Measles Virus Transmembrane Fusion Protein Synthesized De Novo or Presented in Immunostimulating Complexes Is Endogenously Processed for HLA Class I- and Class II-restricted Cytotoxic T Cell Recognition

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Summary

The routes used by antigen-presenting cells (APC) to convert the transmembrane fusion glycoprotein (F) of measles virus (MV) to HLA class I and class II presentable peptides have been examined, using cloned cytotoxic T lymphocytes in functional assays. Presentation by Epstein-Barr virus-transformed B lymphoblastoid cell lines was achieved using live virus, ultraviolet light-inactivated virus, and purified MV-F delivered either as such or incorporated in immunostimulating complexes (MV-F-ISCOM). Only live virus and MV-F-ISCOM allow presentation by class I molecules, while all antigen preparations permit class II-restricted presentation. We observe presentation of MV-F from live virus and as MV-F-ISCOM by class II molecules in a fashion that is not perturbed by chloroquine. Our studies visualize novel presentation pathways of type I transmembrane proteins.

Presentation of protein antigens to MHC class I- and II-restricted T cells by APC requires that APC degrade antigens to peptides, and that peptides combine with class I or II molecules. Two major pathways for antigen processing have been identified (for review see references 1–5).

The endosomal/lysosomal processing pathway in APC is involved in the processing of exogenous antigen taken up by endocytosis in a low pH endocytotic compartment (6–12). Peptides resulting from endosomal processing combine with class II molecules that are subsequently expressed at the surface of APC for recognition by $CD4^+$ T cells (13, 14). The endosomal pathway intersects the biosynthetic route of class II molecules (15). Recent evidence indicates that peptides generated by processing of exogenous antigens may bind to newly synthesized class II molecules in acidic compartments (16–19), leading to the formation of stable class II dimers. Recent immunocytochemical studies with human EBV-transformed human lymphoblastoid cell lines (B-LCL)[1] have demonstrated the acidic compartment highly enriched for class II molecules that is a close relative of lysosomes (20).

The endogenous processing pathway requires that antigens are synthesized de novo within APC (21–26), or that exogenous antigens are experimentally introduced into the cytoplasmic compartment of APC (27–29). Current evidence suggests that the endogenous processing of antigen, which includes cytosolic proteins as well as membrane glycoproteins synthesized in the endoplasmic reticulum (ER), may occur in the cytosol of APC (21, 25, 26, 30–35). At an as yet unidentified site, peptides may combine with class I molecules to form stable complexes (36–44) that are subsequently deported to the cell surface of APC for recognition by $CD8^+$ T cells.

Alternative routes of processing of antigens for presentation to class I- and class II-restricted cells may exist. Several exogenous antigens may be presented to class I-restricted T cells in vivo and in vitro (27–29, 45–52). It is not clear whether the same intracellular protein processing pathway that is operative for de novo synthesized proteins is used in such cases. Second, proteins synthesized de novo in cytosol or ER of APC, can be presented to class II-restricted T cells (53–59).

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; F, fusion; ISCOM, immunostimulating complex; LCL, lymphoblastoid cell line; MOI, multiplicity of infection; MV, measles virus; NP, nucleoprotein.

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The routes of protein breakdown and presentation in such cases require further clarification.

We therefore examined the processing and presentation of the measles virus (MV) transmembrane fusion (F) glycoprotein to class I- and class II-restricted cloned CTL. During biosynthesis the MV-F protein is cotranslationally inserted into the membrane of the ER as a type I transmembrane glycoprotein (60, 61). To distinguish the possible pathways involved in the processing of class I- and class II-restricted epitopes, we used different forms of presentation of MV-F proteins. We used UV-irradiated nonreplicative MV (UV-MV) expected to be processed and presented via the endosomal class II pathway, and purified MV-F protein, likewise expected to be routed via the endosomal class II pathway. Furthermore, we utilized MV-F protein incorporated in immunostimulating complexes (ISCOM) (62, 63) and MV-F protein biosynthesized within APC after infection with live virus. We show that exogenous MV-F protein, when presented in UV-MV, MV-F, or MV-F-ISCOM, can be processed via the endosomal/lysosomal pathway to peptides that are recognized by class II-restricted CTL only, but when presented in MV-F-ISCOM can be processed also via the endogenous pathway to peptides that are recognized by both class I- and class II-restricted CTL. The same class I and class II binding peptides are also generated via the endogenous nonendosomal pathway by processing of MV-F derived by de novo synthesis within the APC.

Materials and Methods

Antigen Preparations

Infectious MV. Plaque-purified MV (Edmonston B strain), cultured in Vero cells and containing 10^7 $TCID_{50}$/ml infectious MV, was routinely used to infect human EBV-transformed B cell lines (B-LCL).

UV-MV. As a source of inactivated MV, plaque-purified MV was propagated in Vero cells in a microcarrier culture (64). Culture supernatants were concentrated 20-fold in a hollow fiber system with a molecular weight cut-off of 10^6 (Amicon Corp., Denver, CO), and the virus was further purified by discontinuous sucrose gradient centrifugation according to methods described (65). The purified MV, containing 300 μg/ml of viral protein was subsequently UV irradiated with a UV dose (1.5×10^{-2} $\mu W/mm^2$, Transilluminator; Ultra Violet Products, San Gabriel, CA) sufficient to eliminate virus infectivity completely, but preserving the hemagglutination and hemolysing/fusion activities of MV.

MV-F. The transmembrane fusion glycoprotein of MV (MV-F) was purified from whole virus by solubilizing purified MV with octyl-β-D glycopyranoside (2% [wt/vol] octylglycoside; Sigma Chemical Co., St. Louis, MO), followed by affinity chromatography using MV-F-specific mAb 7-21 coupled to CNBr-activated Sepharose 4B as described (62). Preparations, containing 300 μg/ml purified MV-F, were maintained in octylglucoside (0.1% [wt/vol]) to preserve a micellar solution of the protein, as determined by electron microscopy.

MV-F Peptides. 12-mer sequences of the MV-F-protein were synthesized on an automated peptide synthesizer according to methods described by Van der Zee et al. (66). Two peptides were used in these studies, corresponding to sequences of MV-F recognized by the two T cell clones JG-F94 and WH-F40: respectively, F 452–463 (amino acid sequence GPPISLERLDVG) and F 437–448 (amino acid sequence SRRYPDAVYLHR).

MV-F-ISCOM. ISCOM were prepared from MV according to the methods described by Morein et al. (63). Briefly, MV was solubilized with 2% Triton X-100 and layered over a 20–60% sucrose gradient in TNE buffer (0.1 M TRIS-HCl, 0.1 M NaCl, and 0.01 M EDTA, pH 7.4) containing 0.2% (wt/vol) Quil A (Spikoside, Iscotec, Sweden) and centifuged in an SW 28 rotor at 20,000 rpm for 18 h at 4°C. Gradient fractions containing ISCOM particles were pooled and analyzed by SDS-PAGE and by a double-antibody ELISA for the quantitation of MV-F, as described by De Vries et al. (62).

Empty ISCOM. The matrix of ISCOM, devoid of any incorporated proteins (empty ISCOM), was constructed from Quil A and cholesterol according to methods described by Fossum et al. (67). Where appropriate, empty ISCOM was used in Quil A-based amounts equivalent to MV-F-ISCOM.

Characterization of ISCOM Structures

The morphologies of MV-F-ISCOM, empty ISCOM, and purified MV-F were analyzed by electron microscopy. Preparations were negatively stained on glow-discharged formvar carbon-coated copper grids using 2% phospho tungstic acid (PTA), pH 5.2. The grids were examined by negative contrast electron microscopy. MV-F-ISCOM, purified MV-F, and whole virus were also analyzed by SDS-PAGE (12.5%) under reducing conditions, followed by immunoblotting. MV proteins were detected using rabbit anti-MV polyclonal antibody Ko35/50 (62).

T Cell Clones

MV-specific human T cell clones described in this paper were established from PBMC of two healthy adult individuals (JG and GR) and of two children (JP and WH) 4 wk after clinical symptoms of measles were observed. All T cell clones were generated from PBMC that were stimulated and cloned with autologous MV-infected B-LCL and were cultured in vitro as described (68, 69). These clones were analyzed for the expression of CD3, CD4, and CD8 in standard cytofluorometry assays as described (69).

Cytotoxicity Assays

B-LCL were cultured and maintained in RPMI 1640 supplemented with 5% (vol/vol) FCS, 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 10^{-5} M 2-ME. Autologous or HLA-matched B-LCL (10^6 to 10^7) were infected with MV at a multiplicity of infection (MOI) of 3.0 for 24 h at 37°C or were left uninfected. Alternatively, B-LCL were pulsed two or three times during a 24- or 48-h culture period, as indicated in the text either with UV-MV (10 μg/ml per 3×10^5 B-LCL), MV-F (1 μg/ml per 3×10^5 B-LCL), or with MV-F-ISCOM (1 μg/ml ISCOM per 3×10^5 B-LCL). When pulsed with peptides, B-LCL were incubated during the course of the CTL-assay (4 h) with synthetic peptides F 437–448 and F 452–463 at a final concentration of 1 μM. The human mutant cell line T2 and the control cell line C1R, both transfected with the gene encoding HLA-B27 (37), were also used as target cells. T2-B27 and C1R-B27 were either infected with MV or left uninfected, incubated for 48 h with MV-F-ISCOM, or incubated during the course of the CTL-assay with the synthetic peptides F 437–448 or NP 380–393 using the same procedures as described for B-LCL target cells. The generation of the HLA-B27-restricted influenza virus-specific CTL line (HF) recognizing a synthetic peptide (nucleoprotein [NP] 380–393)

corresponding to the NP epitope of influenza virus has been described elsewhere (70). T cell clones were subsequently incubated with ^{51}Cr-labeled B-LCL, T2-B27, or C1R-B27 at different E/T ratios. After 4 h of incubation at 37°C, supernatants free from cells were collected from individual cultures and counted in a gamma counter. Spontaneous ^{51}Cr release (target cells only) and maximal ^{51}Cr release (target cells in 2% Triton X-100) were used as control values in all assays. Results are expressed as the mean percentages of specific target cell lysis ± SD of triplicate cultures.

Proliferative Assays

T cell clones were cultured in 96-well round-bottomed microtiter plates (Greiner Labor Technik, Nürtingen, Germany) in 150 μl of RPMI 1640, supplemented with 10% (vol/vol) pooled human AB serum, 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), 10^{-5} M 2-ME plus 20 U/ml rIL-2 (Boehringer Mannheim, Mannheim, Germany), referred to as complete medium. Growing clones were expanded and kept at a density of 3–5 × 10^4 cells/well in the presence of rIL-2 and were restimulated with MV-infected B-LCL every 10–14 d of culture. HLA-typed B-LCL were used as stimulator cells for proliferative responses of T cell clones. B-LCL were incubated with the same antigens and by the same procedures as described for the cytotoxicity assays. In addition, B-LCL were also infected with two vaccinia virus recombinants, either a control vaccinia virus (vv-vsc8) or vaccinia virus containing the full-length cDNA encoding MV-F (vv-F37). Briefly, 10^7 B-LCL were infected with vv-vsc8 or vv-F37 at a MOI of 5.0 for 16 h at 37°C. All B-LCL stimulator cells were then fixed for 15 min with 0.5% (wt/vol) paraformaldehyde in 1 mM PBS, pH 7.2, and subsequently treated for 15 min with 0.2 M glycine in PBS and 60 min with complete medium before they were used as stimulator cells. For proliferative T cell responses, T cell clones (3 × 10^4/well) were cultured for 3 d at 37°C in the absence of rIL-2 with paraformaldehyde-fixed B-LCL and pulsed with 0.5 μCi [^{3}H]Tdr over the last 18 h of culture. Cells were harvested, and the incorporated radioactivity was counted in a flat-bed β-scintillation counter. Results are expressed as the mean cpm ± SD of triplicate cultures.

Chloroquine Inhibition Experiments

2 h before antigen incubation and throughout the culture of B-LCL with the same antigens as described above, B-LCL were cultured in the presence of 50 μM chloroquine (Sigma Chemical Co.). Chloroquine-treated and untreated B-LCL were fixed with paraformaldehyde and used as stimulator cells in proliferative assays as described above.

Results

MV-F-specific CD8$^+$ Class I– and CD4$^+$ Class II–restricted CTL. Our first series of experiments were aimed at the generation of CD8$^+$ class I– and CD4$^+$ class II–restricted CTL clones with specificity for the fusion protein of MV (MV-F). An extensive analysis of the T cell clones that we obtained is described elsewhere (68, and R. van Binnendijk et al., manuscript in preparation).

For the present study, two CD8$^+$, class I–restricted T cell clones (JP-F20 and WH-F40) and two CD4$^+$ class II–restricted T cell clones (GRIM-F99 and JG-F94) were selected. All clones are specific for the MV-F protein and exert CTL activity for appropriately sensitized target cells (Fig. 1). The CD8$^+$ T cell clones JP-F20 and WH-F40 recognized MV-F when presented in association with HLA-Bw62 and HLA-B27, respectively, and the CD4$^+$ T cell clones GRIM-F99 and JG-F94 recognized their epitopes when presented in association with HLA-DQw1 (either DQ5 or DQ6) and HLA-DRw53, respectively (Fig. 1).

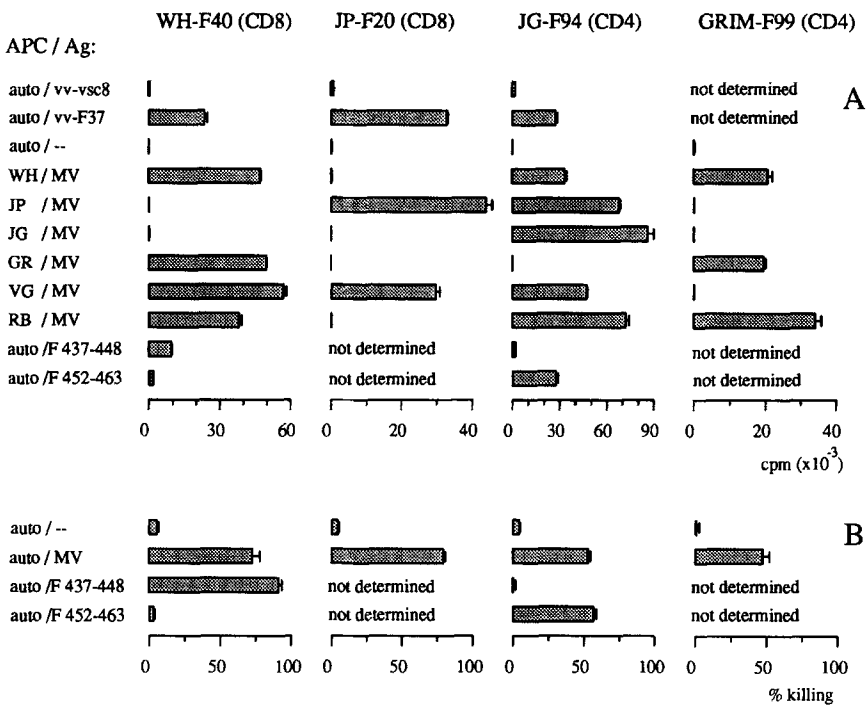

Figure 1. Antigen recognition and HLA-restriction of MV-F-specific CD3$^+$ CD4$^+$ CD8$^-$ (CD4) and CD3$^+$ CD4$^-$ CD8$^+$ (CD8) CTL clones. (*A*) Proliferative T cell responses and (*B*) CTL responses (measured at an E/T ratio of 3 in a 4-h ^{51}Cr-release assay) were carried out as described in Materials and Methods. Autologous (auto) and other HLA-typed B-LCL were used as APC, either uninfected or infected with MV (24 h, MOI 3), control vaccinia virus (vv-vsc8; 18 h, MOI 5), or vaccinia virus expressing MV-F (vv-F37; 18 h, MOI 5). Proliferative and cytotoxic T cell responses of clones WH-F40 and JG-F94 were also conducted with B-LCL that were pulsed for 24 h with 1 μM of the synthetic peptides F 435–446 or F 450–461, or pulsed with 1 μM of these peptides before and during the course of the CTL experiment. HLA-typing: B-LCL WH: A2,11; B27,35; DQw1 (DQ5), 3 (DQ8); DR1,4, w53; B-LCL JP: A2; Bw55,w62; DQw3 (DQ7,DQ8); DR4,w53; B-LCL JG: A1; B8,12,44; DQw2,3; DR3,4,w52,w53; B-LCL GR: A1,3; B7,27; DQw1 (DQ6); DR2, w6,w52; B-LCL VG: A24,31; B27,w62; DQw3; DR4,w53; B-LCL RB: A28,29; B12,27,44; DQw1,2; DR1,7,w53.

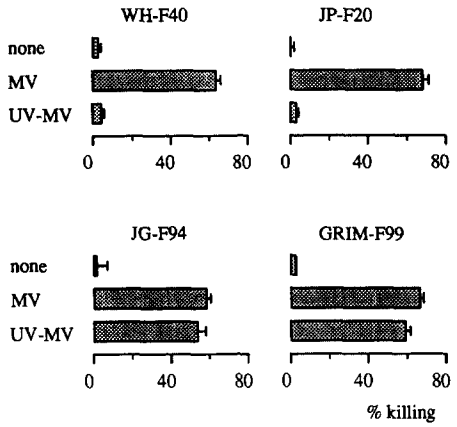

Figure 2. Recognition of UV-MV by MV-F-specific CD8+ (WH-F40, JP-F20) and CD4+ (JG-F94, GRIM-F99) CTL clones. Cytotoxic T cell responses were carried out in a 4-h ^{51}Cr-release assay at an E/T ratio of 3 using B-LCL that were either infected with MV (24 h, MOI 3) or pulsed with UV-MV (30 μg/ml, 24 h).

CD4+ Class II–restricted, but not CD8+ Class I–restricted, CTL Detect the Presentation of Exogenous MV-F. B-LCL target cells, either infected with MV or pulsed with UV-MV, were analyzed for their ability to be lysed by the class I– and class II–restricted T cell clones. Clones JP-F20 and WH-F40, both class I restricted, killed MV-infected B-LCL, but consistently failed to kill B-LCL pulsed with UV-MV (Fig. 2). Clones GRIM-F99 and JG-F94, both class II restricted, killed target cells that presented MV-F either way. These results are concordant with the view that intracellular de novo synthesized proteins (i.e., virus infection of APC) can be processed to yield peptides that may associate with both class I and class II molecules, whereas exogenously added protein antigens are degraded to peptides that associate with class II molecules only.

MV-F Incorporated in ISCOM Is Presented to Both CD8+ Class I–restricted and CD4+ Class II–restricted CTL. ISCOM are stable molecular structures in which detergent-solubilized proteins are incorporated in a matrix of the adjuvant glycoside Quil A (63). From whole virus, we prepared MV-F-ISCOM containing almost exclusively MV-F (Fig. 3). We investigated whether MV-F incorporated in ISCOM could sensitize B-LCL for lysis by the CTL clones JP-F20, WH-F40, and JG-F94. B-LCL incubated for 24 h with 1 μg MV-F-ISCOM are only sensitized for lysis by the class II–restricted clone JG-F94, but not by the class I–restricted clone JP-F20 (Fig. 4, exp. 1). 2 or 3 μg MV-F-ISCOM given at a single dose to B-LCL severely affected the viability of the cells (data not shown). We therefore fed B-LCL two or three times with 1 μg MV-F-ISCOM during a 24 h incubation period. This resulted in the sensitization of target cells for class II–restricted killing by clone JG-F94. However, killing of such targets by class I–restricted clones JP-F20 or WH-F40 was minimal or absent (Fig. 4, exps. 2 and 4). However, when the same MV-F-ISCOM-fed B-LCL were used as APC in a 72-h proliferative T cell response, proliferation of class I–restricted clones

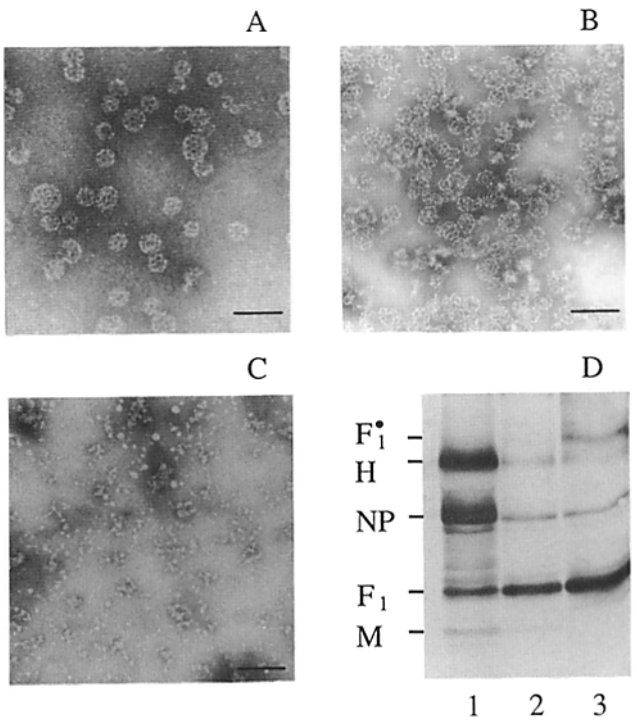

Figure 3. Analyses of MV-F-ISCOM by electron microscopy and by Western blotting. MV-F-ISCOM (*A*) and empty ISCOM (*B*). Purified MV-F protein MV-F (*C*). Bars represent 100 nm. Western blot analyses of the individual polypeptides of MV (*D*, lane *1*), MV-F-ISCOM (*D*, lane *2*), and purified MV-F (*D*, lane *3*) using polyclonal anti-MV antibody Ko35/50. Under reducing conditions, MV-F is a cleaved product of 41 kD with a minor contamination of a dimer in the purified MV-F preparation (indicated as F_1). H, hemagglutinin; F, fusion protein; NP, nucleoprotein; and M, matrix protein.

was noticed, indicating that prolonged incubation of B-LCL with MV-F-ISCOM would be required to sensitize target cells for CTL recognition (data not shown). We therefore pulsed targets one or three times with 1 μg MV-F-ISCOM for 48 h.

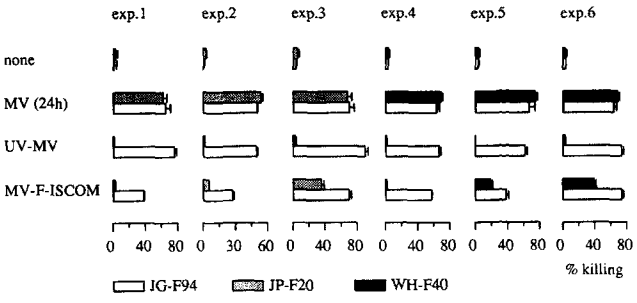

Figure 4. HLA class I– and class II–restricted CTL respond to MV-F-ISCOM. CTL responses of clones JP-F20, WH-F40, and JG-F94 were measured at an E/T ratio of 6, using B-LCL JP as target cells in Exps. 1–3, and B-LCL WH in Exps. 4–6. B-LCL were either infected with MV for 24 h in all experiments, or were feeded with one, two, or three pulses of MV-F-ISCOM (1 μg/ml) or UV-MV (10 μg/ml) during a 24- or 48-h culture period, according to the following regime: one pulse/24 h (Exp. 1); two pulses/24 h (Exp. 2); three pulses/24 h (Exp. 4); one pulse/48 h (Exp. 5); and three pulses/48 h (Exps. 3 and 6).

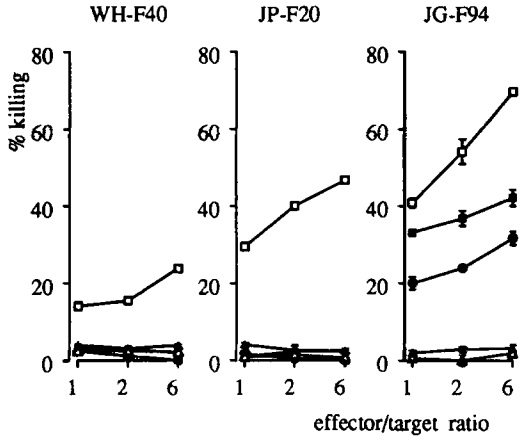

Figure 5. Intact MV-F-ISCOM structure is required to induce class I-restricted CTL responses. B-LCL VG were used as untreated target cells (*open triangles*) or they were incubated during a 48-h culture period with MV-F-ISCOM (1 μg/ml three times; *open squares*), empty ISCOM (1 μg/ml three times; *filled triangles*), MV-F protein (1 μg/ml three times; *filled squares*), or with a mixture of empty ISCOM plus MV-F protein (1 μg/ml each three times; *filled circles*).

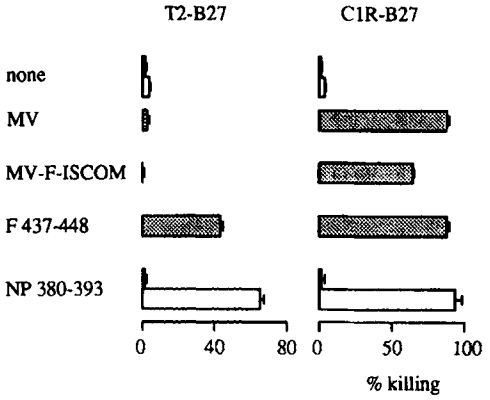

Figure 6. Activation of HLA-B27-restricted CTL clone WH-F40 is not due to the presence of peptide in MV-F-ISCOM. T2-B27 and control cell line C1R-B27, both transfected with the gene encoding HLA-B27, were either uninfected, infected with MV (24 h, MOI 3), or incubated for 48 h with MV-F-ISCOM (1 μg/ml three times). In addition, these cell lines were also incubated with 1 μM of the synthetic peptides F 435–446 or NP 380–393 (influenza virus) during the course of the CTL assay. The CTL response of clone WH-F40 (*dark bars*) and of the influenza virus NP-specific T cell line HF (*light bars*) is demonstrated at an E/T ratio of 3.

As shown in Fig. 4, exps. 3, 5, and 6, this made targets susceptible for both class II- and class I-restricted killing by clones JG-F94, JP-F20, and WH-F40, respectively. In agreement with the CTL responses shown in Fig. 2, all clones lysed MV-infected but not uninfected B-LCL target cells, whereas only the class II-restricted clone JG-F94 killed target cells sensitized with UV-MV (Fig. 4, exps. 1–6). Thus, in contrast to UV-MV, which activates class II-restricted CTL only, MV-F-ISCOM activates both class II- and class I-restricted CTL.

Could the CTL clones have been activated nonspecifically by the ISCOM matrix structure, notably by Quil A? This possibility was addressed by testing the cytolytic capacity of CTL clones towards targets incubated for 48 h with: MV-F-ISCOM, purified MV-F (shown as protein micelles in Fig. 3 *C*), empty ISCOM (Fig. 3 *B*), and empty ISCOM mixed with purified MV-F. The class I-restricted clones JP-F20 and WH-F40 only lysed targets pulsed with MV-F-ISCOM (Fig. 5). In contrast, the class II-restricted clone JG-F94 killed targets pulsed with MV-F presented as an intact MV-F-ISCOM structure, as purified MV-F alone or as purified MV-F mixed with empty ISCOM. Empty ISCOM alone consistently failed to activate either class of CTL (Fig. 5). Thus, only intact MV-F-ISCOM can activate class I-restricted clones.

APC Defective in the Generation of Class I Presentable Peptides from Cytosolic Proteins Do Not Present MV-F-ISCOM to Class I-restricted CTL. We used T2 cells transfected with HLA-B27. These cells have lost the ability to present intracellular viral antigen in the context of HLA-B27 molecules but can be sensitized by extracellular peptides added for cytolysis by HLA-B27-restricted CTL (37). When the T2-B27 cell line was incubated with MV-F-ISCOM for 48 h, no presentation of MV-F to clone WH-F40 was found, whereas control cell line CIR-B27, when pulsed with MV-F-ISCOM, effectively presented MV-F to WH-F40 (Fig. 6). Likewise, presentation of the WH-F40 epitope by the T2-B27 cell line via the classical class I route, i.e., infection of T2-B27 with MV, also failed to activate clone WH-F40, whereas the MV-infected CIR-B27 line did activate the clone, to CTL activity. Cytofluorometric studies showed clear evidence for reproductive MV infection in both T2-B27 and CIR-B27 cell lines (Fig. 7). When pulsed with the HLA-B27 binding peptides spanning the epitope of MV-F recognized by clone WH-F40 (F 437–448) or the epitope of the influenza virus-specific CTL line HF (NP 380–393), both the peptide-pulsed mutant T2-B27 and the CIR-B27 control cell lines were lysed by their respective CTL (Fig. 6). Thus, the integrity of cytosolic enzymes and/or transporter molecules is required to generate class I presentable peptides from MV-F either presented in ISCOM or as de novo synthesized transmembrane MV-F in MV-infected APC.

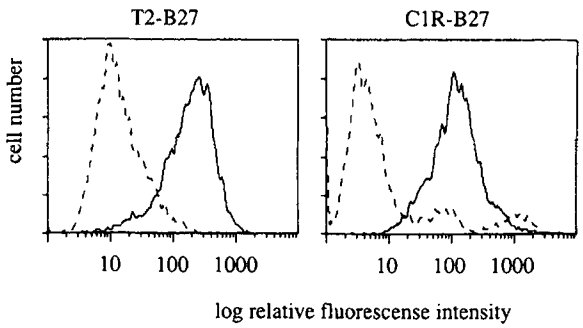

Figure 7. De novo synthesis of MV-F in MV-infected T2-B27 and C1R-B27. The expression of MV-F on the surface of uninfected (*dotted lines*) and on 24-h MV-infected cell lines (*solid lines*) was analyzed by FACS®-measured fluorescence using monospecific anti-MV-F polyclonal antibody Ko-9.

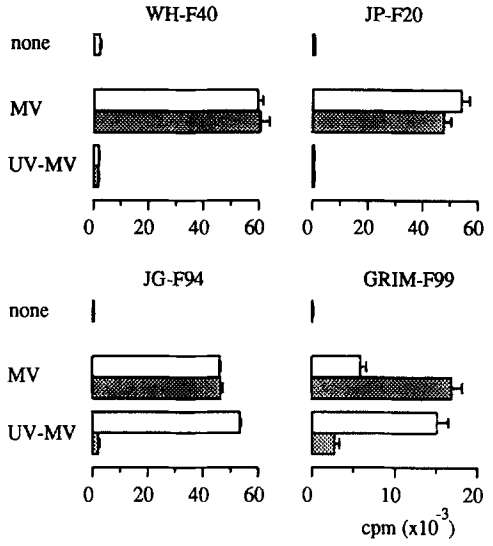

Figure 8. MV-F synthesized de novo is presented by an endogenous processing pathway to both class I- and class II–restricted CTL. B-LCL were infected with MV (24 h, MOI 3) or pulsed with UV-MV (10 μg/ml) in the presence (*dark bars*) or absence (*light bars*) of 50 μM chloroquine. Thereafter, B-LCL were fixed with paraformaldehyde (pfa) before being used as stimulator cells in proliferative assays. B-LCL GR (B27,DQw1) was used as stimulator cell in combination with clones WH-F40 and GRIM-F99, and B-LCL JP (Bw62, DRw53), in combination with clones JP-F20 and JG-F94.

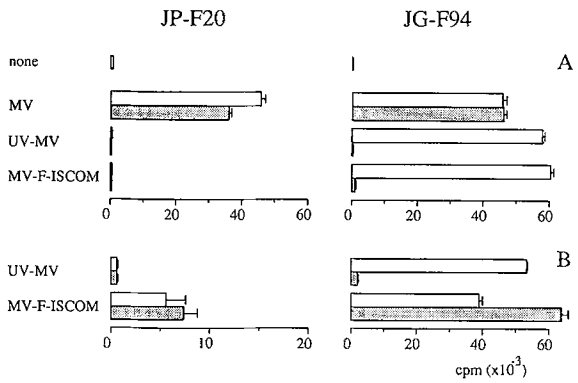

Figure 9. MV-F-ISCOM is presented by an endogenous processing pathway to both class I- and class II–restricted CTL. Proliferative T cell responses were carried out, using pfa-fixed B-LCL JP, either uninfected or infected with MV, or previously incubated for 24 h (*A*) or 48 h (*B*) with UV-MV (10 μg/ml two or three times, respectively) or MV-F-ISCOM (1 μg/ml two or three times, respectively) in the presence (*dark bars*) or absence (*light bars*) of 50 μM chloroquine.

MV-F Peptides Are Presented by Class I and Class II Molecules in MV-infected and MV-F-ISCOM-pulsed B-LCL via the Endogenous Pathway. Exogenously presented proteins are usually degraded in the endocytotic compartment to peptides that associate with class II but not class I molecules, a process sensitive to lysosomotropic agents such as chloroquine. Chloroquine blocked the presentation of UV-MV to CD4$^+$ clones (Fig. 8). The CD8$^+$ CTL clones failed to respond to UV-MV, as also was shown in Fig. 2. Surprisingly, chloroquine had no inhibitory effects on the activation of either type of CTL clones by MV-infected B-LCL, whereas in the same experiment, the drug inhibited the presentation of exogenously added UV-MV to the class II–restricted T cell clones (Fig. 8). These data suggest that an endogenous (nonendosomal) pathway for presenting epitopes of viral membrane glycoproteins to class II–restricted CTL may be operational in virus-infected cells.

The endogenous (nonendosomal) route could also be involved in the breakdown of MV-F presented as MV-F-ISCOM. Thus, we incubated B-LCL JP (HLA-Bw62, HLA-DRw53) with MV-F-ISCOM for 24 or 48 h in the presence or absence of chloroquine. B-LCL were also infected with MV or pulsed with UV-MV in the presence or absence of chloroquine. The ability of class I- and class II–restricted clones to respond to these sensitized stimulator cells was examined (Fig. 9). The results with infected and UV-MV-pulsed B-LCL were similar, as in previous experiments (see Figs. 2, 4, and 8). B-LCL pulsed with MV-F-ISCOM for 24 h (see Fig. 9) generated peptides in a chloroquine-sensitive compartment that could be presented in association with class II molecules only. Prolonged incubation (48 h) of B-LCL with MV-F-ISCOM, in agreement with the CTL assays (See Fig. 4), resulted in stimulation by these cells of both the class I- (JP-F20) and the class II- (JG-F94) restricted clone. This latter presentation of MV-F to either of both clones was not inhibitable by chloroquine.

Thus, an endogenous (nonendosomal) route for presentation of MV-F-ISCOM to both class I and class II molecules must therefore exist. In addition, MV-F-ISCOM can be routed to the endocytotic pathway, yielding the same class II presentable peptide.

Discussion

We have demonstrated the in vitro activation of CD8$^+$ class I–restricted and MV-F-specific CTL clones by MV-F incorporated in ISCOM (Figs. 4 and 5). Clearly, when presented in ISCOM, MV-F is processed via an endogenous nonendosomal pathway for association with class I molecules (Figs. 8 and 9). The experiments with the antigen presentation–defective mutant T2-B27 (71–77) demonstrate that processing of MV-F in MV-F-ISCOM-pulsed B-LCL occurs in the cytosol. MV-F-ISCOM-pulsed T2-B27 cells, but not C1R-B27 control cells, are defective in presentation of MV-F to the HLA-B27-restricted T cell clone WH-F40. However, in the same experiment, T2-B27 cells are able to synthesize functional class I molecules, as revealed by pulsing with peptide (Fig. 6). These results are in agreement with those of other groups, showing defective presentation by T2 cells in the context of class I molecules of proteins, either introduced in the cytosol by osmotic lysis of pinosomes or de novo synthesized in the cytosol (37, 38). Results of the experiments with T2-B27, as well as those presented in Fig. 5, also exclude the fact that free peptides might have been present in the MV-F-ISCOM preparation, as such peptides would have sensitized T2-B27

targets for recognition by class I–restricted CTL. Given these observations, it is very likely that the ISCOM matrix may serve as a vehicle for introducing soluble viral proteins into the cytosol of APC. How would MV-F-ISCOM enter the cytosol? ISCOM do not fuse with cell membranes as liposomes can (B. Morein, unpublished results). We assume they may integrate in membranes, either cell membranes or endosomal membranes, thereby exposing the incorporated protein to the cytosol. This allows the cytosolic degradation of viral proteins, thereby making peptides available for the loading of class I molecules in the ER. Although MV-F-ISCOM are rapidly taken up by endocytosis (see Fig. 4), resulting in degradation of MV-F to class II presentable peptides, their postulated integration in membranes is apparently a slow process (see Fig. 4). We are currently investigating the mechanism involved in the entry of MV-F-ISCOM in APC.

The nonendosomal processing of MV-F into class II presentable peptides, as is evidently shown by the chloroquine-insensitive presentation of MV-F-ISCOM to class II–restricted CTL, again indicates that the ISCOM matrix may facilitate cytosolic introduction of MV-F. In addition, we have obtained evidence for nonendosomal processing of de novo synthesized MV-F (Figs. 8 and 9). These results and those of others (46, 55, 56) apparently contradict recent findings, showing that stable class II–peptide complex formation is a chloroquine-sensitive exogenous antigen-dependent process only occurring with class II α/β dimers free from the invariant chain (Ii) (16, 18). Recent evidence has shown that the biosynthetic route of class II molecules intersects the endosomal/lysosomal pathway of protein degradation (15). Association of peptides, resulting from endosomal processing, with nascent class II molecules may occur in a low pH lysosomal compartment (16). In this compartment, dissociation of the Ii from class II α/β heterodimers may also occur (77), thereby allowing the formation of stable class II–peptide complexes. In our case, it could be postulated that MV-F class II presentable peptides, derived from the cytosol, can enter the ER compartment. Assuming high affinity for certain class II molecules (i.e., HLA-DRw53), such peptides could charge nascent class II molecules under conditions that would otherwise prevent the formation of stable peptide–class II complexes, i.e., neutral pH and presence of Ii. Preliminary results of binding studies have indeed revealed evidence for high affinity peptide binding to HLA-DRw53 bearing B-LCL, using a 10-mer peptide spanning the minimal length of the JG-F94 epitope (R. van Binnendijk, unpublished observations). An alternative explanation would be that after cytosolic processing of MV-F, peptides could combine with class II molecules in a lysosomal compartment where they have arrived, i.e., by a process of autophagy (78).

The assumptions that processing of de novo synthesized transmembrane glycoproteins occurs in the cytosol of APC, as apparently is the case for de novo synthesized nontransmembrane proteins, merely rest on observations that the conversion of such proteins into cytosolic versions by, e.g., deleting the leader-insertion sequences of the genes encoding them, does not affect their presentation by class I molecules (25, 26). In this report, we have shown additional evidence highly suggestive of cytosolic processing of a de novo synthesized transmembrane glycoprotein, i.e., MV-F. FACS® analysis showed that MV-F is expressed on the surface of both MV-infected C1R-B27 and T2-B27 (Fig. 7). However, in contrast to C1R-B27, which do present MV-F to HLA-B27-restricted CTL, T2-B27 cells do not. In a recent report by Anderson et al. (75), it was shown that T2 cells do present a de novo synthesized peptide, provided it was translocated in the ER during biosynthesis. Collectively, these data show that, whereas in T2 cells the secretory pathway for de novo synthesized membrane glycoproteins and class I peptide complexes is functional, proteolytic degradation of ER-synthesized proteins to class I presentable peptides apparently does not occur in the ER or Golgi complex of T2 cells. These results could be explained if one assumes that residence in the cytosol or in another as yet unidentified compartment is required for membrane proteins to be processed to class I presentable peptides.

In conclusion, our studies demonstrate two novel routes for presentation of a type I transmembrane glycoprotein: presentation of exogenously added antigen to class I, and a nonendosomal presentation of either exogenously added or endogenously synthesized antigen to class II molecules. In addition, our findings indicate that processing of type 1 transmembrane glycoproteins to class I presentable peptides occurs in the cytosol of APC.

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