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Viral effects on metabolism: changes in glucose and glutamine utilization during human cytomegalovirus infection

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Abstract

Human cytomegalovirus (HCMV) infection causes dramatic alterations of intermediary metabolism, similar to those found in tumor cells. In infected cells, glucose carbon is not completely broken down by the tricarboxylic acid (TCA) cycle for energy; instead it is used biosynthetically. This process requires increased glucose uptake, increased glycolysis and the diversion of glucose carbon, in the form of citrate, from the TCA cycle for use in HCMV-induced fatty acid biosynthesis. The diversion of citrate from the TCA cycle (cataplerosis) requires induction of enzymes to promote glutaminolysis, the conversion of glutamine to -ketoglutarate in order to maintain the TCA cycle (anaplerosis) and ATP production. Such changes could result in heretofore uncharacterized pathogenesis, potentially implicating HCMV as a subtle co-factor in many maladies, including oncogenesis. Recognition of the effects of HCMV, and other viruses, on host cell metabolism will provide new understanding of viral pathogenesis and novel avenues for antiviral therapy.

The Warburg effect and tumor cell metabolism

Intermediary metabolism has been resurrected in the minds of many of us who left metabolic studies in the 1970s to pursue the molecular biology revolution. This awakening has been spurred by the discoveries over the past five to eight years in the area of cancer biology, where metabolism has been shown to be significantly altered by oncogenesis^{1–6}. Additionally, modern technologies in metabolomics (see Glossary), combined with classical biochemistry, cell biology and molecular biology, provide a great opportunity for these studies, at this time. However, the realization that metabolism is dramatically altered in tumor cells derives from an old and classic observation, the Warburg effect. Otto Warburg and colleagues observed, in 1924, that cancer cells metabolize glucose very differently than normal cells⁷. Cancer cells, like normal cells, use glycolysis to convert glucose to pyruvate (Figure 1); however, normal cells and cancer cells part dramatically in how they utilize pyruvate. Most normal cells allow pyruvate to enter the tricarboxylic acid (TCA) cycle, where it is catabolized to CO₂ and promotes oxidative phosphorylation. Warburg observed that in cancer cells pyruvate is converted to lactate (Figure 1), rather than entering into the TCA cycle, even when there is sufficient oxygen to support mitochondrial oxidative phosphorylation⁸. It is now known that some of the pyruvate is also converted to acetyl

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CoA (AcCoA) in the mitochondrion and begins the TCA cycle by enzymatically combining with oxaloacetic acid (OAA) to form citrate (Figure 1). However, the citrate is shuttled from the TCA cycle and the mitochondrion to be converted back to OAA and AcCoA in the cytoplasm where the AcCoA can be used biosynthetically, primarily for fatty acid synthesis. The development and progression of cancer is often associated with a remarkable increase in de novo fatty acid synthesis⁹⁻¹¹.

Such utilization of glucose has several important implications. First, the production of pyruvate from glucose via glycolysis produces just two ATP molecules per molecule of glucose. In contrast, if glucose-derived pyruvate proceeds through the TCA cycle, the NADH produced would have supported oxidative phosphorylation and an additional 36 ATP molecules would have been made. While this appears to be an inefficient utilization of glucose energetically, the shift from the complete breakdown of glucose by cancer cells (and other rapidly proliferating cells) promotes the use of glucose carbon for macromolecule biosynthesis, for example the synthesis of fatty acids needed to construct progeny cells^{5, 8}.

The second implication of this utilization of glucose is that it deprives the TCA cycle of glucose-derived carbon, which not only limits ATP production, but also limits the production of TCA cycle intermediates which are important biosynthetic precursors, e.g. for amino acids. Cancer cells, or any cells that choose to utilize glucose in this way, must overcome these limitations. It is now known that in many cancer cells exogenous glutamine is used as a carbon source for maintenance of the TCA cycle¹⁻³. This is accomplished via glutaminolysis, which requires the enzyme glutaminase (Glnase) to deaminate glutamine to glutamate, and glutamate dehydrogenase (GDH) to convert glutamate to α -ketoglutarate (Figure 1). Thus glutamine anaplerotically maintains the TCA cycle intermediates (Figure 1) and, in the process, maintains NADH generation for oxidative phosphorylation and ATP production^{1, 2}. In contrast, most normal, quiescent cells use only a small amount of consumed glutamine in this way. Thus both glucose and glutamine metabolism are dramatically altered in tumor cells^{2, 3, 12}.

Increasing evidence suggests that a variety of infectious agents, including bacteria, parasites and viruses, contribute to oncogenesis¹³. Mechanistically these agents might contribute to oncogenesis directly or indirectly. For example, some viruses encode proteins which can transform cells by affecting major cellular growth control proteins such as p53 and the retinoblastoma proteins (RB)¹⁴, while others transform the cell by using modified host genes which have integrated into the viral genomes¹⁵. Other viruses, as well as bacteria and parasites, could function more indirectly, through immunosuppression, chronic inflammation, suppression of apoptosis and induction of genetic instability¹³. In truth, we have only begun to discover the extent to which infectious agents could contribute to oncogenesis, especially via indirect routes.

In this review we will examine the effects of human cytomegalovirus (HCMV) on cellular metabolism; HCMV is one of the first viruses on which modern metabolic analysis has been performed. The startling result is that HCMV's modification of glucose and glutamine metabolism is very much like that seen in tumor cells.

HCMV, potentially a subtle co-factor in many maladies

The basics of HCMV and its known pathogenesis are provided in Box 1. In order for HCMV's replicative cycle to be successful the virus needs to alter the host cell to establish an environment that can accommodate the increased demands for nutrients, energy and macromolecular synthesis that accompany viral infection. These alterations also result in cellular stress that induces stress responses, the consequences of which could be deleterious to the viral infection; for example, most stress responses inhibit translation. Thus during the

long HCMV growth cycle the virus must not only induce means to increase energy and macromolecule synthesis, but also alter stress responses to trick the cell into ignoring stress signaling. HCMV is adept at taking control of key cellular processes, e.g. signaling pathways, stress response signaling and metabolism, that affect broad aspects of cellular synthesis, growth and survival²¹⁻⁴². Thus it is not difficult to see how the pathologic effects of these alterations could implicate HCMV as a subtle cofactor in far more maladies than presently considered. For example, it has long been suggested that HCMV could contribute to atherosclerosis⁴³⁻⁴⁷, and with the advent of more sensitive methods for detection, HCMV has been implicated in the development of several cancers, including glioblastoma^{48, 49}, colorectal cancer⁵⁰ and prostate cancer⁵¹. While there are reports in the literature suggesting that HCMV could be able to transform cells by a hit and run mechanism⁵², the virus is not considered to be able to frankly transform cells. However, the recent data proposing associations with cancer have led to the suggestion that HCMV might mediate more subtle means of manipulating cells towards oncogenesis (oncopotentiation), or tumor cells toward more serious malignancy (oncomodulation)^{53, 54}. The counter argument in any discussion of viral association with tumorigenesis is that tumor tissue could simply provide a favorable tissue niche in which a virus can replicate and that the presence of the virus has little to do with oncogenesis. At this point one cannot rule out this possibility for HCMV; however, during immune suppression HCMV is capable of infecting and replicating in a wide variety of tissues and organs, thus it does not seem to need a favorable tissue niche.

Box 1

HCMV and its pathogenesis: the basics

HCMV is the prototype β -herpesvirus, characterized by slow growth and restricted species and tissue specificity. It is the largest human herpesvirus, with a genome of greater than 200,000 bp, capable of encoding an estimated 200 or more open reading frames¹⁶. The expression of HCMV genes is temporally regulated where groups of genes are activated more or less in unison at different times post-infection. These include the immediate early (IE) or α genes which are expressed first; followed by early (E) or β gene expression; and finally the late (L) or γ genes are expressed¹⁷. Expression of each temporal class affects the initiation of expression of the next class¹⁷. It is well established that IE gene products function in preparing the cell to execute the viral infection; thus they would be expected to have significant effects on cellular processes. The early genes primarily function in viral DNA synthesis, and the late genes primarily encode virion structural proteins needed to encapsidate the DNA and form the final virion.

HCMV is a significant human pathogen which infects most of the human population by puberty, the primary infection can be unnoticed. Like all herpesviruses, HCMV establishes lifelong latency after the primary infection; the primary sites of HCMV latency are the mononuclear cells of myeloid lineage, from CD34+ haematopoietic stem cells through to peripheral blood monocytes¹⁸⁻²⁰. HCMVs subsequent reactivation from latency accounts for much of the morbidity associated with the virus which can have severe outcomes: infection in utero may result in central nervous system damage, severe birth defects, deafness or death; infection of immunocompromised individuals can result in severe disease, transplant rejection, organ damage and death.

In the following discussion we will examine the effects of HCMV infection on glucose and glutamine metabolism and the role these play in potentiating fatty acid synthesis needed by the viral infection. Although it remains to be established how HCMV might be involved in oncopotentiation and oncomodulation, the changes in metabolism induced by HCMV are

very similar to those seen in tumor cells; thus HCMV-mediated alteration in metabolism could benefit a transforming cell.

Glucose transport in HCMV-infected cells

The first indication that HCMV might alter intermediary metabolism came from early studies showing that glucose uptake was significantly increased in infected cells⁵⁵. The transport of glucose requires specific carrier molecules, the glucose transporters (GLUTs). There are 13 different GLUTs in mammalian cells, GLUT 1–12, plus the proton (H⁺)-myo-inositol cotransporter (HMIT)^{56, 57}. Structurally, the GLUTs can be divided into three classes: GLUT1-4 (class I); GLUT5, 7, 9 and 11 (class II) and GLUT6, 8, 10, 12 and HMIT (class III)^{56, 57}; all have a differing tissue specificities and affinities for glucose.

The best characterized are the class I GLUTs, GLUT1-4. GLUT1 is ubiquitously distributed and is abundant in fibroblasts, erythrocytes, and endothelial cells^{58–60}. It is the major GLUT found in human fibroblasts, used in many HCMV studies³⁶. GLUT2 is a low-affinity transporter for glucose, found primarily in the intestines, pancreatic β -cells, kidney and liver⁶¹. GLUT3 protein distribution is restricted to brain and testis⁶², and has the highest affinity for glucose. GLUT4 is the major glucose transporter in adipose tissue, as well as in skeletal and cardiac muscle. Under conditions where GLUT4 is not needed on the cell surface for transport, it resides in intracellular vesicles. Its translocation to the cell surface for glucose transport requires activation of Akt kinase, a consequence of insulin signaling^{63, 64}.

Examination of GLUTs in HCMV-infected human fibroblasts has shown that the predominant GLUT, GLUT1, is eliminated from the infected cells and replaced by GLUT4, which has a greater transport capacity but is normally not expressed in these cells³⁶. This change in GLUTs appears to be vital for the success of the HCMV infection in fibroblasts, since specific inhibition of GLUT4 function not only lowers glucose uptake but also dramatically inhibits formation of infectious progeny virions. The mechanism for the replacement of GLUT1 with GLUT4 utilizes HCMV protein IE72, the 72 kDa major immediate early protein, which appears to modulate the mRNA levels, eliminating GLUT1 mRNA and greatly increasing GLUT4 mRNA³⁶. Previous studies have suggested that overexpression of GLUT4 alone can increase GLUT4 levels on the cell surface⁶⁵, thus circumventing the Akt-mediated translocation step described above. Thus the dramatic increase in GLUT4 in infected cells might drive GLUT4 onto the cell surface, assuring that glucose uptake increases regardless of the activity of Akt.

Glucose utilization in HCMV-infected cells

For many years it was assumed that the increased uptake of glucose resulted in increased glucose breakdown to promote oxidative phosphorylation, supplying ATP to support the viral infection. However, a number of studies beginning in 2006 dramatically altered the view of glucose metabolism in infected cells. Metabolic flux analysis^{34, 35} using liquid chromatography–tandem mass spectrometry (LC-MSMS) analysis and ¹³C-labeled glucose showed that HCMV institutes its own metabolic program, altering the metabolism pattern observed under normal conditions and considerably stimulating glycolysis, the TCA cycle, and the pyrimidine biosynthetic pathway³⁴. Of particular note was the efflux of glucose carbon from the TCA cycle, in the form of citrate, to support fatty acid biosynthesis³⁵ (Figure 1). This sets up a situation which is much like that in tumor cells (Figure 1). Specifically, the increased amounts of glucose taken up by HCMV-infected cells proceed through glycolysis to pyruvate, some of which is converted to lactate in the cytoplasm and some to AcCoA in the mitochondrion. The AcCoA combines with OAA to form citrate, much of which shuttles out of the mitochondrion and is converted back to OAA and AcCoA

in the cytoplasm, thus supplying cytoplasmic AcCoA for fatty acid and sterol synthesis. Microarray analysis of gene expression suggested that the metabolic and biosynthetic enzymes needed to increase glycolysis and biosynthesis are upregulated by HCMV ³⁴. Additionally, it was shown that this diversion of glucose carbon for fatty acid synthesis was critical for HCMV infection. Specifically, pharmacological inhibition of fatty acid biosynthesis suppressed HCMV replication ³⁵. Thus in HCMV-infected cells glucose metabolism is altered such that glucose carbon is used primarily for fatty acid biosynthesis and is not broken down in the TCA cycle to promote oxidative phosphorylation.

Glutamine utilization in HCMV-infected cells

The removal of citrate from the citric acid cycle in HCMV-infected cells has the same consequence as it does in tumor cells: there is a great decrease in glucose-derived carbon in the TCA cycle. This presents virally-infected cells with the same problem as tumor cells, the production of TCA cycle intermediates is limited. Since the biosynthesis of these intermediates is the source of NADH for oxidative phosphorylation, the production of ATP is also limited. However, it has been demonstrated that HCMV-infected cells, similar to tumor cells, activate a means to use glutamine anaplerotically to maintain the TCA cycle ²⁹(Figure 1).

That an alternative carbon source must be used in HCMV-infected cells came from the observation that normal human fibroblasts infected with HCMV for at least 12 hours remained viable upon glucose deprivation ²⁹, while uninfected cells rapidly lose viability without glucose ^{29, 66}. Since tumor cells use glutamine as the alternative carbon source to maintain the TCA cycle, it was suspected that glutamine might be used for anaplerosis in HCMV-infected cells ³⁴. Viral growth analyses supported this hypothesis, showing that glutamine deprivation stopped the formation of infectious virions; in addition, glutamine was found to be a major source of ATP in infected human fibroblasts, but not in uninfected cells ²⁹. HCMV-induced glutamine utilization was also indicated by increased glutamine uptake and increased ammonia production, which results from glutaminolysis. The increase in glutamine utilization corresponded to increased levels and enzymatic activities of glutaminase and glutamate dehydrogenase, the two enzymes that must be activated to convert glutamine to α -ketoglutarate for entry into the TCA cycle (Figure 1). Finally, confirmation that glutamine is used to maintain the TCA cycle was established by the finding that the TCA cycle intermediate α -ketoglutarate, the product of glutaminolysis (Figure 1), could rescue ATP production and viral growth under conditions of glutamine deprivation ²⁹. Thus in HCMV-infected cells, as in many tumor cells, a program is activated whereby glutamine uptake is increased for the purpose of maintaining the TCA cycle and ATP production, thereby allowing glucose to be used biosynthetically, e.g. for fatty acid synthesis.

Mechanisms by which HCMV controls glycolysis, glutaminolysis and citrate cataplerosis

At this point, the mechanisms by which HCMV alters cellular metabolism are largely speculative; however, we can propose models for experimental analysis.

Glycolysis

Microarray analysis suggests that HCMV infection increases the levels of mRNA encoding enzymes in the glycolytic pathway ³⁴, providing one means of increasing glycolysis. Whether this is due to transcriptional activation or increased mRNA stability remains to be determined. The virus might also mediate mechanisms that allosterically activate glycolytic enzymes. In this regard a potential means for HCMV to activate glycolysis is to control the

production of fructose 2,6-bisphosphate (F-2,6-P₂), a potent positive glycolytic signaling molecule (reviewed in ^{67, 68}). As shown in Figure 2, the first step of glycolysis is the conversion of glucose to fructose-6-phosphate (F-6-P). To proceed in glycolysis the enzyme 6-phosphofructo-1-kinase (PFK-1) converts F-6-P to fructose-1,6-bisphosphate (F-1,6-P₂); this is the first committed step in glycolysis, thus it is tightly controlled. Interestingly, negative effectors of PFK-1 include citrate and ATP, two molecules that HCMV-infected cells are striving to produce; thus a mechanism to overcome negative effectors seems appropriate. The positive effector of PFK-1 is F-2,6-P₂ (Figure 2); the activity of PFK-1 is very low unless there are physiological (micromolar) concentrations of F-2,6-P₂ to activate PFK-1 and to relieve inhibition.

Conditions known to increase glycolysis in the heart are accompanied by concomitant increases in F-2,6-P₂. The concentration of F-2,6-P₂ is determined by the bifunctional enzyme 6-phosphofructo-2-kinase (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2), which controls both phosphorylation and dephosphorylation at the 2 position. Mammalian tissues have distinct isozymes of PFK-2/FBPase-2 and in most cases phosphorylation determines whether the predominant activity of the enzyme is as a protein kinase or a phosphatase. Phosphorylation usually leads to increased activity of the PFK-2 kinase activity, thus increasing F-2,6-P₂ levels, which would activate PFK-1 and advance glycolysis. PFK-2/FBPase-2 is the target of many protein kinases, including AMP-activated protein kinase (AMPK), mitogen activated protein kinase activated protein kinase 1 (MAPKAPK-1), p70 ribosomal S6 kinase (p70S6K) and Akt. In HCMV-infected cells many of the above kinases are known to be active, e.g. AMPK ²³, Akt ²⁴⁶⁹ and p70S6K ²². Thus one prediction is that HCMV-infected cells have increased levels of F-2,6-P₂ due to activation of PFK-2, possibly due to HCMV-mediated activation of PFK-2-activating kinases (Figure 2).

In this regard a recent study established that inhibition of calmodulin-dependent kinase kinase (CaMKK) blocks HCMV-mediated activation of glycolysis and restricts HCMV replication ⁷⁰. That a calcium-dependent kinase could be involved is supported by the observations that HCMV infection mobilizes calcium stores ⁷¹ and successful infection is very dependent on the calcium homeostasis set up during infection ²⁷. The mechanism by which CaMKK is utilized by HCMV to activate glycolysis remains to be determined; however, one possibility is CaMKK-mediated activation of calmodulin-dependent kinase 1 (CaMK1), another kinase suggested to be able to phosphorylate PFK-2⁷⁰.

Glutaminolysis

Initially, glutamine transport is increased in infected cells ²⁹; the mechanisms used by HCMV to increase glutamine uptake has not yet been studied. However, it is known that glutaminase (Glnase) and glutamate dehydrogenase (GDH) enzyme levels and enzymatic activities are greatly increased in infected cells to promote the conversion of glutamine to -ketoglutarate (Figure 1). The mechanism by which HCMV increases the levels of these enzymes has not yet been determined; however, the virus could utilize the same mechanism used by tumor cells. Recent studies have shown that oncogenic levels of c-Myc induce a transcriptional program that promotes glutamine uptake and glutamine conversion to -ketoglutarate, resulting in cellular addiction to glutamine as a bioenergetic substrate ³. In this regard, many tumor cells show increased c-Myc levels and glutamine dependence ^{3, 66}. Several studies have suggested that c-Myc mRNA and protein levels are increased in HCMV-infected cells ⁷²⁻⁷⁴.

Citrate cataplerosis

An important step in the metabolic program set up by HCMV is the induction of citrate cataplerosis, specifically the activation of mechanisms promoting the efflux of citrate from

the mitochondria to the cytosolic compartment, where it is converted by ATP-citrate lyase to OAA and AcCoA (Figure 1). Mitochondrion/cytoplasmic shuttling mechanisms have been characterized that shuttle citrate and pyruvate or citrate and malate^{75, 76}; these could be targets for HCMV manipulation. A number of HCMV proteins traffic through the mitochondria-associated membrane (MAM) compartment and localize in mitochondrial membranes (reviewed in⁷⁷). It has been proposed that viral proteins affecting the MAM might manipulate cellular processes including calcium signaling, lipid synthesis and transfer, bioenergetics, metabolic flow, and apoptosis⁷⁷. Thus citrate shuttling could also be affected.

Fatty acid synthesis

The means by which HCMV activate fatty acid synthesis remains undefined. However, the increased fatty acid synthesis detected in HCMV-infected cells³⁵ requires the activation of a number of lipogenic enzymes, similar to the events seen during adipocyte differentiation⁷⁸. Thus it is possible that the virus induces a modified form of adipocyte differentiation in infected cells. This is supported by the fact that HCMV-mediated upregulation of GLUT4³⁶, the glucose transporter found in abundance in adipocytes, is induced during adipocyte differentiation⁷⁸.

Why does the virus need such an increase in fatty acid synthesis?

Since HCMV is an enveloped virus, there are clearly fatty acid requirements for membranes from which the envelopes can be derived. However, examination of HCMV-infected cells suggests that membrane formation is needed for many other aspects of viral infection. It is well known that infected cells are enlarged (cytomegalia), which would require additional plasma membrane. In addition, the virion egress and assembly process appears to require a significant amount of membrane. The immunofluorescence micrographs in Figure 3 show two striking features of HCMV-infected cells. First is the remodeled nucleus, which is much enlarged and often takes on a characteristic concave, kidney shape. The significantly enlarged nucleus in comparison to mock infected cells indicates another requirement for additional membranes for nuclear expansion. The second feature shown in Figure 3 is a perinuclear structure known as the cytoplasmic assembly compartment or assembly complex (AC) nestled against the concave surface of the kidney-shaped nucleus^{79–81}. Current models of HCMV egress and assembly suggest that nucleocapsids, which form in the remodeled nucleus, exit the nucleus and move through the AC where the virion tegument layer and envelope are acquired. The AC is a highly vesicular viral organelle composed of viral proteins as well as components from the endoplasmic reticulum, Golgi, *trans*-Golgi network and early endosomes^{39, 81–83}. Numerous viral structural proteins have been identified as part of the AC, e.g. tegument proteins (pp28, pp65)⁷⁹ and viral glycoproteins (gB, gH, gL, gO, gp65)^{79, 82, 84}. A rigorous study of AC structure⁸¹ resulted in a three-dimensional model proposing that the AC is cylindrical and composed of organelle-specific vesicles which form nested cylinders. Each vesicular cylinder is proposed to contain a specific set of virion structural proteins (tegument proteins) which transfer to nucleocapsids as they move toward the center of the AC⁸¹. In agreement with this model, ultrastructural studies suggest that full tegumentation requires the nucleocapsids to migrate through circularly arrayed vesicles^{85–87}. The electron micrograph in Figure 3 shows the vesicular nature of the AC. Thus another need for membranes in the infected cells is for the vesicles concentrated within the AC and found throughout the cytoplasm of infected cells.

Considering the enlargement of the infected cell and the infected cell nucleus, as well as the vesicular nature of the AC, it is not difficult to understand why the virus must alter glucose and glutamine utilization in order to allow glucose to be used biosynthetically for the production of fatty acids to fulfill the significant membrane requirement in infected cells.

Concluding remarks

HCMV infection dramatically alters cellular metabolism, and in this review we have focused on how the virus alters glucose and glutamine metabolism and fatty acid synthesis (Figure 4). Changes to glucose metabolism include increased glucose uptake through the induction of the glucose transporter GLUT4, upregulation of glycolytic enzymes and likely allosteric activation of glycolysis. Glucose is not completely broken down by the TCA cycle but maintained intact for biosynthetic purposes. This is primarily accomplished by the transport of citrate from the mitochondrion to the cytoplasm. In order to counter this loss of glucose carbon from the TCA cycle, HCMV affects glutamine metabolism by increasing exogenous glutamine uptake and inducing glutaminolysis enzymes to convert the imported glutamine to α -ketoglutarate to anaplerotically maintain the TCA cycle. In the cytoplasm, HCMV upregulates fatty acid and sterol synthesis so that AcCoA, derived from the cytoplasmic citrate, is used in fatty acid synthesis. The induction of fatty acid synthetic enzymes and the induction of GLUT4 could be related, as each would occur if HCMV infection induces a form of adipocyte differentiation.

While these changes are quite dramatic they are likely to represent only a fraction of the metabolic changes wrought by HCMV infection. Such changes are likely to contribute to forms of HCMV pathogenesis that could be very different from those that have been studied and documented to this point. For example, HCMV and other viruses which induce metabolic changes similar to HCMV might play a greater role in oncogenesis than previously recognized.

At this point our understanding of the mechanisms used by HCMV to alter metabolic processes is very limited and requires targeted study. It is essential that the metabolic alterations wrought by HCMV infection be worked out using a combination of modern mass spectrometric based metabolic flux analysis, classical biochemistry and enzymology, and cellular and molecular biological techniques. In a broader sense, similar studies must be initiated with all viral families; it is likely that other viruses will take very different approaches in their interactions with host metabolism. The complete understanding of viral interactions with host metabolism will provide new targets for antiviral therapy, for example drugs that might inhibit GLUT4, glutamine uptake or key metabolic enzymes with which the viral infection is dependent. As metabolism is experiencing a renaissance, it is unfortunate to find that many find it intimidating or see it as boring, we hope that this review shows it otherwise.

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Glossary

Anaplerosis and cataplerosis

anaplerosis is the process of replenishing TCA cycle intermediates that have been removed for cataplerosis, i.e. removed from the cycle for biosynthetic reactions. For example, in HCMV-infected cells citrate is cataplerotically removed from the TCA for fatty acid synthesis, while glutamine is anaplerotically used to fill the TCA cycle to compensate for the lost citrate

Glutaminolysis	the set of metabolic reactions that converts glutamine to -ketoglutarate, allowing glutamine to be used anaploretically to form the intermediates of the TCA cycle and support oxidative phosphorylation
Glycolysis	the metabolic pathway that converts glucose to pyruvate
Metabolomics	the systematic study of a cell's or organism's small-molecule metabolite profiles. Using isotopically-labeled compounds, for example ^{13}C -labeled glucose or glutamine, the metabolites resulting from metabolism can be detected by techniques involving gas chromatography-mass spectrometry (GC-MS) or nuclear magnetic resonance (NMR) spectroscopy. The resulting data can define the metabolic flux and the metabolome, the collection of all metabolites in a biological sample
Tricarboxylic acid (TCA) cycle	also known as the Krebs's cycle and the citric acid cycle and was established by the work of Albert Szent-Györgyi and Hans Krebs. It is a succession of enzyme-catalyzed reactions that occur in the mitochondria which are part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate usable energy. The cycle begins with combination of the two-carbon acetyl group from acetyl-CoA (derived from pyruvate via glycolysis) to the four carbons of oxaloacetate to form the six-carbon citrate molecule. The citrate is biochemically altered as it moves through the TCA cycle, losing two carboxyl groups as CO_2 , returning to the four carbon oxaloacetate to allow the cycle to run again with more AcCoA derived from glucose by glycolysis. As a result of the oxidative reactions of the TCA cycle, electrons are transferred to NAD^+ to form NADH. In turn, NADH transfers its electrons to the oxidative phosphorylation pathway which, along with oxygen, forms ATP

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Figure 1. Aspects of glucose and glutamine metabolism and fatty acid synthesis. Reactions within the circle occur in the mitochondrion (MITO), while those outside the circle occur in the cytoplasm (CYTO). Movement across the mitochondrial membrane is indicated by dashed arrows. Enzymes and general enzymatic pathways are shown in green. Abbreviations: TCA Cycle, tricarboxylic acid cycle; Glnase, glutaminase; GDH, glutamate dehydrogenase; AcCoA, acetyl coenzyme A; CoA, coenzyme A.

Figure 2.

The control of glycolysis by fructose-2,6-bisphosphate. The bifunctional enzyme 6-phosphofructo-2-kinase (PFK-2)/fructose-2,6-bisphosphatase (FBPase-2) can both phosphorylate and dephosphorylate fructose-6-phosphate at the 2 position. A number of kinases (some of which are activated during HCMV infection) can phosphorylate and activate the kinase activity of the enzyme. This increases the levels of fructose-2,6-bisphosphate, which allosterically activates 6-phosphofructo-1-kinase (PFK-1), a key enzyme in glycolytic control. Activation steps are indicated by dashed red arrows.

Figure 3.

The enlargement and structural changes in the nucleus and the formation of the assembly compartment during HCMV infection. The two immunofluorescence micrographs show normal nuclei in mock infected cells (left) and the significantly enlarged and kidney shaped nuclei seen in HCMV-infected cells (middle, the nuclei are visualized by staining for lamin B, red). In infected cell nuclei, a perinuclear structure known as the cytoplasmic assembly compartment or assembly complex (AC; visualized by staining for the HCMV tegument protein pp28, green) is seen nestled against the concave surface of the kidney-shaped nucleus^{79–81}. The immunofluorescence micrographs are reproduced from Ref. 38 with permission from the *Journal of Virology*. As shown in the electron micrograph (right), the AC is made of many vesicular structures; the bar indicates 2 μ m.

Figure 4.

HCMV-mediated modifications in cellular processes that lead to alterations of glucose and glutamine metabolism and fatty acid synthesis. (1) Glucose uptake increases through the induction of the glucose transporter GLUT4 which replaces the transporter GLUT1. (2) Glycolytic enzymes are upregulated and it is likely that glycolysis is allosterically activated by infection. (3) Glucose carbons are diverted from the TCA cycle through transport of citrate from the mitochondrion to the cytoplasm. (4) Fatty acid and sterol synthesis are upregulated so that AcCoA derived from cytoplasmic citrate can be used for fatty acid synthesis. The induction of fatty acid synthesis enzymes and the induction of GLUT4 may be related, as each would occur if HCMV infection induces a form of adipocyte differentiation. (5) Exogenous glutamine uptake is increased in infected cells. (6) Glutaminolysis enzymes are induced to convert the imported glutamine to α -ketoglutarate to anaplerotically maintain the TCA cycle. Abbreviations: CYTO, cytoplasm; MITO, mitochondrion.