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Low-dose human cytomegalovirus infection of human fibroblast cultures induces lymphokine-activated killer cell resistance: interferon-β-mediated target cell protection does not correlate with up-regulation of HLA class I surface molecules

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SUMMARY

We have investigated the susceptibility of human foreskin fibroblast (HFF) monolayers infected at low level with human cytomegalovirus (HCMV) strain AD 169, or a clinical HCMV isolate, to lysis mediated by interleukin-2 (IL-2)-activated killer cells (LAK). HFF cultures inoculated with a multiplicity of infection (MOI) dose of 0·001–0·01 had significantly decreased susceptibility to lysis by IL-2-activated non-adherent blood cells (PNAC) or purified $CD56^+$ cells in comparison to mock-infected cultures. By 12 h post incubation HFF cultures showed diminished susceptibility to LAK cell cytotoxicity when HFF cultures were incubated with HCMV (MOI 0·01) or interferon-β (IFN-β; 100 U/ml). Cytofluorometric analysis of HCMV-infected HFF cultures showed a modulation of HLA class I expression on single cells 3 days post-infection; namely, segregation of the cells in low- and high-density HLA class I-expressing cells depended on the dose of HCMV inoculum. However, up-regulation of HLA class I surface molecules was not significantly enhanced 12 h post-incubation with HCMV inoculum or IFN-β. Anti-IFN-β antibodies prevented both the development of the resistance and the increase of HLA class I expression in infected HFF cultures. In summary, the comparison of HLA class I expression and the LAK susceptibility of HCMV-infected HFF cultures may lead to the following conclusions: IFN-β mediates the protection of neighbouring uninfected fibroblasts, but the modulation of HLA class I expression on uninfected cells does not correlate with the diminished susceptibility.

INTRODUCTION

Human cytomegalovirus (HCMV), a double-stranded DNA virus belonging to the group of β1 herpes viruses, is an important pathogen in the immunocompromised host or the immunologically immature fetus. In immunocompetent humans this virus rarely causes symptomatic disease. However, in immunocompromised patients such as transplant recipients or acquired immune deficiency syndrome (AIDS) patients it causes pneumonitis, gastrointestinal disease, retinitis, encephalitis and disseminated HCMV infection. As with the other herpes viruses, HCMV establishes a life-long persistence after primary infection and has the ability to cause recurrent infection after reactivation (reviewed in ref. 1). HCMV as well as other herpes viruses plays its role as an immune 'escape artist', using different strategies for immune evasion.[2] HCMV establishes persistence in monocytes[3] and is able to infect primary differentiated macrophages.[4] Furthermore, HCMV induces immunoglobulin (Ig) Fc receptor synthesis[5] and virus-infected cells can bind β_2-microglobulin.[6] The UL 18 gene of HCMV encodes for a putative glycoprotein that shares 20% homology with the major histocompatibility complex (MHC) class I α chain,[7] which may bind to β_2-microglobulin[8] and facilitate viral attachment and penetration. Another sequence homology exists between the HCMV immediate early (IE) region 2 and the HLA-DR β chain,[9] which might contribute to graft rejection after transplantation. All these immune evasion mechanisms of CMV-infected cells also strongly influence cell-mediated immunity.

Recently Biron *et al.*[10] reported about an adolescent patient with a complete lack of natural killer (NK) cells and without inducible NK activity. This patient suffered from severe herpes virus infections (HSV), though was able to mount a normal cytotoxic T lymphocyte (CTL)-mediated proliferative response to HSV. *In vivo*, NK cells seem to exert their protective cytotoxicity against virus-infected cells early, and may provide interim protection until a specific CTL response arises. NK cells

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have been defined as $CD3^-$, T-cell receptor (TcR) $\alpha\beta^-$ and $\gamma\delta^-$, $CD56^+$ large granular lymphocytes (LGL) that mediate MHC unrestricted cytotoxicity against certain sensitive tumours and virus-infected cells.[11] Lymphokine-activated killer (LAK) cells are at the progenitor and effector cell stage NK cells that, upon activation with interleukin-2 (IL-2), also exhibit cytotoxicity against NK-resistant target cells.[12,13]

An inverse correlation between the level of MHC class I antigen expression of target cells and their susceptibility to NK/LAK cytotoxicity has been found.[11] Transfected class I HLA genes protected target cells from NK cytotoxicity but not from LAK-mediated cytotoxicity.[14] One strategy viruses use to evade immune surveillance may be down-regulation of class I MHC expression on infected cells.[15] Recently a HCMV-induced down-regulation of the surface expression of class I MHC proteins on infected cells has been reported.[16–18]

In this study we analysed the susceptibility of human HCMV-infected foreskin fibroblasts (HFF) to lysis mediated by IL-2-activated effector cells from healthy blood donors. In contrast to previous studies, HFF monolayers were infected with low multiplicities of infection (MOI 0·001–0·1). In order to investigate the influence of HLA class I expression of target cells on susceptibility to LAK-cell mediated lysis, we analysed the HLA class I expression of infected HFF cultures by flow cytometry.

MATERIALS AND METHODS

Cell cultures

Primary cultures of HFF cells grown out of trypsin-digested skin biopsy material after circumcision (Department of Pediatric Surgery, University of Tübingen, Tübingen, Germany) were cultivated in Hanks' minimal essential medium (MEM; Gibco BRL, Eggenstein, Germany) supplemented with 10% fetal calf serum (FCS) (Gibco BRL), 0·1 mg/ml streptomycin (Grünenthal, Stolberg, Germany) and 100 U/ml penicillin (Grünenthal). Fibroblast cells and virus stock solutions used in this study were mycoplasma free (Mycoplasma detection kit; Boehringer Mannheim, Mannheim, Germany). HFF cells were derived from passage levels 10–20.

Virus strains and virus titration

The HCMV strain AD 169 (ATCC) and a clinical isolate of HCMV from a patient after bone marrow transplantation were propagated on HFF cells. Virus stock solutions were made of cell-associated virus by sonication of trypsinized fibroblast monolayers exhibiting up to 90% cytopathic effect (CPE). After centrifugation, aliquots of virus-containing supernatants were kept at −70° until use. Mock solution was prepared analogously using uninfected HFF cultures. Eight replicates of each dilution were assayed 24 hr post-infection. The minimum virus dose which caused brownish stained nuclei by immunoperoxidase staining of the 72,000 MW major immediate early antigen (IEA) in 50% of the replicates, the 50% tissue culture infective dose ($TCID_{50}$), was calculated by the Speerman–Kärber method.[19] This method compares favourably with the classical $TCID_{50}$ assay.[16]

CMV infection of fibroblasts

HFF cells were seeded at $2–4 \times 10^4$ cells/well in MEM–10% FCS in 96-well plates (Nunc, Roskilde, Denmark) and grown 48–96 hr to confluence. Culture medium was removed and 50 μl of the appropriate dilution of HCMV strain AD 169 ($10^{6·1}$ $TCID_{50}$/ml) or the patient virus isolate ($10^{6·2}$ $TCID_{50}$/ml) was added, yielding MOI from 0·001 to 0·1. Controls included mock-infected HFF cultures and cultures incubated with heat-inactivated virus preparations (56°, 30 min). After adsorption of virus for 1 hr at 37°, the inoculum was removed and 200 μl medium containing 3% FCS alone or with anti-interferon-β (IFN-β) (400 U/ml; Boehringer Mannheim) added. HFF cultures were also incubated with different dilutions of human IFN-β (huIFN-β; ICN Biochemical, High Wycombe, U.K.).

Immunoperoxidase staining of HCMV–IEA

The fibroblast monolayers were fixed with methanol (−20°) for 10 min. After washing, 100 μl of primary monoclonal antibody (mAb) anti-IEA (mAb E13; Paesel, Frankfurt, Germany diluted 1/1000) was added followed by incubation at 37° for 60 min. After repeated washing steps, 100 μl of 1/1000 diluted secondary antibody (rabbit anti-mouse IgG conjugated to horseradish peroxidase; Dako, Hamburg, Germany) was added, followed by incubation at 37° for 60 min. Thereafter, the substrate solution [(1 mM 3-amino-9-ethylcarbazol (AEC), 0·1% (v/v) hydrogen peroxide (H_2O_2)] was added. Brownish-stained nuclei in fibroblast monolayers scanned with an inverted microscope indicated HCMV infection.

IFN-β assay

The quantity of huIFN-β was measured with an immunoenzymetric assay (IFN-β–EASIA; Medgenix Diagnostics, Fleurus, Belgium). Briefly, samples and standard dilutions were incubated overnight together with horseradish peroxidase (HRP)-labelled anti-IFN-β mAb. The substrate solution (tetramethylbenzidine; TMB) was added and colour development was stopped with H_2SO_4 after 30 min of incubation. The IFN-β assay was standardized against an international reference of huIFN-β.

Preparation of peripheral blood mononuclear cells (PBMC) and their subpopulations

Human PBMC were prepared from heparinized blood of healthy volunteers using the method described by Bøyum.[20] Monocytes were depleted from PBMC by plastic adherence followed by a passage through a nylon wool column resulting in a population of peripheral non-adherent cells (PNAC). $CD56^+$ cells were isolated from PNAC by indirect immunomagnetic cell separation using Dynabeads M-450 (sheep anti-mouse IgG; Dynal, Hamburg, Germany) following the outlines given by the manufacturer. Culturing of PBMC, PNAC or $CD56^+$ was performed in RPMI-1640 supplemented with 10% FCS, L-glutamine (200 mmol), α-thioglycerol (5×10^{-5} mol), penicillin (100 U/ml) and streptomycin (0·1 mg/ml). For induction of LAK activity effector cell populations were incubated with IL-2 (50 U/ml; Cetus Corp., Emeryville, CA) for 2 days. Phenotype analysis of the cell samples was performed by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA).

Colorimetric cytotoxicity assay

A modified lactic acid dehydrogenase (LDH) release assay of natural cytotoxicity,[21] originally described by Korzeniewski and Callewaert,[22] was used as short-term (4 hr) colorimetric cytotoxicity assay. According to these authors[22] and our own

findings, the results of LDH release assay are comparable to those of the classical ^{51}Cr-cytotoxicity assay. Briefly, adherent HFF target cells (2×10^4/well) were cultivated in 96-well microtitre plates at 37° with 5% CO_2 in a humidified incubator for 48–72 hr. Subsequently, monolayers were HCMV- or mock-infected or incubated with IFN-β. At the end of the incubation time culture supernatants were removed and the monolayers were washed once with 100 μl RPMI-1640 without phenol red, supplemented with 2% bovine serum albumin (BSA). In a total volume of 200 μl, the adherent fibroblasts were co-cultured with the effector cells. Furthermore, target cells and effector cells were also cultured separately. At the end of the cytotoxicity assay, aliquots of cell-free supernatants (100 μl) were transferred into corresponding wells of flat-bottomed microtitre plates. The red-coloured formazan reaction product was monitored in an ELISA reader at 490 nm with a reference wavelength of 630 nm. Under these conditions, the spontaneous LDH release of both effector and target cells was less than 5% of total cell lysis. The percentage of specific cytotoxicity (C) was calculated using the formula:

$$\%C = \frac{E - S_{EC+TC}}{M - S_{TC}} \times 100.$$

E, experimental LDH release resulting from co-culture of effector cells with target cells; S, spontaneous LDH release of separate cultures of effector (EC) and target (TC) cells; M, maximum LDH release of target cells achieved by addition of 100 μl 0·1% Triton X-100.

Cell preparation, fixation and staining for flow cytometry

For staining of HLA class I surface molecules, the mAb B9.12.1 was used, which is directed against a monomorphic determinant of human class I HLA molecules (HLA-ABC) associated with β_2-microglobulin (diluted 1/20; Dianova, Hamburg, Germany). Briefly, adherent HFF were trypsinized (saline/0·05% trypsin/0·025% EDTA), washed in phosphate-buffered saline (PBS)/2% FCS/0·01% NaN_3 (washing buffer) and fixed with paraformaldehyde (PFA; 1% w/v in PBS) for 15 min on ice. Fixed cells (1×10^5/100 μl) were incubated for 30 min with 100 μl of the primary antibodies in 96-well V-bottomed plates at room temperature. Subsequently the cells were washed three times and resuspended with 100 μl goat anti-mouse (gam)–FITC. After 30 min cells were washed again three times.

Flow cytometry

Samples were analysed using a laser cytofluorometer (FACScan; Becton Dickinson). The data were stored in list mode fashion and data analysis was performed with Consort 30. The stored data were displayed as a two-parameter dot-plot using forward scatter versus side scatter. Gates were established to exclude cellular debris from analysis. Gated cells were analysed with histograms for single fluorescence (mean fluorescence channel; MFC).

RESULTS

Low-dose HCMV-infected HFF cultures develop resistance to lysis by LAK cells

In this study HFF cultures were HCMV infected with MOI from 0·001 to 0·1. These low infection doses, particularly MOI of 0·01, mimics as far as possible the *in vivo* situation. Under these conditions we analysed the susceptibility of HCMV-infected HFF cultures against lysis mediated by LAK cells.

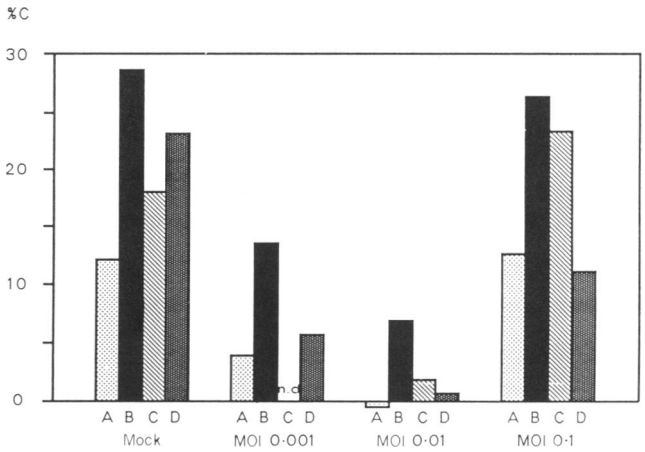

Figure 1. LAK cell-mediated lysis of HFF monolayers mock-infected or infected with different MOI (0·001–0·1) of HCMV strain AD 169 (A–C) and a clinical isolate (D) (3 days post-infection) by PNAC ($8·0 \times 10^5$/well) from four different donors (A–D) preincubated with IL-2 (2 days, 50 U/ml). Cytotoxicity was tested in a 4-hr LDH release assay.

Figure 1 depicts the cytotoxicity of IL-2-activated non-adherent blood cells (LAK) from four different donors (A–D) against HFF cultures infected with HCMV strain AD 169 (A–C) and a clinical isolate (D) 3 days post-infection. An infection dose of 0·001 MOI yielded less than 0·5% infected cells 3 days post-infection, and led to a decrease of lysis by LAK cells. The infection with 0·01 MOI resulted in about 5–10% infected cells 3 days post-infection and decreased further the susceptibility to lysis by LAK cells. However, HCMV infection with MOI 0·1 hardly influenced the LAK activity in comparison to mock-infected HFF cultures. In these cases about 60–80% of HFF cells were infected, as recently reported elsewhere.[18]

Positively selected CD56$^+$ NK cells did not exert any cytotoxicity against HFF cultures in a 4-hr LDH release assay

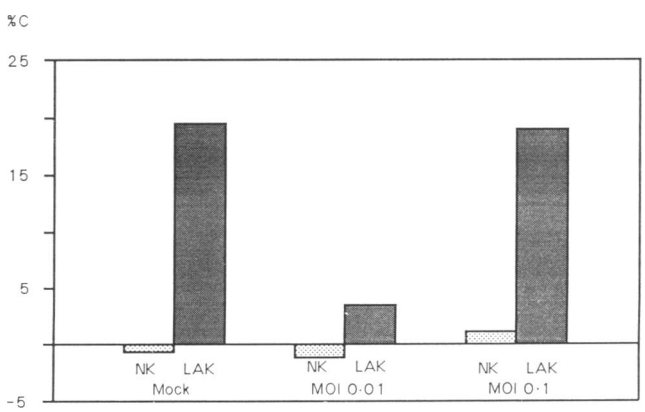

Figure 2. Lysis of mock-infected or HCMV-infected (MOI 0·01–0·1) HFF monolayers (3 days post-infection) by resting (NK) or IL-2-activated (LAK) CD56$^+$ effector cells ($1·0 \times 10^5$/well). Cytotoxicity was tested in a 4-hr LDH release assay.

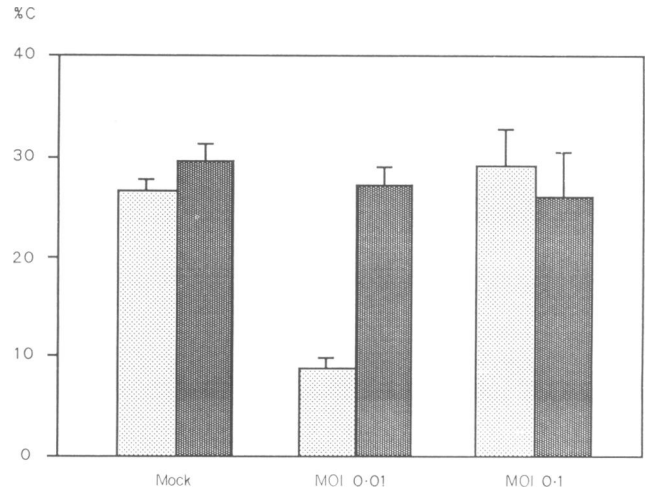

Figure 3. Lysis of mock-infected or HCMV strain AD 169 (MOI 0·01–0·1)-infected HFF monolayers (□) and co-incubation with anti-IFN-β antibodies (■) (400 U/ml) (3 days post-infection) by PNAC ($8·0 \times 10^5$/well) preincubated with IL-2 (2 days, 50 U/ml) ($n = 3$). Cytotoxicity was tested in a 4-hr LDH release assay.

(Fig. 2). On the contrary, activation of $CD56^+$ cells with IL-2 generated an effector cell population that exhibited cytotoxicity against HFF cultures either mock-infected or HCMV (MOI 0·1)-infected. However, low-dose HCMV (MOI 0·01)-infected HFF cultures also developed resistance to lysis by IL-2-activated purified $CD56^+$ cells (Fig. 2).

Effect of anti-IFN-β on development of LAK resistance

In order to determine if the development of the resistance was due to the release of IFN-β, anti-IFN-β serum (400 U/ml) was added to HCMV-infected or mock-infected HFF cultures and incubated for 3 days (Fig. 3). Mock-infected and HFF cultures infected with MOI 0·1 were hardly influenced by the addition of anti-IFN-β. However, the decline of susceptibility of low-dose HCMV (MOI 0·01)-infected HFF cultures was restored totally by anti-IFN-β. Therefore, the formation of resistance seems to be mediated by the release of IFN-β from target cells.

IFN-β release during the infection period

The amount of human IFN-β in the supernatants of HFF cultures 72 hr post-infection was determined with an enzyme immunoassay (Table 1). Whereas there was 30·4 IU/ml IFN-β in the virus stock solution, the supernatants of HFF cultures inoculated with a 1:100 dilution of HCMV stock solution (MOI 0·1) contained between 30 and 75 IU/ml IFN-β, and the supernatants of HFF cultures infected with 1:1000 dilution (MOI 0·01) contained between 2 and 5 IU/ml IFN-β. Thus the level of IFN-β secreted by infected HFF monolayers increased during 72 hr about 100-fold. Mock-infection or infection with heat-inactivated HCMV induced no detectable release of IFN-β.

Table 1. IFN-β enzyme immunoassay of HCMV stock solution and HFF culture supernatants after 72 hr of incubation

Sample	IFN-β (IU/ml)
HCMV stock solution	30·4
Mock infection	Not detectable
MOI 0·1 (diluted 1:100*)	30–75
MOI 0·01 (diluted 1:1000)	2–5
Heat-inactivated (diluted 1:100)	Not detectable

*Dilution of the HCMV stock solution.

Fluorescence analysis of class I MHC expression by flow cytometry

HFF were infeced with different MOI of HCMV strain AD 169 and, parallel to cytotoxicity experiments, HLA class I expression was analysed at day 3 post-infection. Figure 4 depicts the histograms of a representative experiment. An inoculum of MOI 0·001 led to a slight increase of surface expression of HLA class I (MFC 93·0, Fig. 4B) in comparison to mock-infected HFF cultures (MFC 78·9, Fig. 4A). When

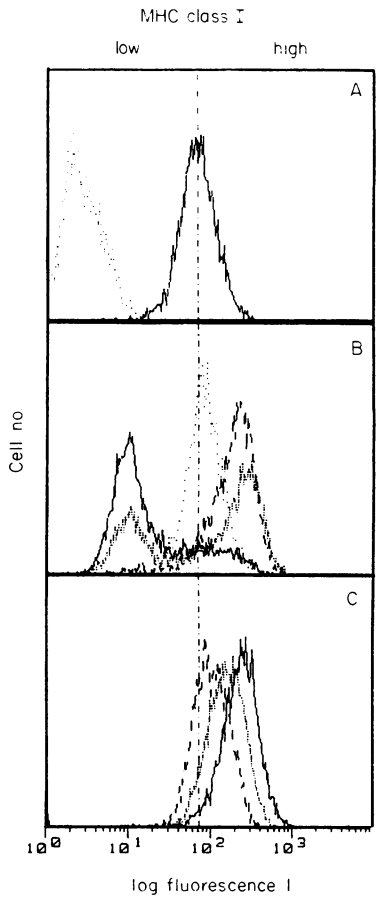

Figure 4. Single histograms of fluorescence intensity demonstrate segregation of HLA class I expression (mAb B 9.12.1; diluted 1/20) of HFF cells 72 hr after addition of: (A) mock infection: (——) MFC 78·9; (B) HCMV strain AD 169: (····) MOI 0·001, MFC 93·0 (----) MOI 0·01, low MFC 45·2 (11·7%), high MFC 234·4 (88·3%), (·····) MOI 0·1, low MFC 20·5 (39·7%), high MFC 280·3 (60·3%), (——) MOI 1·0, low MFC 16·6 (83·4%), high MFC 181·4 (16·6%); (C) IFN-β: (----) 1 U/ml, MFC 118·3, (·····) 10 U/ml, MFC 177·3, (——) 100 U/ml, MFC 261·4.

HFF cultures were infected with MOI 0·01, however, the HFF culture was divided into high-density and low-density HLA class I-expressing fibroblasts. For analysis of the MFC of these two populations, a boundary was chosen corresponding to the MFC of the mock-infected HFF cultures. In this experiment the MFC of the low-density HLA class I cells (11·7%) was 45·2 and MFC of high-density HLA class I-expressing cells (88·3%) was 234·5. Infection with MOI 0·1 divided the HFF culture into a population (39·7%) with MFC 20·5 and a population (60·3%) with MFC 280·3, and infection with MOI 1·0 segregated the fibroblasts in 83·4% with MFC 16·6 and 16·6% with MFC 181·4 3 days post-infection (Fig. 4B).

Figure 4C depicts the increase of HLA class I expression of fibroblasts incubated with different concentrations of IFN-β in the same experiment. One unit per millilitre of IFN-β increased the MFC of HLA class I expression to 118·3, 10 U/ml IFN-β to 177·3 and 100 U/ml to 261·4. The addition of anti-IFN-β antibodies at the beginning of the infection period prevented the increase of class I HLA expression in comparison to controls (see ref. 18).

Kinetics of target cell susceptibility to LAK cytotoxicity and the expression of MHC class I surface molecules

In Fig. 5 the kinetics of susceptibility to LAK cytotoxicity induced by low-dose HCMV infection or addition of IFN-β to uninfected HFF monolayers was correlated to the increase of MHC class I surface molecules. Both HCMV infection (MOI 0·01) and IFN-β addition (100 U/ml) did not significantly increase MHC class I expression in comparison to control cultures 12 hr post-incubation. IFN-β induced a slight increase of MHC class I expression 24 hr after addition and reached a maximum level 72 hr post-incubation. Infection with HCMV (MOI 0·01) caused an increased level of MHC class surface molecules not before 24 hr post-infection. A significant enhancement of HLA class I expression could be registered 48 hr post-infection.

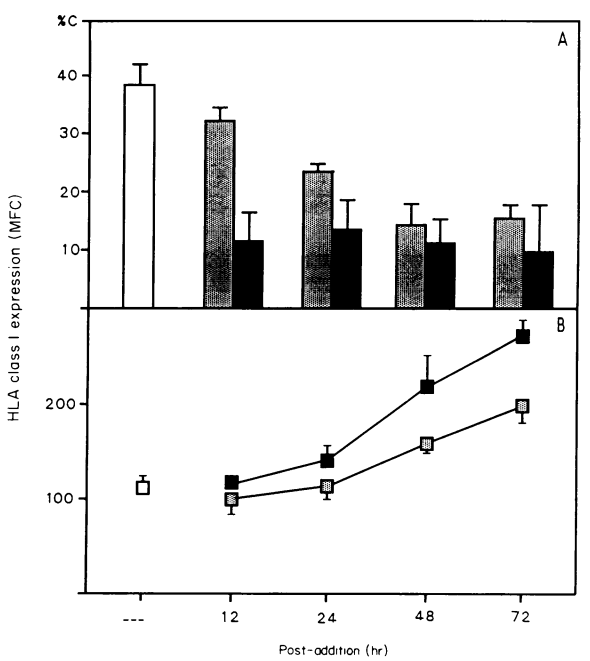

Figure 5. Kinetics (12–72 hr) of LAK cytotoxicity (A) and HLA class I expression (B) ($n = 3$). (A) Lysis of HFF monolayers by PNAC preincubated with IL-2 (2 days, 50 U/ml); (B) Average of HLA class I cell-surface expression (MFC) of the whole cell population. (□) Mock; (▨) HCMV (MOI 0·01); (■) IFN-β (100 U/ml).

However, 12 hr of incubation with IFN-β (100 U/ml) protected the HFF cultures from lysis by LAK cells, similar to that achieved after 72 hr. Also, HCMV infection (MOI 0·01) diminished slightly the susceptibility of HFF cultures 12 hr post-infection. However, a significant LAK resistance of HCMV-infected HFF monolayers could be monitored only 24 hr post-infection.

In summary, by 12 hr post-incubation a clear difference was seen between the susceptibility of mock-infected, HCMV-infected or IFN-β-incubated HFF cultures to LAK cytotoxicity, but no significant differences could be registered in the MHC class I expression.

DISCUSSION

In this study the ability of IL-2-activated NK cells to lyse HCMV-infected HFF cultures was investigated in the context of MHC class I expression. For this purpose HFF cultures were infected with infective doses ranging from MOI 0·001 to 0·1 of HCMV, and target cell lysis of monolayers was read out in a colorimetric enzyme release assay. In parallel, HLA class I expression of the fibroblasts was shown by flow cytometry.

NK cell activity has been defined as MHC-unrestricted cytotoxicity against certain tumours and virus-infected cells.[11] In comparison to NK cytotoxicity against sensitive tumour cells, the presence of HLA-DR$^+$, IFN-α-producing cells is necessary to get NK activity against HSV-, VSV- or CMV-infected target cells.[23,24] Therefore, it seems that NK cells are activated in the long-term cytotoxicity assay by the release of IFN or other cytokines. IFN-α as well as IFN-β is known to potentiate the NK activity against uninfected and CMV-infected target cells.[25] When we infected HFF cultures with low MOI (0·001–0·01) of HCMV, we found that these target cultures were significantly less susceptible to lysis by LAK cells than mock-infected or high-dose HCMV (MOI 0·1)-infected target cells (Figs 1–3). A possible explanation of the resistance against LAK cell-mediated lysis could be the release of IFN-β by infected fibroblasts. It is known that IFN induce cytolytic resistance to NK and LAK cell-mediated cytolysis to some target cells,[26,27] but not all. For example, target cells infected with cytopathic viruses like adenovirus, murine CMV or HSV are not protected by IFN against lysis.[28–30] In the case of HSV, IFN even enhance the susceptibility of infected target cells to lysis by NK cells.[30]

The results of this report demonstrate clearly that IFN-β was produced by HCMV-infected fibroblasts during the infection period (Table 1) and that uninfected fibroblasts in the culture were protected against lysis by LAK cells by IFN-β, because the addition of anti-IFN-β antibodies abrogated the decline of susceptibility to LAK cytotoxicity (Fig. 3). However, HCMV-infected fibroblasts were not generally protected against LAK cytotoxicity by IFN-β (Fig. 1; MOI 0·1), while high levels of IFN-β (30–75 IU/ml) could be detected in cell-free supernatants. In this case the virus inoculum used for

adsorption (stock solution diluted 1/100) contained only 0·3 IU/ml IFN-β (Table 1).

In tumour target cells an inverse relationship between MHC class I expression and NK sensitivity has been found.[31] Target cells which are treated with IFN or other MHC class I-enhancing cytokines (IL-1, tumour necrosis factor) become refractory to NK activity, while cytokine-induced MHC class I expression may reduce, not alter or even increase susceptibility to LAK activity.[13] So the exact role of MHC class I antigens expressed on the surface of target cells in the regulation of NK or LAK sensitivity remains unclear. Different reports suggest that MHC molecule expression on tumour- and virus-infected target cells might potently influence their susceptibility to NK and LAK cell-mediated lysis. According to this assumption, the 'missing self' hypothesis[11,32] suggests that the absence of MHC class I or the loading of MHC class I complex with foreign peptides[33,34] may activate the killing cascade. The 'missing self' hypothesis is compatible with data of virus-infected target cells presented by other authors.[35–37] Recognition of class I–virus peptide complexes by NK cells may be mediated by groups of receptor molecules that are selectively expressed on different NK clones.[35] Enhanced NK lysis of vaccinia virus-infected cells is concomitant with a decrease in H-2 class I antigen expression.[36] HSV-infected, HLA class I^+ targets were more susceptible to NK-mediated cytotoxicity than non-infected, HLA class I^+ targets, although HSV infection did not alter significantly the level of HLA class I expression.[37]

Two hypotheses exist to explain how MHC expression protects target cells against lysis by NK/LAK cells: the 'effector inhibition' model and the 'target interference' model (reviewed by ref. 11). The 'effector inhibition' model supposes that MHC class I molecules have a negative regulatory influence on NK/LAK activation; the 'target interference' model assumes that MHC class I molecules mask target structures on cells. However, experiments to cancel the protective effect of class I HLA expression gave different results. In contrast to some investigators,[38,39] Lin *et al.*[40] were not able to increase the susceptibility to target cell lysis by the addition of $F(ab')_2$ molecules directed to HLA class I molecules. In this investigation, the cleavage of class I MHC antigenes by citric acid treatment enhanced susceptibility of IFN-treated, but not untreated, target cells, though both target cells lost class I MHC surface molecules.

The investigation reported here using IL-2-activated effector cell populations found a decline of LAK susceptibility of low-dose HCMV-infected or IFN-β-treated cultures 3 days post-addition, correlating to an increase of MHC class I surface molecules on uninfected HFF cells. However, already 12 hr post-infection an increased resistance was measured to LAK cytotoxicity although no significantly enhanced MHC class I expression was detectable in comparison to control cultures. Further, uninfected HFF cultures were killed to a similar extent as infected fibroblasts, although MHC class I detection of infected target cells was significantly reduced in comparison to uninfected HFF cells, as demonstrated in detail in a previous report.[18]

In summary, LAK effector cells resulting from PNAC and purified $CD56^+$ NK cells killed both fibroblasts of uninfected HFF cultures and HCMV-infected fibroblasts to a similar extent. In contrast, low-dose infected HFF cultures showed a significantly reduced susceptibility to lysis by LAK cells. The protection of HFF cells was mediated by the release of IFN-β, but the resistance to LAK activity initially did not correlate with the increase of MHC class I expression. These findings are in agreement with results from Reiter *et al.*,[41] that the protective effect of IFN on Daudi cells against IFN-activated NK cells is not mediated by MHC class I expression.

Further investigations have to be done to illuminate the mechanism of how uninfected neighbouring cells are protected against LAK cytotoxicity by virus-induced IFN-β.

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