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Original Research Communication

Redox Control of EBV Infection: Prevention by Thiol-Dependent Modulation of Functional CD21/EBV Receptor Expression

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ABSTRACT

CD21 serves as a receptor for the Epstein–Barr virus (EBV). In this report, surface expression of CD21 on B and T cells was shown to be suppressed by a thiol-antioxidant, *N*-acetylcysteine (NAC), in a dose- and time-dependent manner. In contrast, expression of other surface markers, CD25 and CD4 for T cells and CD19 and surface IgM for B cells, was not affected by NAC. When an EBV-negative B-cell line B104 was treated with NAC, the cells were not susceptible to infection with B95-8-derived EBV. The effect of NAC was shown to be irrelevant to the transcriptional levels of CD21 mRNA and the intracellular glutathione levels. Immunoprecipitation study revealed that NAC causes a loss of anti-CD21 monoclonal antibody (HB5) binding to both membrane and soluble CD21, suggesting that NAC modulates the structure of CD21. Other thiol-antioxidants, such as 2-mercaptoethanol, pyrrolidine dithiocarbamate, and glutathione, showed similar effect to NAC on CD21 expression. These results suggest the possible modulation of EBV infection *via* thiol-dependent redox control of CD21, and thiol-antioxidants may be good candidates for controlling EBV infection. Antioxid. Redox Signal. 3, 1075–1087.

INTRODUCTION

THE SECOND COMPLEMENT RECEPTOR, CR2, was first described as a receptor for C3 fragments (30, 36) and has been designated as CD21. CD21, a 145-kDa glycoprotein, is also a receptor for Epstein–Barr virus (EBV) (22) and a member of an intracellular signaling pathway, which modulates B-cell activation, growth, and differentiation (1, 12). Structurally, CD21 consists of an extracellular domain of 15 or 16 repetitive units of 60–75 amino acids, named short consensus repeats (SCR), followed by a 28-amino acid transmembrane domain and a 34-amino acid intracytoplasmic domain (1). It is also described as a receptor for interferon-α (7) and CD23 (2). CD21 is primarily expressed on B cells, human T-cell-leukemia virus type I (HTLV-1) infected T-cell lines, and to a lesser extent on normal T cells and follicular dendritic cells (47). The cellular function of CD21 has been described well for normal B

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cells, but remains largely unknown for other cell types.

EBV, a gamma human herpesvirus, is associated with a variety of lymphoproliferative and autoimmune diseases (34, 44). EBV is causally linked to lymphoid tumors of B- and T-cell origins, particularly among immunosuppressed persons who have received immunosuppressive agents after transplantation, as well as in AIDS patients, in whom there is defective regulation of EBV infection (3). The virus enters cells through its specific receptor, CD21, which binds the EBV envelope glycoprotein, gp350/220 (29, 42), and establishes latent infection in B-lymphocytes. Recent studies have shown that CD21 plays a role as an enhancing factor in human immunodeficiency virus (HIV)-I infection of EBV-infected B cells and T cells (40, 46, 48). In addition, it has been reported that EBV infects nonlymphoid cell types *in vivo via* CD21 (14, 20, 24). Thus, it is of importance to elucidate the mechanism by which CD21 expression is regulated, so that not only EBV infection but also CD21-associated secondary infection, can be controlled.

Redox regulation of cellular responses has been widely studied and accumulating evidence has suggested that cellular redox status is a critical factor in multiple cellular function, signal transduction, and gene expression (31). A number of reports have shown therapeutic effects of antioxidants in oxidative stress-associated diseases, including viral infection (19, 28, 31, 38). Thiol-antioxidants have been reported to have cytoprotective effects (6, 9). A member of thiol-antioxidants, *N*-acetylcysteine (NAC) has been used for the treatment of pulmonary diseases (16) and HIV (10, 19, 35) on the basis of its property of restoring intracellular glutathione (GSH) and neutralizing reactive oxygen species. In the present study, we have investigated the effect of NAC and other thiol-antioxidants on the expression of CD21 and assessed the susceptibility of EBV infection.

MATERIALS AND METHODS

Antibodies and reagents

Anti-CD21 mouse monoclonal antibodies (mAbs), OKB7 (Ortho Diagnostics, Raritan, NJ, U.S.A.), HB5 (American Type Culture Collection, Rockville, MD, U.S.A.) and BE5 (Serotec Ltd., Oxford, U.K.) were obtained as indicated. NAC, L-buthionine-*S*,*R*-sulfoximine (BSO), GSH, and oxidized GSH were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 2-Mercaptoethanol (2-ME), pyrrolidine dithiocarbamate (PDTC), and catalase were purchased from Nacalai Tesque (Kyoto, Japan).

Cell lines

MT2 (26) is a human T-cell line derived from the co-cultivation of cord blood mononuclear cells with adult T-cell leukemia cells, generating HTLV-I. Raji is an EBV-transformed B cell line from Burkitt's lymphoma. B104 is an EBV-negative B-lymphoma line (21). B95-8 is an EBV-producer marmoset lymphoblastoid line (25). All cell lines in this study were maintained in RPMI 1640 (GibcoBRL, Rockville, MD, U.S.A.) supplemented with 2 m*M* glutamine, antibiotics, and 10% heat-inactivated fetal calf serum.

Flow cytometric analysis

Cells (1×10^6 cells) were washed with fluorescence-activated cell sorter (FACS) buffer [0.1% bovine serum albumin and 0.1% sodium azide in phosphate-buffered saline (PBS)] and incubated with 20 μl of each mAb diluted with FACS buffer for 1 h on ice. After washing, the cells were incubated with fluorescein isothiocyanate-conjugated anti-mouse Ig antibody (Amersham Pharmacia Biotech, Uppsala, Sweden) for 30 min on ice. The specific binding was analyzed on a FACS Calibur (Nippon Becton Dickinson Co., Ltd, Tokyo, Japan). Nonspecific staining was assessed with an irrelevant antibody of identical isotype. The percent inhibition was calculated by the following formula: % inhibition = [(MFI of control cells) − (MFI of NAC-treated cells)] × 100 ÷ (MFI of control cells), where MFI is the mean fluorescence intensity.

EBV preparations and infection

B95-8 cells were cultured at 1×10^6 cells/ml for 5 days. The B95-8 cell supernatant was filtered through a 0.45-μm pore size membrane to remove debris, and the filtrate was used as a source of EBV. B104 cells (1×10^6) were incubated with 1 ml of the source of EBV at 37°C

for 2 h, washed with PBS twice, and cultured for 4 days. Infection of EBV was assessed with the detection of EBV nuclear antigen-2 (EBNA2) transcripts by reverse transcription–polymerase chain reaction (RT-PCR), as described below.

Detection of EBNA2 transcripts by RT-PCR

Total RNA from each cell preparation was extracted using TRIzol (Gibco-BRL) according to the manufacturer's protocol. First-strand cDNA was prepared using the Superscript preamplification system (GibcoBRL) with oligo(dT)$_{(12–18)}$. To detect EBNA2 transcripts, the following PCR primers were used: 5′-CCCCATGTAACGCAAGATAG-3′, position 48,534–48,515; and 5′-GCGCCAATCTGTCTACATAG-3′, position 47,940–47,959. PCR was conducted in a GeneAmp PCR system 9700 (PE Applied Biosystems, Foster City, CA, U.S.A.) for 35 cycles (denaturing at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 2 min). The primers for EBNA2 amplify a 209-bp product (32).

Northern blot analysis

Total RNA was extracted using TRIzol (GibcoBRL). Fifteen micrograms of total RNA per lane was resolved by electrophoresis on a 1% agarose gel containing formaldehyde and then transferred to nylon membranes by capillary blotting. A 4.4-kb insert of human CD21 from pSFFV.CR2 (a gift from Dr. V. Michael Holers, Washington University School of Medicine) (4), was radiolabeled by a random priming method using Probe Quant G-50 Micro Columns (Amersham Pharmacia Biotech) and hybridized to membranes.

Determination of intracellular GSH

Cells were washed twice with ice-cold PBS and lysed by the addition of 5-sulfosalicylic acid (final concentration, 1%). After centrifugation, the supernatant was assayed for total GSH by the enzymatic recycling assay (45).

Detection of membrane or soluble CD21 with immunoprecipitation

Cells (2.4×10^7 cells/ml) were reacted with PBS containing Sulfo-NHS-biotin (0.5 mg/ml) for 30 min at room temperature, and extensively washed with PBS. The cells were lysed with 1% NP-40, 10 m*M* Tris, 150 m*M* NaCl, 1 m*M* EDTA buffer, pH 7.4, containing protease inhibitors. The supernatant was concentrated by 80% ammonium sulfate precipitation and dialyzed against 10 m*M* Tris, 150 m*M* NaCl, 1 m*M* EDTA buffer, pH 7.4. Immunoprecipitation was then performed with HB5 or with an isotype-matched mAb for 2 h at 4°C. Immunoprecipitates were electrophoresed on 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and blotted onto membranes. CD21 was detected with peroxidase-labeled streptavidin (Amersham Pharmacia Biotech).

Statistical analysis

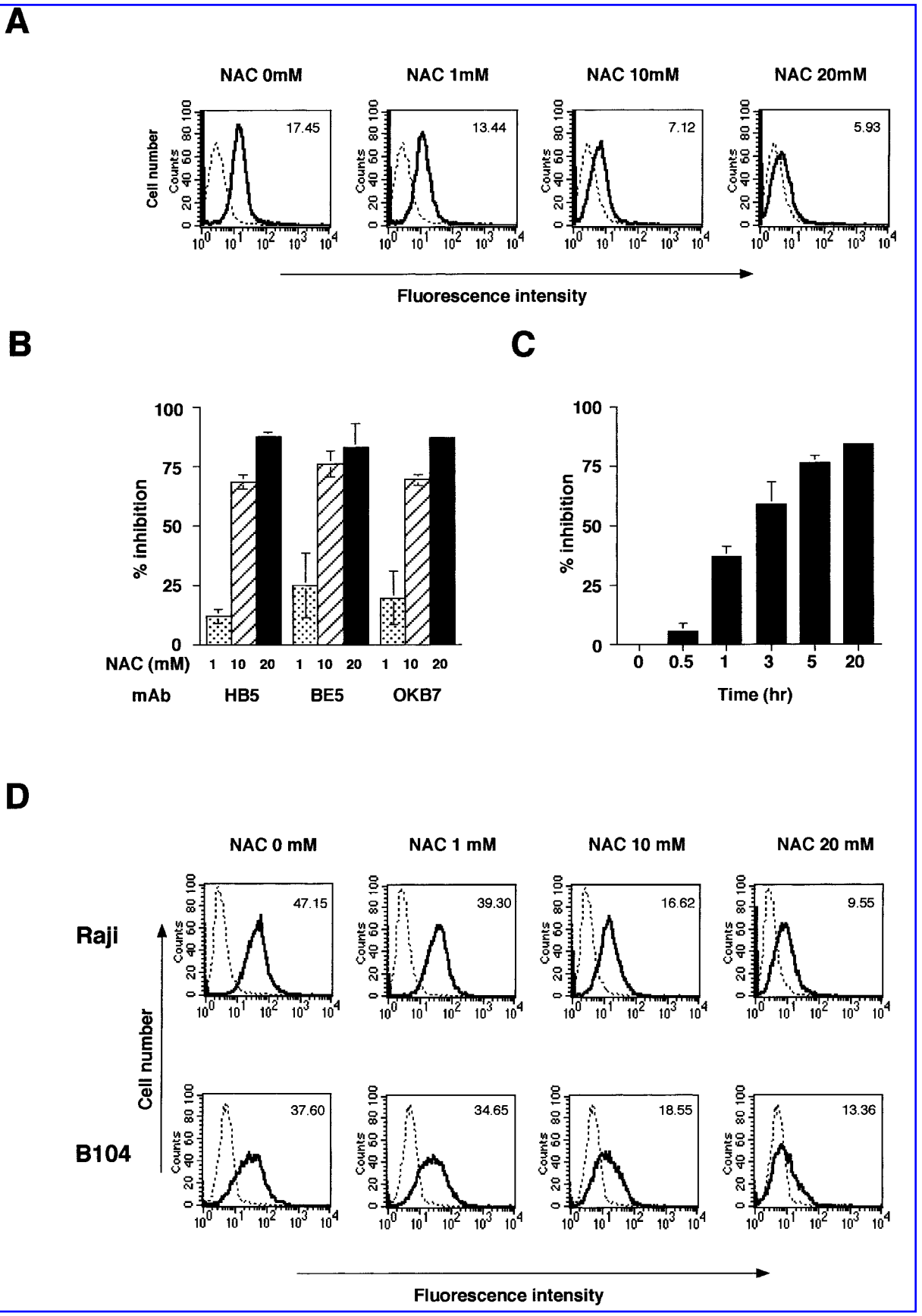
Statistical analysis was performed using a two-tailed Student's *t* test.

RESULTS

NAC suppresses the surface expression of CD21 on both T- and B-cell lines

Expression of CD21 on the HTLV-I infected cell line MT2 was examined by FACS analysis. When MT2 cells were incubated with various concentrations of NAC, a thiol-antioxidant, CD21 expression was markedly reduced in a dose-dependent manner (Fig. 1A and B). The results were essentially the same among experiments using three mAbs (HB5, BE5, or OKB7), which recognize different epitopes on CD21 (Fig. 1B). The suppression of CD21 expression by NAC was also shown to be time-dependent (Fig. 1C). Suppressive effect of NAC on CD21 expression was observed not only in HTLV-I-infected T-cell lines, but B-cell lines including Raji (EBV-positive) and B104 (EBV-negative) (Fig. 1D).

To evaluate the specificity of the effect, we next examined expression of other surface molecules, such as CD4 and CD25 for T cells and CD19 and surface IgM (sIgM) for B cells. Expression of CD21 was suppressed by NAC treatment, but not that of CD4 and CD25 on the HTLV-I transfected T-cell line MT2 (Fig. 2A). Similarly, expression of CD21 was suppressed by NAC treatment, but not that of CD19 and sIgM for the B-cell lines, B104 and Raji (Fig. 2B).


A
NAC 0mM
NAC 1mM
NAC 10mM
NAC 20mM
17.45
13.44
7.12
5.93
Cell number
Counts
Fluorescence intensity
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% inhibition
NAC (mM)
1 10 20 1 10 20 1 10 20
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HB5
BE5
OKB7
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0 0.5 1 3 5 20
Time (hr)
D
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Raji
B104
47.15
39.30
16.62
9.55
37.60
34.65
18.55
13.36
Cell number
Counts
Fluorescence intensity

The expression of sIgM was not detected on Raji cells, which is consistent with earlier reports (17). These results indicate that the suppressive effect of NAC is selective for CD21.

NAC can inhibit in vitro *EBV infection*

EBV infection is initiated by the binding of EBV to CD21. Thus, we investigated whether a NAC-induced decrease of CD21 expression affects EBV infection. B104 (EBV-negative B-cell line) is susceptible to EBV infection with B95-8 EBV, and EBNA2 is one of the first viral proteins that is expressed upon *in vitro* infection of normal B cells. Therefore, we analyzed EBNA2 transcripts following infection of B104 cells with B95-8-derived EBV. Prior to infection, the expression of CD21 on B104 cells was reduced by an overnight treatment of 20 m*M* NAC (Fig. 3A). Cells were then incubated with B95-8-derived EBV, as described in Materials and Methods, and RNA extracts were subjected to RT-PCR analysis. A 209-bp product was amplified from nontreated B104 mRNA with specific primers for EBNA2. However, no EBNA2 transcript was amplified from NAC-treated B104 mRNA (Fig. 3B), indicating that B104 cells with decreased expression of CD21 are not susceptible to infection with EBV. To exclude the possibility that NAC directly inhibits the expression of EBNA2, we examined the expression of EBNA2 in EBV-positive B-cell lines, Raji and B104/EBV. B104/EBV was established from EBV-negative B104 cells by infection with B95-8-derived EBV. The expression of EBNA2 was not abolished by NAC treatment in both cell lines (Fig. 3C).

NAC has no effect on the level of CD21 mRNA transcript

To investigate the mechanisms involved in the suppression of CD21 expression by NAC, we first analyzed transcriptional levels of CD21 expression. MT2 cells were treated with 20 m*M* NAC for various periods of times, and RNAs extracted from cells at each time point were subjected to northern blot analysis. No significant change was observed in the levels of CD21 mRNA expression at any time points (Fig. 4). Moreover, cycloheximide and actinomycin D, which inhibit protein synthesis, did not inhibit the suppression of CD21 (data not shown). These results collectively suggested that the suppressive effect of NAC occurred at post-transcriptional levels.

Decreased expression of CD21 by NAC is independent of intracellular GSH levels

NAC is known to be a precursor of GSH and is capable of restoring levels of intracellular GSH. An increase of intracellular GSH has been shown to initiate signal transduction, thereby enhancing proliferation and interleukin-2 production by T cells (11). To evaluate the involvement of the GSH-dependent signaling pathway in CD21 expression, the effect of NAC on CD21 expression was examined in the presence of BSO, an inhibitor of GSH synthesis. When MT2 cells were treated with BSO, an intracellular GSH level was markedly reduced. However, whether or not cells were treated with BSO, NAC significantly decreased the CD21 expression (Fig. 5). BSO treatment by it-

FIG. 1. NAC suppresses the surface expression of CD21 on both T- and B-cell lines. (**A**) MT2 cells were incubated with NAC for 16 h at the indicated concentrations, and CD21 expression was analyzed by FACS. The cell number (ordinate) is depicted against intensity of staining (abscissa): dotted lines, control staining; bold lines, anti-CD21 stained cells. Mean fluorescence intensity (MFI) of the population is indicated in the upper right corner of each histogram. The results shown are representative of three independent experiments. (**B**) A dose-dependent inhibition of CD21 expression by NAC. MT2 cells were incubated with NAC at the indicated concentrations for 16 h, and CD21 expression was analyzed by FACS using anti-CD21 mAb (HB5, BE5, or OKB7). Data are shown as % inhibition. (**C**) A time-dependent inhibition of CD21 expression by NAC. MT2 cells were incubated with 20 m*M* NAC for the indicated times, and CD21 expression was analyzed by FACS using HB5 mAb. Data are shown as % inhibition. The % inhibition was calculated by the following formula: % inhibition = [(MFI of control cells) − (MFI of NAC-treated cells)] × 100 ÷ (MFI of control cells). Each column represents the mean ± SD of three experiments. (**D**) EBV-negative B-cell line B104 or EBV-positive B cell line Raji cells were incubated with NAC for 16 h at the indicated concentrations, and CD21 expression was analyzed by FACS. The cell number (ordinate) is depicted against intensity of staining (abscissa): dotted lines, control staining; bold lines, anti-CD21 stained cells. The results shown are representative of three independent experiments.

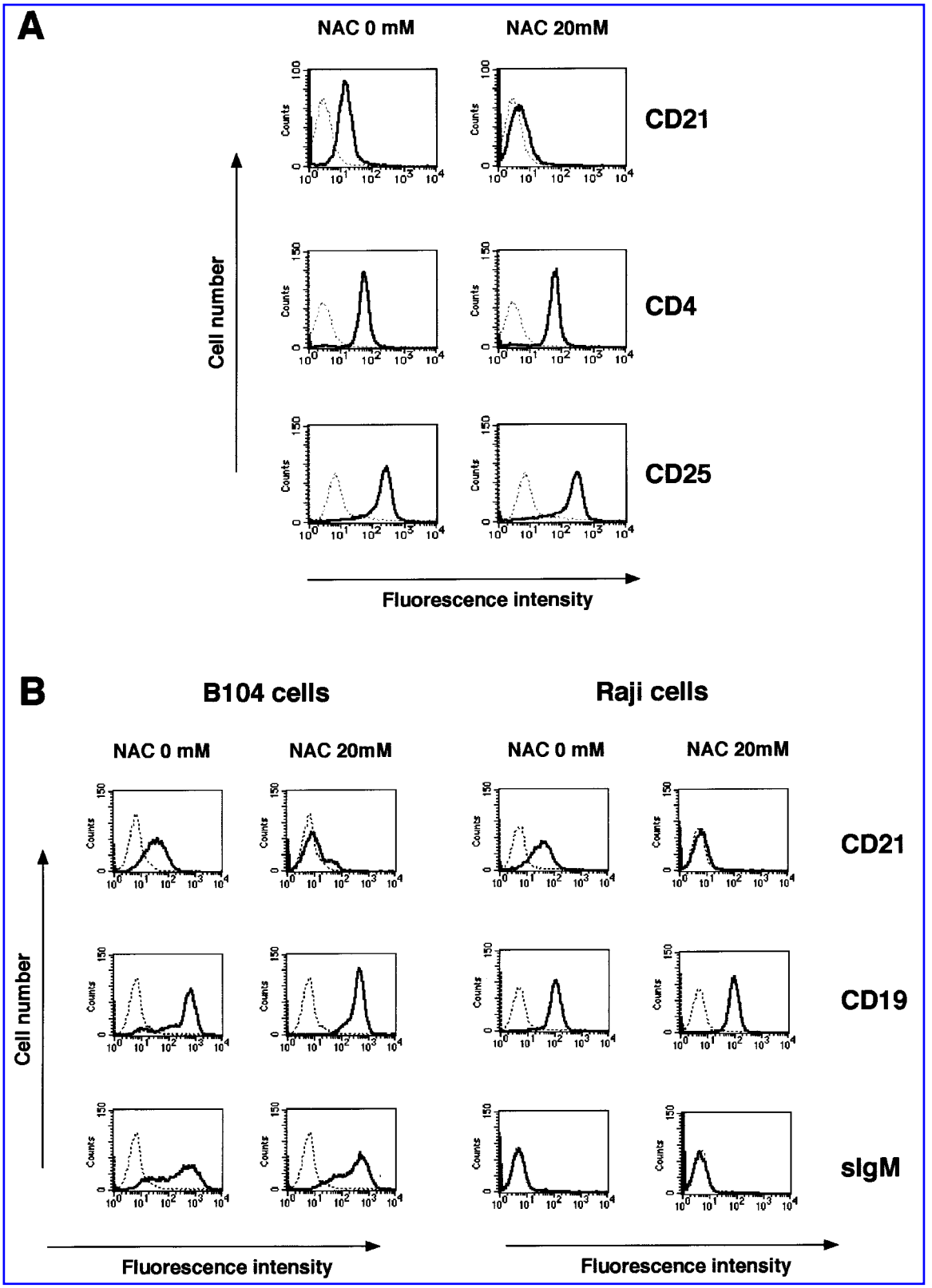

FIG. 2. NAC suppresses the surface expression of CD21 but not CD4 or CD25 on MT2 cells (A), and not CD19 or IgM on B104 and Raji cells (B). The cell number (ordinate) is depicted against intensity of staining (abscissa): dotted lines, control staining; bold lines, anti-CD21, anti-CD4, anti-CD25, anti-CD19, or anti-sIgM stained cells. The results shown are representative of three independent experiments.

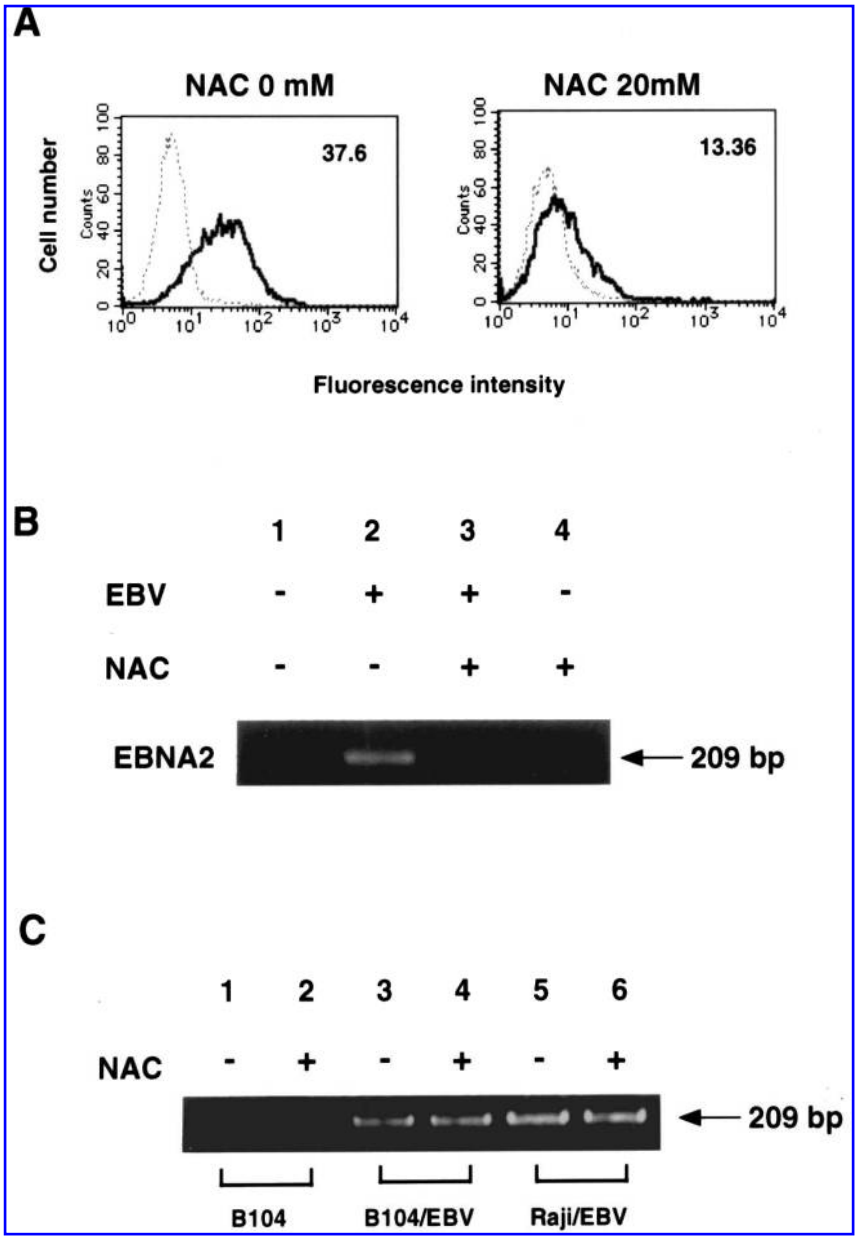

FIG. 3. NAC can inhibit *in vitro* EBV infection. (**A**) Expression of CD21 on B104 cells with or without treatment of 20 m*M* NAC. (**B**) Detection of EBNA2 transcripts within NAC-treated and -untreated cells. B104 cells were treated without or with NAC for 16 h before *in vitro* EBV infection. Then cells were infected with B95-8 EBV and cultured for 4 days. Each mRNA of B104 control cells (lane 1), B104 infected with EBV (lane 2), B104 infected with EBV after NAC treatment (lane 3), and B104 control cells with NAC treatment (lane 4) was reverse-transcribed, and a 209-bp product was amplified using EBNA2-specific primers as described in Materials and Methods. (**C**) B104 (lanes 1 and 2), B104/EBV (lanes 3 and 4), which was infected with EBV prior to NAC treatment, or Raji (EBV-positive cell line) cells (lanes 5 and 6) were treated with (lanes 2, 4, and 6) or without (lanes 1, 3, and 5) 20 m*M* NAC for 16 h before RNA was extracted. Each extracted RNA was reverse-transcribed, and a 209-bp product was amplified using EBNA2-specific primers.

self did not affect the CD21 expression. These results indicate that the decreased expression of CD21 by NAC is independent of intracellular GSH levels.

NAC modulates the structure of CD21

A soluble form of CD21 is spontaneously released from human B and T lymphocytes. To examine the possibility that a release of mem-

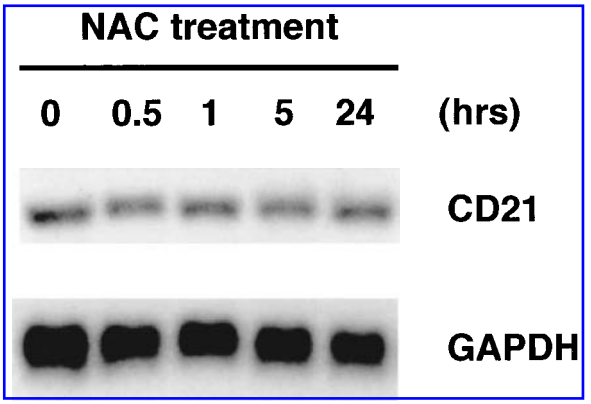

FIG. 4. NAC has no effect on the expression levels of CD21 mRNA. MT2 cells were cultured in the presence of 20 m*M* NAC for the indicated times. Total RNA (15 μg) of each sample was extracted, separated on a 1% agarose formaldehyde gel, and transferred to a nylon membrane. Blots were hybridized with ^{32}P-labeled human CD21 cDNA probe. Hybridization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe was used to normalize for equal loading and blot blotting efficiency.

brane CD21 is enhanced by NAC treatment resulting in decreased expression of surface CD21, we next analyzed the presence of membrane and soluble CD21 in Raji cells. Cells were first surface-labeled with biotin and cultured with or without NAC for 5 h. The cells and supernatant were collected and subjected to immunoprecipitation with HB5 mAb followed by SDS-PAGE analysis. Molecules of 145 kDa (membrane form) and 135 kDa (soluble form) were detected respectively in the cell lysate and in the supernatant of NAC-untreated cells (Fig. 6A, lanes 1 and 3), although neither form was detected in the NAC-treated cells (Fig. 6A, lanes 2 and 4). No band was detected in the immunoprecipitates with control IgG (Fig. 6A, lanes 5 and 6). These data suggest that NAC modulates the structure of both membrane and soluble CD21, including epitopes recognized with HB5 mAb. To determine whether the observed effect is a direct effect of NAC on the binding between CD21 and HB5 mAb, the immunoprecipitated complex of CD21 and HB5 mAb was treated with NAC for 5 h and subjected to SDS-PAGE analysis. CD21 was detected at a similar intensity in both NAC-treated and -nontreated immunoprecipitated complex (Fig. 6B), suggesting that NAC does not directly dissociate the binding between CD21 and HB5 mAb.

Other thiol-antioxidants also suppresses the surface expression of CD21

As NAC is a member of the thiol-antioxidant family, we tested effects of other thiol-antioxidants, 2-ME, GSH, and PDTC, on the surface expression of CD21. All three showed the ability to suppress CD21 expression in a dose-dependent manner (Fig. 7), whereas oxidized GSH (GSSG) and a nonthiol antioxidant catalase had no activity (data not shown), suggesting an involvement of a thiol-dependent mechanism.

DISCUSSION

We have shown that thiol-antioxidants, including NAC, suppressed the functional ex-

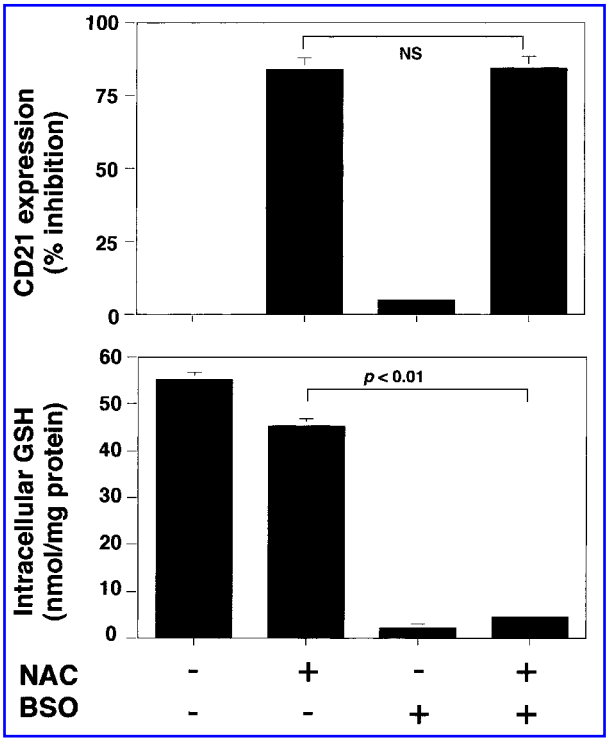

FIG. 5. Suppression of CD21 by NAC is independent of intracellular GSH levels. MT2 cells were incubated with or without 5 m*M* BSO for 4.5 h prior to NAC treatment. After an overnight incubation with 20 m*M* NAC, cells were lysed and subjected to measurement of intracellular GSH. Intracellular GSH levels were measured according to the method described in Materials and Methods. CD21 expression was analyzed by FACS, and data are expressed as % inhibition. The % inhibition was calculated by the following formula: % inhibition = [(MFI of control cells) − (MFI of NAC-treated cells)] × 100 ÷ (MFI of control cells). Each column represents the mean ± SD of three experiments. NS, not significant. A p value of <0.01 was considered significant.

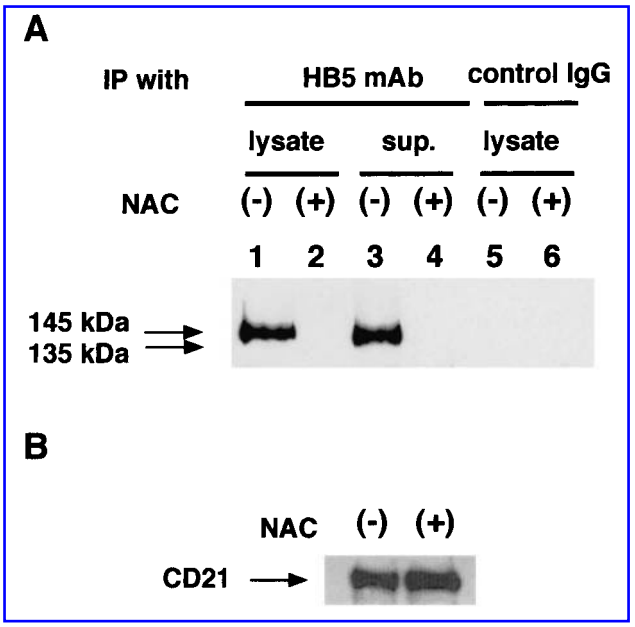

FIG. 6. Immunoprecipitation (IP) of biotin surface-labeled Raji cells using anti-CD21 mAb (HB5). Raji cells were labeled with biotin as described in Materials and Methods. (**A**) Biotin surface-labeled Raji cells were incubated with or without 20 m*M* NAC for 5 h, lysed and immunoprecipitated as follows: lane 1, NAC-untreated cell lysate with HB5; lane 2, NAC-treated cell lysate with HB5; lane 3, culture supernatant of NAC-untreated cells with HB5; lane 4, culture supernatant of NAC-treated cells with HB5; lane 5, NAC-untreated cell lysate with isotype-matched mAb; lane 6, NAC-treated cell lysate with isotype-matched mAb. The immunoprecipitates were electrophoresed and blotted. The membrane was revealed with HRP-SA followed by enhanced chemiluminescence detection (Amersham Pharmacia Biotech). (**B**) Biotin surface-labeled Raji cells were lysed and immunoprecipitated with HB5. The immunoprecipitates were then treated with or without NAC for 5 h at 37°C, and electrophoresed followed by visualization with HRP-SA on blotted membranes.

pression of CD21 on both T- and B-cell lines. The suppressive effect of NAC was shown to be selective for CD21 and to be regulated by posttranscriptional mechanisms.

Adequate intracellular GSH levels are required for the biological function of lymphocytes (5, 11, 18). As NAC modulates the activity of several redox-sensitive cellular signal transduction components by restoring the intracellular GSH levels, the association between the suppressive effect of NAC and intracellular GSH levels was analyzed. In our experiments using MT2, intracellular GSH levels were not affected by NAC, but depleted by BSO. In addition, NAC retained the ability to suppress the surface expression of CD21 in the GSH-depleted cells (Fig. 5). These results indicate that intracellular GSH levels are not involved in the suppressive effect of NAC on CD21 expression.

A soluble form of CD21 is spontaneously released from human B and T lymphocytes by unknown mechanisms (15, 23). Thus, we examined the possibility that NAC enhances the release of CD21, thereby decreasing the surface expression of CD21. However, immunoprecipitation studies showed that both membrane and soluble CD21 were not immunoprecipitated with HB5 mAb after NAC treatment. CD21 is composed of 15–16 SCRs in the extracellular domain followed by a transmembrane domain and a C-terminal cytoplasmic tale (1). Each SCR has two sets of internal disulfide bonds formed with four cysteine residues. As epitopes of OKB7 and HB5 mAbs are defined to be within SCR1–2 and SCR3–4, respectively, one might think that NAC reduces the internal disulfide bonds in SCRs, thereby disrupting the recognition of CD21 by those mAbs. However, NAC did not directly dissociate the binding between HB5 mAb and immunoprecipitated CD21 (Fig. 6B). These results suggest that the effect is not due to the direct reducing activity of NAC. There may be an involvement of cel-

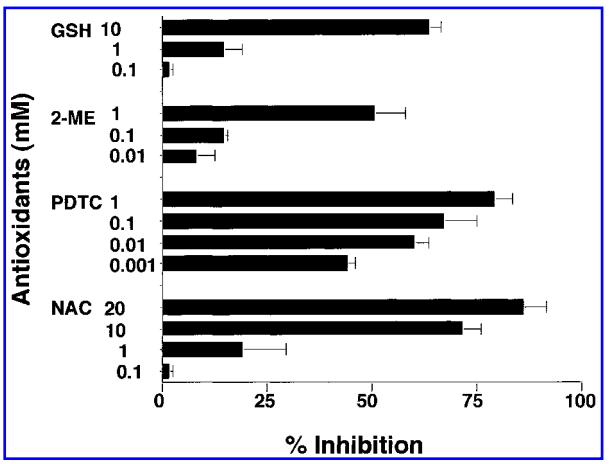

FIG. 7. Thiol-antioxidants suppress the expression of CD21 in a dose-dependent manner. MT2 cells were cultured with each compound for 16 h, and the surface expression of CD21 was analyzed by FACS. Data are expressed as % inhibition. The % inhibition was calculated by the following formula: % inhibition = [(MFI of control cells) − (MFI of NAC-treated cells)] × 100 ÷ (MFI of control cells). Each column represents the mean ± SD of three experiments.

lular components. As the activity of numerous enzymes is redox-sensitive, we hypothesized that NAC enhanced the activity of proteases specific to CD21. We tested protease inhibitors including leupeptin, E-64, aprotinin, pepstatin A, EDTA, and some metalloprotease inhibitors, but none of them inhibited the suppressive effect of NAC on CD21 expression (data not shown). However, we cannot yet rule out the possibility that an unknown protease is involved in the modulation or cleavage of CD21 by NAC. The precise mechanism needs to be further investigated.

EBV infects normal B-lymphocytes *via* CD21 and establishes latent viral infection. EBV infection is associated with a number of human diseases (34, 44). In particular, EBV-induced posttransplant lymphoproliferative disease (EBV-PTLD) continues to be a major complication following solid organ transplantation (33). We demonstrated that the suppression of CD21 expression by NAC resulted in the prevention of EBV infection using the EBV-negative B-cell line B104. As NAC had no effect on the constitutive expression of EBNA2 in EBV-positive cell lines (Fig. 3B), the preventive effect is most likely due to the inhibition of EBV entry *via* CD21. In addition to primary infection, an activation of lytic replication in latently infected cells is a major concern for triggering EBV-associated diseases. In *in vitro* experiments, lytic replication of viral DNA can be induced by cross-linking of surface Ig (41), or by stimulation of protein kinase C (49). However, the *in vivo* mechanism by which latently infected lymphocytes make a transition to a lytic cycle is not defined. When cells enter a lytic phase, viral DNA is amplified and a large amount of infectious virus particles is produced (34). Therefore, the prevention of subsequent infection is important to avoid progression to the EBV-associated disease state. Furthermore, recent reports have shown that EBV binding to CD21 enhances the production of several cytokines including interleukin-6, which is capable of inducing the lytic viral cycle (43). Thus, it would be beneficial to inhibit EBV binding to CD21, even after the establishment of latent infection. Our data suggest that the treatment of posttransplant or idiopathic immunosuppressive patients with NAC offers the possibility of reducing the frequency of EBV-PTLD or other EBV-associated diseases.

Recently, CD21 has been implicated as an enhancing factor in HIV infection of EBV-infected B cells and of T cells, independently of CD4 antigen molecule (27, 40, 48). Thus, control of CD21 expression may offer the advantage to prevent HIV infection as well as EBV infection. NAC has been used for the treatment of HIV-infected patients. The observed antiviral effect of NAC in the treatment of HIV-infected patients has been demonstrated to be due to inhibition of viral replication by reactive oxygen species and its ability to restore intra- and extracellular GSH levels (5, 10, 19, 35). Our finding in this report may raise the additional possibility that the suppression of functional CD21 expression by NAC is involved in the mechanism of therapeutic effect observed in HIV-infected patients.

In this report, the prevention of EBV infection by a thiol-antioxidant, NAC, was clearly demonstrated. Although the precise mechanism is not yet clear, it should be noted that the other members of the thiol-antioxidant family showed a similar effect to NAC (Fig. 7), suggesting the involvement of a thiol-dependent mechanism. The cytoprotective effect of the thiol-antioxidant family and its therapeutic potential have been well documented (6, 8, 9, 11, 35). We have previously reported that an intracellular redox regulatory protein, thioredoxin, suppressed the lytic replication of EBV (39). Recently, protein disulfide isomerase, which catalyzes the thiol–disulfide interchange reaction on the cell surface, has been shown to be important for triggering HIV entry (13, 37). The process of viral infection and replication may be closely associated with a thiol-dependent redox control mechanism. In conclusion, this study may have relevance for understanding the regulatory mechanism of viral infection and prevention of virus-associated diseases. Furthermore, thiol compounds similar to or better than NAC may be good candidates for the therapy of virus infectious diseases such as HIV and EBV.

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ABBREVIATIONS

BSO, L-buthionine-*S*,*R*-sulfoximine; EBNA2, Epstein–Barr virus nuclear antigen-2; EBV, Epstein–Barr virus; FACS, fluorescence-activated cell sorter; GSH, glutathione; HIV, human immunodeficiency virus; HTLV-I, human T-cell-leukemia virus type I; mAb, monoclonal antibody; 2-ME, 2-mercaptoethanol; MFI, mean fluorescence intensity; NAC, *N*-acetylcysteine; PBS, phosphate-buffered saline; PDTC, pyrrolidine dithiocarbamate; PTLD, posttransplant lymphoproliferative disease; RT-PCR, reverse transcription–polymerase chain reaction: SCR, short consensus repeat; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; sIgM, surface IgM.

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