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Suppression of monocyte functions by human cytomegalovirus

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SUMMARY

The role of the monocyte in human cytomegalovirus (HCMV)-induced immunosuppression was examined by assessing the ability of the virus to directly suppress various monocyte accesory cell functions. Both patient-derived and laboratory-adapted strains of HCMV were capable of impairing antigen-presenting functions of purified human monocytes. In seven of 12 virus-infected samples, there was a significant decrease ($P < 0{\cdot}05$) in the ability of HCMV-infected monocytes to present tetanus toxoid to autologous lymphocytes compared with mock-infected controls; similar results were obtained with *Candida albicans* and mumps. In contrast, the response to PHA was impaired in only one of eight HCMV-infected samples. The increased expression of MHC class II Ia antigens (HLA-DQ and HLA-DR) by monocytes after stimulation by interferon-gamma was impaired in approximately one-third of the 43 virus-infected samples tested. Interleukin-1 (IL-1) production after incubation with the stimulating antigens, however, was unaffected. Attempts to augment immunosuppression by co-stimulation of monocytes with lipopolysaccharide (LPS), heat-killed *Escherichia coli* or *Listeria monocytogenes* were not successful; however, dramatically increased levels of immunsuppression was obtained with HCMV preparations containing mycoplasma. Thus, although HCMV is capable of directly perturbing monocyte accessory cell functions, the variability and partial suppression observed suggests that infection of monocytes by HCMV alone is not sufficient to produce the levels of immune hyporesponsiveness observed in HCMV-infected patients.

INTRODUCTION

Human cytomegalovirus (HCMV) is reported to cause transient immunosuppression during acute infection. Proliferative responses to viral antigens (Levin *et al.*, 1979) as well as non-specific mitogens (Carney & Hirsch, 1961; Rinaldo *et al.*, 1980; Quinnan *et al.* 1983) are reduced in patients with HCMV mononucleosis. After *in vitro* infection multiple immunological functions are impaired, despite the detection of HCMV in only a small proportion of lymphocytes and monocytes (Einhorn & Ost, 1984; Rice, Schrier & Oldstone, 1984; Dudding & Garnett, 1987). HCMV-infected peripheral blood mononuclear cells exhibit reduced T-cell proliferation in response to mitogens (Rice *et al.*, 1984; Schrier, Rice & Oldstone, 1986; Wahren *et al.*, 1986), impaired natural killer cell activity, and reduced cytotoxic lymphocyte responses (Schrier & Oldstone, 1986). While the mechanisms and cell types involved in these reactions have not been identified, an adherent cell has been implicated as being responsible for reduced T-cell proliferation (Carney & Hirsch, 1981; Dudding & Garnett, 1987). Since the monocyte is a pivotal cell in the induction of immune responses (Unanue, 1981), HCMV infection of monocytes which produced a reduction in antigen presentation function or a decrease in monokine production (Nathan, 1987) would contribute to a generalized immunological hyporesponsiveness. For these reasons, we examined the effect on several monocyte immunological functions of incubating HCMV with purified monocytes in order to determine the contribution of the monocyte to HCMV-induced immunosuppression.

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MATERIALS AND METHODS

Virus

The HCMV laboratory-adapted strains, AD-169, Towne and Davis were obtained from the ATCC (Rockville, MD). Low passage patient-derived strains of HCMV were isolated from bone marrow tranplant patients with an active HCMV infection. Such specimens were received from Dr S. Spector, UCSD Medical Center, expanded, and used between passages 4 and 10. All HCMV strains were grown in human foreskin fibroblasts.

Monocytes and peripheral blood mononuclear cells (PBMC) were infected with the following sources of HCMV: (i) virus-containing supernatants of infected fibroblasts harvested 2 days after 100% cytopathic effect (CPE); (ii) virus-containing pellets (43,000 ***g*** for 1 hr) of homogenized lysates of infected fibroblasts, initially clarified by low-speed centrifugation (650 ***g*** for 15 min); or (iii) virally infected, viable fibroblasts, used when

exhibiting 75–95% CPE. Cells were infected by incubating with 2×10^6 to 2×10^7 plaque-forming units (PFU)/10^7 cells of laboratory-adapted HCMV strains, 2×10^3 to 2×10^4 PFU/10^7 cells of patient HCMV strains, or 2×10^5 HCMV-infected fibroblasts/10^7 monocytes for the times indicated. Because most low-passage patient strains of HCMV produce about 1000-fold less virus (10^3–10^5 PFU/ml) than laboratory-adapted strains, attaining higher infecting doses with the patient-derived strains was not possible. Attempts to infect with greater numbers of fibroblasts proved toxic to the monocytes. Monocytes were also incubated with control inocula (mock virus) comparably prepared from uninfected fibroblasts. Tests for mycoplasma were performed by a commercial mycoplasma-testing service (Bionique Laboratories, Saranac Lake, NY) using the Hoescht stain and direct culturing techniques. Using these assays, mycoplasma was not detected in the HCMV strains or preparations of foreskin fibroblasts except where noted.

Monoclonal antibodies (mAb)

Anti-HLA-DQ and anti-HLA-DR were purchased from Becton-Dickinson (Mountain View, LA). AB-1 (anti-CR2) was a gift from Dr G. Nemerow, Research Institute of Scripps Clinic. MAbs F4-5C and F12-1G, directed against early HCMV proteins, and F2-8B, reactive with a late HCMV protein, were generated in this laboratory; mAb L-14-94 directed against an HCMV immediate early (IE) protein was a gift of Dr J. Nelson, Reseach Institute of Scripps Clinic.

Preparation and culture of cells

PBMC were prepared from normal donors by centrifugation over lymphopaque (Nyegaard and Co., Oslo, Norway). Human monocytes and lymphocytes were purified from PBMC by centrifugal elutriation employing endotoxin-free conditions (Esparza, Fox & Schrieber, 1986). The elutriated lymphocyte pool contained no myeloperoxidase-positive cells (Kaplow, 1965) (per 400 cells counted) while the monocyte pool contained greater than 90% myeloperoxidase-positive cells.

Monocytes and PBMC were cultured in teflon vials (Pierce Chemical, Rockford, IL) or in polypropylene tubes (Fisher Scientific, Pittsburgh, PA) at a density of $1\text{–}4 \times 10^6$/ml in endotoxin-free RPMI media supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate and either 15% heat-inactivated (HCMV-negative) autologous or AB-positive (HCMV-negative) serum. Interferon-gamma (IFN-γ)-containing supernatants were prepared by culturing PBMC at 5×10^6/ml with 5 μg/ml of concanavalin A (Con A) (Sigma, St Louis, MO) for 3 days in RPMI media plus 1% fetal calf serum. The supernatants, which contained 75–150 U/ml of IFN activity as assessed by their antiviral activity on WISH cells, were used at a 1/5 dilution.

Lymphocyte proliferation

PBMC were incubated with HCMV or mock virus for 1–7 days, after which 2×10^5 cells in 200 μl of RPMI containing 10% heat-inactivated autologous serum were incubated in flat-bottomed 96-well tissue culture plates (Costar, Cambridge, MA) with the experimentally determined optimal dose of tetanus toxoid (1·3LF/ml; Public Health Lab, Boston, MA) or phytohaemagglutinin (PHA-L) (1 μg/ml, Sigma). The cells were further incubated for 6 days, pulsed the final 18 hr with 1 μC/ml of [^{3}H]thymidine (Amersham, Arlington Heights, IL), and harvested. The mean of quadruplicate wells for each sample was determined and the significance of variation between the means of the mock-infected and HCMV-infected cultures was determined using the Student's *t*-test. A value of $P < 0{\cdot}05$ was considered to be significant. Only samples with a positive response to the respective antigens were included in the analysis.

Antigen presentation

Purified monocytes were incubated with HCMV for from 1 to 7 days, washed three times, irradiated (2000 rads) and 2×10^4 viable monocytes were added to 2×10^5 freshly obtained purified autologous lymphocytes. The optimal dose of tetanus toxoid, PHA, *Candida albicans* (1·5 μl/ml, Hollister-Stier, Elkhart, IN) or mumps viral and soluble antigen (1·5 μl/ml MA Bioproducts, Walkerville, MD) was added to each well and incubation continued for 6 days. Incorporation of [^{3}H]thymidine and data analysis were as described above.

Immunofluorescence

Purified monocytes were incubated with HCMV or mock virus for 1–7 days after which they were stimulated for 2 days with IFN-γ-containing supernatants. After washing, the cells were incubated with anti-HLA-DQ or HLA-DR mAbs for 30 min on ice, washed twice and then incubated with FITC-labelled anti-mouse IgG (H+L) (KPL, Gaithersburg, MD) for an additional 30 min. The cells were again washed twice and resuspended in 1% paraformaldehyde in phosphate-buffered saline, pH 7·4 (PBS). Ten thousand cells were analysed by flow cytometry using a FACS IV (Becton-Dickinson). Dead cells were excluded from analysis by the criteria of either forward and side scatter or the exclusion of propidium iodide (Jacobs & Pipho, 1983). The level of background staining for the positioning of the flow cytometry marker was determined with cells incubated with antibody to CR2 which is not present on monocytes.

In order to detect the presence of HCMV antigens, cytocentrifuge smears of HCMV-infected monocytes were air-dried and acetone-fixed for 10 min at −20°. Non-specific staining was blocked by incubating with 2% normal rabbit serum in PBS for 30 min at room temperature. The cells were then incubated with the various mAbs to HCMV for 30 min, washed and then incubated with FITC-labelled anti-murine IgG (H+L). The entire smear was examined for positive cells using a Nikon fluorescent microscope.

Production of IL-1

Monocytes were infected with HCMV or mock virus for 1 day and then further incubated with tetanus toxoid, *Candida* or mumps antigens at concentrations used for lymphocyte proliferation for 2 or 6 days. The supernatants were harvested and assayed for IL-1 activity by the C3H/HEJ murine thymocyte assay (Tiku *et al.*, 1986). The mean value for each sample was determined from triplicate wells.

RESULTS

Lymphocyte proliferation

PBMC incubated with HCMV for 1–7 days were significantly impaired ($P < 0{\cdot}05$) in their ability to respond to tetanus toxoid in 12 of 17 HCMV-infected samples included in seven experiments. HCMV-infected PBMC also exhibited a reduced mitogenic response to PHA in five of 21 infected samples tested in seven

Table 1. Effect of HCMV on lymphocyte proliferation

Inoculum	Medium	Tetanus toxoid	PHA
(a) Free virus*			
Mock virus†	4338	81,366	200,316
HCMV-L	8210	5141‡	87,512‡
AD-169	4555	9899‡	153,891
(b) Cell associated virus*			
Mock virus†	2620	87,761	121,681
HCMV-L	2908	9130‡	63,372‡
HCMV-P	5776	10,216‡	57,733‡
AD-169	1768	2213‡	134,117

* PBMCs were preincubated for 7 days with mock virus or HCMV strains followed by 6 days with antigen or phytohemagglutinin (PHA). Values are expressed as the mean c.p.m. of four values.

† Uninfected fibroblasts.

‡ $P < 0.05$, compared to mock virus using Student's t-test.

experiments. In each case, values were compared to identical samples treated with mock virus. Table 1 presents the data from one experiment in which PBMC were preincubated for 7 days with either concentrated free virus (a) or infected fibroblasts (b) before stimulation with antigen. The response to tetanus toxoid was reduced in PBMC infected with AD-169 as well as two low-passage patient strains (HCMV-L and HCMV-P). The response to PHA was significantly reduced in three of the five virus-infected samples. Infecting with cell-associated virus produced comparable levels of reduction to that obtained with free virus. A summary of the results from seven experiments produced no obvious correlation between the ability to reduce PBMC proliferative responses and the strain of HCMV used, infection with free or cell-associated virus or time of incubation with virus.

Antigen presentation

Monocytes incubated with HCMV for 1–7 days exhibited reduced ability to present antigen to autologous lymphocytes. The response to tetanus toxoid was reduced in 11 of 12 HCMV-infected samples included in five experiments; of these seven were statistically significant ($P < 0.05$). Similar results were obtained when the response to *Candida albicans* and mumps virus was assessed. In each case, values were compared to identical samples treated with mock virus. In only one of eight HCMV-infected monocyte samples was there a significant ($P < 0.05$) reduction in responsiveness to PHA. Table 2 presents the results of two separate experiments in which monocytes were pretreated with either free virus for 6 days (a) or with fibroblast-associated virus for 4 days (b) before addition, together with antigen, to autologous lymphocytes. In Table 2, the response was significantly reduced ($P < 0.05$) in five of the seven HCMV-infected monocyte samples examined for presentation of tetanus toxoid or *Candida albicans*. The observed response to these antigens was monocyte dependent since negligible proliferation was observed in samples lacking monocytes. In contrast, HCMV infection of monocytes had no effect on the response to PHA. This may reflect the lack of dependence on the addition of monocytes to generate a PHA response in these experiments.

Table 2. Effect of human cytomegalovirus HCMV on antigen presentation by monocytes to autologous lymphocytes

Inoculum	Medium	Tetanus toxoid	Candida	PHA
Exp. (a) Free virus*				
No monocytes	433	1131	1086	26,766
Mock virus†	2421	20,740	24,690	30,021
AD-169	507	13,414	11,304‡	26,203
HCMV-P	3567	11,124‡	14,043	23,070
Exp. (b) Cell-associated virus*				
No monocytes	329	457	NT§	284,353
Mock virus†	467	27,958	NT	187,073
AD-169	294	1099‡	NT	175,622
HCMV-L	1405	6489‡	NT	169,330
HCMV-P	952	5171‡	NT	187,105

* Monocytes were preincubated with mock virus or HCMV six (Exp. a) or four (Exp. b) days before the addition to autologous lymphocytes together with antigen or PHA for an additional 6 days. Values are expressed as the mean c.p.m. of four samples.

† Uninfected fibroblast.

‡ $P < 0.05$, compared to mock virus using Student's t-test.

§ Not tested.

The cumulative results from five experiments which included 12 HCMV-infected samples compared to mock virus controls suggest that preincubation of the monocytes with HCMV for longer than 4 days is associated with a greater reduction in their ability to stimulate lymphocytes. The ability to suppress monocyte antigen presentation did not correlate with the strain of HCMV used or the type of virus inoculum.

MHC class II antigen (Ia) expression

The ability of monocytes to function as antigen-presenting cells depends on their ability to present antigen to histocompatible lymphocytes in association with MHC II molecules which are up-regulated by IFN-γ during an immune response and their capacity to produce IL-1. Both of these functions were analysed individually in HCMV-infected monocytes. The ability of monocytes to respond to IFN-γ by increased MHC II expression was assessed after HCMV infection of purified monocytes. Cells were stained for HLA-DQ and HLA-DR expression and examined by flow cytometry. Cells stained for CR2, which is absent on monocytes, were used to position the marker for the determination of positive cells. Since the background level of monocyte HLA-DQ expression is low (5–15% positive cells), the quantification of IFN-γ-induced HLA-DQ was expressed as the increase in percentage of HLA-DQ-positive cells. Background levels of monocyte HLA-DR expression are higher (85–95%) and therefore the IFN-γ-stimulated increase in HLA-DR staining was expressed in mean channel fluorescence units (FL-1), which is a measurement of fluorescence intensity.

Fifteen independent experiments, which included more than 40 samples of monocytes infected with various strains of HCMV for varying periods of time, are presented in summary form in Table 3. The expression of HLA-DQ increased by 21 percentage points in mock virus-infected monocytes stimulated with IFN-γ, while HLA-DR expression increased by an average of 99 mean

Table 3. Effect of human cytomegalovirus HCMV on IFN-γ-stimulated monocyte Ia expression

HLA-DQ*	No change	10–20%	21–30%	31–40%	>40%
No. HCMV-infected samples	16	15	7	6	2
HLA-DR†	<20	20–40%	41–60%	60–100%	>100
No. HCMV-infected samples	17	11	1	7	5

* Expressed as the reduction in percentage of HLA-DQ-positive cells observed with HCMV-infected monocytes compared with mock-infected monocytes 2 days after stimulation with IFN-γ.

† Expressed as the reduction in the mean FL1 observed with HCMV-infected monocytes compared to mock-infected monocytes 2 days after stimulation with IFN-γ.

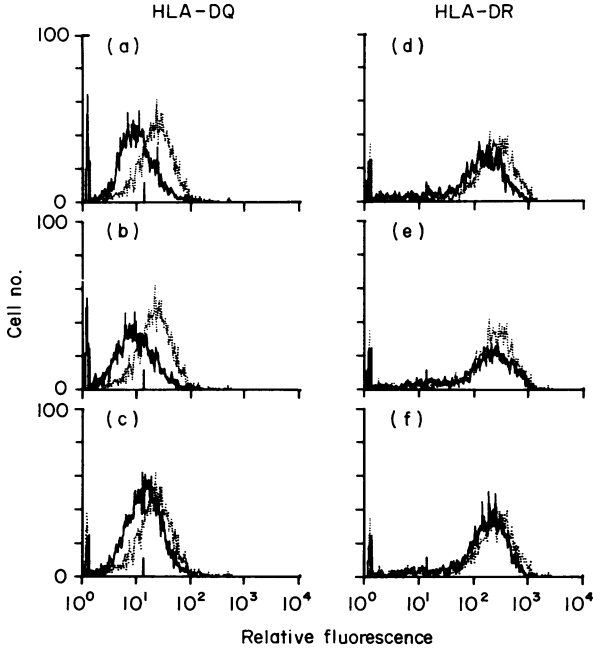

Figure 1. Effect of human cytomegalovirus on MHC class II antigen expression. Monocytes were cultured for 7 days with HCMV, stimulated for 2 additional days with IFN-γ, stained and examined by flow cytometry. (a) and (d) AD169, (b) and (e) HCMV-P, (c) and (f) HCMV-L. Dotted line, mock virus; solid line, HCMV.

fluorescence units. Incubation with HCMV reduced HLA-DQ expression in response to IFN-γ by more than 20 percentage points in 15 of 46 virus-infected samples, while no change or a decrease of less than 20% was observed in 31 of 46 virus-infected samples. HCMV reduced HLA-DR expression in response to IFN-γ by more than 40 FL-1 units in 13 of 41 virus-infected samples, while little or no effect was observed in 28 of 41 virus-infected samples. The FACS profile of one experiment in which moderate changes were observed is illustrated in Fig. 1. In this experiment, 73% of the mock-infected monocytes were positive for HLA-DQ after 2 days stimulation with IFN-γ. This was

Table 4. IL-1 production by HCMV-infected monocytes

		Monocytes incubated with*		
Inoculum	Medium	Tetanus toxoid	Candida	Mumps
Medium	27,350	19,005	25,356	29,041
Mock virus†	17,952	16,908	22,737	13,593
HCMV-P	29,262	30,979	25,982	30,624
Davis	22,622	28,723	25,133	20,583
rIL-1 (0·83 U/ml)	21,134			
Thymocytes	887			
Thymocytes-PHA	5040			

* Monocytes were incubated for 1 day with medium, mock virus or HCMV followed by 2 days wtih antigen to assess their continued level of IL-1 production after HCMV infection. The IL-1 activity of a 1/4 dilution of monocyte supernatant was measured using the mouse thymocyte assay. Values are mean c.p.m., determined from triplicate samples.

† Uninfected fibroblast lysate.

reduced to 30% for monocytes infected for 7 days with either AD-169 (a) or HCMV-P (b), and to 50% for monocytes infected with HCMV-L (c). In the same experiment, over 90% of the cells expressed HLA-DR after IFN-γ treatment and exhibited an FL-1 value of 289. AD169, HCMV-P and HCMV-L infection reduced HLA-DR FL1 values of IFN-γ-stimulated monocytes to 108, 210 and 252 FL-1 units, respectively.

In general, there was a greater reduction in the ability to express Ia antigens when infected cells were used as the inoculum as opposed to HCMV-containing supernatants or virus concentrated by high-speed centrifugation. Increasing the incubation period with virus to 7 days prior to the addition of IFN-γ also tended to elicit greater reductions in MHC class II expression. There was no correlation between the behaviour of the three laboratory-adapted and two patient-derived HCMV strains used. In most cases a decrease in HLA-DQ expression was associated with a reduction in HLA-DR expression.

IL-1 production

The continued production of IL-1 by monocytes after incubation with the antigens used to assay for antigen presentation was unaffected by HCMV infection. There was no decrease in IL-1 activity in supernates of HCMV-infected monocytes incubated for 2 days with the antigens tetanus toxoid, *Candida* or mumps virus when tested at a 1/4 dilution (Table 4). Reductions in IL-1 activity were also not observed at dilutions of 1/2 or 1/32 or in samples incubated for 6 days (not shown).

HCMV infection

We were unable to detect HCMV antigens in monocytes infected wtih laboratory or patient-derived strains for 1–7 days by indirect immunofluorescence of the infected monocytes using mAb directed against IE, early and late HCMC antigens. No definitive staining of 10^5 infected monocytes with any of the anti-HCMV antibodies was observed in cytocentrifuged preparations. In contrast, fibroblasts infected with similar amounts

Table 5. Effect of HCMV contaminated with mycoplasma on monocyte functions

	Antigen presentation*		IL-1† production	HLA-DQ‡ expression
	Tetanus toxoid	PHA		
Mock virus§	139,917	108,314	28,951	75·1%
HCMV-D (mycoplasma contaminated)	2809	1030	1095	10·7%

Monocytes were infected for 1 day with HCMV supernatants containing mycoplasma and their functions analysed as previously described.

* Expressed as the mean c.p.m. of four samples.

† Mean c.p.m. determined from triplicate samples in mouse thymocyte assay.

‡ Expressed as the percentage of monocytes expressing HLA-DQ, 2 days after stimulation with IFN-γ.

§ Uninfected fibroblast lysate.

of the patient HCMV strains exhibited numerous foci of infection as detected by mAb to IE and early antigens by 3 days after infection, while fibroblasts infected with AD-169 displayed 90% staining within 24 hr after infection.

Effect of mycoplasma contamination

When HCMV strains contaminated with mycoplasma were used to infect monocytes, there was nearly a complete loss of antigen-presenting functions. Experiments with four patient strains and one laboratory-adapted strain of HCMV contaminated with mycoplasma showed consistent elimination of HLA-DQ expression in response to IFN-γ. A loss in the ability to produce IL-1 activity and an almost complete inability to stimulate lymphocytes were also observed (Table 5). All five of these strains were found to contain mycoplasma by Hoescht stain. Five additional mycoplasma-free strains of HCMV were obtained and were repeatedly tested to assure that they remained mycoplasma-free. These five mycoplasma-free HCMV strains which were used in the studies described earlier, never produced the dramatic changes in monocyte functions observed with the mycoplasma containing virus preparations. Attempts to mimic the effect of the mycoplasma by the addition of lipopolysaccharide (0·1 ng/ml to 10 ng/ml) (*Salmonella typhosa*, Sigma L-6386), heat-killed (56°, 45 min) *E. coli* (5×10^5–5×10^7 particles/ml) or heat-killed *Listeria monocytogenes* (5×10^5–5×10^7 particles/ml) to the mycoplasma free HCMV strains proved unsuccessful (not shown).

DISCUSSION

We have examined the role of human monocytes in HCMV-induced immunosuppression. Our experiments demonstrate that incubation of purified human monocytes with HCMV reduced in a variable manner their ability to present antigen to autologous lymphocytes. Seven of 12 monocyte samples incubated with HCMV were impaired ($P > 0.05$) in ability to present tetanus toxoid, and similar results were obtained with *Candida albicans* and mumps. Individual components of the antigen-presentation function were examined in order to identify specific defects in the capacity of the monocyte to respond to immunological stimuli. Moderate reductions in the expression of MHC II after IFN-γ stimulation were noted in approximately one-third of the infected samples. There was no detectable change in IL-1 activity of HCMV-incubated monocyte supernatants after incubation with stimulating antigens. The replication of HCMV is relatively slow with maximum amounts of virus produced from 72 to 96 hr after infection of fibroblasts (Ruebner *et al.*, 1965). The production of infectious particles is also inefficient with virus spreading more readily by cell to cell contact. The growth of newly isolated patient strains of HCMV is less efficient than laboratory-adapted strains with virus particles being produced from 5 to 7 days after infection and at very low levels (10^3–10^5 PFU/ml). Attempts to enhance the infection of monocytes included concentrating the virus 100-fold by ultracentrifugation, infecting with HCMV-infected fibroblasts to allow for cell to cell virus spread, and incubating monocytes for up to 7 days with virus before assaying for individual monocyte functions. We were unable to define a combination of experimental conditions which gave both consistent and significant reductions in either antigen presentation by monocytes or MHC II expression. Differences in infecting virus titre is not responsible for the variation between experiments since the infectious titre on human foreskin fibroblasts among the laboratory-adapted strains and among the patient-derived strains was similar.

We were unable to obtain evidence of productive infection of monocytes by HCMV using immunofluorescence to detect immediate early, early or late HCMV proteins and yet some experiments demonstrated a sizable proportion of monocytes with reduced MHC class II expression (up to 70%). Other investigators have reported only a small minority of infected monocytes after *in vitro* infection: 4–13% (Einhorn & Ost, 1984) and 10% (Rice *et al.*, 1984). This suggests that the effect on the monocyte may be mediated by only a few infected cells. The production of immunosuppressive factors which depress Con A responses have been described after incubation of macrophages with other viruses such as avian retrovirus (Isreal, Beiss & Wainberg, 1980).

HCMV cultures contaminated with mycoplasma produced an almost complete abolition of monocyte antigen presentation, MHC II expression and IL-1 activity. The suppressive effect of mycoplasma on lymphocyte proliferation has been known for some time. Early studies by Copperman & Morton (1966) and Barile & Levanthal (1968) have described the inhibition of lymphocyte mitogenic responses when incubated in the presence of mycoplasma. In another report, five of 10 species of mycoplasma were found to inhibit human lymphocyte blastogenic responses to PHA and antigens (Simberkoff, Thorbecke & Thomas, 1969). Our studies suggest that mycoplasma may also interfere with monocyte functions such as increased MHC II expression after IFN-γ stimulation, in the absence of lymphocytes. Our experiments emphasize the importance of continually monitoring the purity of viral cultures for mycoplasma, especially in studies involving immunological responsiveness. The pervasiveness of mycoplasma in HCMV viral stocks is suggested in a study by Darai *et al.* (1981) where mycoplasma was detected in 23 out of 30 HCMV stocks obtained from laboratories in five countries. Recently mycoplasma has been reported to be responsible for the suppression in IL-1 activity which had

been previously attributed to HCMV (Scott & Sissons, 1987).

The variability of results using the same virus samples to infect multiple monocyte preparations supports the conclusions that direct infection of monocytes by HCMV is not the sole cause of HCMV-induced immunosuppression. There may be multiple reasons for the range of results observed. Although the monocytes were obtained from normal, healthy HCMV antibody-negative donors taking no medication, a range of responses can be expected using human cells. This is especially true if other factors such as subacute infections and the immune status of the donor influence the interaction of HCMV with PBMC. The variable results of our *in vitro* infection experiments reflect a similar variability in published studies performed with PBMC samples after *in vivo* infection. Among patients with HCMV mononucleosis, PHA responsiveness was depressed in patients with transfusion-acquired disease but normal in patients with community-acquired disease (Rinaldo *et al.*, 1980). A range of hyporesponsiveness to Con A has been reported for acute HCMV mononucleosis patients (Carney & Hirsch, 1981). This suggests that immunosuppressive co-factors such as transfusion (Shenton *et al.*, 1979), surgery (Park *et al.*, 1971), chemotherapy and infection with other agents may contribute to the immunosuppression observed in HCMV-infected patients. In conclusion, these studies indicate that incubation of purified monocytes with HCMV is capable of partially reducing several monocyte accessory cell functions but may not be sufficient to account for the immune hyporesponsiveness reported in HCMV-infected patients.

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