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Epstein–Barr Virus Infection of CR2-Transfected Epithelial Cells Reveals the Presence of MHC Class II on the Virion

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Epithelial cell lines transfected with the Epstein–Barr virus (EBV) receptor CR2 are susceptible to infection by EBV. Following infection with certain EBV strains we found that these cells became positive for MHC class II. The class II was confirmed as being of viral and not target cell origin by immunostaining with HLA-specific monoclonal antibodies. Electron microscopic immunogold staining confirmed the presence of MHC class II on the surface of the virion. While some MHC class I was also found on the EB virion, other cell surface molecules were absent. Dual color immunofluorescence and confocal microscopy analysis demonstrated colocalization of class II with EBV-encoded structural proteins (MA and VCA) in infected epithelial cells. However, preincubation of EBV with antibodies against either MHC class I or MHC class II failed to affect either EBV binding or EBV infection. The presence of MHC on the surface of the EB virion may be a consequence of the intracellular pathways through which productive virus exits from the cell and may influence the target cell tropism of EBV.

INTRODUCTION

Epstein–Barr virus (EBV) is implicated in the pathogenesis of a number of human malignancies including Burkitt's lymphoma (BL), B cell lymphomas seen in immunosuppressed individuals, and the epithelial malignancy nasopharyngeal carcinoma (Miller, 1990). EBV infects two target tissues *in vivo:* B lymphocytes where infection is largely nonproductive (Nilsson *et al.,* 1971) and stratified squamous epithelium in which virus replication occurs (Sixbey *et al.,* 1984; Greenspan *et al.,* 1985). The interaction of EBV with B cells is initiated through virus binding to the B cell surface molecule CR2 (Fingeroth *et al.,* 1984) and, *in vitro,* results in virus-induced growth transformation. Conversely, EBV infection of epithelium remains poorly understood, reflecting the lack of an appropriate cell culture model. We have previously described a culture system in which human epithelial cell lines expressing a transfected EBV receptor (CR2) gene are rendered susceptible to EBV infection (Li *et al.,* 1992). This system highlighted significant differences between certain EBV strains in their ability to infect epithelial cells that did not correlate with their B cell transforming activity. This suggests that CR2 is not the only prerequisite for infection and that other molecules may influence EBV fusion and entry into epithelial cells.

The external envelope of EBV is dominated by the virus-encoded glycoprotein gp350/220 through which virus binding to the B lymphocyte receptor CR2 is mediated (Fingeroth *et al.,* 1984; Nemerow *et al.,* 1985). Another viral glycoprotein, gp85, is also present on the viral envelope and, although a relatively minor virus component, it is thought to be critical for fusion between the virus and the cell membrane (Miller and Hutt-Fletcher, 1988; Haddad and Hutt-Fletcher, 1989). The glycoprotein gp42, encoded by the BZLF2 open reading frame, has recently been reported to form a complex with gp85 and gp25 (BKRF2); this glycoprotein complex appears be involved in EBV fusion and may influence virus tropism (Li *et al.,* 1995). Studies of other enveloped viruses have suggested that virus-encoded proteins are not the only constituents of the envelope; host-cell proteins have previously been shown to be selectively represented on virus particles and may influence the infectious nature of the virion (Gelderbolm *et al.,* 1987; Henderson *et al.,* 1987). Thus, in this study we examined whether host cell-derived molecules are carried on EBV and whether this influences the efficiency with which certain viral strains infect CR2-transfected epithelial cells.

MATERIALS AND METHODS

Cell lines

SVK-CR2 (an SV40-transformed keratinocyte cell line), SCC12F-CR2 (a squamous cell carcinoma line), and BHK-CR2 were previously transfected with the EBV receptor CR2/CD21 (Li *et al.,* 1992). SVK-CR2 were maintained on

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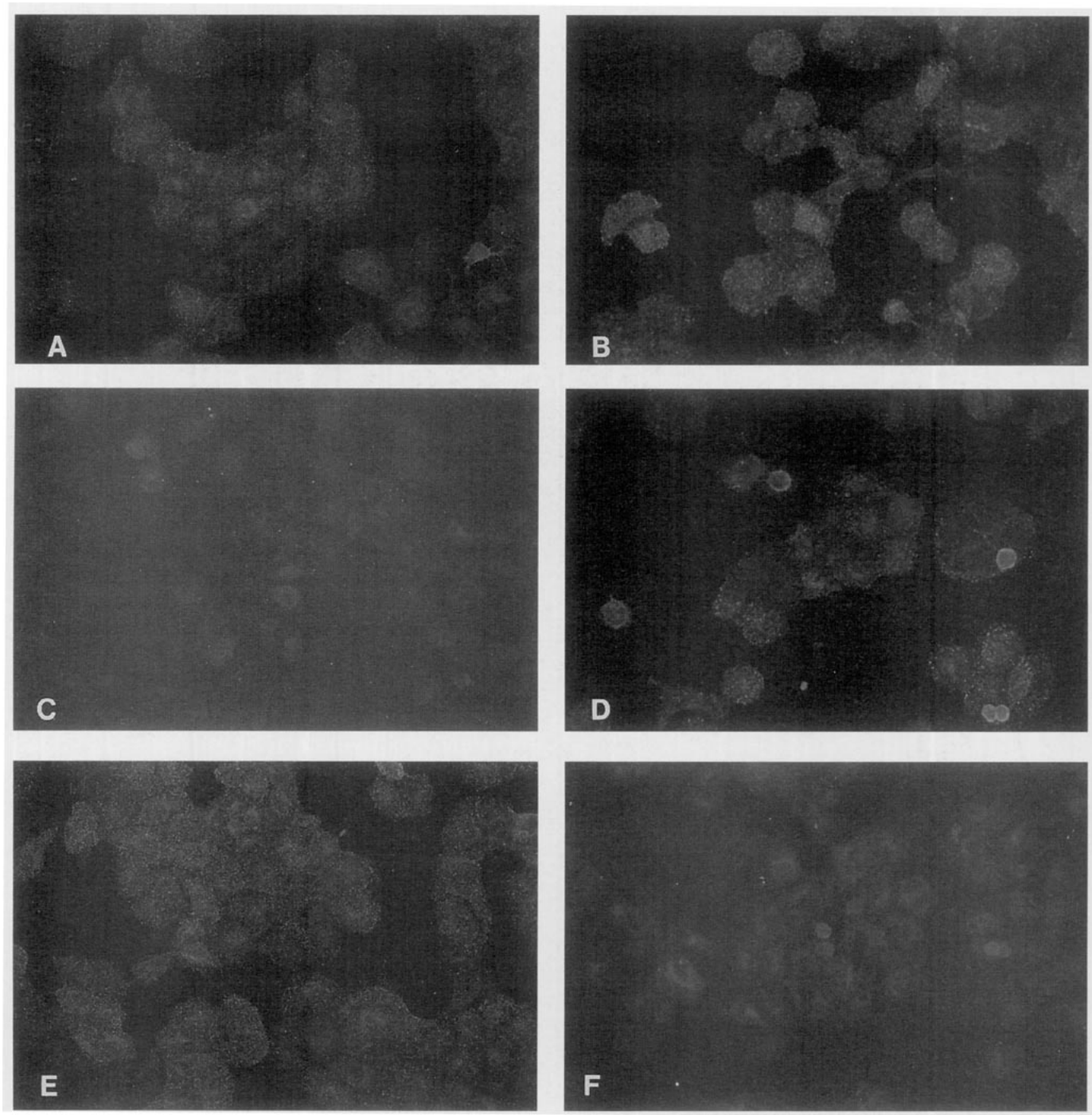

FIG. 1. Detection of MHC class II on Akata and AG876 virus-exposed epithelial cells. SVK-CR2 cells grown on glass slides were incubated with either (A, B, C) Akata or (D, E, F) AG876 strains of EBV at 4° for 1 hr and subsequently fixed in 1% paraformaldehyde. Positive punctate staining for MHC class II was seen on cells with bound virus using the anti-MHC class II MAbs L243 (A, D) and BU26 (B, E) but no staining was detected using the anti-MHC invariant chain MAb LN2 (C, F). SVK parental cells incubated with virus stained negative using the L243 and BU26 MAbs (not shown). Magnification, ×165.

Joklik's cell growth medium supplemented with 10% FCS, 2 m*M* glutamine, 0.4 μg/ml hydrocortisone, 10 ng/ml cholera toxin, and antibiotics. SCC12F-CR2 were maintained with γ-irradiated fibroblast feeder layers on DME:F12 (3:1) growth medium supplemented with 10% FCS, 2 m*M* glutamine, 0.4 μg/ml hydrocortisone, and antibiotics. BHK-CR2 were maintained on DME growth medium supplemented with 10% FCS, 2 m*M* glutamine, and antibiotics.

Antibodies

The following HLA-DR antibodies were used: L243, anti-MHC class II β chain (Lampson and Levy, 1980);

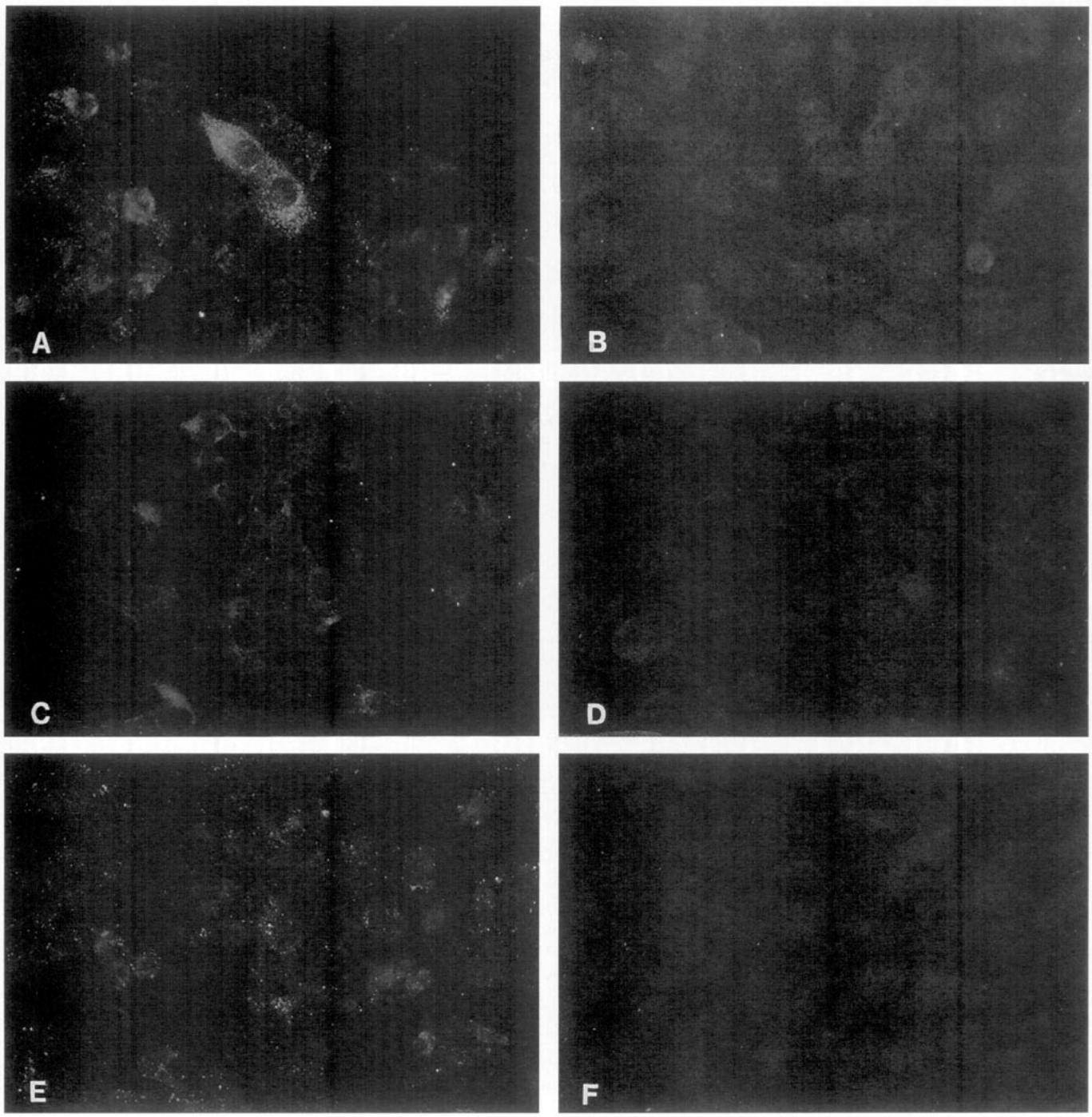

FIG. 2. Detection of MHC class I and MHC class II in BHK-CR2 cells following incubation with EBV. (A, C, E) BHK-CR2 and (B, D, F) BHK parental cells were grown on glass slides prior to incubation with Akata strain EBV, as described under Materials and Methods and were fixed for staining at Day 3 postincubation. BHK-CR2 cells incubated with Akata virus stained positive for MHC class II (A, L243 MAb), MHC class I (C, W6/32 MAb), and gp350/220 (E, BOS-1 MAb). BHK parental cells incubated with virus stained negative using the same MAbs (B, L243 MAb; D, W6/32 MAb; F, BOS-1 MAb). Magnification, ×165.

BU26, anti-MHC class II β chain (Birmingham University, Dept. Immunology); 16-23 and Hu-30, anti-MHC class II specific for HLA-DR 3, 52a and HLA-DR 1, 2, 7, 9, 10, 103, respectively. In addition, MAb W6/32 recognizing HLA-A, -B, and -C locus products (Parham *et al.*, 1979) and the anti-invariant chain MAb LN2 (Epstein *et al.*, 1984) were used. Antibodies used to detect structural components of EBV were the anti-160-kDa VCA MAb F-323 (Sera-Lab) and the anti-MA (gp350) MAb BOS-1 (Nemerow and Cooper, 1984).

Immunofluorescence staining

Cells grown on glass slides were washed in PBS and fixed in −20° acetone unless stated otherwise. After rehydrating in PBS for 5 min, primary antibody was added, diluted to working concentration in PBS. After 1 hr of incubation at 37°, the slides were washed in PBS and stained using FITC-conjugated IgG (Sigma). For dual color immunofluorescence, a mix of primary MAbs was applied and second-step antibodies [FITC−IgG1 and biotin−IgG2a (Southern Biotechnology Associates, Inc.)] were mixed together to give a final concentration of 1:50. Following a further hour of incubation slides were finally incubated with streptavidin−Texas Red (Southern Biotechnology Associates, Inc.) at a dilution of 1:25 for 20 min at 37°. Slides were washed thoroughly in PBS before mounting with anti-fadant mountant DABCO (1,4-diazabicyclo-[2,2,2]-octane) (Johnson *et al.*, 1972).

Immunogold labeling for transmission electron microscopy

SVK-CR2 cells grown on glass coverslips were washed in PBS and incubated with 50 μl 100× Akata EBV for 1 hr at 4°. Cells were washed twice in washing buffer [0.8% bovine serum albumin (BSA), 0.1% fish gelatin in PBS] and incubated for 30 min at 4° with blocking buffer (0.8% BSA, 0.1% fish gelatin, 5% FCS in PBS). Primary antibody/sera diluted in washing buffer was then applied for 1 hr at 4°, followed by colloidal gold-labeled second-step Ig [either 5 nm goat anti-human gold-labeled IgG (Bio-Rad) or 10 nm goat anti-mouse gold-labeled IgG (Amersham)]. Cells were fixed in 2.5% glutaraldehyde for 1 hr, followed by 1% osmium tetroxide fixation for a further hour. Samples were dehydrated through an alcohol series and treated with propylene oxide for 30 min. A 1:1 mixture of propylene oxide/resin was used for embedding and samples were placed under vacuum for 20 min. Resin was polymerized at 60° overnight and ultrathin sections were mounted on copper grids and stained with 2% uranyl acetate and Reynolds lead citrate.

Cytofluorimetric analysis

Cytofluorimetric analysis of bound EBV was performed on SVK-CR2 cells as previously described (Dawson *et al.*, 1990).

Infection of CR2-transfected cells with EBV

Monolayer cultures at 70−80% confluency were washed twice in PBS and incubated with EBV [×100 filtered cultured supernatant from induced Akata-BL/untreated AG876 cell cultures/Akata-marmoset cultures treated with 100 μM butyrate, 5×10^{-8} M TPA (12-*O*-tetradecanoyl phorbol-13-acetate) for 4 days] for 4 hr at 37°, then fed again with normal growth medium. Cultures were refed with fresh medium every 2−3 days. After 4 days, cell smears were made and stained for EBNA1 by anticomplement immunofluorescence (ACIF) using a monospecific human serum AMo (Reedman and Klein, 1973). To investigate the effect of preincubating virus with anti-MHC antibodies, equal volumes of virus preparation and MAb were incubated with agitation at 37° for 1 hr and this virus/MAb mixture was used in virus binding or infection assays.

TABLE 1

Specificity of MHC Class II MAbs on Infected SVK-CR2 Cells

	MHC class II antibody			
Cell line	16.23	Hu-30	L243	LN2
SVK-CR2	−	−	−	−
SVK-CR2 + IFNγ[a]	+	+	+	+
AR LCL	+	+	+	+
SVK-CR2 + Akata EBV	−	−	+	−

MHC Class II Typing of Cell Lines and Specificity of MAbs

Akata-BL	DR4 DR14
AR (AR LCL and SVK-CR2)	DR2 DR3
MAb 16.23	DR3 DR52a
MAb Hu-30	DR1, 2, 7, 9, 10, 103

[a] SVK-CR2 cells were treated with 15 ng/ml IFNγ for 3 days.

RESULTS

Detection of MHC class II in EBV-infected SVK-CR2 cells

Following incubation with Akata and AG876 strains of EBV, SVK-CR2 cells became positive for MHC class II, as detected by indirect immunofluorescence using the anti-MHC class II MAbs L243 and BU26 (Fig. 1). MHC class II was detectable in cells as soon as 30 min postinfection and levels peaked between Day 1 and Day 2 postinfection. Although small amounts of class II were still visible at Day 4 postinfection, little staining was seen at Day 8 postinfection (data not shown). No staining was observed with the anti-invariant chain MAb LN2, suggesting that the MHC class II detected after EBV infection is not the result of *de novo* class II synthesis. The timing of the appearance of class II was similar to that observed for other virus-encoded components of the virion (MA and VCA) (data not shown). MHC class II staining was not observed on mock-infected SVK-CR2 controls. Similar patterns of MHC class II staining were observed following incubation with Akata in another CR2-transfected epithelial cell line, SCC12F-CR2 (data not shown).

Confirmation of the viral source of MHC class II in EBV-infected SVK-CR2 cells

The source of MHC class II observed following EBV infection of SVK-CR2 cells could be (i) cellular, induced

in response to virus binding/internalization, or (ii) from the virion itself. To investigate these possibilities, MAbs specific for the HLA-DR type of SVK (DR2 DR3) were used to determine the source of MHC class II in EBV-infected SVK-CR2 cells (Table 1). The MAb 16.23, specific for DR3, 52a, and MAb Hu-30, specific for DR 1, 2, 7, 9, 10, 103, were selected to detect MHC class II of SVK origin (DR2, DR3). As expected, these specific MAbs did not react with MHC class II-positive Akata BL cells (DR4, DR14) (data not shown). Uninfected SVK-CR2 cells did not react with a general HLA-DR β chain-specific MAb (L243). SVK-CR2 cells treated with IFNγ prior to staining reacted with L243 and MAbs 16.23 and Hu-30, specific for HLA-DR of SVK origin. Additionally, an EBV-transformed lymphoblastoid cell line from the same human donor as SVK stained positive with MAbs L243, 16.23, and Hu-30. SVK-CR2 cells infected with Akata EBV stained positive for MHC class II only with the general anti-β-chain L243 but no staining was observed using the HLA-DR-specific MAbs, implying that the class II present on infected cells was delivered by EBV.

Confirmation that EBV was the source of MHC class II in infected SVK-CR2 cells was observed when CR2-transfected BHK cells, which bind EBV but do not sustain virus infection as determined by ACIF, were incubated with EBV. Following exposure to Akata virus, MHC class II was detected in BHK-CR2 cells using the MAb L243 (Fig. 2). MHC class II in these cells was confirmed to be of viral and not BHK origin, as the anti-human MHC class II MAb L243 does not cross-react with endogenous hamster MHC class II in BHK cells pretreated with IFNγ (data not shown). BHK-CR2 cells incubated with Akata EBV also showed low levels of reactivity when stained using an anti-class I MAb, W6/32 (Fig. 2). However, MAbs against ICAM-1, LFA-1, CD19, and CD23 did not react with Akata/AG876-treated BHK-CR2 cells, suggesting that these molecules are not carried on the virion.

MHC class II distribution in SVK-CR2 cells following infection

A more detailed analysis of MHC class II localization in EBV-infected SVK-CR2 cells was obtained using confocal microscopy. From 30 min postinfection, MHC class II staining was observed in discrete patterns within cells. Taking a series of optical sections through the depth of a cell (Z series) illustrated a change in the pattern of MHC class II staining with increasing depth. As seen in Fig. 3, MHC class II was present in ring-like structures near the upper surface of the cell. These circular structures became smaller in diameter and more intense in the level of MHC class II staining with increasing depth through the cell.

Detection of MHC class II on EBV by electron microscopy (EM)

SVK-CR2 cells were used in binding assays to trap virus particles and then stained with an EBV-antibody-positive human serum, EE, to detect gp350 or with anti-MHC class II mouse MAbs. Following incubation with anti-human and anti-mouse colloidal gold-labeled IgG, cells were processed for EM. Virus particles of approximately 100 nm bound to the cell surface were confirmed to be EBV by immunogold labeling for gp350/220, using the human serum EE (Fig. 4). No gold labeling was visible using a control EBV-negative human serum, CD (not shown). The anti-MHC class II MAb BU26 gave a similar pattern of gold particle distribution on the virion (Fig. 4). While a second MHC class II MAb, L243, also showed some staining on EB virions (data not shown) the level of gold labeling was significantly less than that detected for the major envelope glycoprotein gp350/220. A control anti-invariant chain MAb, LN2, showed no reactivity against bound EBV particles (data not shown).

Colocalization of class II and gp350/VCA in EBV-infected SVK-CR2 cells

Dual color immunofluorescence was used to determine the extent of colocalization of MHC class II and gp350 in Akata-infected SVK-CR2 cells. At Day 1 postinfection, MHC class II (BU26) gave a more dispersed pattern of staining than gp350 (BOS-1) but areas of colocalization were clearly evident (Figs. 5A and 5B). Similar patterns of granular staining, localized to discrete areas in the cell, were observed in double staining for MHC class II and VCA (Fig. 5C). Using confocal microscopy to merge VCA and MHC class II staining illustrates that class II staining was highly coincident with that of VCA (Fig. 5D). X/Z confocal sections giving a side-on view through each cell demonstrated the extent of colocalization between MHC class II and VCA with depth through the cell (Fig. 6). The regular "funnel" pattern of staining of MHC class II observed after infection of SVK-CR2 cells was not seen in similar side-on sections of Akata-treated BHK-CR2 cells which were refractory to EBV infection; instead class II staining was present through the depth of the cell in a more irregular pattern (Fig. 6).

Heterogeneity in levels of MHC class II on different viral strains

Although SVK-CR2 cells become positive for MHC class II following infection with Akata and AG876 strains of EBV, the same effect was not observed using the marmoset-derived B95.8 strain which has previously been shown to be unable to establish infection in SVK-CR2 cells (Li *et al.*, 1992). However, incubation of SVK-CR2 cells with B95.8 virus resulted in the detection of gp350/220 and VCA in the cells but class II was not observed; these data suggest that B95.8 virus is internalized even though infection is not established. The anti-human MHC class II MAb L243 was shown to cross-react with MHC class II of marmoset origin, as the class II-positive B95.8 virus-producing marmoset B cell line

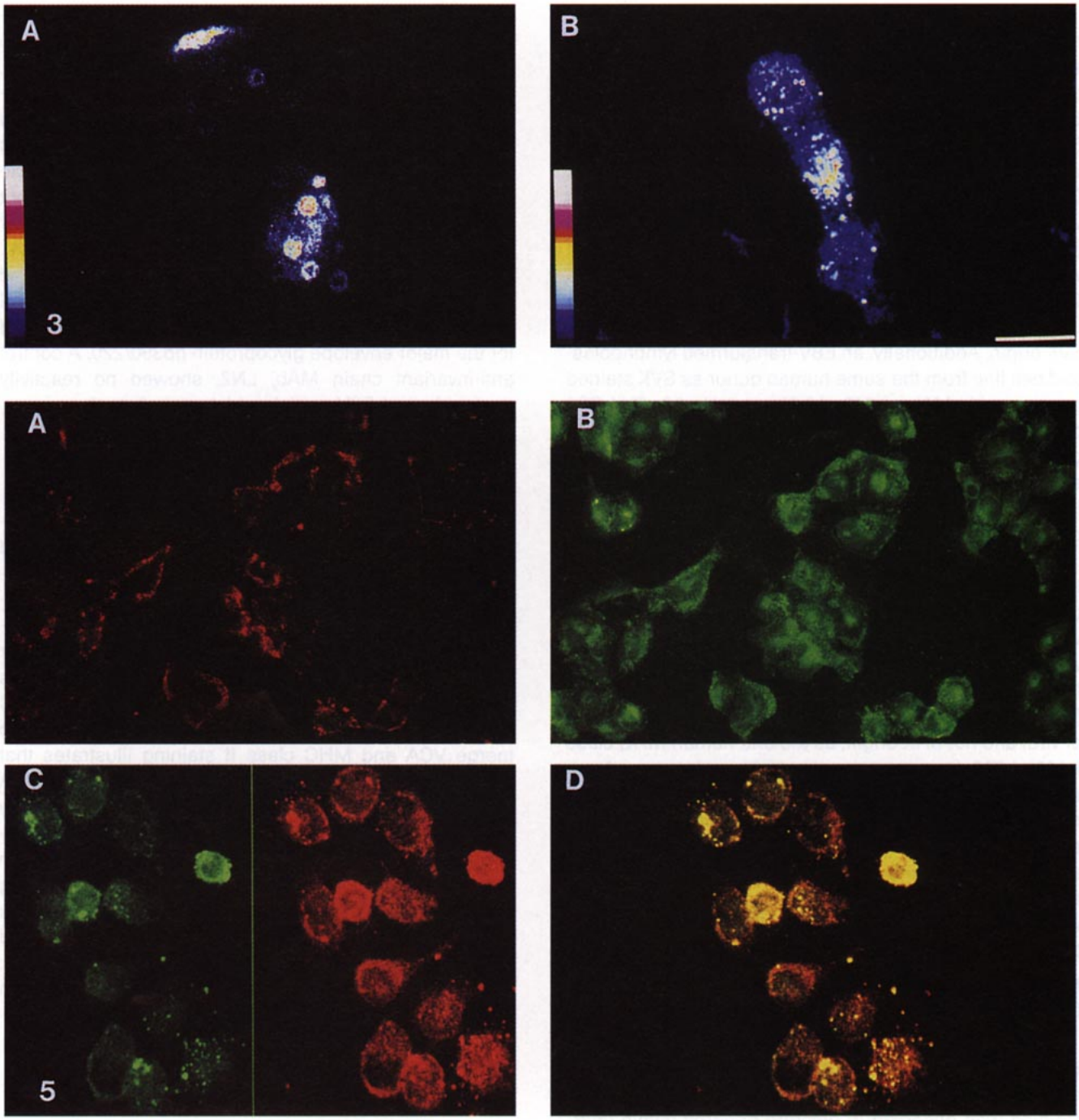

FIG. 3. MHC class II staining of SVK-CR2 cells detected by confocal microscopy. SVK-CR2 cells grown on glass microdot slides were infected with Akata EBV as described under Materials and Methods. Slides were stained for MHC class II using MAb L243 and were examined using confocal microscopy. Z series analysis was performed, viewing serial sections through the entire depth of the cell. (A) A horizontal section through two typical cells near the upper surface shows MHC class II staining in infected cells, appearing in ring-like patterns while (B) toward the lower surface of the same cells the patterns of class II staining change in shape and appear more intense (lower magnification). The color gradient (purple to white) reflects the intensity of staining (low to high). Bar, 25 μM.

FIG. 5. Dual-color immunostaining for gp350220/VCA and MHC class II in EBV-infected SVK-CR2 cells. SVK-CR2 cells were infected with EBV as described under Materials and Methods. At Day 1 postinfection, cells were fixed in −20° acetone and were double-stained either for (A) gp350/220 (MAb BOS-1, IgG_{2a}, red fluorescence) and (B) MHC class II (MAb BU26, IgG_1, green fluorescence) or (C, left) VCA (MAb F-323, IgG1, green fluorescence) and (C, right) MHC class II (MAb L243, IgG2a, red fluorescence). The yellow fluorescence seen in D represents coincident VCA and MHC class II staining detected by confocal microscopy. Magnification, ×160.

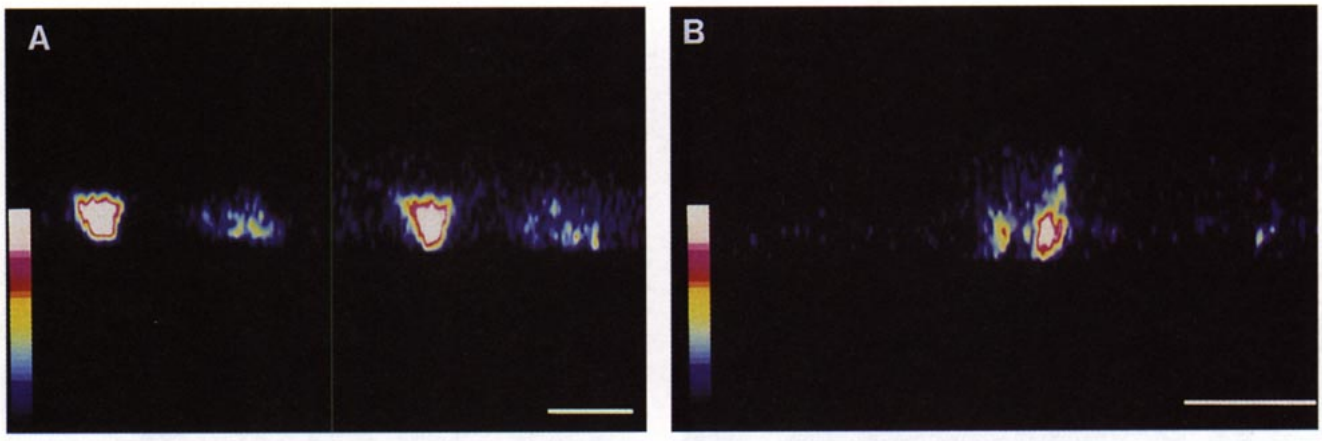

FIG. 6. Patterns of MHC class II staining in SVK-CR2 and BHK-CR2 cells incubated with EBV. CR2-transfected cells were incubated with EBV as described under Materials and Methods and examined by confocal microscopy. X/Z sections giving a "side-on" view of single cells show class II/VCA staining through the depth of a typical cell. In Akata-infected SVK-CR2 cells, (A, left) class II staining (MAb L243) appeared in a regular pattern and was highly coincident with (A, right) VCA staining (MAb F-323). (B) However, patterns of class II staining in BHK-CR2 cells incubated with EBV were of a more irregular nature. The color gradient (purple to white) reflects the intensity of staining (low to high). Bar, 1 μm.

stained positive using this MAb (data not shown). Subsequent experiments using SVK-CR2 cells in binding assays to trap virus particles revealed that although B95.8 virus carries MHC class II, it appears to be present at significantly lower levels than found on Akata virus (Fig. 7). Lower levels of class II on B95.8 virus may explain the apparent absence of class II in virus-exposed cells but does not appear to be a consequence of the marmoset origin of this virus strain, since other marmoset-derived strains of EBV (Akata-Marmoset, Fig. 7, and M-ABA, not shown) carry comparatively high levels of MHC class II.

Effect of preincubating EBV with anti-class I/II antibodies on virus binding and infection

The possible functional significance of the presence of MHC class I and II on the EB virion was investigated in a series of experiments. Any involvement of MHC molecules in virus binding and infection may be detected by blocking this function through preincubation of the virus with anti-MHC class I/II MAbs. Equal volumes of concentrated Akata virus and either a class II antibody (L243) or a control antibody BU12 (anti-CD19) were preincubated at 37° for 1 hr and the virus/antibody mixture was then used in a virus binding assay with SVK-CR2 cells. Binding of MHC class II MAb to the EBV virion was confirmed using an FITC-labeled anti-mouse IgG to stain cells after virus binding (Fig. 8). This interaction was specific as significant levels of binding were not observed using a control MAb. Akata virus preincubated with MHC class II antibodies bound to SVK-CR2 cells with no detectable change in the levels of bound EBV (Fig. 8). In a further series of experiments to determine a possible role for MHC molecules in infection, Akata virus was preincubated with MAbs against MHC class I and class II and then used to infect SVK-CR2 cells. At Day 3 postinfection, no change in the level of infection, as detected by ACIF staining for EBNA1, was observed as a result of preincubation of virus with the MHC-specific MAbs (Table 2).

DISCUSSION

Results from this study suggest that molecules originating from the host cell are present on the EB virion. Soon after infection with EBV, MHC class II was detected in CR2-transfected epithelial cells by indirect immunofluorescence using a number of anti-class II MAbs. MHC class I was also detected to a lesser extent in BHK-CR2 cells incubated with EBV. The pattern of staining for MHC class II in infected cells was similar to gp350/220 staining and a high degree of colocalization of MHC class II and VCA/gp350/220 was observed in infected cells by dual color immunofluorescent staining. To demonstrate that these observations were not simply a response of the target cell to EBV infection, antibodies specific for the HLA-DR type of SVK-CR2 were used in immunostaining and these confirmed the viral source of MHC class II.

The detection of MHC class I and II on the EB virion has not been previously described. However, the presence of MHC class II on virions is not restricted to EBV, having also been documented for HIV (Gelderbolm *et al.*, 1987; Schols *et al.*, 1992) and for HTLV (Henderson *et al.*, 1987). Other viruses carrying host cell molecules include Friend leukemia virus (Chen and Lilly, 1979), avian leukosis virus (Young *et al.*, 1990), vesicular stomatitis virus (Hecht and Summers, 1976; Calafat *et al.*, 1983), murine leukemia virus (Calafat *et al.*, 1983), Sindbis virus (Strauss, 1978), and influenza virus (Holland and Kiehn, 1970). However, the mechanism and implications of such virus/host cell molecule interactions are at present unclear.

In searching for a functional role for MHC class II in

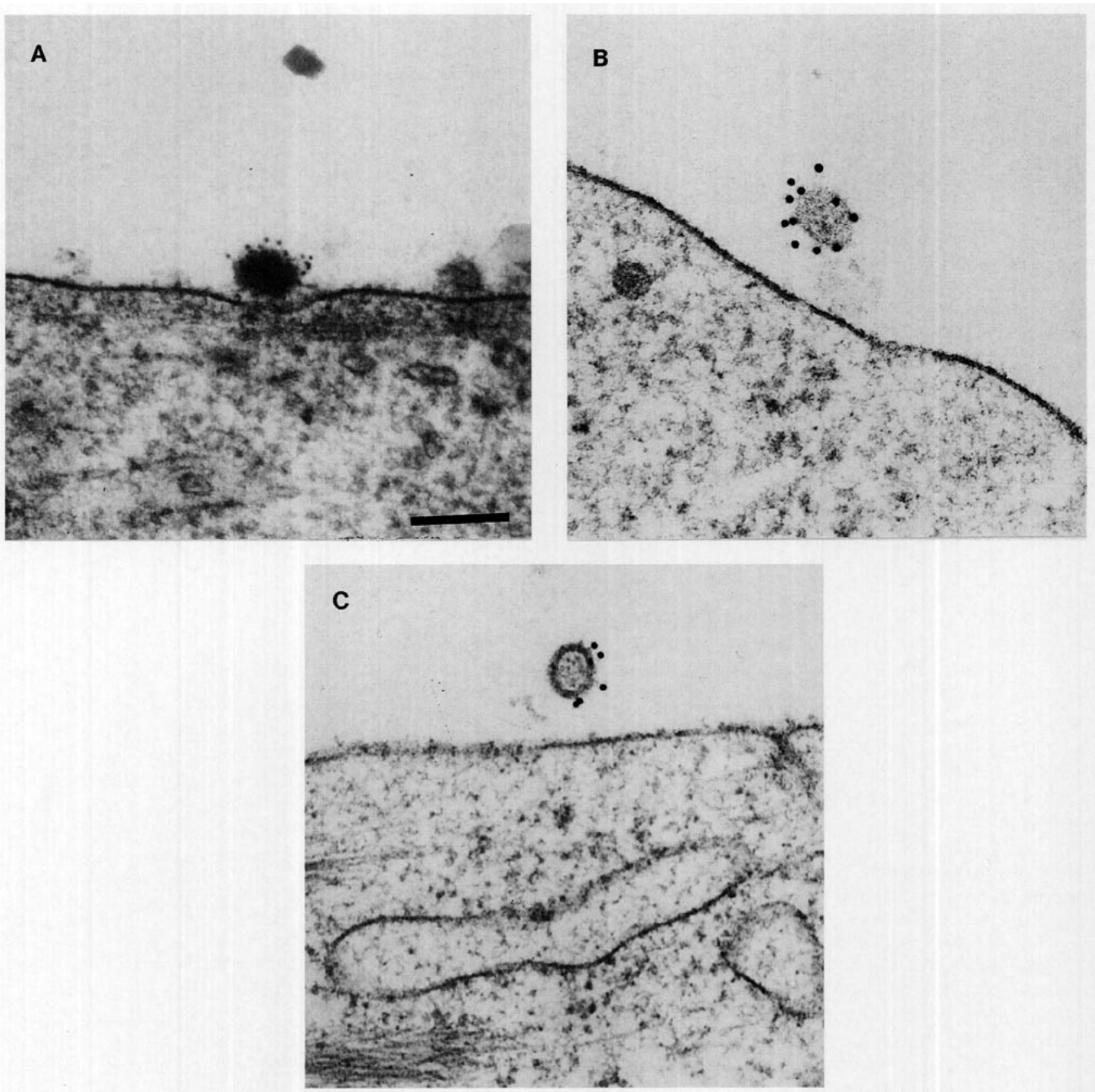

FIG. 4. Immunoelectron microscopy of EBV virions bound to SVK-CR2 cells. SVK-CR2 cells grown on glass coverslips were incubated with Akata EBV and prepared for immunogold labeling as described under Materials and Methods. (A) Particles of approximately 100 nm bound to the cell surface were confirmed to be EBV by staining with the EBV-antibody-positive human serum EE. (B, C) MHC class II was detected on virions using the mouse MAb BU26. No staining was visible on virions or cells using the anti-invariant chain MAb LN2 (not shown). Bar, 200 nm.

EBV binding, it is interesting to consider previous studies linking EBV and MHC class II with infectivity. Reisert *et al.* (1985) proposed a role for class II in EBV binding by demonstrating that EBV infection could be blocked by incubation of Jijoye-BL cells with the anti-class II MAb L243. Since incubation of Jijoye with three other anti-class II MAbs had no effect, the blocking action of L243 may depend on the antibody binding to a specific epitope on MHC class II to cause a conformational change. A study by Di Renzo *et al.* (1993) also linked class II with EBV infection; a series of BL cell lines induced into virus replication were found to display increased levels of class II, suggesting that MHC class II may be required for some aspect of the EBV lytic cycle.

As the exact pathways for intracellular trafficking of maturing EB virions in late lytic infection are still disputed, it is not immediately apparent how EBV could incorporate host molecules such as MHC class II into

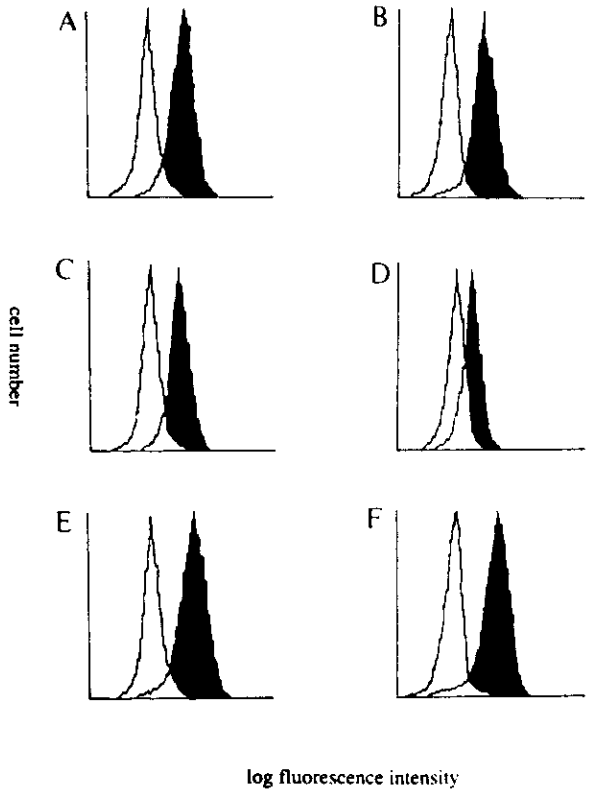

FIG. 7. Heterogeneity in levels of MHC class II present on different EBV strains. Cytofluorimetric analysis was used to detect EBV binding on SVK-CR2 cells (A, B) Akata strain, (C, D) B95.8 strain, (E, F) Akata-Marmoset); shaded areas of profiles show staining of cells with bound virus and unshaded areas show background staining in the absence of virus. Mean fluorescence intensity (MFI) and percentage positive cells are expressed as values above background staining. Virus binding was confirmed by incubating cells with an EBV antibody-positive human serum EE (A, C, E). Cells with bound virus stained positive for MHC class II using the MAb L243 (B, D, F). While class II was present on all three virus strains, B95.8 virus (D; 59% cells positive, MFI 19) appeared to carry less in comparison to Akata (B; 89% cells positive, MFI 43) and another marmoset-derived EBV strain, Akata-Marmoset (F; 91% cells positive, MFI 55).

its structure. However, a number of recent reports describing MHC class II assembly and peptide loading in antigen presenting cells (APCs) have revealed some possible routes by which EBV could acquire class II from the host cell. Gong and Kieff (1990) previously proposed two pathways for EBV envelopment and egress in B95.8 cells. Nucleocapsids assembled in the nucleus bud through the nuclear membrane and are released into the cytoplasm. A definitive envelope is acquired either by (i) budding through the plasma membrane which contains Golgi-processed viral glycoproteins or by (ii) budding into post-Golgi vesicles where the virus can acquire gp350/220 and other viral glycoproteins before exocytosis. Gong and Kieff (1990) have suggested that the first pathway is the most likely route, as naked nucleocapsids can be detected by EM in the cytoplasm budding through the plasma membrane. Although no firm evidence exists by EM for the second pathway, involving fusion of EBV into vesicles, EBV has been detected in cytoplasmic membrane structures which could be part of the cell secretory pathway (Gong and Kieff, 1990). Further evidence for this route has been demonstrated in infected epithelial cells from oral hairy leukoplakia (HL), a focus of EBV replication occurring in tongue epithelium of immunosuppressed patients (Rabanus *et al.*, 1990). In the upper epithelial layers of HL lesions, EM studies have suggested that gp350/220 is processed and undergoes its

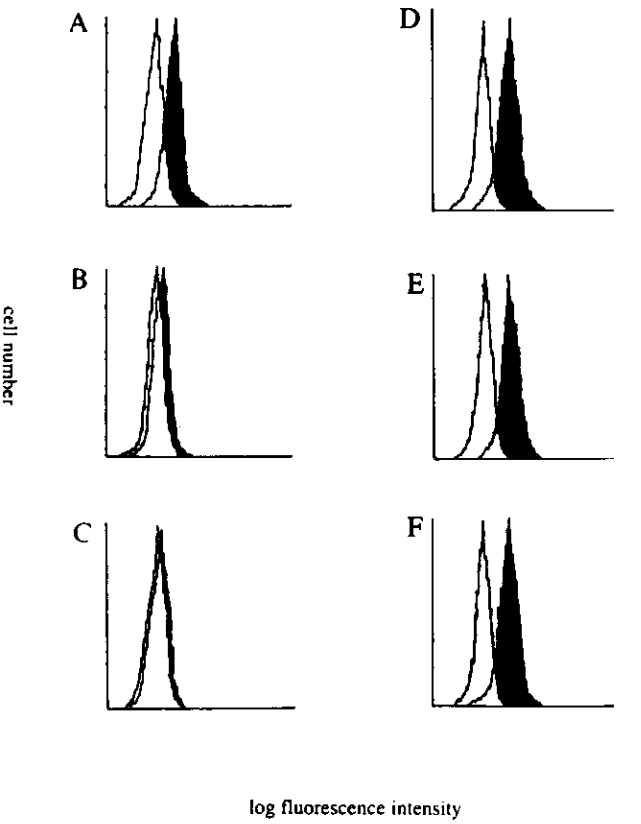

FIG. 8. Effect of preincubating virus with MHC class II and class I MAbs on virus binding. Akata virus was preincubated with various MAbs and was then added to SVK-CR2 cells. The shaded area of each profile shows staining detected using virus/MAb mixtures; staining of cells incubated with MAb L243 but in the absence of virus is seen in the unshaded areas of the profiles. Mean fluorescence intensity (MFI) and percentage positive cells are expressed as values above background staining. (A, B, C) Confirmation of an interaction between EBV and L243 was obtained by staining cells with bound virus using anti-mouse IgG only. (A) Seventy-eight percent of cells (MFI 24) exposed to virus preincubated with L243 stained positive for the presence of mouse Ig. (B) In comparison, only background levels of staining were detected with virus preincubated with a control anti-CD19 MAb BU12 (36% cells positive, MFI 12) and also with (C) normal growth medium (3% cells positive, MFI 2). (D, E, F) The effect of preincubating virus on its ability to bind to SVK-CR2 cells was assessed by staining for bound EBV with the EBV-antibody-positive human serum EE. (D) Virus preincubated with the MHC class II MAb L243 bound to cells with no detectable change in the levels of bound EBV (88% cells positive, MFI 34) when compared (E) to binding after preincubation with a control MAb BU12 (89% cells positive, MFI 33) or (F) with growth medium (88% cells positive, MFI 33).

TABLE 2

Effect of Preincubating EBV with MHC Class I/II MAbs on EBV Infection of SVK-CR2 Cells

Preincubating MAb		% EBNA1-positive cells
None (growth medium)		41
L243	anti-MHC class II	47
BU-26	anti-MHC class II	51
W6/32	anti-MHC class I	44
16.23[a]	anti-MHC class II	47

Note. Virus was preincubated with MAbs as described under Materials and Methods and cells were harvested at Day 4 postinfection, fixed in −20° methanol:acetone (1:1) and stained for EBNA1 by ACIF using the monospecific human serum AMo.

[a] MAb 16.23 is specific for HLA-DR type of SVK origin only.

final modification in the Golgi complex of virus-producing cells. Furthermore, mature virions were found to be released from the cell by fusion of Golgi-associated secretory vesicles with the plasma membrane. These observations suggest that EBV follows the same physiological pathway as cellular proteins during their final processing and egress. In this context, the recent identification of a unique endosome-related subcellular compartment in APCs where peptide loading to MHC class II molecules occurs is of particular interest (Amigorena *et al.,* 1994; Qiu *et al.,* 1994; Tulp *et al.,* 1994; West *et al.,* 1994). In this compartment for peptide loading (CPL), invariant chain-free class II molecules are thought to be held in a receptive state, or floppy conformation (Qiu *et al.,* 1994), while antigens are proteolysed and loading occurs. If, during virus maturation, EBV gained access to either the CPL or an early endosome containing class II molecules, it is conceivable that class II could be incorporated into the virion. The recent observation that cellular MHC class II binds with high affinity to gp42, an EBV-encoded viral envelope glycoprotein involved in virus:cell fusion, provides a possible mechanism for specific incorporation of MHC class II into the virion (Li *et al.,* 1995; M. Spriggs, personal communication).

In this study, no evidence was found linking MHC molecules on EBV with a functional role in binding or infection. Preincubation of virus with MAbs against class I/II resulted in binding of MAb to the virus but subsequent virus binding and infection of CR2-transfected epithelial cells was not impaired. Lower levels of MHC class II on marmoset-derived B95.8 virus, in contrast with those found on Akata and AG876 strains, are especially interesting in view of the heterogeneity between EBV strains in infectivity of CR2-transfected epithelial cells (Li *et al.,* 1992), suggesting that high levels of class II on the virion may be important for efficient infection of SVK-CR2. However, other marmoset-derived virus strains, which have previously been shown to be unable to infect SVK-CR2, were found to carry levels of MHC class II similar to those found on Akata virus. Additional verification that class II is not an essential requirement for infection was obtained from NPC-KT virus which is produced from an MHC class II-negative human epithelial cell line and has been shown to infect SVK-CR2 cells (Knox and Young, unpublished observations).

Although results from this study have suggested that MHC class II on the EB virion is not directly essential for infection, it may have a more subtle role in establishing infection in the host. The recent observations concerning the interaction between MHC class II and gp42 (M. Spriggs, personal communication) point to a possible role for class II in either virus assembly and/or postbinding events necessary for efficient infection. Furthermore, host cell molecules may be used by the virus in a subtle strategy of immune evasion. Most individuals become infected with EBV in early childhood and EBV establishes a lifelong asymptomatic carrier state kept in check by cytotoxic T lymphocytes. While the mechanism for EBV persistence in the body is disputed, it is likely that a small number of EBV-infected cells replicate occasionally at some site in the body. It is possible that during progeny virion assembly MHC class II molecules are incorporated into the virus envelope by binding to gp42 and that this interaction may interfere with peptide binding to MHC class II, thereby reducing the immunogenicity of productively infected cells. Given the results of previous studies (Reisert *et al.,* 1985; Di Renzo *et al.,* 1993), the possibility that MHC class II may also facilitate EBV infection of other cell types such as T cells is particularly attractive.

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