Differential Expression of the Major Histocompatibility Antigen Complex (MHC) on a Series of Burkitt's Lymphoma Lines

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Abstract We compared the expressions of class I and class II major histocompatibility antigen complex (MHC) on the surface of Jijoye and P3HR-1 cells of Burkitt's lymphoma sublines. Jijoye cells had a large amount of class I and class II MHC antigens, whereas these antigens were less expressed on P3HR-1 cells. On a subline of P3HR-1 K cells the expression of class I antigen markedly diminished and class II antigen was undetectable. On the other hand, Jijoye, P3HR-1, and P3HR-1 K cell lines were confirmed to be Epstein-Barr virus (EBV) nonproducer, low producer, and high producer, respectively. The chemical activation of EBV genome by treating P3HR-1 cells with 12-O-tetradecanyl phorbol-13 acetate (TPA) and n-butyrate resulted in inhibition of the expression of class I and II antigens, while the addition of retinoic acid, an inhibitor of virus replication, blocked the decrease in the MHC antigen expression. These findings suggested that there might be an inverse correlation between the virus production and the expression of class I and II MHC antigens.

The major histocompatibility antigen complex (MHC) is a key element in restricting immunological recognition of viral antigens (24, 25). Virus infection often modulates the cell surface expression of class I (7, 16, 17) and class II MHC antigens (12). Epstein-Barr virus (EBV) selectively infects and transforms human resting B cells expressing both class I and class II antigens of MHC. Usually EBV-transformed B lymphoblastoid lines and Burkitt's lymphoma lines express human leukocyte antigens (HLA) and can be killed by MHC class I-specific cytotoxic T cell lines (18). The Burkitt's lymphoma subline of P3HR-1 cells has been obtained by cloning a high virus-producing cell from the parental Jijoye cells (6). The P3HR-1 cells express only low level of MHC class II antigen, in contrast to the parental Jijoye (19). In this paper we present the various patterns of MHC antigen expression in sublines of a Jijoye-P3HR-1 family and discuss the relationship between the MHC antigen expression and EBV production.

MATERIALS AND METHODS

Cell lines. Jijoye, P3HR-1, and P3HR-1 K lines were maintained in PRMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. Jijoye and

P3HR-1 lines were obtained from the American Tissue Culture Collection. P3HR-1 K, BJAB, Daudi, and 6.1.6 cl4 lines were provided by Dr. Edward Clark at the University of Washington.

Immunofluorescence assay. Specific antigens were stained by the indirect immunofluorescence method. Briefly, cells were incubated with anti-class I (E3b) or class II (B10a) monoclonal antibody (2), washed three times, and stained with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (Tago Inc., Burlingame, Calif., U.S.A.). Subsequently, the cells were washed twice and fixed in a 1% paraformaldehyde solution. Suspensions of immunofluorescence-positive cells were analyzed with the aid of a laser flow cytometer (Cell sorter CS-20, Showa Denko K.K., Tokyo). Examination with a monoclonal antibody to transferrin receptor was also carried out. EBV-associated antigens in acetone-fixed cells were stained by EBV-seropositive serum from a nasopharyngeal carcinoma (NPC) patient (a kind supply of Dr. Y. Hinuma at Kyoto University) and followed by FITC-conjugated rabbit anti-human IgG. The cells were examined under a fluorescence microscope. The staining titers of human NPC serum against EBV viral capsid antigen, membrane antigen, and early antigen were 1,280, 1,280 and 320, respectively. In the present study the antiserum was used at a 1:100 dilution.

Labeling of proteins, immunoprecipitation, and gel electrophoresis. Twenty million cells of Jijoye, P3HR-1, and P3HR-1 K were respectively labeled by the lactoperoxidase method using 1 mCi of ^{125}I (20). Cell lysates were prepared and adjusted to a concentration of 0.1% sodium dodecyl sulfate (SDS) (23). The lysate was immunoprecipitated with a monoclonal antibody and protein A-Sepharose CL-4B (Pharmacia Fine Chemical, Uppsala, Sweden). Radiolabeled proteins were analyzed by polyacrylamide gel electrophoresis in the presence of SDS in 12.5% slab gels under reducing conditions (11). Radiolabeled bands in the gels were identified by autoradiography. Molecular weights were determined by calculating the correlation of log molecular weight to migration distance. The Pharmacia low molecular protein standard kit was used as reference.

Activation and inhibition of the EBV genome. For an activation of the EBV genome, cells $(2 \times 10^5/ml)$ were incubated in fresh culture medium containing 100 ng/ml 12-*O*-tetradecanyl phorbol-13 acetate (TPA) and 2 mM *n*-butyrate for 2 days (22). In inhibition experiments retinoic acid was further added to the medium at a concentration of 10 μM/ml (21).

RESULTS

The Expression of MHC Class I and Class II Antigens on Jijoye, P3HR-1, and P3HR-1 K Cell Lines

Two methods were employed to determine the expression of MHC class I and II antigens. First, the analysis by immunoprecipitation and gel electrophoresis was carried out. The anti-class I antibody precipitated the polypeptide with a molecular weight of 43,000 dalton from the three different cell lines (Fig. 1a). However, another subunit, β_2 microglobulin, was indistinguishable from the dye front.
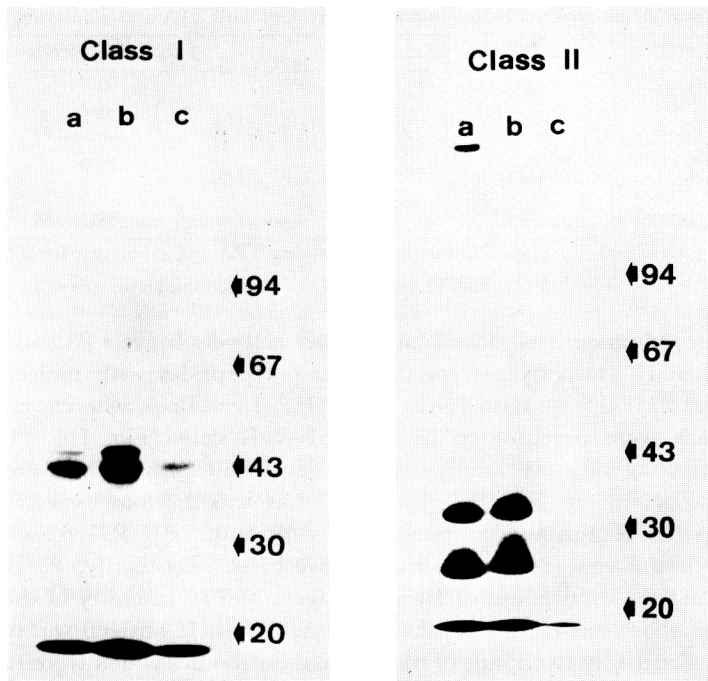
Fig. 1.   Autoradiograph of the SDS/polyacrylamide gel electrophoresis of ^{125}I-labeled class I and class II antigens. P3HR-1 cells (a); Jijoye cells (b); P3HR-1 K cells (c).
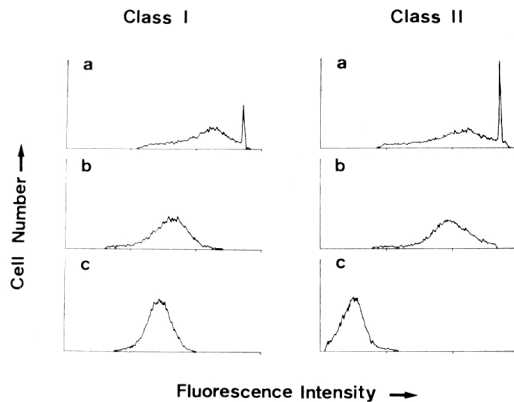
Fig. 2.   Flow cytometry analysis of class I and class II antigen expression on Jijoye (a), P3HR-1 (b), and P3HR-1 K cells (c). The histogram of the negative control treated with an irrelevant monoclonal antibody was the same as that of P3HR-1 K cells stained by anti-class II monoclonal antibody. The intensity of fluorescence is expressed on a log scale.

Table 1.   The frequency of EBV-associated antigen-positive cells on a series of Burkitt's
           lymphoma sublines before and after treatment with TPA and n-butyrate

Cell line	Treatment[a]	% Positive cells[b]
Jijoye	−	0
	+	0
P3HR-1	−	0
	+	12.0
P3HR-1 K	−	0.8
	+	39.1

[a] Cells were incubated in medium containing TPA and n-butyrate for 3 days.
[b] Five hundred cells per test.

The order in the amount of class I antigen per cell was Jijoye > P3HR-1 > P3HR-1 K.   Anti-class II antibody precipitated two polypeptides with molecular weight of 34,000 and 27,000 from both Jijoye and P3HR-1 cell lines, whereas no such polypeptide bands were precipitated from P3HR-1 K cells (Fig. 1b).   In the next experiment, the quantitative analysis of their surface expression was done with the aid of a flow cytometer (Fig. 2).   Jijoye cell line was the most reactive with anti-class I and class II antibodies among three cell lines.   P3HR-1 cells had a lesser amount of class I and II antigens than Jijoye cells.   Especially, P3HR-1 K cells expressed the smallest amount of class I antigen, and its intensity of the peak fluorescence was about one-eighth of that of Jijoye.   Class II antigen was undetectable on P3HR-1 K cells by two kinds of monoclonal antibodies which were reactive with the different antigenic determinant sites of class II molecule.   On the other hand, there was no difference among these three cell lines in the expression of transferrin receptors detected by a monoclonal antibody (data not shown).

Virus Productivity of Jijoye, P3HR-1, and P3HR-1 K Cell Lines

Virus-producing activity was measured by detecting the EBV-associated antigens in the cells (Table 1).   P3HR-1 K cell line contained a small percent of immunofluorescence-positive cells, while no such positive cells were detected in either P3HR-1 or Jijoye cells.   After activation of the EBV genome by treatment with TPA and n-butyrate for 3 days, the frequency of fluorescence-positive cells in P3HR-1 K cell culture increased up to about 40% and also a significant number of fluorescence-positive cells appeared in P3HR-1 line.   However, no positive cells were detected in Jijoye line even after the same treatment.   This finding confirmed the fact that the order in virus productivity among these cell lines is P3HR-1 K > P3HR-1 > Jijoye (9).

Changes in the Expression of Class I and II MHC Antigens by Activation and Inhibition of the EBV Genome

The results in the preceding section suggested that the virus production might be inversely correlated with the expression of MHC class I and II antigens.   Therefore, we investigated the MHC antigen expression after chemical activation of EBV genome (Fig. 3, a and b).   When virus production in P3HR-1 cells was stimulated
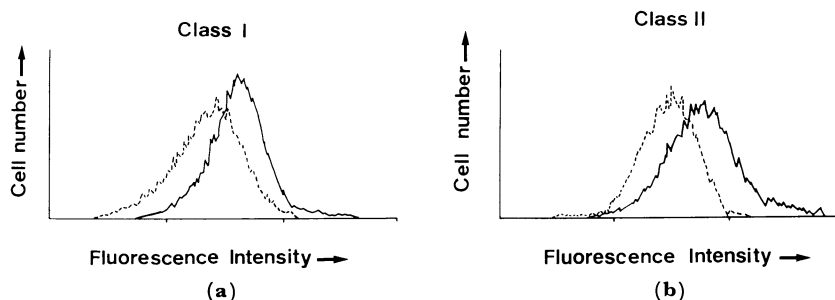
Fig. 3. Flow cytometry analysis of class I and class II antigen expression in TPA and n-butyrate-treated ($\cdots\cdots$) and untreated control P3HR-1 cells (———). The intensity of fluorescence is expressed on a log scale. Note the significant leftward displacement of chemically treated cells.

by TPA and n-butyrate treatment, the expression of MHC class I and class II antigens was significantly reduced and was less than half that of untreated control cells. The additional treatment with retinoic acid blocked this reduction of MHC expressions (data not shown). In contrast to P3HR-1 cells, no reduction of MHC antigens was found on Jijoye cells, EBV-negative BJAB Burkitt's lymphoma cells, and some EBV-transformed B lymphoblastoid cell lines even after chemical stimulation of virus genome.

DISCUSSION

In the present study we have found an inverse correlation between the expression of class I and II MHC antigens and the EBV productivity. Klein et al (9) have also reported that certain nonproducer revertant cells from P3HR-1 line again express a high level of class II antigen (9). Although virus structural proteins have been detected in only a limited number of cells even in virus producer P3HR-1 K cells (Table 1), a significant number of virus DNA copies are synthesized in these cells (1). Further, chemical treatment markedly enhances the copy number of virus genomes per cell (4). Therefore, it seems likely that the amplification of virus genome may participate in the decrease of MHC antigen expression.

The parental Jijoye and other EBV-positive Burkitt's lymphoma lines release viruses with transforming activity (15). When normal peripheral blood lymphocytes are transformed by these EBV (13), class I molecules markedly become increased. On the contrary, P3HR-1 cells produce nontransforming EBV, and this variant virus is unable to immortalize normal B lymphocytes and shows cytopathic effects on EBV receptor-carrying cells (14). The P3HR-1 EBV DNA has a deletion of 2×10^6 dalton out of the Jijoye EBV DNA and the deleted part of the DNA sequence encodes the mRNAs detected in restringently-infected, growth-transformed cells (5, 8). Therefore, this transformation-related DNA might be involved in the regulation of MHC antigen expression.

On the other hand, the disappearance of MHC antigens from the cell surface does not necessarily mean activation of the EBV genome, since class I antigen-negative Daudi Burkitt's lymphoma line and class II antigen-negative EBV-transformed line, 6.1.6 cl 4 (3), hardly show EBV productivity even after treatment for chemical induction (data not shown).

Besides MHC class I and II antigens, the P3HR-1 sublines differ from the parental Jijoye in several surface molecules; first, P3HR-1, unlike Jijoye, does not express the receptor for EBV and C3d (10). Second, the B lymphoblast antigen (BB-1) is expressed on the Jijoye but few on P3HR-1 (23). The differential expression of B cell differentiation markers raises another possibility that the diminished expression of MHC antigens may reflect the difference at a differentiation stage in a B cell lineage.

It has been established that cytotoxic T cell killing is restricted by class I molecules, and that inducer T cells in the process of antibody production are restricted by class II molecules. Therefore, it is of interest to speculate that EBV may try to escape from cellular and humoral immune surveillance by affecting expression of the MHC antigens on virus-infected cells. Although EBV is closely associated with Burkitt's lymphoma, infectious mononucleosis, nasopharyngeal carcinoma, and X-linked lymphoproliferative syndrome, it is uncertain whether this phenomenon may happen *in vivo*.

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