Stealth-Adapted Viruses and Viteria: Insights into Virus Construction, Replication and Potential Therapies Based on DNA Sequence Analysis of an African Green Monkey Simian Cytomegalovirus-Derived Stealth Virus

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Abstract

Stealth-adaptation is a mechanism that allows cytopathic viruses to evade immune elimination through the deletion of genes coding the major antigens targeted by the cellular immune system. A prototype stealth-adapted virus, repeatedly cultured from a patient with chronic fatigue syndrome (CFS) was cloned and partially sequenced. It has a fragmented, genetically unstable, genome. It has retained numerous viral sequences that can be aligned to various regions of the genome of human cytomegalovirus (HCMV). Where the comparison can be made, the sequences match much more closely to those of African green monkey simian cytomegalovirus (SCMV) indicating an unequivocal origin from SCMV. Kidney cells from cytomegalovirus seropositive African green monkeys were, until recently, routinely used to produce live poliovirus vaccine. The SCMV-derived stealth-adapted virus has five adjacent, but divergent, open reading frames that potentially code for molecules related to the US28 CC chemokine receptor protein of HCMV. In addition, the virus has acquired cellular sequences from infected cells, including a set of three divergent genes that potentially code for proteins related to the putative oncogenic CXC chemokine known as melanoma growth stimulatory activity (MGSA/Gro-alpha). The genes in the prototype SCMV-derived stealth-adapted virus, supports current experimental therapeutic approaches based on chemokine suppression. Interestingly, the MGSA-related genes generally lack introns and were, therefore, presumably assimilated into viral DNA from cellular RNA through reverse transcription. The virus has also acquired genetic sequences from various bacteria. This finding has led to the secondary designation of this type of novel microorganism as viteria. Molecularly heterogeneous viruses, inducing similar cytopathic effects in culture (and when examined, non-inflammatory vacuolating cellular damage in brain and tissue biopsies), have been cultured from numerous patients with severe neurological, psychiatric, immunological and neoplastic diseases. In controlled, blinded, studies, cytopathic effects were recorded in 9% of healthy individuals donating blood for transfusion; in contrast to the positive results recorded in virtually all blood samples from patients with various illnesses. The differing clinical manifestations in infected patients may reflect the assimilation of different cellular and other sequences in various stealth-adapted viruses. Stealth–adapted viruses (and viteria) pose a major threat to Public Health. Further information is available on the internet at www.ccid.org.

Background Information

A. There is an increasing incidence of diseases with accompanying signs and symptoms of brain damage. These include neurological and psychiatric illnesses, childhood behavioral disorders, and such common conditions as chronic fatigue, Gulf War Syndrome, so-called “chronic Lyme disease”, and many cancers. Altogether, these diseases have an enormous social impact.

B. An infectious cause of many of these chronic illnesses has not been considered primarily because there is no inflammation in the involved tissues.

C. Brain biopsies do, however, show cells with damaged mitochondria, lipid vacuoles, and irregular inclusions. Examples are shown in the figures 1-5.

D. Viral cultures from patients with neuropsychiatric and other illnesses, regularly develop clusters of foamy vacuolated cells. These cellular changes are consistent with infection by actively cytopathic viruses. Figures 6-7.

E. The cultures are also remarkable in the production of large quantities of lipids, including cholesterol esters, and pigmented, protease-resistant, aggregates and ribbon-shaped materials, some of which incorporate metals. Figures 8-15.

F. Viral cultures can induce severe, non-inflammatory, widespread illness when inoculated into cats. The cytopathic effect (CPE) seen in tissues of infected animals is comparable to that seen in the tissue cultures.

G. While the viruses causing CPE in viral cultures differ in different patients, one viral isolate was unequivocally derived from an African green monkey simian cytomegalovirus (SCMV). The issue of probable SCMV contamination of live polio virus vaccines produced in kidney cells of African green monkeys was identified by Industry and FDA in 1972. Unfortunately, this potential problem with live polio virus vaccines was not publicly disclosed, nor scientifically addressed.

H. Continued sequencing of DNA isolated from this cultured virus shows intriguing genetic modifications. Apparent loss of critical viral genes can explain how the virus evades the cellular immune system. Sequencing also reveals the surprizing presence of an assortment of bacterial genes, including genes very closely related to those of Brucella, Mycoplasma, Streptococcus, and other bacterial species. This finding shows the capacity of such viruses to pass, and possibly, to have been passed, through bacteria. Stealth viruses can also potentially incorporate cancer causing cellular genes, as shown by the presence of a cancer-related chemokine gene in the SCMV-derived stealth-adapted virus.

Brain Biopsies From Stealth Virus Infected Patients

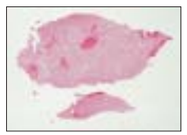

Figure 1. Brain biopsy obtained in 1991 from a stealth virus culture positive school teacher. Her illness began as a chronic fatigue-like syndrome and progressed to a more severe cognitive disorder. Conventional neurological examination was, nevertheless, essentially normal. Periventricular white matter changes were detected using MRI. The pink color of the biopsy is an indication of the absence of inflammatory cells, (lymphocytes and macrophages) that stain blue.

Figure 2. Electron micrograph of an abnormal cell seen in the above brain biopsy. The pale staining material in the vacuoles is lipid. The striking irregularly shaped dark staining materials (inclusions) do not correspond to any normal cellular structures. The long fibers are filaments, typical of glial cells. The round structure at the bottom of the photo is an axon surrounded by a myelin sheath. Mitochondria, seen elsewhere in the biopsy showed degenerative changes.

Figure 3. Brain biopsy obtained in 1998 from a stealth virus culture positive 8 year old boy from the Mohave Valley. His illness began as an attention deficit, behavioral problem. Even when gross abnormalities were detected on MRI, there were no clinical signs of motor, sensory or autonomic nervous system dysfunction. The markedly vacuolated appearance, without signs of inflammation, is reminiscent of the changes seen in diseases attributed to prions, such as mad cow disease. The child's mother had previously been shown to be infected. She continues to have repeated bouts of a severe personality disorder.

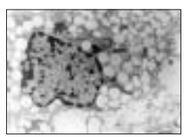

Figure 4. Electron micrograph of a foamy, vacuolated cell in the boy's brain biopsy. Viral particles were not seen.

Figure 5. Electron micrograph of another cell showing marked disruption of mitochondria and the presence of an unusual, irregularly staining inclusion. The myelin sheaths show extensive blebing. The child showed a clinical response to ganciclovir, but subsequently died.

Stealth Virus Cultures

Figure 6. Normal MRC-5 fibroblasts seen under phase contrast microscopy. Note the rather bland appearing, closely interdigitating spindle shaped cells. Many cell types can be used to demonstrate the cytopathic effects of stealth viruses.

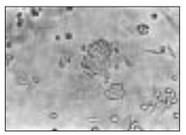

Figure 7. MRC-5 fibroblasts following exposure to mononuclear cells from a stealth virus infected patients. The cells become enlarged and rounded, with a tendency to form large clusters. The cytoplasm develops a foamy vacuolated appearance. Intracellular pigmented material will commonly develop, especially in long term cultures.

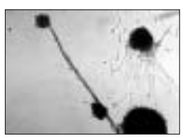

Figure 8. Cell clusters in a long-term stealth virus culture showing the accumulation of dark pigmented material and formation of long ribbon shaped structures. Relatively normal appearing cells, that grew out from the top left cluster, can be seen.

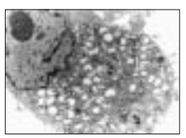

Figure 9. Electron microscopic appearance of a stealth virus infected MRC-5 cell. The culture was from a patient involved in a1996 outbreak of stealth virus infection in the Mohave Valley region of the US. The marked vacuolization is similar to that shown in Figure 4.

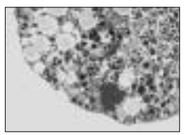

Figure 10. Electron microscopy of a stealth virus infected cell showing widespread accumulations of particulate materials and lipid-filled vacuoles. Unlike infections with many conventional viruses, it is rather uncommon to see intact viral particles.

Structures Developing in Stealth Virus Cultures

Figure 11. Formation of free-cholesterol-like solid crystals in a stealth virus culture.

Figure 12. Massive formation of cholesterol ester-like needle shaped crystals in a stealth virus culture. The crystals are best seen under dark field illumination. The culture was obtained from the patient known to be infected with the SCMV-derived stealth virus.

Figure 13. Stealth virus culture examined under dark field illumination showing a floating aggregation of insoluble material and the presence of cholesterol-ester-like needles.

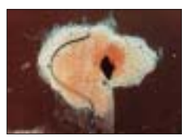

Figure 14. Complex cell cluster developing in a stealth virus culture. It shows a blue colored thread, fine reddish intracellular material and an irregularly shaped dark deposit.

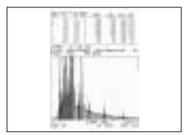

Figure 15. X-ray spectroscopic analysis of aggregated particulate matter in a stealth virus culture. It has accumulated several minerals, including aluminum, titanium, iron and zinc. Other aggregates from the same culture showed different spectroscopic patterns.

Methods and Brief Summary of Sequencing Study

DNA isolated from the stealth virus culture was cloned and sequenced. Nucleotide sequences were analyzed using Blast Programs at NCBI.

While the complete genome of human (HCMV) is known, only partial sequence data are available for African green monkey (SCMV), Baboon (BaCMV) and rhesus monkey (RhCMV) cytomegaloviruses.

Most of the clones aligned to CMV sequences. Near identity of some clones to SCMV.

Several clones contained atypical sequences of bacterial origin.

Other clones partially matched to human proteins, including to a cancer associated chemokine, and to various highly reiterated genes present in the human genome.

The sequence data clearly establish the existence of atypically structured viruses.

Structure of Human Cytomegalovirus

(>230,000 nucleotides)

UL (Unique Long) Region — US Region

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Genes UL 1-154 — US 1-32

Note: The majority of anti-CMV cytotoxic T cells are directed against UL 83 gene product. Other major antigens are UL 55 and UL 123. Deletion/mutation of these genes would enable a virus to evade effective CTL mediated immunity.

SCMV Related Genes in the Cultured Stealth-Adapted Virus

CMV-Related Contiguous Sequences		Matching of Stealth Virus Sequences to SCMV nucleotide matching (Expect value)		BaCMV	RhCMV	HCMV
Genes	**Length**					
UL 14	1,458					
UL 28-48	28,199					
UL 48-54	8,407	UL50	571/598	422/465	384/453	159/185
UL 56	767		(0.0)	(e-180)	(e-95)	(e-38)
UL 57 + ori	2,748	UL57	1226/1246	499/577	Not avail.	370/492
UL 61-69	4,459		(0.0)	(0.0)		(e-62)
UL 70	1,729					
UL 71-76	6,328					
UL 77-78	1,884					
UL 84-104	25,023	UL93	630/656	575/647	368/406	266/317
UL 104-105	1,464		(0.0)	(0.0)	(e-137)	(e-57)
UL 111-112	1,955	UL111	760/807	709/901	74/89	75/89
UL 115-132	8,628		(0.0)	(e-171)	(e-7)	(e-15)
UL 141-144	5,820					
US 20-29	16,011	*5 copies of US28 related gene: a chemokine (and also HIV) receptor.				
US 30-32	3,978					

Analysis based on >300 clones of DNA of stealth virus culture. There is good evidence that the virus has a fragmented, unstable genome. Note apparent absence of UL 83 and UL 55. The UL 123 showed significant mutations. The products of these three genes would ordinarily provide the major antigens recognized by anti-CMV cytotoxic T cells. The nucleotide homology data establish that this particular stealth-adapted virus was derived from SCMV. It most likely arose from an SCMV-contaminated batch of live polio virus vaccine.

Analysis of the Bacteria Derived Genes

Clones obtained from stealth virus culture that match to bacterial genes		Bacterium showing the highest level of sequence homology	Nucleotide (amino acid) identity Expect value	Bacterial proteins/genes identified by sequence homology to the cloned DNA obtained from the SCMV derived stealth virus culture
Clone	**Size**			
3B43	3,620	Brucella melitensis	0.0	ribosomal RNA operon C
3B23	8,916	"	0.0	sorbose dehydrogenase, hippurate hydrolase
3B313	7,985	"	0.0	ABC transporter, metal chelation, secreted protein
3B614	5,062	"	0.0	acetyl-CoA carboxylase, dehydroquinate synthase, shikimic acid kinase, recombinase
3B534	612		3e-101	enolase
3B315	4,495	"	(4e-97)	glutathione S-transferase, transcriptional regulator
C16134	4,142	"	0.0	dihydrodipicolinate synthase, guanosine pyrophosphohydrolase, DNA to RNA polymerase
C1616	4,626	"	e-120	UDP-epimerase, 3-demethylubiquinone-9-3 methyltransferase, sodium/bile cotransporter, transposon from plasmid of Agrobacterium (0.0)
3B41	2,869	Agrobacterium tumefaciens	(2e-155)	sensory transduction histidine kinase
3B47	2,024	Sinorhizobium meliloti	(8e-139)	ABC transporter,
C16122	4,915	Zymomonas mobilis	(5e-63)	dihydrodipicolinate synthase, cystathionine gamma-synthase
3B513	8,106	Alpha-proteobacteria sp.	(e-30)	Fe binding, nitrogen fixating, invertase, others
3B512	2,345	Mycoplasma	(5e-98)	ATP-transporter p115-like
3B520	2,797	"	(e-37)	ABC transporters p29 and p69
3B35	2,142	"	(5e-81)	unknown function
3B632	1,396	Streptococcus	(8e-33)	ribonuclease H
3B528	2,049	"	(3e-33)	glucuronyl hydrolase, sugar transporter

Conclusion: Modified bacteria-derived sequences present in stealth virus culture. For many proteins there are structural similarities with related proteins from other bacterial species, leading to the probability of antigenic cross-reactivity.

Example of Cellular Derived Gene

Clone 3B516: (5,820 nt). Codes for UL141, UL144, truncated UL145, and 3 divergent copies of gene matching to human MGSA/Gro-alpha. This is a chemokine with potential oncogenic activity (in melanomas and other tumors). Two of the 3 copies of the gene lack a major intron present in genomic DNA. This suggests recombination with RNA rather than DNA.

Alignments of Predicted Amino Acids of Stealth Virus Genes With MGSA/Gro-alpha

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Stealth Gene A 4938   NPRFLGVTLLLMSLIAY------CQSTTELRCQCTQTVQGIHPKNIQSVSIKDKGPNCPN 5099
                      NPR L V LLL+ L+A          TELRCQC QT+QGIHPKNIQSV++K  GP+C
Human MGSA Gene 12    NPRLLRVALLLLLLVAAGRRAAGASVATELRCQCLQTLQGIHPKNIQSVNVKSPGPHCAQ 71

Stealth Gene A 5100   QEVIATLKNGQKVCLNPTAPMVQKILKKTITDN 5198
                       EVIATLKNG+K  CLNP +P+V+KI++K  + +
Human MGSA Gene 72    TEVIATLKNGRKACLNPASPIVKKIIEKMLNSD 104

Stealth Gene B 5469   LLVATLLGTLLASTMVFADK----EERCLCPKTIQGIHPKNIQSVELHEPRDMCPNVEVM 5636
                      L VA LL  L+A+    A       E RC C +T+QGIHPKNIQSV +  P   C   EV+
Human MGSA Gene  16   LRVALLLLLLVAAGRRAAGASVATELRCQCLQTLQGIHPKNIQSVNVKSPGPHCAQTEVI 75

Stealth Gene B 5637   *VCWYCVIIGKLAHEITYNSLYFSYLHSAKLKNGNEVCLNTEGPMVKKIIEKM 5795
                                 intron           A LKNG + CLN   P+VKKIIEKM
Human MGSA Gene  76    -------------------------ATLKNGRKACLNPASPIVKKIIEKM 100

Stealth Gene C 4583   SPRFLAVALLIVSLIAYSESSQG------IRCECKKGTQKIPENKIVVKKMKRPSGPNHP 4744
                      +PR L VALL++ L+A +    G       +RC+C +  Q I    I    +K P GP+
Human MGSA Gene 12    NPRLLRVALLLLLLVAAGRRAAGASVATELRCQCLQTLQGIHPKNIQSVNVKSP-GPHCA 70

Stealth Gene C 4745   RTEVKDSTKQPGRDPMGRPVS 4807
                      +TEV  +T + GR       P S
Human MGSA Gene  71   QTEV-IATLKNGRKACLNPAS 90
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Additional cellular genes have been identified in several clones obtained from viral DNA isolated from the stealth virus culture. Many of the genes have highly reiterated/repeat sequences. Some of the sequences match to endogenous reverse transcriptase, suggesting a possible mechanism whereby recombinations invoving cellular RNA can be back translated into viral incorporated cellular DNA.

Conclusions

Atypically structured, non-inflammation inducing cytopathic viruses definetely exist.

Some of these viruses were derived from simian CMV and have presumably entered the human population from SCMV contaminated batches of live polio vaccines.

Non-inflammatory cytopathic viruses are grouped under the term “stealth.” They can be regularly cultured from patients with complex multi-system illnesses, including various cancers. Positive stealth virus cultures were found in approximately 10% of University students donating blood for transfusion. Community outbreaks do occur.

Stealth-adaptation is considered to be a generic process that can involve many types of cytopathic viruses. It presumably occurs through the loss of genes coding for major antigens normally targeted by the cellular immune system.

Tissue culture provides the best method to screen for stealth-adapted viruses. Viral cultures can also provide useful insights into pathology, including formation of lipids, and of protease-resistant protein complexes.

The production of lipids and pigmented materials is viewed as a reparative process helping to maintain cell viability. There is a marked reduction in the intensity of the CPE if the culture medium is not frequently replaced.

Bacteria and cell-derived genes are present in the SCMV-derived stealth virus culture. This important finding indicates the potential intermixing of cellular, viral and bacterial genes in the creation of new highly pathogenic organisms. Viteria is used to define viruses with bacterial sequences. Atypical bacteria can commonly be cultured from stealth virus infected patients.

Stealth viruses are found in cancer patients, many of who have symptoms of an underlying neuropsychiatric illness. The prospect of bacteria transmitting cancer causing viruses is a very serious and urgent public health concern.

Bacterial genes can help explain partial and inconsistent serological and/or PCR diagnostic findings for mycoplasma, (in CFS, Gulf War Syndrome): Borrelia (in “chronic Lyme disease”), streptococcus (in PANDAS), etc.

Apparent expansion of chemokines and chemokines-receptor genes provide an adjunctive approach to anti-stealth virusl therapy. Many therapeutic agents are available that can lead to cytokine/chemokine suppression.

Ongoing Research Program

- Complete the sequencing of SCMV and SCMV-derived stealth virus
- Test for passage of this virus through bacteria using molecular methods
- Characterize lipids and proteins synthesized in stealth virus cultures
- Survey patient populations for evidence of stealth virus infections and for any disease-related characteristics of their positive cultures
- Determine if vaccines can activate a stealth virus infection and/or pathology
- Sequence additional stealth virus isolates, especially from cancer patients
- Conduct clinical trials on substances shown to inhibit stealth virus CPE
- Educate clinicians on the multi-system nature of stealth virus infections

Guiding Quotes:

One can only see what one observes, one observes only the things that are already in the mind. *Alphonse Bertollin, 1853-1914.*

The more I look, the more I see, and the more I see, the more I look for. *Teihard de Chardin, 1881-1914.*

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