Biochem. J. (1987) 241, 313–324 (Printed in Great Britain)

REVIEW ARTICLE

Molecular biology and immunology of cytomegalovirus

Paul D. GRIFFITHS and Jane E. GRUNDY
Virology Unit, Department of Medical Microbiology, Royal Free Hospital School of Medicine, London NW3 2PF, U.K.

INTRODUCTION

During their lifetime, 60–80% of individuals in developed countries and virtually 100% of those in developing countries will become infected with cytomegalovirus (Sarov *et al.*, 1982; Peckham *et al.*, 1983; Griffiths *et al.*, 1985). That most of these infections remain entirely asymptomatic is a tribute to the ways in which cytomegalovirus (CMV) has evolved to live in equilibrium with the host immune defences which have developed for the control of virus infections. Yet the attempts of the virus to live peacefully with its host are incomplete where the immune responses of the latter are either immature (fetus and neonate) or compromised (allograft recipients; patients with acquired immune deficiency syndrome). As a result, intrauterine CMV infections are second only to Down's syndrome as a known cause of mental retardation (Stern & Tucker, 1973; Stagno *et al.*, 1983), CMV pneumonitis is the most common single cause of death following bone marrow transplantation (Watson, 1983) and disseminated CMV infection is a major cause of mortality and morbidity in patients with renal allografts (Glenn, 1981) or with acquired immune deficiency syndrome (Macher *et al.*, 1983). In modern medical practice CMV is thus a major pathogen whose ultimate control by means of immunization (Plotkin *et al.*, 1984) or drug therapy (Meyers *et al.*, 1982) has become an important objective. At the time of writing, control of disease produced by CMV is still a distant target due to the complexity of the biochemistry of the virus and the immunology of its natural history.

MOLECULAR BIOLOGY

The Virion

Structure. The virion structure of CMV is typical of a member of the herpes virus family (reviewed by Roizman, 1982). The nucleic acid is complexed helically with protein to form a core, which is enclosed in a protein capsid consisting of a total of 162 capsomere subunits. Each of 150 capsomeres is hollow for half of its long axis and is hexagonal in cross-section, while the remaining 12 capsomeres are pentameric. The capsid measures 100 nm in diameter and is surrounded by a poorly defined area, the tegument. This is enclosed by a lipid bilayer envelope containing peplomers to give a final diameter of 180 nm for the whole virion (see Fig. 1). The envelope has been reported to be derived from both the internal nuclear membrane (Smith & De Harven, 1973) and from the endoplasmic reticulum (Severi *et al.*, 1979). The recent description of two distinct lamellae to the lipid envelope may lead to these earlier observations being reconciled (Farrar & Oram, 1984).

Assembly. The assembly of proteins to form progressively more mature A, B and C capsids is depicted schematically in Fig. 2. When CMV is passaged in cell cultures, two morphological forms other than the virion can be identified by means of positive-density negative-viscosity ultracentrifugation gradients (Talbot & Almeida, 1977). One form is the dense body (Sarov & Abady, 1975) which is much larger (300 nm) and more

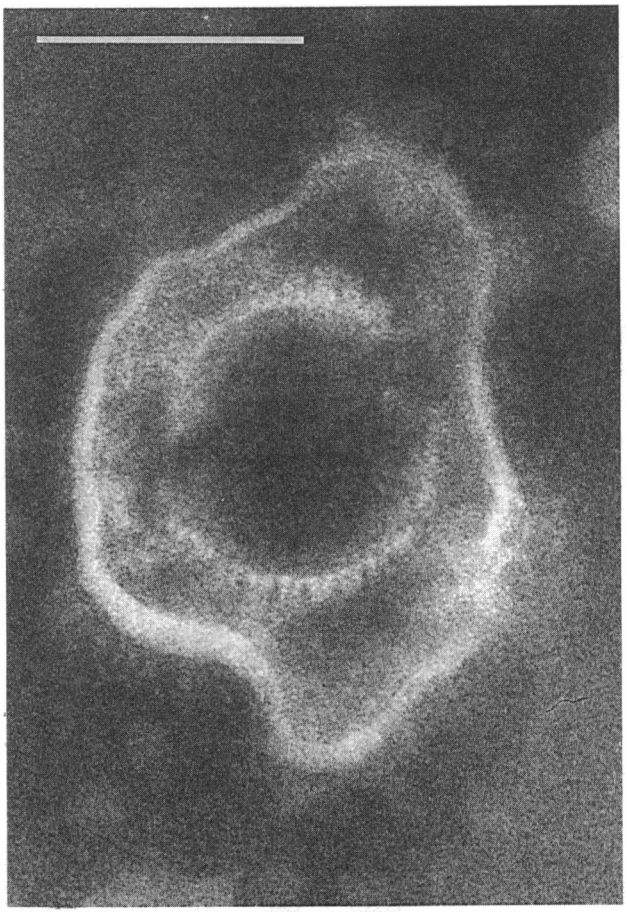

Fig. 1. The electron-microscopic appearance of cytomegalovirus

Photograph prepared by Mrs D. Roy. The white bar represents 100 nm.

Abbreviations used: CD, cluster designation (of lymphocyte surface antigen); CMV, cytomegalovirus; gA, gB, gC and gD, glycoproteins of families A, B, C or D; H-2, major histocompatibility complex of the mouse; HLA, major histocompatibility complex of human; HSV, herpes simplex virus; IR_L, IR_S, inverted repeats of long and short portions of genome; NIEP, non-infectious enveloped particle; NK, natural killer cell; MCMV, murine cytomegalovirus; Tc, cytotoxic T-lymphocyte; TR_L, TR_S, terminal repeats of long and short portions of genome; U_L, U_S, unique long and short regions.

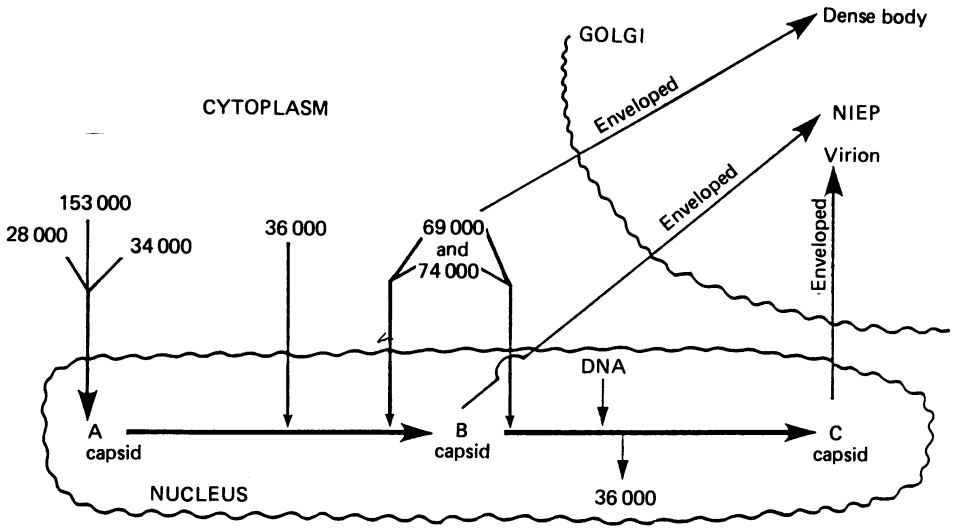

Fig. 2. Schematic outline of assembly of cytomegalovirus particles

Numbers (e.g. 36000) represent the M_r of identified proteins.

pleomorphic than the virion; 90% of its protein is the matrix protein of M_r 69000 together with envelope glycoproteins (Irmiere & Gibson, 1983) and it does not contain nucleic acid or a nucleocapsid. The second form (Irmiere & Gibson, 1983) has been termed non-infectious enveloped particle (NIEP). It resembles a virion without its central core containing the DNA. As depicted in Fig. 2 the dense body and NIEP are aberrant forms of CMV. The NIEP is probably an enveloped B capsid which contains an excess of the assembly protein of M_r 36000 while the dense body consists of enveloped matrix protein (Irmiere & Gibson, 1985).

Nucleic acid

The nucleic acid in the virion is linear double-stranded DNA of M_r 1.5×10^8. Within the nucleus of an infected cell the DNA is probably synthesized by 'rolling circle' replication (Stinski, 1983) using a DNA polymerase coded for by the virus (Huang, 1975*a*). The DNA is synthesized as a long concatemeric molecule and is later cleaved into individual genomes ready for packaging inside daughter capsids. The genome contains sequences of repeated genetic information and unique sequences (see Fig. 3). The unique sequences are contained in two portions of the genome, termed long and short, each of which can be inverted relative to the other (Westrate *et al.*, 1980; Oram *et al.*, 1982; Fleckenstein *et al.*, 1982). There are thus four genomic isomers, each of which is found in equimolar concentrations following cell culture passage of CMV (De Marchi, 1981; Oram *et al.*, 1982; Fleckenstein *et al.*, 1982; Spector *et al.*, 1982).

Repeated sequences. Repetitive sequences of the genome are highly rich in guanine and cytosine (Honess,

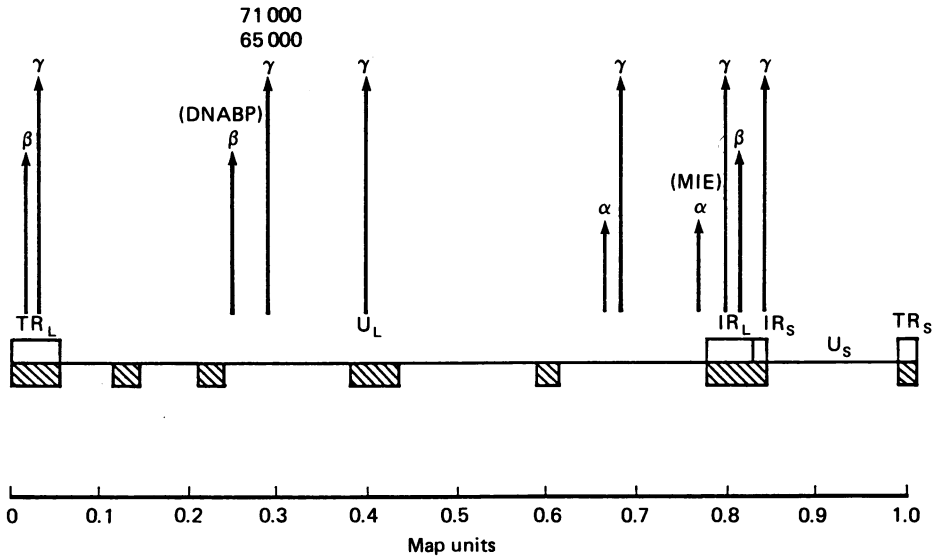

Fig. 3. Identification of some important regions of the cytomegalovirus genome

Further abbreviations: DNABP, DNA-binding protein; MIE, major immediate-early protein; α, β, γ, proteins of α, β or γ class; 71000 and 65000 represent the M_r values of two proteins. Hatched areas of the genome cross-react with human DNA.

1984). These G+C-rich segments may contain some of the sequences which control expression of eukaryotic genes. They bind avidly to host cell DNA, even under conditions of relatively high stringency (Peden *et al.*, 1982; Ruger *et al.*, 1984), and so DNA probes containing these regions must not be used to test for the presence of CMV genomes in human cells. The areas of the CMV genome which cross-react in this way with host cell DNA are shown in Fig. 3. It is possible that reports of the presence, even under stringent conditions, of the oncogene v-*myc* (Spector & Vacquier, 1983) within the CMV genome may be attributed to cross-reactivity with such highly G+C-rich sequences.

The function of the repeat sequences shown in Fig. 3 is not known. However, a sequence termed 'a' which exists as a direct repeat in the TR_L and TR_S and as an inverted repeat in the IR_L of the analogous genome of herpes simplex virus (HSV) functions as a cleavage and packaging signal for encapsidation of virion DNA (Mocarski & Roizman, 1982; Vlazny & Frenkel, 1981). Since the substitution of CMV 'a' region for that in HSV has been reported still to allow HSV encapsidation it seems likely that this may represent the role of 'a' during natural CMV infection (Spaete & Mocarski, 1985*a*). Furthermore, since in HSV the presence of the direct 'a' repeat and inverted 'a' at the junctional region provides a *cis* signal for circularization of the genome (Wadsworth *et al.*, 1975) and for inversion of the long and short segments (Mocarski *et al.*, 1980) it is to be expected that 'a' will also subserve these functions in CMV.

Typing of strains. The genome of CMV strains isolated from patients can be labelled with ^{32}P, digested with restriction endonucleases (Garrett & Warren, 1985) and compared with the DNA from other strains (see Fig. 4). An alternative method is to digest the unlabelled wild-type DNA with restriction enzymes and, following electrophoresis and transfer to nitrocellulose filters, probe with a labelled cloned fragment from the junction of the long and short regions of the genome (Spector *et al.*, 1985). Such work has revealed that, although there is an average of 80% homology between strains (Huang *et al.*, 1976; Kilpatrick *et al.*, 1976), many thousands of different strains can be recognized.

Transcription and translation

Cascade expression. Following infection of a susceptible cell the temporal expression of the genome of the input virus is closely controlled. Expression proceeds by a cascade synthesis of mRNA and proteins termed α (or immediate-early), β (or delayed early) and γ (or late). In general terms, the products of the α genes are those the virus requires to take over control of host cell macromolecular synthesis, the β-products are required to control production of daughter virions, while the γ-proteins form the structural components of the virion.

The genome expression is termed cascade because the presence of the earlier products is required for expression of the later products (Wathen & Stinski, 1982). Thus as shown in Fig. 5, α-proteins permit β-mRNA synthesis, β-proteins permit DNA replication which is followed by γ-mRNA synthesis. Direct biochemical evidence for this cascade sequence can be obtained by culturing infected cells in the presence of various inhibitors of transcription or translation. As shown in Fig. 5, the cellular presence of cycloheximide at the time of inoculation of CMV will prevent translation of α-mRNA. If medium containing this inhibitor is replaced by medium containing actinomycin D, then α-proteins can be produced in the absence of β-mRNA. Expression of this latter class of genes can

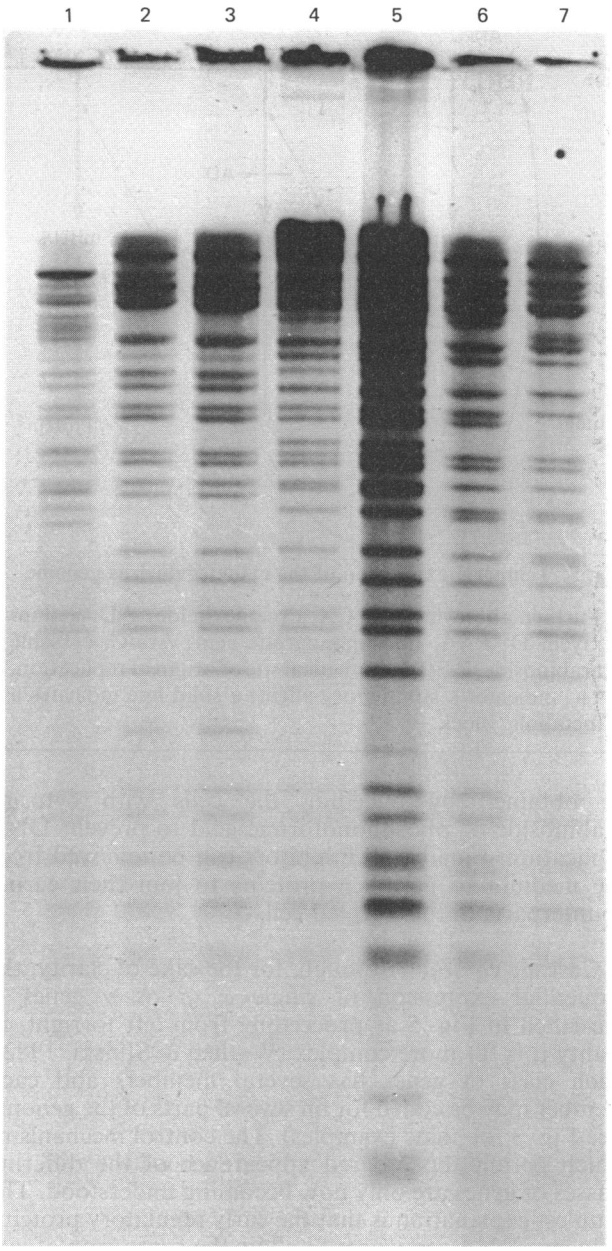

Fig. 4. Identification of strains of cytomegalovirus by means of restriction enzyme analysis

Isolates were propagated in the presence of [^{32}P]. The labelled DNA was digested with the restriction enzyme *Bam*H1 and the resulting oligonucleotides separated by gel electrophoresis and visualized by autoradiography. Seven isolates (numbered from the left) are shown. The first is the laboratory-adapted strain AD169. The second and third are from patients whose CMV infection was epidemiologically related. The patterns seen are identical but are different from those in lanes 4 and 5 which are epidemiologically related and identical to each other. Lanes 6 and 7 contain isolates which were not epidemiologically related to any of the other patients and these patterns are distinct from all the others. Experiment performed by Mr M. Super.

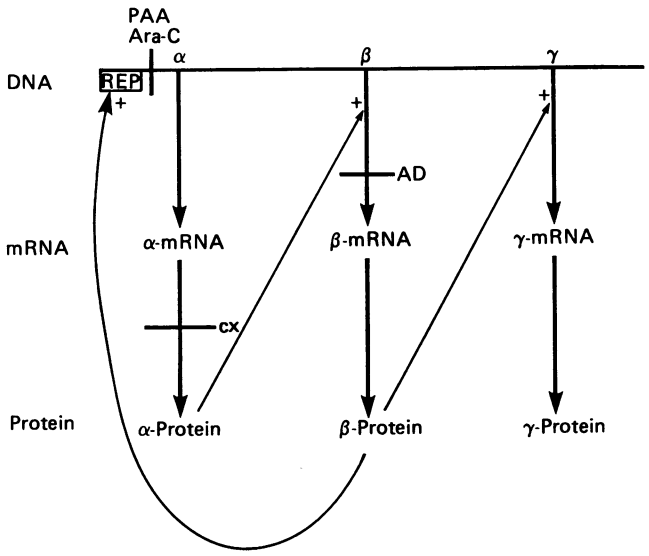

Fig. 5. Temporal expression of the cytomegalovirus genome

Further abbreviations: CX, cycloheximide; AD, actinomycin D; PAA, phosphonoacetic acid; Ara-C, cytosine arabinoside; REP, hypothetical site of origin of replication. '+' indicates a stimulatory effect; a solid line indicates a metabolic block.

be obtained by refeeding the cells with cytosine arabinoside or phosphonoformic acid to prevent DNA replication. Finally, all inhibitors can be removed from the medium to permit γ-proteins to join their earlier counterparts in the infected cell.

Cascade control. Although, for the sake of clarity, the sequential expression of single α, β or γ genes is presented in Fig. 5 as proceeding from left to right, in reality it is far more complex (Wathen & Stinski, 1982). Each class of genes has several members and each member may be coded for on several parts of the genome (see Fig. 3 for some examples). The control mechanisms which permit the ordered appearance of the differing classes of genes are only now becoming understood. The simplest explanation is that the early regulatory proteins influence DNA sequences to allow the expression of late genes. The α and β genes are transcribed by host cell RNA polymerase II (Thomsen *et al.*, 1984; Spaete & Mocarski, 1985*b*; Stinski & Roehr, 1985). Their expression is controlled by sequences proximal to the promoter which are *cis*-activated by a *trans*-acting viral function (Spate & Mocarski, 1985*b*). It seems likely that the viral function concerned will turn out to be a structural protein, as has been described for HSV (Batterson & Roizman, 1983). The advantages to the virus of such an arrangement are obvious; uncoating of the virus particle could yield a structural protein which could immediately activate the genes. This mechanism could also explain how the α genes can be *trans*-activated by a virion protein even in the absence of protein synthesis *de novo* (Geballe *et al.*, 1986). A different mechanism may control expression of γ genes, however. Recent evidence suggests that late genes are transcriptionally active at early times so that their delayed expression may depend upon post-transcriptional influences such as transcript transport to the cytoplasm, transcript accumulation, differential polysome association or different stability of mRNA (De Marchi, 1983*a*; Geballe *et al.*, 1986). This could explain why whole cell RNA is homologous to many regions of the virion DNA at early and late times although mRNAs associated with polysomes show differential expression (Chua *et al.*, 1981; De Marchi, 1983*a*; Wathen & Stinski, 1982).

Identification of coding regions. The regions which encode each gene class have been determined either by electrophoresing restriction enzyme fragments of CMV DNA, performing Southern transfer and reacting with radiolabelled viral RNA (De Marchi *et al.*, 1980; Wathen & Stinski, 1982) or by electrophoresing mRNA, performing Northern transfer and reacting with radiolabelled viral DNA (Wathen & Stinski, 1982; McDonough *et al.*, 1985). Recently, Mocarski *et al.* (1985) have used an expression vector to produce a β-galactosidase fusion product from a randomly generated DNA library including the β-gene which encodes a DNA-binding protein. By immunoprecipitating the product with a monoclonal antibody specific for the DNA binding protein, the location of the gene could be identified with certainty (see Fig. 3).

One recent report (Nowak *et al.*, 1984*b*) has provided convincing evidence that two late proteins are produced by an essentially identical length of virion DNA (see Fig. 3). The matrix protein (M_r 65000) and a phosphoprotein of M_r 71000 were not immunoprecipitated by the same monoclonal antibody and so are presumably not immunologically related. It seems likely that these two distinct proteins are either produced by different DNA reading frames or that the relevant mRNAs undergo different splicing patterns (Nowak *et al.*, 1984*b*).

Infected-cell-specific proteins. Major problems have been encountered by workers attempting to identify the proteins within infected cells which are CMV specific. The virus has a genome theoretically capable of coding for over 100 proteins of average size, each of which may be subject to post-translational modification by cleavage, phosphorylation, glycosylation or sulphation. The frequent cleaving of proteins requires that precursor–product relationships be defined and that artefacts due to exogenous proteinases are avoided. The glycosylated proteins appear to be translocated in clathrin-coated vesicles through the Golgi apparatus in order to effect this biochemical modification. It is therefore not surprising to find morphologically that the Golgi region becomes rather prominent in CMV-infected cells. Such glycosylation does produce proteins of variable *M*r and there is evidence that several of the CMV-specific intracellular glycopolypeptides thought to be distinct are in fact related proteins with polymorphic glycosylation (Pereira *et al.*, 1984). This has been shown by using monoclonal antibodies to immunoprecipitate virus-coded proteins from infected cells and analysing their relative molecular masses. Such work has identified four immunologically distinct families of proteins termed gA, gB, gC and gD (Pereira *et al.*, 1984). Several members are found within each family and pulse–chase experiments have identified the series of processing events which lead ultimately to the production of a mature gA product destined to be a structural component of the virion (Pereira *et al.*, 1984).

Structural proteins. When virions are purified by physical methods and then chemically denatured, the

Table 1. Structural proteins of virus particles

Data from Gibson (1983) and Irmiere & Gibson (1983).

$10^{-3} \times M_r$	Provisional name	Present in: Virion	NIEP	Dense body
212	High molecular weight protein	+	+	
153	Major capsid protein	+	+	
149	Basic phosphoprotein	+	+	
115	—	+	+	
74	Matrix protein	+	±	
69	Matrix protein	+	±	+
36	Assembly		+	
34	Minor capsid protein	+	+	
8	Minor capsid protein	+	+	

relative molecular masses, biochemical modifications and immunological relationships of the structural proteins can be examined. Many of the structural proteins in the tegument appear to be phosphorylated while those peplomers embedded in the lipid envelope are usually glycosylated (Gibson, 1983) with evidence from endoglycosidase treatment for both *O*- and *N*-linked oligosaccharides (Farrar & Oram, 1984). The availability of monoclonal antibodies has provided specific reagents, but it appears that several distinct structural proteins can be co-immunoprecipitated because they aggregate spontaneously to form complexes (Nowak *et al.*, 1984*a*) or because they associate covalently by way of disulphide bonds (Britt & Auger, 1986). These problems can be bypassed by using the monoclonal antibodies in Western blot experiments under denaturing conditions, but then many of the antibodies fail to react (Nowak *et al.*, 1984*a*; Rasmussen *et al.*, 1985) presumably because the reactive epitopes are formed by conformational protein changes in many cases.

Interpretation of previous work is compounded by authors reporting different strains of CMV passaged in different fibroblast cell lines with variable attempts to separate virions from dense bodies or NIEPs and by the use of differing nomenclature. Nevertheless it is possible to assign tentative functions to some structural proteins, as is summarized in Table 1. It should be emphasized that in attempting to produce such a 'consensus' table for this Review we have assumed that obvious discrepancies in the literature will ultimately be resolved.

Envelope proteins. When the published reports of glycosylated envelope proteins are reviewed, however, there is far greater disagreement (see Table 2). This might be expected for proteins which undergo extensive post-translational modification. For example, Britt has used monoclonal antibodies in pulse–chase experiments to show that a glycosylated M_r 150000 precursor is modified to a glycosylated M_r 160000 protein which is then cleaved to form subunits of M_r 55000 and 116000 which are found in purified virions (Britt, 1984; Britt & Auger, 1986). The mature proteins of M_r 55000 and 116000 appear to share no structural homology or antigenic determinants and so their relationship could easily have been missed. Similarly, Law *et al.* (1985) have shown that three glycoproteins of M_r 52000, 95000 and 130000, together with a protein of M_r 50000 which may not be glycosylated, are co-precipitated by monoclonal antibodies because disulphide bonds link the various proteins. Under reducing conditions two distinct bands of M_r 50000 and 52000 were found which could be reproduced by the action of endoglycosidase H on the glycosylated protein of M_r 52000. These two examples should serve to illustrate why the results in the literature are often variable and to show how much effort will be required to identify the remaining glycoproteins of CMV.

Classification of proteins. Ultimately, each protein encoded by CMV will be identified as α, β or γ. Most of the structural proteins described above will be γ, although this remains to be proven formally. An example of a β-protein is the virion DNA polymerase which can be distinguished from the host enzyme by its requirement for high salt concentrations, its sedimentation properties (Huang, 1975*a*) and its reversible susceptibility to phosphonacetic acid (Huang, 1975*b*). Other β-proteins are the phosphorylated DNA-binding protein of M_r 50000 (Gibson, 1983) and the non-phosphorylated protein of M_r 140000 which binds single-stranded DNA (Anders *et al.*, 1986). As regards α-proteins, Blanton & Tevethia (1981) immunoprecipitated four proteins from CMV-infected cells at immediate-early times (M_r values 78000, 77000, 75000 and 31000). In contrast, Wathen *et al.* (1981) isolated polyadenylated mRNAs and translated them *in vitro* to yield nine proteins (M_r values 75000, 72000, 59000, 56000, 42000, 39000, 27000, 16500 and 6700). The extent to which such translation products would be modified *in vivo* is not

Table 2. Reported M_r values of CMV envelope glycoproteins

Abbreviation: ND, not determined.

Number of distinct protein bands described	$10^{-3} \times$ Apparent M_r of protein bands											References
8			131	115	108	81	66	62	ND	ND		Stinski (1976)
4	210				100		62	57				Fiala *et al.* (1976)
6		175/165	140/130		105/84		66		52/44		22	Kim *et al.* (1976)
3			145				62	57				Gibson (1983)
5	250		130		95		67	52				Farrar & Oram (1984)
7			130		94		68	58	43	30	21.5	Nowak *et al.* (1984*a*)

known. However, there is good evidence from several authors that the most prominent α-protein is phosphorylated (Gibson, 1981) and has an M_r of between 70000 and 79000 (Michelson-Fiske *et al.*, 1977; De Marchi *et al.*, 1980; Wathen & Stinski, 1982; Gibson, 1981; Cameron & Preston, 1981; Stinski *et al.*, 1983). The gene encoding this protein (see Fig. 3) has recently been sequenced (Sternberg *et al.*, 1984). The gene contains four exons with a spliced mRNA beginning in the second exon and extending to encode a protein of 491 amino acids. The predicted M_r of this protein is 64000 which, even allowing for phosphorylation, is rather less than that described in earlier reports. The protein is rich is proline residues and one possibility is that extensive β-pleating may give it an erroneously high M_r value in denaturing gels (Sternberg *et al.*, 1984).

Effect of CMV on host cells

Most viruses alter host cell macromolecular synthesis to reduce potentially inhibitory effects such as secretion of interferons. In contrast, CMV has been reported to stimulate synthesis of host DNA, RNA and proteins, even in the absence of viral DNA synthesis (Yamanishi & Rapp, 1979). This apparent paradox can probably be explained by the results of De Marchi (1983*b*). She found that cells productively infected with CMV produced an α-protein which stimulated host cell functions and that a β-protein subsequently switched off this reaction. Thus, in cells which were destined to produce progeny viruses, a transient stimulation of host macromolecular synthesis was apparent. Since one of the host functions which was activated was thymidine kinase (De Marchi, 1983*b*), it is plausible to suggest that CMV employs this mechanism of increasing intracellular thymidine monophosphate concentrations in contrast to the related HSV and varicella-zoster viruses which encode their own thymidine kinases for this purpose. If this reasoning is correct, then the continuous activation of host cellular synthesis in cells which were abortively infected can be explained since, in such cells, CMV genome expression cannot proceed as far as the β-protein required to switch off such stimulation.

Latency

It is generally assumed that CMV frequently establishes latent infection in its host, since therapeutic immunosuppression leads to active virus excretion in most individuals who have serological evidence of past infection (Glenn, 1981). In such patients CMV is most frequently isolated from urine and/or saliva, leading to the possibility that the kidney tubules or salivary gland may be the sites of latency. Yet, it may be that these sites are simply accessible to clinical sampling and that reactivation in an internal organ leads to virus dissemination to the peripheral organs.

CMV can also be transmitted by blood transfusion so that some cellular blood element could be another (or the major) site of latency. Attempts to culture CMV from donor blood have, with the exception of one isolated report (Diosi *et al.*, 1969), all given negative results (Mirkovic *et al.*, 1971; Kane *et al.*, 1975), even when co-cultivation techniques were employed. There is thus no evidence to support blood cells as a site of CMV latency, although a recent publication (Schrier *et al.*, 1985) describes the detection of mRNA from the major gene of CMV in some lymphocytes from 8/8 seropositive and 1/12 seronegative individuals. In immunosuppressed patients with viraemia, CMV has been cultured from a variety of leukocytes including monocytes and granulocytes (Garnett, 1982). However, this finding does not implicate such cells as a site of latency, since CMV may have been present simply as a result of their phagocytic activity.

Work *in vitro* with fibroblasts, which are the most permissive cells identified, has however shown that cells not expressing CMV antigens but which contain on average 45 genome equivalents each of CMV DNA can be selected (Mocarski & Stinski, 1979). Persistence was favoured by a low multiplicity of infection using fresh isolates but a high multiplicity when laboratory-adapted strains were used (Mocarski & Stinski, 1979). The latter result probably reflects the artificial propagation of viruses with defective genomes in stocks of laboratory strains maintained by passage at high multiplicity (Stinski *et al.*, 1979*a*, *b*). CMV DNA can also persist in non-permissive mouse cells from which it can be recovered by fusion with fully permissive cells (Boldogh *et al.*, 1977).

THE IMMUNOLOGY OF CMV INFECTION

The interaction between the host's immune system and CMV is a complex one as the virus can itself cause suppression or enhancement of host responses and can infect cells of the lymphoid system. Furthermore, although the host has evolved mechanisms of controlling virus replication, the virus has evolved ways of avoiding them. An understanding of this complex interaction has come from studies *in vivo* in a murine model, using murine CMV as well as from measurements of parameters *in vitro* with both the human and murine viruses.

Non-specific host defence mechanisms

Natural killer cells. Evidence for the protective role of natural killer (NK) cells in murine CMV (MCMV) infection is substantial. NK cells are activated rapidly after MCMV infection and the level of activity correlates with the degree of resistance in susceptible and resistant strains of mice (Bancroft *et al.*, 1980, 1981). Mice which have a defect in NK function, homozygous beige mice, are more susceptible to MCMV (Shellam *et al.*, 1981, 1985). Administration of anti-asialo-G_{M1} antibody, which depletes NK cells, was found to increase the severity of MCMV infection (Bukowski *et al.*, 1984). Furthermore, adoptive transfer studies showed that resistance to MCMV could be induced in susceptible suckling mice, which have low levels of NK cells, by transfer of the NK fraction of spleen cells or cloned NK cells (Bukowski *et al.*, 1985). Thus these studies *in vivo* support the role of the NK cells in protection against MCMV, although the mechanism is not clear. Some workers have reported that nonactivated NK cells preferentially lyse MCMV-infected cells compared with uninfected cells (Quinnan & Manishewitz, 1979; Lee & Keller, 1982) while others have reported that MCMV-infected cells are less susceptible to NK lysis than uninfected cells when using NK cells activated by infection *in vivo* (Bancroft *et al.*, 1981; Bukowski & Welsh, 1985). However, interferon was able to protect uninfected cells from lysis by activated NK cells, whereas this effect was not seen with infected cells (Bukowski &

Welsh, 1985). Thus, in the presence of interferon, the virus-infected cell may be a good NK target. Since interferon is produced in MCMV infection (see below) and activates NK cells, NK lysis of infected cells *in vivo* is probably the mechanism of protection.

NK cells have been shown to lyse cells infected with human CMV (Starr & Garrabrant, 1981; Borysiewicz *et al.*, 1985). Such lysis was greater than that seen with uninfected cells and required the expression of α and β genes (Borysiewicz *et al.*, 1985). The target structure recognized by the NK cells was not thought to be a viral antigen, but a cellular structure present in greater amounts on the infected cell. A candidate structure, the transferrin receptor, which was increased in CMV-infected cells, was later shown not to be the NK recognition structure (Borysiewicz *et al.*, 1986). Another group have found differences in the susceptibility to NK lysis of cells infected with different laboratory strains of CMV (Waner & Nierenberg, 1985). Similar studies have not been carried out using clinical isolates of CMV.

Interferon. The production of α and β interferon is an important part of the host's non-specific defence against viruses, particularly in the early stages of infection. Early studies presented contradictory evidence on the role of interferon in infection with cytomegaloviruses. MCMV was reported to be a poor inducer of interferon *in vitro* and *in vivo* (Osborn & Medearis, 1966) and to be relatively insensitive to its antiviral action (Osborn & Medearis, 1966; Oie *et al.*, 1975, Kern *et al.*, 1978). Other reports described interferon production during MCMV infection (Henson & Smith, 1964; Henson *et al.*, 1966, Kelsey *et al.*, 1977; Stringfellow *et al.*, 1977; Tarr *et al.*, 1978) and assumed this to have a protective role. Definitive evidence of such a protective role for interferon came from the studies by Grundy (Chalmer) *et al.* (1982) in which it was shown that administration of an antiserum specific for α and β interferon significantly reduced the resistance of mice to MCMV infection, and resulted in increased viral titres in blood and liver. These studies also demonstrated that MCMV induced a partially acid-labile α/β interferon, which may explain the disparate reports referred to above, since samples were usually treated at pH 2 before assay for interferon. The production of interferon early in MCMV infection was under genetic control with high, intermediate and low producer strains [Grundy (Chalmer) *et al.*, 1982; Shellam *et al.*, 1983] and may provide the basis for the differences in activation of NK cells following MCMV infection in various mouse strains referred to above. It was not possible to protect low interferon producer strains with exogenous α/β interferon [Grundy (Chalmer) *et al.*, 1982] and it was apparent that strains of mice differed in their ability to utilize interferon in defence against MCMV as well as in its production (Shellam *et al.*, 1983). Administration of interferon to newborn mice was found to increase the resistance to MCMV of resistant strains but not of susceptible strains (Shellam *et al.*, 1983).

The role of interferon in CMV infections in man is not well established. Human CMV can induce α/β interferon *in vitro* (Cruz *et al.*, 1981) and we have recently found a partially acid-labile α interferon in the bronchoalveolar lavage fluid and plasma of patients with CMV pneumonitis (Grundy *et al.*, 1986). Both laboratory strains of CMV and clinical isolates have been reported to be sensitive to the antiviral action of interferon (Postic & Dowling, 1977).

The production of γ or immune interferon by sensitized lymphocytes from CMV-seropositive donors on restimulation with CMV antigen *in vitro* is well described (Starr *et al.*, 1980), but the role *in vivo* of this lymphokine as an antiviral agent or as an immunomodulator is not known. Therapeutic trials of interferon therapy for prophylaxis against CMV infection in renal transplant recipients have resulted in a reduction of CMV excretion (Cheeseman *et al.*, 1979) together with some evidence of reduced clinical severity of disease attributable to CMV infection (Hirsch *et al.*, 1983).

Host genetics. The resistance of adult mice to lethal infection with MCMV has been found to be controlled by genes within the H-2 complex of the mouse (equivalent to the HLA region in man) as well as by non-H-2-linked genes [Chalmer *et al.*, 1977; Chalmer 1980; Grundy (Chalmer) *et al.*, 1981]. Two genes within the H-2 complex were involved, mapping to the Class I regions [Grundy (Chalmer) *et al.*, 1982]. The mechanism of the H-2 control of resistance has not yet been fully elucidated but was not found to relate to levels of antibody [Grundy (Chalmer) *et al.*, 1981], interferon [Grundy (Chalmer) *et al.*, 1982], or NK cell activation (Bancroft *et al.*, 1981), following MCMV infection. The H-2-associated resistance to lethal infection of adult mice is also seen with newborn mice (Shellam & Flexman, 1986) and a similar pattern is seen with the replication of MCMV *in vitro* in fibroblasts from different strains of mice (Harnett & Shellam, 1982). Thus the mechanism of H-2 control of resistance appears to relate to viral replication at the cellular level rather than to any component of the host immune response to MCMV. Recent studies with human CMV have demonstrated that the virus can use Class I HLA molecules as a receptor to bind to cells and initiate infection (Grundy *et al.*, 1986*b*) and we have postulated that the H-2 control of resistance to MCMV relates to different affinities of binding of MCMV to the Class I molecule in the various H-2 haplotypes. The fact that susceptibility is a dominant trait in F_1 hybrid mice between resistant and susceptible haplotypes [Grundy (Chalmer) *et al.*, 1981] is consistent with a receptor hypothesis.

The non-H-2-linked genes controlling resistance to MCMV have not yet been genetically mapped. The genetic control of interferon induction by MCMV (referred to above) resides in multiple non-H-2-linked genes (J. E. Grundy & G. R. Shellam, unpublished work). NK activation is similarily controlled by non-H-2-genes (Bancroft *et al.*, 1981; Shellam *et al.*, 1982) but whether or not this is a secondary effect of differences in interferon induction has not been determined.

A genetic basis of resistance has not been demonstrated in CMV infections in man. In one study of CMV infection in renal transplant recipients, no correlation between HLA type and disease was found (Patel *et al.*, 1978); however, such patients are known to be at high risk from CMV infection due to their immunosuppressive therapy, and the latter may well have obscured any differences in innate resistance patterns. Another study documented a higher level of antibody in normal individuals with the Bw 15 haplotype (Pereira *et al.*, 1978), but whether this represented more frequent

infections or the ability to produce higher levels or antibody after infection was not determined. We have observed differences in levels of CMV replication in fibroblasts from different individuals (J. A. McKeating, J. E. Grundy & P. D. Griffiths, unpublished work) and are currently investigating whether this correlates with HLA type in a similar manner to that found for MCMV and H-2 type.

Humoral immune response to CMV

Antibody as a marker of infectivity. Humoral immune responses are thought not to play an important role in defence against CMV. The fetus can be infected by intrauterine transmission of CMV in women known to possess antibodies prior to conception and the neonate can be infected by CMV in breast milk despite the presence of passively acquired maternal antibodies (Stagno *et al.*, 1977, 1980). Seropositive transplant recipients can be reinfected with CMV from the donor (Grundy *et al.*, 1986*c*), again suggesting that pre-existing antibodies do not confer protection. The presence of antibody to CMV should therefore not be considered as a measure of immunity but as a marker of previous infection with the virus. Since seropositive patients have latent CMV infection which may reactivate, antibody is a marker of potential infectivity.

Nevertheless, there is some evidence that infections during pregnancy (Stagno *et al.*, 1982) or following renal transplantation (Glenn, 1981) may be less severe in the presence of pre-existing antibody. Thus specific antibodies cannot prevent CMV infection but they may moderate its pathogenicity. Passive administration of antibodies to CMV have been shown to modify or prevent disease if given before infection in mice (Araullo-Cruz *et al.*, 1978; Shanley *et al.*, 1981) and man (Winston *et al.*, 1982) and has had apparently beneficial effects in the treatment of established CMV pneumonitis in man (Blacklock *et al.*, 1985). However, the role of antibody in prophylaxis or treatment of CMV infections remains to be resolved.

Virtually all of the viral proteins described earlier have been immunoprecipitated by human immune sera (Schmitz *et al.*, 1980; Pereira *et al.*, 1982) or recognized by Western blotting with human immune sera (Landini *et al.*, 1985). Similar results were obtained using sera from seropositive individuals (Pereira *et al.*, 1982), from infants with congenital or perinatal infection (Pereira *et al.*, 1983) or from recipients of bone marrow transplants (Zaia *et al.*, 1986). Some authors have reported that the same range of proteins were immunoprecipitated by IgG and IgM antibodies (Pereira *et al.*, 1982), whilst others have found that some proteins were preferentially recognised by IgG and others by IgM classes of antibody (Landini *et al.*, 1985). Using antigens captured by CMV-specific monoclonal antibodies from a crude lysate of infected cells, Cremer *et al.* (1985) have shown that the antibody response directed towards gA glycoproteins (Pereira *et al.*, 1984) was present at a higher concentration in primary and secondary infection, and persisted longer, than did antibody to other antigens. Antibody to the major capsid protein was present at a lower concentration, rose more slowly in infection, and persisted for a shorter time, whilst antibodies to gC and gD (Pereira *et al.*, 1984) showed an intermediate pattern in both levels and temporal appearance (Cremer *et al.*, 1985).

Nature of neutralization. Antibodies that neutralize CMV can be demonstrated in human sera *in vitro* (Chiba *et al.*, 1972). As with many other viruses, the addition of complement enhances viral neutralization (Graham *et al.*, 1971). The appearance of neutralizing antibodies following primary CMV infection is reported to be slow (Stalder & Ehrensberger, 1980).

A viral protein recognized by murine monoclonal antibodies which had neutralizing activity *in vitro* was a glycoprotein of M_r 55000 (Britt & Auger, 1986). Other workers have reported that proteins of M_r 86000, 55000 and 130000 were recognized by neutralizing antibodies raised in the guinea pig, the latter two requiring the presence of complement for neutralization (Rasmussen *et al.*, 1985).

Neutralization of virus *in vivo* has not been demonstrated; indeed, Rundell & Betts (1980) have shown that CMV complexes were still infectious in cell culture. Furthermore, infectious virus can be found in saliva (Tamura *et al.*, 1980) or cervical secretions (Waner *et al.*, 1977) despite the presence of antibody in these secretions. Thus neutralization of CMV appears to be inefficient *in vivo*. We have recently suggested that this is due to the masking of the viral antigenic determinants by the binding of a host protein, β_2-microglobulin (see below).

Primary versus secondary infection. After primary CMV infection in normal individuals, IgM antibodies rise rapidly but do not persist (Griffiths, 1981). A rise in IgG antibodies is seen slightly later than IgM, achieving peak levels within 2 months and then falling slightly to remain at a stable but lower level throughout life. In immunocompromised patients a rise in IgG titres is common during secondary episodes of CMV infection (Pass *et al.*, 1983). Secondary CMV infections may be accompanied by a rise in IgM antibodies in a proportion of patients (Kangro *et al.*, 1982; Pass *et al.*, 1983). It is possible that these infections represent reinfections whilst those without an IgM response represent reactivations.

Cellular immune response to CMV

The cell-mediated immune response to CMV is believed to be important in host defence because patients with deficiencies of cell-mediated immunity are at high risk of CMV disease. In the murine model, T-cell-deficient nude mice are more susceptible to MCMV infection [Starr & Allison, 1977; Grundy (Chalmer) & Melief, 1982].

Cytotoxic T cells. Cytotoxic T (Tc) cells capable of specifically lysing MCMV-infected fibroblasts *in vitro* have been found in the spleens of mice from day 3 of infection, peaking on day 7–8 (Quinnan *et al.*, 1978; Sethi & Brandis, 1979). However, other workers have not been able to demonstrate Tc cells against MCMV-infected targets without restimulating the spleen cells *in vitro* in the presence of virus-infected stimulator cells (Ho, 1980), or with lymph node cells after a period of culture *in vitro* (Sineckas *et al.*, 1985). Passive transfer of T cells primed *in vivo* and restimulated *in vitro* could protect mice from MCMV infection (Ho, 1980), although it was not demonstrated that it was the Tc component that mediated the protective effect. The predominant viral epitope recognized by Tc cells was an

immediate early antigen expressed on the surface of infected fibroblasts (Reddehase & Koszinowski, 1984).

Tc cells that lyse human fibroblasts infected with human CMV have been demonstrated after stimulation *in vitro* of peripheral blood lymphocytes from seropositive donors with CMV-infected fibroblasts (Borysiewicz *et al.*, 1983) or CMV antigen (Gehrz & Rutzick, 1985) or directly by lymphocytes from patients undergoing an active CMV infection *in vivo* (Quinnan *et al.*, 1981; Rook *et al.*, 1984; Gehrz & Rutzick, 1985). Such Tc cells are usually class I HLA restricted and are of the CD 8 positive phenotype (Borysiewicz *et al.*, 1983). Recently a class II HLA restricted CD 4 positive T cell has been found in the peripheral blood of seropositive donors which, after restimulation *in vitro* with CMV antigen, could kill monocytes expressing CMV antigens (Lindsley *et al.*, 1986). A relationship between Tc activity against CMV and protection against CMV infection *in vivo* has been postulated, but not proven, in renal transplant patients (Rook *et al.*, 1984).

Evasion of host defence mechanisms by CMV

Induction of Fc receptors. CMV induces a receptor for the Fc portion of human IgG in infected cells (Keller *et al.*, 1976; Westmoreland *et al.*, 1976; Rahman *et al.*, 1976). This appears to be a glycoprotein of M_r 42000 (Sakuma *et al.*, 1977) which appears from 36 h post-infection (Keller *et al.*, 1976) and is found in the perinuclear region (Keller *et al.*, 1976) as well as on the surface (Westmoreland *et al.*, 1976) of infected fibroblasts. Similar observations have been made with HSV, where the viral glycoprotein E has been identified as being responsible for the Fc-receptor activity (Para *et al.*, 1982). The glycoprotein E of HSV is a constituent of the viral envelope, so that the virus, as well as virally infected cells, expresses Fc receptor activity. The Fc receptor induced by CMV has not yet been demonstrated to be on the viral particle.

It has been postulated that the Fc receptor plays an important role in the pathogenesis of HSV infection (Lehner *et al.*, 1975). It is assumed that the non-specific binding of IgG molecules via their Fc portion by CMV-infected cells will protect such cells from specific lysis by cytolytic antibody or cytotoxic cells *in vivo*, and thus this phenomenon represents an important strategy of the virus to evade host responses. Recently Mackowiak *et al.* (1984) have shown significantly enhanced binding of antibody-coated bacteria to CMV-infected cells as a consequence of the Fc receptor. These authors concluded that enhanced adherence of secondary pathogens to non-phagocytic cells *in vivo* might explain the predisposition to secondary bacterial infections seen in patients with CMV infections.

Binding of β_2-microglobulin. We have recently shown that, in body fluids such as urine, CMV is coated with the host protein β_2-microglobulin (McKeating *et al.*, 1986*a*). Such binding masked the antigenic sites recognized by murine monoclonal antibodies (McKeating *et al.*, 1986*b*) and virus from such fluids could not be neutralized by murine monoclonal antibodies or human immune sera, which had good neutralizing activity against CMV grown in cell culture (McKeating *et al.*, 1986*a*). We have shown *in vitro* that CMV has a strong binding capacity for β_2-microglobulin, which it binds after release from cells (Grundy *et al.*, 1986*d*). We have postulated that CMV has evolved this mechanism of coating itself in a host protein as a means of evading the host's humoral immune response and facilitating transmission of the virus.

Effect of CMV on the host immune response to other antigens

Immunosuppression. CMV is commonly perceived to be an immunosuppressive agent and indeed there is much laboratory data to support this. In the murine model the humoral immune response to other antigens has been found to be suppressed during the acute phase of MCMV infection (Osborn *et al.*, 1968; Howard & Najarian, 1974), as has the interferon response to another virus (Osborn & Medearis, 1967). The proliferative responses of T lymphocytes to stimulation with mitogens is reported to be suppressed in both patients with CMV mononucleosis (Rinaldo *et al.*, 1980) and mice with acute MCMV infection (Howard *et al.*, 1974; Booss & Weelock, 1975; Selgrade *et al.*, 1976; Kelsey *et al.*, 1977; Allan *et al.*, 1982). This defect has been attributed to the accessory macrophage both in man (Carney & Hirsch, 1981) and mouse (Loh & Hudson, 1980). Recently Rodgers *et al.* (1985) have shown that the ability of monocytes to produce interleukin 1 is abrogated by CMV infection *in vitro*, due to the release of an inhibitor of interleukin 1 by the monocytes. These authors could not detect CMV replication, or the production of early antigens, in these monocytes. There are several other reports which also suggest a direct effect of the virus in inducing immunosuppression in both the murine (Ho, 1980; Sineckas *et al.*, 1985) and human (Schrier *et al.*, 1986; Wahren *et al.*, 1986) systems.

The induction of immediate-early or early proteins of CMV in lymphocytes and monocytes has been described after infection *in vitro* with clinical isolates of CMV (Einhorn & Ost, 1984; Rice *et al.*, 1984), and this has been postulated to play an important role in the suppression of immune responses. However, the precise mechanism by which CMV induces immunosuppression remains to be determined.

MCMV has also been reported to affect the phagocytic function of macrophages (Shanley & Pesanti, 1980) and the phagocytic and migratory activities of neutrophils (Bale *et al.*, 1985).

Immunoenhancement. Enhancement of Tc cell activity towards allogeneic or hapten-modified syngeneic target has been described following MCMV infection in certain strains of mice (Grundy & Shearer, 1984). This enhancement of the cytotoxic response to Class I H-2 antigens was even more marked after secondary MCMV infection (Grundy & Reid, 1985) and was proposed by these authors as an explanation for the reported link between episodes of graft rejection and CMV infection in transplant patients (Lopez *et al.*, 1974; Simmons *et al.*, 1974). Enhancement of the antibody response to sheep erythrocytes has been described following secondary MCMV infection (Howard & Najarian, 1974). There is one report that human CMV can induce polyclonal B cell activation *in vitro* with nonspecific immunoglobulin production (Hutt-Fletcher *et al.*, 1983).

Autoimmune responses. The induction of autoantibodies has been observed following both human CMV (Kantor *et al.*, 1970) and MCMV (Bartholomaeus *et al.*,

1983) infection. It is not clear whether this is a result of polyclonal B cell activation or due to effects of the virus on regulatory T cells. Cytotoxic T cells which could lyse self targets have been described following MCMV infection (Sinickas *et al.*, 1985).

SUMMARY

The application of modern biochemical techniques has led to a rapid improvement in our knowledge of the molecular biology of CMV. Several coding regions of the DNA genome have been identified with certainty and major virus-coded proteins have been given provisional names. The cascade expression of the CMV genome has been shown to be controlled by mechanisms similar to those found in other herpes viruses, together with novel post-transcriptional controls which remain to be defined.

The control of CMV replication by the host involves both non-specific and specific defence mechanisms. The induction of natural killer cells and interferon early after CMV infection appears to be the most important aspects of the non-specific host defence against the virus. The cell-mediated immune response, in particular the generation of Tc cells against CMV early antigens, is probably the most important facet of the specific immune defence against CMV. When intact these defence mechanisms appear to be efficient in restricting viral replication; however, when such immunity is compromised, the balance rapidly swings in favour of the virus. As our understanding of the interaction between the host and the virus increases, it may be possible to redress the balance in such cases in favour of the host.

REFERENCES

Allan, J. E., Shellam, G. R. & Grundy (Chalmer) J. E. (1982) Infect. Immun. **36**, 235–242

Anders, D. G., Irmiere, A. & Gibson, W. (1986) J. Virol. **58**, 253–262

Araullo-Cruz, T. P., Ho, M. & Armstrong, J. A. (1978) Infect. Immun. **21**, 840–842

Bale, J. F., O'Neil, M. E. & Greiner, T. (1985) J. Leuk. Biol. **38**, 723–734

Bancroft, G. J., Shellam, G. R. & Chalmer, J. E. (1980) in Genetic Control of Natural Resistance to Infection and Malignancy (Skamene, E., ed.), pp. 277–282, Academic Press, New York

Bancroft, G. J., Shellam, G. R. & Chalmer, J. E. (1981) J. Immunol. **126**, 988–994

Bartholomaeus, W. N., Shellam, G. R., Allan, J. E., Reed, W. D. & Joske, R. A. (1983) Clin. Exp. Immunol. **52**, 89–93

Batterson, W. & Roizman, B. (1983) J. Virol. **46**, 371–377

Blacklock, H. A., Griffiths, P., Stirk, P. & Prentice, H. G. (1985) Lancet **ii**, 152–153

Blanton, R. A. & Tevethia, M. J. (1981) Virology **112**, 262–273

Boldogh, I., Gonczol, E., Gartner, L. & Vaczi, G. (1977) Arch. Virol. **53**, 101–108

Booss, J. & Wheelock, E. F. (1975) Proc. Soc. Exp. Biol. Med. **149**, 443–446

Borysiewicz, L. K., Morris, S. M., Page, J. D. & Sissons, J. G. P. (1983) Eur. J. Immunol. **13**, 804–809

Borysiewicz, L. K., Rodgers, B., Morris, S., Graham, S. & Sissons, J. G. P. (1985) J. Immunol. **134**, 2695–2701

Borysiewicz, L. K., Graham, S. & Sissons, J. G. P. (1986) Eur. J. Immunol. **16**, 405–411

Bukowski, J. F. & Welsh, R. M. (1985) J. Immunol. **135**, 3537–3541

Bukowski, J. F., Woda, B. A. & Welsh, R. M. (1984) J. Virol. **52**, 119–128

Bukowski, J. F., Warner, J., Dennert, G. & Welsh, R. M. (1985) J. Exp. Med. **161**, 40–52

Britt, W. J. (1984) Virology **135**, 369–378

Britt, W. J. & Auger, D. (1986) J. Virol **58**, 185–191

Cameron, J. M. & Preston, C. M. (1981) J. Gen. Virol. **54**, 421–424

Carney, W. P. & Hirsch, M. S. (1981) J. Infect. Dis. **144**, 47–54

Chalmer, J. E., Mackenzie, J. S. & Stanley, N. F. (1977) J. Gen. Virol. **37**, 107–114

Chalmer, J. E. (1980) in Genetic Control of Natural Resistance to Infection and Malignancy (Skamene, E., ed.), pp. 283–290, Academic Press, New York.

Cheeseman, S. H., Rubin, R. H., Stewart, J. A., Tolkoff-Rubin, N. E., Cosimi, A. B., Canteli, K., Gilbert, J., Winkle, S., Herrin, J. T., Black, P. H., Russell, P. S. & Hirsch, M. (1979) New Engl. J. Med. **300**, 1345–1349

Chiba, S., Striker, R. L. & Benyesh-Melnick, M. (1972) Appl. Microbiol. **23**, 780–783

Chua, C. C., Carter, T. H. & St Jeor, S. (1981) J. Gen. Virol. **56**, 1–11

Cremer, N. E., Cossen, C. K. & Pereira, L. (1985) J. Clin. Microbiol. **21**, 517–521

Cruz, J. R., Dammin, G. J. & Waner, J. L. (1981) Infect. Immun. **32**, 332–342

De Marchi, J. M. (1981) Virology **114**, 23–38

De Marchi, J. M. (1983*a*) Virology **124**, 390–402

De Marchi, J. M. (1983*b*) Virology **129**, 274–286

De Marchi, J. M., Schmidt, C. A. & Kaplan, A. S. (1980) J. Virol. **35**, 277–297

Diosi, P., Moldovane, E. & Tomescu, N. (1969) Br. Med. J. **4**, 660–662

Einhorn, L. & Ost, A. (1984) J. Infect. Dis. **149**, 207–214

Farrar, G. H. & Oram, J. D. (1984) J. Gen. Virol. **65**, 1991–2001

Fiala, M., Honess, R. W., Heine, J. W., Murnane, J., Wallace, R. & Guze, L. B. (1976) J. Virol. **19**, 243–254

Fleckenstein, B., Muller, I. & Collins, J. (1982) Gene **18**, 39–46

Forman, S. J., Zaia, J. A., Clark, B. R., Wright, C. L., Mills, B. J., Pottahil, R., Racklin, B. C., Welte, K. & Blume, K. G. (1985) J. Immunol. **134**, 3391–3395

Garnett, H. M. (1982) J. Lab. Clin. Med. **99**, 92–97

Garrett, A. J. & Warren, D. E. (1985) J. Virol. Methods **10**, 187–194

Geballe, A., Leach, F. S. & Mocarski, E. S. (1986) J. Virol. **57**, 864–874

Gehrz, R. C. & Rutzick, S. R. (1985) Clin. Exp. Immunol. **61**, 80–89

Gibson, W. (1981) Virology **112**, 350–354

Gibson, W. (1983) Virology **128**, 391–406

Glenn, J. (1981) Rev. Infect. Dis. **3**, 1151–1178

Graham, B. J., Minamishima, Y., Dressman, G. R., Haines, H. G. & Benyesh-Melnick, M. (1971) J. Immunol. **107**, 1618–1630

Griffiths, P. D. (1981) Br. J. Obstet. Gynaecol. **88**, 582–587

Griffiths, P. D., Baboonian, C. & Ashby, D. (1985) Int. J. Epidemiol. **14**, 447–452

Grundy (Chalmer), J. E. & Melief, C. J. M. (1982). J. Gen. Virol. **61**, 133–136

Grundy (Chalmer), J. E., Mackenzie, J. S. & Stanley, N. F. (1981) Infect. Immun. **32**, 277–286

Grundy (Chalmer), J. E., Trapman, J., Allan, J. E., Shellam, G. R. & Melief, C. J. M. (1982) Infect. Immun. **37**, 143–150

Grundy, J. E. & Shearer, G. M. (1984) Transplantation **37**, 484–490

Grundy, J. E. & Reid, M. F. (1985) Transpl. Proc. **17**, 592–594

Grundy, J. E., Lever, A. M. L., Milburn, H. J., Pratt, P. & Griffiths, P. D. (1986*a*) ISIR–TNO Interferon Symp. Proc., in the press

Grundy, J. E., McKeating, J. A., Ward, P. J., Sanderson, A. R. & Griffiths, P. D. (1986*b*) J. Gen. Virol., in the press

Grundy, J. E., Super, M. & Griffiths, P. D. (1986*c*) Lancet **i**, 159–160

Grundy, J. E., McKeating, J. A. & Griffiths, P. D. (1986*d*) J. Gen. Virol., in the press
Harnett, G. B. & Shellam, G. R. (1982) J. Gen Virol. **62**, 39–47
Henson, D. & Smith, R. D. (1964) Proc. Soc. Exp. Biol. Med. **197**, 517–520
Henson, D., Smith, R. D. & Gehrke, J. (1966) Am. J. Pathol. **49**, 871–888
Hirsch, M. S., Schooley, R. T. & Cosimi, A. B. (1983) New Engl. J. Med. **308**, 1489–1493
Ho, M. (1980) Infect. Immun. **27**, 767–776
Honess, R. W. (1984) J. Gen. Virol. **65**, 2077–2107
Howard, R. J., Miller, J. & Najarian, J. S. (1974) Clin. Exp. Immunol. **18**, 119–126
Howard, R. J. & Najarian, J. S. (1974) Clin. Exp. Immunol. **18**, 109–118
Huang, E.-S. (1975*a*) J. Virol. **16**, 298–304
Huang, E.-S. (1975*b*) J. Virol. **16**, 1560–1565
Huang, E.-S., Kilpatrick, B. A., Huang, Y.-T. & Pagano, J. S. (1976) Yale J. Biol. Med. **49**, 29–43
Hutt-Fletcher, L. M., Balachandran, N. & Haswell-Elkins, M. (1983) J. Exp. Med. **158**, 2171–2176
Irmiere, A. & Gibson, W. (1983) Virology **130**, 118–133
Irmiere, A. & Gibson, W. (1985) J. Virol. **56**, 277–283
Kane, R. C., Rousseau, W. E. & Noble, G. R. (1975) Infect. Immun. **11**, 719–723
Kangro, H. O., Griffiths, P. D., Huber, T. J. & Heath, R. B. (1982) J. Med. Virol. **10**, 203–212
Kantor, G. L., Goldberg, L. S., Johnson, B. L., Derechin, M. M. & Barnett, E. V. (1970) Ann. Intern. Med. **73**, 553–558
Keller, R., Peitchel, R., Goldman, J. & Goldman, M. (1976) J. Immunol. **116**, 772–777
Kelsey, D. K., Olsen, G. A., Overall, J. C. & Glasgow, L. A. (1977) Infect. Immun. **18**, 754–760
Kern, E. R., Olsen, G. A., Overall, J. C. & Glasgow, L. A. (1978) Antimicrob. Agents Chemother. **13**, 344–346
Kilpatrick, B. A., Huang, E.-S. & Pagano, J. S. (1976) J. Virol. **18**, 1095–1105
Kim, K. S., Sapienza, V. J., Carp, R. I. & Moon, H. M. (1976) J. Virol. **20**, 604–611
Landini, M. P., Mirolo, G., Baldassarri, B. & La Placa, M. (1985) J. Med. Virol. **17**, 303–311
Law, K. M., Wilton-Smith, P. & Farrar, G. H. (1985) J. Med. Virol. **17**, 255–266
Lee, G. D. & Keller, R. (1982) Infect. Immun. **35**, 5–12
Lehner, T., Wilton, J. M. A. & Shillitoe, E. J. (1975) Lancet **ii**, 60–62
Lindsley, M. D., Torpey, K. J. & Rinaldo, C. R. (1986) J. Immunol. **136**, 3045–3051
Loh, L. & Hudson, J. B. (1980) Infect. Immun. **27**, 54–60
Lopez, C., Simmons, R. L., Maver, S. M., Najarian, J. S., Good, R. A. & Gentry, S. (1974) Am. J. Med. **56**, 280–289
Macher, A. M., Reichert, C. M. & Strauss, S. E. (1983) New Engl. J. Med. **309**, 1454–1457
Mackowiak, P. A., Marling-Cason, M. & Luby, J. P. (1984) J. Clin. Invest. **73**, 987–991
McDonough, S. H., Staprans, S. I. & Spector, D. H. (1985) J. Virol. **53**, 711–718
McKeating, J. A., Griffiths, P. D. & Grundy, J. E. (1986*a*) J. Gen. Virol., in the press
McKeating, J. A., Grundy, J. E., Varghese, Z. & Griffiths, P. D. (1986*b*) J. Med. Virol. **18**, 341–348
Meyers, J. D., McGuffin, R. W., Bryson, Y. J., Cantell, K. & Thomas, E. D. (1982) J. Infect. Dis. **146**, 80–84
Michelson-Fiske, S., Horodniceanu, F. & Guillon, J. C. (1977) Nature (London) **271**, 615–617
Mirkovic, R. J., Werck, J., South, M. A. & Benyesh-Melnick, M. (1971) Infect. Immun. **3**, 45–50
Mocarski, E. S. & Roizman, B. (1982) Cell **31**, 89–97
Mocarski, E. S., Post, L. E. & Roizman, B. (1980) Cell **22**, 243–255
Mocarski, E. S., Pereira, L. & Michael, N. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 1266–1270
Mocarski, E. S. & Stinski, M. F. (1979) J. Virol. **31**, 781–775
Nowak, B., Sullivan, C., Sarnow, P., Bricout, F., Nicolas, K. C., Fleckenstein, B. & Levine, A. J. (1984*a*) Virology **132**, 325–338
Nowak, B., Gmeiner, A., Sarnow, P., Levine, A. J. & Fleckenstein, B. (1984*b*) Virology **134**, 91–102
Oie, H. K., Easton, J. M., Ablashi, D. V. & Baron, S. (1975) Infect. Immun. **12**, 1012–1017
Oram, J. D., Downing, R. G., Akrigg, A., Dollery, A. A., Duggleby, C. J., Wilkinson, G. W. G. & Greenaway, P. J. (1982) J. Gen. Virol. **59**, 111–129
Osborn, J. E. & Medearis, D. N. (1966) Proc. Soc. Exp. Biol. Med. **121**, 819–824
Osborn, J. E. & Medearis, D. N. (1967) Proc. Soc. Exp. Biol. Med. **124**, 347–353
Osborn, J. E., Blaskoree, A. A. & Walker, D. L. (1968) J. Immunol. **100**, 835–844
Para, M. F., Baucke, R. B. & Spear, P. G. (1982) J. Virol. **41**, 129–136
Pass, R. F., Griffiths, P. D. & August, A. M. (1983) J. Infect. Dis. **147**, 40–46
Patel, R., Fiala, M., Berne, V. & Chatterjee, N. (1978) New Zeal. Med. J. **87**, 393–394
Peckham, C. S., Chin, K. S., Coleman, J. C. & Preece, P. M. (1983) Lancet **i**, 1352–1355
Peden, K., Mounts, P. & Hayward, G. S. (1982) Cell **31**, 71–80
Pereira, R. A., James, D. C. O. & Stern, H. (1978) Br. Med. J. **2**, 126
Pereira, L., Hoffman, M., Gallo, D. & Cremer, N. (1982) Infect. Immun. **36**, 924–932
Pereira, L., Stagno, S., Hoffman, M. & Volanakis, J. E. (1983) Infect. Immun. **39**, 100–108
Pereira, L., Hoffman, M., Tatsuno, M. & Dondera, D. (1984) Virology **139**, 73–86
Plotkin, S. A., Smiley, M. L., Friedman, H. M. & Starr, S. E. (1984) Lancet **i**, 528–530
Postic, B. & Dowling, J. N. (1977) Antimicrob. Agents Chemother. **11**, 656–660
Quinnan, G. V. & Manischewitz, J. E. (1979) J. Exp. Med. **150**, 1549–1554
Quinnan, G. V., Manischewitz, J. E. & Ennis, F. A. (1978) Nature (London) **273**, 541–543
Quinnan, G. V., Kirmani, N. & Esker, E. (1981) J. Immunol. **126**, 2036–2041
Rahman, A. A., Teschner, M., Sethi, K. K. & Brandis, H. (1976). J. Immunol. **117**, 253–258
Rasmussen, L., Mullenax, J., Nelson, M. & Merigan, T. C. (1985) Virology **145**, 186–190
Reddehase, M. J. & Koszinowski, U. H. (1984) Nature (London) **312**, 369–371
Rice, G. P., Schrier, R. D. & Oldstone, M. B. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 6134–6138
Rinaldo, C. R., Carney, W. P., Richter, B. S., Black, P. H. & Hirsch, M. S. (1980) J. Infect. Dis. **141**, 488–495
Rodgers, B. C., Scott, D. M., Mundin, J. & Sissons, J. G. P. (1985) J. Virol. **54**, 527–532
Roizman, B. (1982) in The Herpesviruses (Roizman, B., ed.), vol. 1, pp. 1–23, Plenum Press, New York and London
Rook, A. H., Quinnan, G. V., Frederick, W. J. R., Manischewitz, J. F. & Kirmani, N. (1984) Am. J. Med. **76**, 385–392
Ruger, R., Bornkamm, G. W. & Fleckenstein, B. (1984) J. Virol. **65**, 1351–1364
Rundell, B. B. & Betts, R. F. (1980) J. Immunol. **124**, 337–342
Sakuma, S., Furukawa, T. & Plotkin, S. A. (1977) Proc. Soc. Exp. Biol. Med. **155**, 168–172
Sarov, I. & Abady, I. (1975) Virology **66**, 474–473
Sarov, B., Naggan, L., Rosenveig, R., Katz, S., Haikin, H. & Sarov, I. (1982) J. Med. Virol. **10**, 195–201
Schmitz, H., Muller-Lantzsch, N. & Peteler, G. (1980) Intervirology **13**, 154–161
Schrier, R. D., Nelson, J. A. & Oldstone, M. B. (1985) Science **230**, 1048–1051

Schrier, R. D., Rice, G. P. A. & Oldstone, M. B. (1986) J. Infect. Dis. **153**, 1084–1091
Selgrade, M. K., Ahmed, A., Sell, K. W., Gershwin, M. E. & Steinberg, A. D. (1976) J. Immunol. **116**, 1459–1463
Sethi, K. K. & Brandis, H. (1979) Arch. Virol. **60**, 227–238
Severi, B., Landini, M. P., Musiahi, M. & Zerbini, M. (1979) Microbiologica **2**, 265–273
Shanley, J. D. & Pesanti, E. L. (1980) Infect. Immun. **29**, 1152–1159
Shanley, J. D., Jordan, M. C. & Stevens, J. G. (1981) J. Infect. Dis. **143**, 231–237
Shellam, G. R., Allan, J. E., Padadimitriou, J. M. & Bancroft, G. J. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 5104–5108
Shellam, G. R., Grundy (Chalmer), J. E. & Allan, J. E. (1982) in Natural Cell Mediated Immunity (Herberman, R., ed.), vol. 2, pp. 1451–1458, Academic Press, New York
Shellam, G. R., Grundy (Chalmer), J. E., Allan, J. E. & Harnett, G. B. (1983) Prog. Immunol. **5**, 1209–1217
Shellam, G. R., Flexman, J. P., Farrell, H. E. & Papadimitriou, J. M. (1985) Scand. J. Immunol. **22**, 147–155
Shellam, G. R. & Flexman, J. P. (1986) J. Virol. **58**, 152–156
Simmons, R. L., Lopez, C., Balfour, M., Rattazzi, L. C. & Najarian, J. S. (1974) Ann. Surg. **180**, 623–628
Sinickas, V. B., Ashman, R. B. & Blanden, R. V. (1985) J. Gen. Virol. **66**, 747–755
Smith, J. D. & DeHarven, E. (1973) J. Virol. **12**, 919–930
Spaete, R. R. & Mocarski, E. S. (1985*a*) J. Virol. **54**, 817–824
Spaete, R. R. & Mocarski, E. S. (1985*b*) J. Virol. **56**, 135–143
Spector, D. H. & Vacquier, J. P. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 3889–3893
Spector, D. H., Lock, L. & Tamashiro, J. C. (1982) J. Virol. **42**, 558–582
Spector, S. A., Neuman, T. R. & Hirata, K. K. (1985) J. Infect. Dis. **152**, 755–759
Stagno, S., Reynolds, D. W., Huang, E.-S., Thames, S. D., Smith, R. J. & Alford, C. A. (1977) New Engl. J. Med. **296**, 1254–1258
Stagno, S., Reynolds, D. W., Pass, R. F. & Alford, C. A. (1980) New Engl. J. Med. **302**, 1073–1076
Stagno, S., Pass, R. F., Dworsky, M. E., Henderson, R. E., Moore, E. G., Walton, P. D. & Alford, C. A. (1982) New Engl. J. Med. **306**, 945–949
Stagno, S., Pass, R. F., Dworsky, M. E. & Alford, C. A. (1983) Semin. Perinatol. **7**, 31–42
Stalder, H. & Ehrensberger, A. (1980) J. Infect. Dis. **142**, 102–105
Starr, E. S. & Allison, A. (1977) Infect. Immun. **17**, 458–462
Starr, S. E. & Garrabrant, T. (1981) Clin. Exp. Immunol. **46**, 484–492
Starr, S. E., Dalton, B., Garrabrant, T., Paucker, K. & Plotkin, S. A. (1980) Infect. Immun. **30**, 17–22
Stenberg, R. M., Thomsen, D. R. & Stinski, M. F. (1984) J. Virol. **49**, 190–199
Stern, H. & Tucker, S. M. (1973) Br. Med. J. **2**, 268–270
Stinski, M. F. (1976) J. Virol. **19**, 594–609
Stinski, M. F. (1983) in The Herpesviruses (Roizman, B., ed.), vol. 2, pp. 67–133, Plenum Press, New York and London
Stinski, M. F. & Roehr, T. J. (1985) J. Virol. **55**, 431–441
Stinski, M. F., Mocarski, E. S. & Thomsen, D. R. (1979*a*) J. Virol. **31**, 231–239
Stinski, M. F., Mocarski, E. S., Thomsen, D. R. & Urbanowski, M. L. (1979*b*) J. Gen. Virol. **43**, 119–129
Stinski, M. F., Thomsen, D. R., Stenberg, R. M. & Goldstein, L. (1983) J. Virol. **46**, 1–14
Stringfellow, D. D., Kern, E. R., Kelsey, D. K. & Glasgow, L. A. (1977) J. Infect. Dis. **135**, 540–551
Talbot, P. & Almeida, J. D. (1977) J. Gen. Virol. **36**, 345–349
Tamura, T., Chiba, S., Chiba, Y. & Nakao, T. (1980) Infect. Immun. **29**, 842–845
Tarr, G. C., Armstrong, J. A. & Ho, M. (1978) Infect. Immun. **19**, 903–907
Thomsen, D. R., Sternberg, R. M., Goins, W. F. & Stinski, M. F. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 659–663
Vlazny, D. A. & Frenkel, N. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 742–746
Wadsworth, S., Jacob, R. J. & Roizman, B. (1975) J. Virol. **15**, 1487–1497
Wahren, B., Ljungman, P., Paulin, T. & Ringde, O. (1986) J. Virol. **58**, 909–913
Waner, J. L. & Nierenberg, J. A. (1985) J. Med. Virol. **16**, 233–244
Waner, J. L., Hopkins, D. R., Weller, T. H. & Allred, E. N. (1977) J. Infect. Dis. **136**, 805–809
Wathen, M. W. & Stinski, M. F. (1982) J. Virol. **41**, 462–477
Wathen, M. W., Thomsen, D. R. & Stinski, M. F. (1981) J. Virol. **38**, 446–459
Watson, J. G. (1983) J. Clin. Pathol. **36**, 683–692
Westmoreland, D., St Jeor, S. & Rapp, F. (1976) J. Immunol. **116**, 1566–1570
Westrate, M. W., Geelen, J. L. M. C. & Van der Noordaa, J. (1980) J. Gen. Virol. **49**, 1–21
Winston, D. J., Pollard, R. B. & Winston, G. (1982) Ann. Intern. Med. **97**, 11–18
Yamanishi, K. & Rapp, F. (1979) Virology **94**, 237–241
Zaia, J. A., Forman, S. J., Ting, Y., Vanderwal-Urbina, E. & Blume, K. G. (1986) J. Infect. Dis. **153**, 780–787