient described her own cats as having aberrant behaviors and that some of her earlier cats had died from undiagnosed neurological illnesses.

Positive stealth adapted virus cultures were subsequently obtained using blood samples from symptomatic cats and dogs of several CFS patients. Moreover, direct inoculation of the blood from sick into healthy cats caused a similar illness as when using CFS patient infected cultured cells. Necropsy of a newborn kitten of a virus inoculated pregnant cat showed widespread histological changes within its various tissues, including the brain. Moreover, milk collected from the stomach of the kitten tested positive by culture for stealth adapted virus. Similar events are predicted for infants born of stealth adapted virus infected humans.

Initial sequencing studies on stealth virus-1

DNA sequencing of PCR products amplified from the stealth virus-1 culture, provided direct support for an atypical herpesvirus. As with the patient's blood sample, a strong PCR response was seen using HTLV I directed primer SK43 in conjunction with the HTLV II directed primer SK44. The PCR reactions yielded two distinct bands on agarose gel electrophoresis.[29] One band migrated into the agarose gel with an estimated size of approximately 1.5 kilobase (kb) and the other band migrated further into the gel with an estimated size of approximately 0.5 kb. The PCR products were cloned into pBluescript plasmids and sequenced. The sequencing was performed at the City of Hope Molecular Core Facility (Duarte, CA).

The results showed that the larger band comprised two separate products (15-5-2 and 15-5-4) with the SK44 primer flanking both sequences. Using BlastN analysis (discussed later), the 15-5-4 sequence showed significant DNA matching to a region within the UL36 gene of HCMV.[29] The sequence was not, however, identical to HCMV. Moreover, the 15-5-2 product could not be matched to HCMV, or indeed to any sequences then available within GenBank; a repository of DNA sequences maintained by the National Center for Biotechnology Information (NCBI). The smaller PCR product, comprising 507 nucleotides was flanked by primer SK43. Its sequence matched to a cellular gene, although there was no amplification of this sequence in performing the PCR assay on uninfected cells.

In experiments using two different cultures obtained from the CFS patient, DNA was extracted, cut with a restriction enzyme and cloned into pBlusscript plasmids. EcoR1 was used on DNA obtained from the ultracentrifuged pellet of Millipore filtered culture supernatant. The resulting clones were designated as the 3B series. DNA pellet from a later culture was further purified by agarose gel electrophoresis as an approximate 20 kb band, cut with SacI restriction enzyme and cloned into pBluescript plasmids. These clones were designated as the C16 series. Sequencing of the majority of the clones was performed either at the City of Hope Molecular Core Facility or Lark Technologies, a major DNA sequencing corporation in Houston Tx. Other commercial sequencing facilities occasionally used were US Biochemical (Cleveland, OH) and BioServe Biotechnology (Wheaton, MD). Complete definitive sequencing was performed on most of the clones referenced in this paper. For a few clones, however, only the initial T3 and T7 readouts are available, with occasional nucleotide uncertainties occurring beyond 500-600 bases. These extended regions were not included in the sequence analysis presented in this or in prior publications.

Many of the newly sequenced clones confirmed the relatedness, but non-identity, to HCMV. One of the clones corresponded to a region of HCMV for which there was also a known sequence for the Colburn strain.[35] of African green monkey simian cytomegalovirus (SCMV). Although, not 100% identical, the clone matched far more closely to SCMV than to HCMV or other sequenced animal herpesviruses. The translated amino acid sequence matched even more closely to that of SCMV. By using different primer sets, similar but still distinguishable reactivity could be shown between the patient's virus and the Colburn strain of SCMV.[36] It was concluded that the patient's virus had unequivocally arisen from SCMV, but had undergone further genetic changes. As more sequence data of the Colburn and other strains of SCMV.[37] became available, it was also possible to assign the sequence of PCR product 15-5-2 to SCMV. It clearly matched to the region of SCMV, which corresponds to the UL20 gene of HCMV.

The nucleotide alignments were performed using BLAST (Basic Local Alignment Search Tool) of the National Center for Biotechnology Information (NCBI). The blastN program provides a statistical measure of the probability of a sequence alignment occurring simply by chance. It is expressed as a "Score," which relate to the percentage of identical nucleotides, size of the aligned regions and the requirement to place "gaps" for missing nucleotides within one or both sequences to obtain the optimal alignment. The higher

the score, the more significant is the alignment, with limited meaning for Scores below 100. Very high Score values are recorded as an exponential to base "e." The statistics of the sequence alignment is also expressed as a function of the total number of available sequences within the database being searched, which is the entire non-redundant collection of all sequences within GenBank. This probability value is recorded as an "Expect" value and is stated as 0.0 when it is beyond the upper limit of 1 in 10179 probability of the alignment occurring by chance.[38,39] The alignments of clones 15-5-4 and 15-5-2 (minus the primers) with Colburn SCMV sequences yielded very high Scores of 2,379 and 2,140 respectively; (95% and 93% nucleotide identity), and both alignments had an Expect value of 0.0. No comparable matching occurred with any virus other than SCMV, including rhesus cytomegalovirus (RhCMV).

Evidence for genetic instability and recombination of stealth virus-1

Further sequencing of the clones revealed some remarkable findings. First, individual clones corresponding to essentially the same region of the putative SCMV derived stealth adapted virus, showed minor nucleotide differences, including deletions, substitutions and apparent duplications.[40] Examples of matching occurring at only one of the two ends, were also found within sets of both the EcoRI and SacI derived plasmids. An artifact of the rejoining of different restriction fragments in the cloning procedure was excluded by ensuring that there were no internal sequences corresponding to the restriction enzyme being used. Rather the heterogeneity was explained by mutations that affected restriction sites. Occasional examples were also seen of major recombination. For example, in three of the C16 series clones, a region matching to SCMV nucleotides 202,128 to 201,415 (coding for US18) was contiguous with a region matching to SCMV nucleotides 220,153 to 223,156 (coding for US32 and beyond). The sequence at the point of recombination was unrelated to the specificity of SacI restriction enzyme. Other clones of the C16 series shared one but not both ends of the clones containing the apparent recombination. The deduced coded proteins of these clones were as expected and corresponded to US18 adjoining to US19 in some of the clones and US31 adjoining to US32 in the other clones. Within the 3B series of clones, one showed recombination linkage between the UL57 and UL69 regions, while other 3B clones contained regions corresponding to portions of the intervening sequences deleted by the apparent recombination.

As more partial sequence data were obtained from unrelated clones, it became clear that the entire genome far exceeded the predicted ~20kb size as suggested by the agarose gel electrophoresis of the purified, uncut, viral DNA. It was concluded, therefore, that the virus genome existed as fragments, as well as being genetically unstable.[40]

Also consistent with a fragmented genome, was the finding that the clones were non-randomly distributed when matched against corresponding genes of HCMV.[41] Five HCMV genes (UL 36, 47, 52, 86, and US28) were each matched by 10 or more of the initially sequenced clones. Ten additional HCMV genes were each matched by 5-9 clones. The majority of the represented HCMV genes were matched by only 1-4 of the partially sequenced clones. No clones were identified with sequences matching to two of the three major immunogenic coding regions of HCMV (UL83 and UL55). The inability to identify clones corresponding to a region within the virus could arise if it was contained within a portion of the virus genome without flanking EcoRi and SacI sites closer than ~9 kb, the practical upper size limit of cloned fragments within the pBluescript plasmid. This was not the case with UL55, since clones containing sequences corresponding to the UL54 and UL56 genes were found. It may still, however, be the case with the UL83 gene. Thus, there is a span of 11,080 and 16,726 nucleotides, respectively, between identified EcoRI and SacI sites, in the cloned sequences corresponding to the UL76 and UL84 coding genes, respectively. The distance between the cutting SacI sites is particularly long because a SacI cutting site present in the UL84 coding gene of SCMV was mutated in stealth adapted virus-1. The SCMV genome actually shows two additional SacI cutting sites within the unidentified region of stealth adapted-1 virus between UL76 and UL84. Again, however, mutation in one or both of these sites cannot be excluded. If the UL83 related gene is present and not deleted in stealth adapted virus-1, , its sequence is presumably mutated in order to explain the lack of an immunogenic product.

A clone (3B546) of stealth adapted virus-1 was identified, which corresponded to the UL123 gene, which in HCMV codes for the major immediate antigen-1, one of the three major immunogenic targets for anti-CMV CTL.[13,14] Sequencing of the clone from stealth adapted virus-1 revealed several major mutations when compared with the sequence of the Colburn and other strains of SCMV.[41] The major immunogenic regions of the UL123 coded protein in HCMV is in the third exon of the major immediate antigen 1 (MIE-1). The deduced amino acid sequence of stealth adapted virus-1 shows only 82% identity with SCMV and interestingly lacks a stretch of 10 amino acids, within the third exon, just distal to a relatively highly conserved region in primate and human cytomegaloviruses.[42] The major deletion was confirmed in a second clone (C16139). While specific T cell epitopes of the MIE-1 gene in HCMV have been defined.[13,14] no comparable data are available for SCMV. Nevertheless, it can be concluded that the apparent MIE-1 coding sequence in stealth adapted virus-1 is not coding for an antigen capable of evoking an inflammatory response in either the patient from which the virus was isolated or in inoculated cats.

Long stretches of contiguous sequences could be assembled using representative examples of the cloned genes, e.g. UL28-48; UL48-54; UL84-102; UL115-132; UL141-146 and US19-28. Overall, the sequences showed statistically far greater homology to SCMV than to any other cytomegalovirus of human or primate origin. Still, throughout the assembled sequences, there were widespread deviations from SCMV, again confirming the remarkable genetic instability of the stealth adapted virus. For all but one of the stretches of sequences from stealth adapted virus-1, the level of nucleotide identity was only 94-95% when compared with the fully sequenced GR2715 strain of SCMV. Optimal alignments also required the insertion of numerous gaps into one or other of the sequences. Moreover, some of alignments did not extend throughout the entire stretch of the stealth adapted virus-1 sequence. In part, this reflected significant differences among some of the fully and partially sequenced SCMV isolates, but in no case did the alignment exceed 95% or not require the insertion of numerous gaps. For one stretch of the stealth adapted virus-1 sequence, the nucleotide identity with SCMV was only 80%, again reflecting widespread mutation. Yet, even with this sequence, the alignment with SCMV is still highly significant with a Score values well beyond 100 and an Expect values of 0.0. While, it is unequivocal that stealth adapted virus-1 originated from SCMV, is has clearly distinguishable from any of the known isolates of SCMV and should not be disregarded as a possible laboratory contaminant.[43]

Are cellular sequences involved in stealth adapted virus replication?

As noted earlier, the sequence of the smaller PCR product generated from the culture of stealth adapted virus-1, matched to cellular DNA and not to known SCMV sequences. The matching was unique to an intron within the Rho guanine neuleotide exchange factor 10 on chromosome 8. Still the matching was not 100%. Indeed, optimal alignment showed only 89% nucleotide identity and requiring 18 gaps. Still the Score was highly significant at 568 and an Expect value of 2e-158. Moreover, partially matching products were generated in PCR assays on stealth adapted virus cultures from two other patients. No such product was ever generated using the PCR assay on uninfected cells. Upon cloning of the DNA isolated from stealth adapted virus-1, approximately 5% of the clones contained sequences, which showed significant homologies to cellular sequences.[44] While these clones could have been dismissed as simply being contaminating cellular DNA, consideration was given to the possibility of cellular DNA being somehow involved in the virus replication process. Supporting this possibility was that cellular DNA related sequences were present in both the 3B and C16 series of clones; the latter being derived from agarose banded virus DNA. Furthermore, the actual sequences of the cloned DNA were not always an exact match to the apparent corresponding normal cellular genes. While, the lack of exact matching could presumably be because the actual cellular sequence of origin had not yet been identified, it is also consistent with genetic instability if the DNA had become incorporated into the virus replication process. It is quite possible that some of the DNA is of monkey origin.

An originally reported stealth adapted-1 DNA sequence was mistakenly identified as newly acquired chemokine-related cellular genes. As additional sequence data of intact SCMV isolates became available, it became apparent that these viruses too had essentially the same cellular sequences as reported for stealth adapted virus-1. The acquisition of these particular sequences was not, therefore, involved in the stealth adaptation process. Specifically, it was reported that sequences adjacent to the gene corresponding to the UL145 gene of HCMV matched closely three copies of an alpha CXC chemokine coding gene. The gene had been loosely implicated as a possible cause of melanoma and is, therefore, designated melanoma growth stimulatory activity (MGSA). The third copy of the gene contains an intron, indicating that this gene had probably been incorporated from cellular DNA rather than as reverse transcribed RNA. As mentioned above, it was incorrectly assumed in the earlier publication.[45] that the cellular-derived gene had been incorporated into the virus as part of the stealth adaptation process. Instead, it is now apparent that the incorporation of MGSA-related cellular genes has occurred much earlier and even before the divergence of SCMV and RhCMV.

Interestingly, different strains of SCMV vary in the retention of the incorporated MGSA-related cellular genes.[43] The pattern in the stealth adapted virus was similar to that in a partially sequenced SCMV, but differed from that in the completely sequenced SCMV strains (Colburn, 2715 and GR2715). It is also worth noting that slight differences exist between the MGSA related sequences in the stealth adapted virus from those of the sequenced SCMV, consistent with the genetic instability of the stealth adapted virus.

The complete sequencing of SCMV viruses isolates has also led to a reinterpretation of the presence of five copies of the gene coding US28 chemokine receptor-related genes in stealth adapted virus-1. HCMV and RhCMV have a single copy of the US28 gene. In an earlier published paper.[46] it was incorrectly suggested that US28 gene amplification had occurred in the stealth adapted virus. This is not so since some other SCMV isolates also contain five copies of these genes.[43,47] Other isolates have fewer copies, indicating once again the unexpected heterogeneity among SCMV isolates. The US28 gene of human and primate cytomegaloviruses is particularly noteworthy since it has been implicated as a potential oncogene.[48] a major co-factor for HIV infection.[49,50] and a stimulus for aberrant vascular proliferation.[51]

The issue still persists, however, that among the apparently amplified and potentially mutated cellular gene sequences there are potentially genes with cell growth regulatory and even oncogenic activity. Furthermore, for some herpesviruses, oncogenic potential may not normally be seen because of the cell killing caused by the virus.[52] It may emerge, however, if the cell killing activity is lost due to gene deletion or mutation, as can seemingly occur in stealth adapted viruses. Sequencing and biological studies are, therefore, indicated for stealth adapted viruses isolated from cancer patients to help better define the occurrence of such possibilities.

Are bacteria-derived sequences involved in stealth adapted virus replication?

The biggest surprise with sequencing of the clones derived from DNA extracted from the stealth adapted virus cultures came with the identification of occasional clones that matched not to virus or to cellular DNA sequences, but to sequences of bacteria.[53] Rather than being dismissed as coming from contaminating bacteria, the sequences were considered part of the patient's disease process for several compelling reasons. First, cells from parallel cultures to those used for DNA extraction were subsequently maintained for several months in antibiotic free media with no evidence of bacterial overgrowth. Second, fecal cultures from some virus positive patients have grown bacteria with unusual metabolic activities. For example, the API and Vitek typing systems,[54] which rely on slightly different metabolic profiles, would sometimes yield discordant identifications of the same fecal-derived bacterial colony. These typing systems rely on somewhat different metabolic profiles and can be misled when bacteria either gain or lose a metabolic activity. Furthermore, in some culture plates, a particular metabolic activity, such as hydrogen sulfide formation would seemingly occur sequentially along a line of non-touching bacterial colonies. It appeared as if an infectious agent was progressively transforming the bacteria and, thereby, inducing changes in their metabolic activities. Finally, filtered extracts from fecal cultures of some stealth adapted virus infected patients were able to produce the same vacuolating CPE as seen with their blood.

The bacteria-related sequences from the stealth adapted virus-1 cloned DNA were entered into GenBank to try to identify their bacterial origins. Both BlastN (nucleotide comparisons) and BlastX (comparison of deduced amino acid sequences) were employed. The preliminary analysis indicated that the sequences were clearly not derived from a single type of bacteria. Some sequences initially appeared to be derived from brucella, an alpha proteobacterium.[55] Some other clones matched to mycoplasma, while several remained unmatchable against the existing databases. Some of the sequences were also suggestive of possible genetic instability. For example, significant differences were noted between a cloned ribosomal sequence and highly conserved sequence in a broad family of matching bacteria. This observation also argued against the cloned bacterial DNA coming from contaminating bacteria. The possibility of genetic recombination was further suggested by the finding of discrete stretches of sequences matching to either different types of bacteria or to widely disparate regions within a known type of bacteria. The term viteria was introduced to describe the possible inclusion of bacteria sequences in the virus replicating process.[53]

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It has now been more than a decade since the preliminary findings were published. Many more bacterial sequences are now available in GenBank allowing for more extensive sequence comparisons. The basic conclusions still hold. Essentially, the bacteria matching sequences can be divided into three groups (Table 1-4). Clearest is the extremely close matching of seven of the 3B series clones to regions of mycoplasma fermentans. The nucleotide matching covered the entire sequences of the clones with from 97 - 99% identity and only occasional gaps. None of the C16 series of clones matched to mycoplasma, raising the possibility that the organism was a secondary contaminant of only one of the cultures from which the DNA cloned. Upon review, however, mycoplasma fermentans has very few Sac1 restriction sites and those that are present are widely dispersed over the nearly million nucleotide base genome.

Table 1 SCMV related sequences in cloned DNA from cultures of stealth adapted virus-1. *The nucleotide numbers shown include the gaps, which were inserted for optimal alignment using the BlastN program of NCBI. ‡The entire length of the sequence did not align to SCMV GR2715 as a contiguous sequence, allowing for short gaps. The non-aligned regions could, however, generally be aligned to the Colburn strain of SCMV and/or to other SCMV strains for which only partial sequence data are currently available on GenBank

Genbank Accession Number: GI	Length (bp)	Coding Region (BlastX)	Matching to SCMV Strain GR2715				
			Nucleotide Identity*	Expect	%	Gaps	Score
121531683	28,199	UL28-UL48	26,523/28,245	94	210	43,062	0.0
121531687	8,407	UL48-UL54	8,027/8,414	95	17	13,402	0.0
121531684	6,328	UL72-UL76	5,112/6,367	80	168	5,615	0.0
121531682	25,023	UL84-UL102	23,941/25,092	95	104	40,010	0.0
121531685	12,375	UL115-UL132	6,222/6,654‡	94	67	10,013	0.0
121531686	16,011	US19-US28	11,583/12,242‡	95	54	19,111	0.0

*The nucleotide numbers shown include the gaps, which were inserted for optimal alignment using the BlastN program of NCBI

‡The entire length of the sequence did not align to SCMV GR2715 as a contiguous sequence, allowing for short gaps. The non-aligned regions could, however, generally be aligned to the Colburn strain of SCMV and/or to other SCMV strains for which only partial sequence data are currently available on GenBank

Table 2 Mycoplasma fermentans species related sequences in culture of stealth adapted virus-1. Note: All sequences aligned over their entire lengths to matching mycoplasma sp. Sequences

Clone	Length	Matching to Mycoplasma Fermantans			
		% Identity	Gaps	Score	Expect
3B35	2,142	99	2	3,833	0.0
3B512	2,345	99	1	4,213	0.0
3B520	2,797	99	0	4,985	0.0
3B528	2,043	99	1	3,577	0.0
3B622-T3	600	97	1	1,003	0.0
3B622-T7	600	97	2	1,003	0.0
3B627	328	99	0	587	2e-164
3B632	1,396	99	1	2,475	0.0

Note: All sequences aligned over their entire lengths to matching mycoplasma sp. sequences

The second grouping of bacteria related sequences comprise those, which originally matched best to brucella. As sequences of the closely related ochrobactum bacterial species became available, many but not all of the brucella matching clones showed a significantly greater homology to ochrobactrum (taxid 528) and most specifically to ochrobactrumanthropi. Ochrobactrum belongs to the family Brucellaceae within the alpha proteobacteria order Rhizobales.[56] In contrast to the mycoplasma related sequences, the nucleotide identities of the ochrobactrum-matching sequences were generally in the range of 80-90% homology with many gaps required for optimal alignments. Moreover, the pairing of the sequences of these clones with ochrobactrum/brucella species did not, with one exception (clone 3B629 of only 165 nucleotides), extend over the entire clone. Regions of statistically significant homology were separated by regions, which could not be reasonably aligned to ochrobactrum. For example, clone 3B23 contained a 1,201 nucleotide sequence that best matched to the chromosomal region of Agrobacterium radiobacter and clone C1616 contained a 884 nucleotide sequence, which best matched to a plasmid identified in Agrobacterimrhizogenes.

With several clones, e.g. C16134, portions of the sequences still statistically matched slightly better to brucella rather than ochrobactum, but were sufficiently close to the latter to probably reflect this species. For clones 3B47 however, the matching was clearly much more to a plasmid associated with a more distant rhizobia, than to members of the Brucellaceae family.[56] Similarly, the T3 and T7 readouts from clone C16125 matched to a plasmid of Chelativorans sp., (Mesorhizobium), rather than to an ochrobactum plasmid.

The DNA data suggest acquired genetic recombinations and/or insertions, rather than indicative of an existing novel, single bacterial species. In further support of this suggestion and consistent with the marked genetic instability observed with the SCMV-matching sequences, are the many discrete intervening regions within several of the ochrobactun matching clones, which are unrelated to any known bacteria, cellular or viral sequences. The nucleotide homologies of the clones with ochrobactrum bacteria related sequences are summarized in Table 3. Some of these clones had intervening sequences, which matched to rhizobium (Ribobiaceae family) or to plasmids of these bacteria, while other regions could not be aligned to any of the available sequences in GenBank. These intervening sequences, which did match to Rhizobia and some other clones with rhizobium plasmid-related sequences are listed in Table 4. Included is clone 3B513 in which widely separated 596 and 202 nucleotide regions within the entire length of 8106 nucleotides roughly matched to sequences within distinct plasmids of a methylobacterium and a rhizobium species, respectively..

Of special interest with clone 3B513, is the matching of a region of clone 3B513 with the T3 sequence of clone 3B525. The continuation of the T3 readout beyond the 3B513 matching region, as well as the T7 readout of this clone, matched to sequences of SCMV. The T7 sequence also matched to several other clones, none of which overlapped with clone 3B513.

Table 3 Ochrobactrum sp. related DNA sequences in culture of stealth adapted virus-1. **When two scores are listed, the clone contained at least one sequence, which showed a higher matching score to Brucella sp., than to Ochrobactrum sp. The first of the two scores is that to Ochrobactrum sp. (generally Ochrobacttrum anthropi) and the second score is to Brucella sp

Clone NCBI Accession	Length	Matching to *Ochrobactrum* sp.*				
		Region	% Identity	Gaps	Score	Expect
3B23 U27612	8916	1-5016	84	16	5,380	0.0
		6777-8242	79	34	1,256	0.0
		8329-8915	78	17	450	1e-121
3B313 U27616	7985	1-6263	76	79	4493/4607**	0.0
		6497-7972	72	56	800/493	0.0
3B41 AF191072	2869	2575-2868	78	1	230	6e-56
3B43 AF191073	3620	1-1785	97	6	2,969	0.0
		1796-2303	91	7	690	0.0
		2378-3620	93	18	1,855	0.0
3B534 U27900	612	1-555	83	3	572	9e-160
		573-603	90	0	42.8	2.8
3B614 U27645	5062	1-378	83	0	385	2e-102
		935-5061	77	103	3,140	0.0
3B629 AF191078	165	1-165	93	0	248	1e-62
C1616 AF065660	4626	2-1346	77	16	1,058	0.0
		1460-1628	88	0	215	2e-51
		1719-2668	78	2	771	0.0
		2896-3702	75	3	547	3e-151
C16116-T3 AF065678	587	292-573	81	5	260/289	7e-66
		25-175	85	0	168/134	3e-38
C16116-T7 AF065679	548	11-112	85	1	114	5e-22
		218-539	79	8	255	3e-64
C16118-T3 AF065682	780	20-713	82	16	675	0.0
C16118-T7 AF065683	675	1-629	85	3	639	0.0
C16134 AF065710	4142	196-1099	82	1	890/911	0.0
		1246-1761	82	3	495/491	1e-135
		1864-3301	79	50	1,211/1,321	0.0
		3582-4142	86	0	655/645	0.0

**When two scores are listed, the clone contained at least one sequence, which showed a higher matching score to *Brucella* sp., than to *Ochrobactrum* sp. The first of the two scores is that to *Ochrobactrum* sp. (generally *Ochrobacttrum anthropi*) and the second score is to *Brucella* sp

Table 4 DNA Sequences in culture of stealth adapted virus-1, which match better to Rhizobiaceae than to Brucellaceae-related sequences available on Genbank

Clone Accession #	Length	Region of Greatest Alignment to Sequences of Rhizobiaceae or their Plasmids					
		Region	Best Match	% Identity	Gaps	Score	Expect
3B23 U27612	8,916	5210-6411	*Agrobacterium radiobacter* K84	69	24	444	5e-120
3B41 AF191072	2,869	627-970	*Rhizobium leguminosarum* Plasmid pR132502	69	8	116	1e-21
3B47 AF191074	2,024	3-366	*Sinorhizobium meliloti* AK83	69	6	145	1e-30
		555-1917		66	54	324	3e-84
3B513 U27894	8,106	2688-2890	*Rhizobium* sp. Str. NT-26 plasmid	81	0	214	1e-50
		6101-6697	*Methylobacterium extorquens* AM1 (plasmid)	75	20	396	2e-105
C1616 AF065660	4,626	3741-4625	*Agrobacterium rhizogenes*, plasmid pRi1724	90	1	1,175	0.0
C16125-T3 AF065692	655	9-650	*Chelativorans* sp. BNC1 plasmid	81	3	607	4e-170
C16125-T7 AF065693	634	1-633		85	9	673	0.0

There is a third grouping of the cloned bacteria-related sequences, which cannot yet be even partially typed with any confidence to any specific bacteria. The BlastN searching was extended beyond the "nr/nt nucleotide collection" of NCBI to include various shotgun genomic sequences, microbial genomes and environmental samples. The BlastX program was also employed to see if the possible translations of the DNA sequence resulted in recognizable amino acid sequences. In doing so, only marginal and limited partial homology was seen to some bacterial proteins of certain actinomyces (gamma proteobacteria). Interpretation of these sequences may become easier as GenBank continues to acquire more data.

As will be discussed elsewhere, several of the matching bacterial genes code for proteins with rather interesting metabolic activities. If actually expressed in infected cells, these proteins might facilitate further passage of the virus within bacteria. The presumed ability of stealth adapted viruses to infect and to be transmitted by bacteria has enormous public health implication. Moreover, the bacterial coded proteins may also contribute to the CPE observed when stealth adapted viruses are grown in eukaryotic cells and also to the production of extraneous materials, as previously described.[32]

The bacterial sequence data are relevant to possible over interpretation of positive PCR and serology-based assays for putative bacteria pathogens in various chronic illnesses. Mycoplasma fermentans has been reported as commonly positive in CFS patients.[57-60] as well as AIDS patients.[61] often in the absence of confirmatory serology. Some CFS patients have also tested positive in assays brucella reactive antibodies.[62] Little consideration is usually given to the potential antibody cross reactivity among bacteria. Certainly, the reports of finding antibodies reactive with Borreliabundgoferi in patients with chronic illness similar to CFS.[63] is not a convincing argument that the patients have Lyme disease, especially since large numbers of patients said to have chronic Lyme disease have tested positive in cultures for stealth adapted viruses. Similar considerations should be given to whether bacteria are the actual cause of PANDAS (Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection.[64]

Scmv origin of stealth adapted viruses cultured from other patients

Sealth adaptation is regarded as a generic process potentially applicable to all forms of cytopathic viruses. It is apparent that several are derived from SCMV. For instance, a SCMV-derived stealth adapted virus, similar but not identical to stealth virus-1, was also isolated in 1991 from the CSF of a 23 year old woman with a 4 year history of bipolar psychosis .[62] Her clinical condition had recently deteriorated with gross hallucinations and delusions. She had repeated seizures during an ambulance trip to the Los Angeles County Hospital and a brief cardiac arrest while in the Emergency Room. Acyclovir was administered from day 2-13 after admission. She remained comatose for several days and when she did awake showed only vegetative activity. Her illness was tentatively assumed to have resulted from a drug overdose although no supporting toxicology data were even obtained. The patient's vegetative state persisted till her death 6 years later. Six repeat blood samples obtained over this period and a repeat CSF sample one year prior to her death all showed the same CPE. Numerous virus particles were seen on electron microscopy of virus-infected cultures. Her culture and a subsequently CSF sample collected 4 years later yielded positive PCR using primer sets based on stealth virus-1. Partial sequencing of a PCR product showed 94% and 87% nucleotide identity, with clone 15-5-2 of stealth adapted virus-1 and the SCMV genome, respectively.[65]

Another gentleman had his blood analyzed for SCMV by PCR at a primate laboratory testing facility. He had returned from a business trip that involved some partying. He became ill, as did his son and visiting father. Learning of the published work on SCMV derived stealth adapted viruses; he sent his and his father's blood to a primate PCR testing facility under the guise that they were monkey-derived specimens. Both blood samples tested positive for SCMV using primers designed to amplify part of the DNA polymerase coding gene. The laboratory provided assurance on the validity of the results and that the specificity of the assay excluded human, rhesus or baboon CMV. The Centers for Disease Control and Prevention (CDC) was informed of the finding. A blood sample was requested for culture, apparently to be performed by an outside laboratory. The CDC would not disclose the name of the laboratory when I offered to provide input into the culturing method. Nor did the CDC make any contact with the PCR testing laboratory. Interestingly, the gentleman's blood also tested positive for anti-brucellosis antibodies in a commercial laboratory.

PCR positivity on blood samples using stealth adapted virus-1 reactive primers was also noted among patients affected by a stealth adapted virus community outbreak occurring in the Mohave Valley region of Western Arizona and involving the town of Needles,

California.[66] Among these patients was a boy whose brain biopsy showed a severe vacuolating encephalopathy.[67] His illness presented as a behavioral/learning disorder for many months prior to his showing objective neurological signs. A second brain biopsy confirmed the non-inflammatory histological findings and on electron microscopy, showed markedly vacuolated cells with structured intracellular inclusions.[68] The child responded somewhat to ganciclovir but subsequently died in the second year of his severe, yet fluctuating illness.

Several other patients, including children with autism and a patient whose illness was complicated by the development of cerebral vasculitis.[69] have shown positive PCR on blood and/or CSF samples using primers reactive with stealth adapted virus-1. Many other isolates, however, have not shown reactivity with these primers. Yet some may possibly still be derived from SCMV, but may have undergone far more deletions and other changes than stealth adapted virus-1. Consistent with multiple origins of stealth adapted viruses, weak positive PCR have occasionally been observed with primers designed to react more specifically with other DNA viruses, including HSV, HHV-6, EBV and adenoviruses.

A limitation of the regular PCR assay for stealth adapted DNA viruses was revealed during PCR assays on a positive culture obtained from the CSF of a patient who became ill when working as an Emergency Room nurse. Her PCR assay was negative using three stealth virus-1 reactive primer sets directed against portions of the SCMV genome. A positive PCR was obtained using a primer set if the PCR was preceded by reverse transcribing RNA sequences into DNA.[70] While the sizes of the PCR products were slightly different from SCMV, the strong reactivity using the SCMV-reactive primers strongly supported the presence of SCMV, but mainly in the form RNA rather than DNA. This finding is consistent with RNA forms of SCMV, undergoing replication by endogenous retroviruses, which can potentially be induced by various herpesviruses.[71,72] Alternatively, it is possible that some stealth adapted viruses may remain as RNA and utilize their own RNA polymerases or a polymerase incorporated from other RNA viruses.

RNA replication lacks the editing functions, which generally accompany DNA replication. RNA replication via reverse transcriptases of endogenous retroviruses or by RNA virus polymerases could well account for the observed heterogeneity of viral, cellular and bacterial DNA sequences as seen in stealth adapted virus-1. Even with DNA replication, the polymerase and the editing functions involve different regions of the enzyme, such that error prone synthesis of DNA can proceed because of a lack or defectiveness in the editing component.[73,74] Whatever the cause, the genetic instability of DNA sequences isolated from cultures of stealth adapted virus-1, is truly remarkable.

Because of genetic variability, within related stealth adapted viruses and even more so because of the multiple potential origins from different DNA and RNA viruses, the PCR assay, even with prior reverse transcription, is an unreliable initial diagnostic test for stealth adapted viruses. Virus cultures, while less easily quantified, provide a more useful screening method. Moreover, as shown in this paper, virus DNA or RNA can be extracted from the positive cultures and used to identify portions of the virus on which sensitive molecular probes can be developed. Infected cells or cell extracts can also be useful in developing individual patient's antibody based assays since, as noted above, antibody responses can occur even without direct T cell recognition of virus-infected cells.

Confirmation of scmv contamination of earlier batches of poliovirus vaccines

FDA officials have now examined prior batches of poliovirus vaccines for DNA of SCMV using PCR. Three of 8 batches released in the mid 1970's tested positive, but they were unable to retrieve infectious virus.[75] More extensive PCR testing for SCMV, was performed on earlier poliovirus vaccines by the United Kingdom Bureau of Standards.[76] Of over 90 vaccines tested, nearly half showed the presence of SCMV, Again, they were unsuccessful in attempts at culturing live virus from the retrieved polio vaccine lots. Neither FDA nor CDC has been forthright in attributing polio vaccines as a probable major source of stealth adapted viruses affecting humans and animals. Some FDA officials are mindful of a 1972 joint study with Lederle (part of Wyeth, which is now a component of Pfizer). In this study, kidney cell cultures from 11 African green monkeys, which would otherwise have been used to produce polio vaccines, were instead tested for SCMV. All 11 cultures were SCMV positive, confirming an earlier finding of Smith and his colleagues.[77] No public notification was provided on the Lederle/FDA results.

An incidental finding included in the United Kingdom Bureau of Standards study were the continued use of rhesus monkeys by one poliovaccine manufacture, as evidenced by the detection of DNA of RhCMV. Even more relevant was the confirmation of RhCMV DNA in the CHAT poliovirus vaccine.[76] This vaccine was developed by Dr. Hilary Koprowski and extensively tested in Africa. Dr. Albert Sabin had expressed his concern regarding a residual, unexplained CPE caused by the CHAT vaccine.[78]

Conclusion

Stealth adaptation is envisioned to be a generic process, which allows viruses to bypass effective recognition by the cellular immune system. It requires deletion or mutation of the relatively few virus antigens, which are normally processed and presented at the surface of virus-infected cells for functional engagement by antigen specific T lymphocytes. While stealth adapted viruses have presumably been in existence since the inception of the cellular immune system, the development of live vaccines has provided additional opportunities for their transmission to humans. Specifically, poliovirus vaccines produced in kidney cell cultures of rhesus and African green monkeys have allowed for the introduction of stealth adapted CMV from these species to humans. The infecting, vaccine-derived, stealth adapted viruses can subsequently spread among humans and along with other stealth adapted viruses, can explain the growing incidence of many illnesses, including CFS, autism and psychiatric disorders. The unique susceptibility of the brain to exhibit clinical signs from even limited and localized cellular damage is consistent with the propensity of neuropsychiatric manifestations in many stealth adapted virus infected individuals. A prototype stealth adapted virus unequivocally derived from African green monkey simian cytomegalovirus (SCMV) seemingly exists as genomic fragments of DNA. It has an extraordinary wide range of infectivity for cells of different species and is pathogenic when inoculated into cats. The cats developed a severe encephalopathy, from which clinical recovery ensures in spite of the lack of an inflammatory response. A striking feature of the genetic analysis of the prototype stealth adapted virus is instability of the virus replication process, with widespread minor nucleotide differences between this virus and conventional SCMV isolates. The data are consistent with replication occurring via an RNA intermediate; a suggestion supported by the need to use reverse transcriptase prior to

DNA based PCR detection of virus in one of the tested cultures. Stealth adapted virus replication appears also to involve the assimilation of cellular genes and also genes of bacterial origin. The dual prospects of stealth adapted viruses acquiring oncogenic cellular genes and of being transferrable within bacteria potentially pose a very serious public health challenge, which will be difficult to address. Overall, the process of stealth adaptation greatly extends the scope of viral illnesses and places an important emphasis on better understanding of the non-immunological anti-virus defense mechanism operative against these viruses in cultures and in inoculated animals.

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Conflict of interest

None.

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