Differential Expression of HLA-DR and HLA-DC/DS Molecules in a Patient With Hairy Cell Leukemia: Restoration of HLA-DC/DS Expression by (12-0-Tetradecanoyl Phorbol-13-Acetate), 5 Azacytidine, and Sodium Butyrate

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Biosynthesis and molecular structure of major histocompatibility complex (MHC) class II antigens of DR2/DR7 hairy cells were analyzed by two-dimensional polyacrylamide-gel electrophoresis (2D-PAGE). Two anti-human Ia monoclonal antibodies (mAb) were used to immunoprecipitate DR and DR-linked DC/DS molecules. Monoclonal antibody VI 15 C recognizes DR (I-E-like) molecules and CA 2.06 precipitates DR and DR-linked DC/DS (I-A-like) molecules in DR7 allootypes. Studies were performed on a pure population of hairy cells before and after culture with phorbol ester: 12-0-tetradecanoyl phorbol-13-acetate (TPA). 5 azacytidine (5 Aza), sodium butyrate (NA-BU), and phytohemagglutinin (PHA-P). Before any treatment, hairy cells expressed and synthesized DR antigens: DR α and β subunits appeared both qualitatively and quantitatively normal by 2D-PAGE profile. In contrast, the hairy cells failed to express and synthesize any DC/DS molecule. The lack of DC/DS molecular expression was restored after culture in presence of TPA, sodium butyrate, and 5 azacytidine, but not after PHA-P treatment. Differential molecular expression of MHC class II antigens in leukemic cells provides a model to define further discrete stages of hemopoietic differentiation and study the role of these molecules in the cellular interactions occurring during differentiation.

THE HUMAN HLA-D region encodes a family of polymorphic cell surface glycoproteins composed of two noncovalently linked subunits of 34 kilodalton (kd) (α) and 29 kd (β). Numerous biochemical studies of major histocompatibility complex (MHC) class II antigens have been performed since the introduction of monoclonal antibodies (mAb). Based on homology at the amino acid sequence levels with murine Ia antigens, several studies have supported the conclusions that at least two pairs of loci encode human class II antigens: HLA-DR antigens, which are equivalent to murine I-E products, and HLA-DC/DS molecules, which are homologous to I-A products. MHC class II antigens are mainly expressed on B lymphocytes, monocytes, and activated T cells. Because of their role in cellular interactions and immune responses, it is of importance to examine their expression on the surface of leukemic cells. Moreover, they could also be regarded as differentiation markers in hemopoiesis. DR antigens have been initially shown to be present on colony-forming units, granulocyte/monocyte (CFU-GM). Although also expressed on erythroid burst-forming units (BFU-E), DR is lost during erythroid differentiation to erythrocyte CFU (CFU-E). More recent studies have shown a lack of DC antigens in both BFU-E and CFU-E, thus demonstrating a differential expression of DR and DC antigens along the normal erythroid pathway. Similar data have been recently obtained in leukemic cells of immature origin, in particular, in acute myeloid leukemia (AML) and in an acute lymphocytic leukemia (ALL) cell line. Altogether, these results suggest a differential expression of DR and DC/DS molecules on immature versus mature hemopoietic cells and their leukemic counterparts. Assessment of expression, biosynthesis, and structure of MHC class II antigens would be of value in defining discrete stages of differentiation in hemopoietic malignancies of B lymphocyte and monocyte lineages.

Origin of the cells in hairy cell leukemia (HCL) remains controversial, but is predominantly thought to be the B lymphocyte. However, the expression and biochemical structure of Ia-related molecules has not yet been extensively studied in this disease. Because Ia antigens have been shown by serology to increase after in vitro TPA treatment in chronic lymphocytic leukemia (CLL), we investigated the expression, biosynthesis, and structure of DR and DC/DS antigens in hairy cells and their possible modification after in vitro treatment with several differentiation agents, such as TPA, sodium butyrate, 5 azacytidine, and PHA-P.

MATERIALS AND METHODS

Cells and Cell Cultures

Peripheral blood hairy cells were collected before any treatment from a hypercellular case of HCL with a WBC count >100,000/μL, with 98%-100% hairy cells as observed in phase contrast microscopy and confirmed by the presence of acid phosphatase resistant to

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surface of the cells. DR typing was performed by microcytotoxicity techniques (V. LePage, L. Degos, and J. Dausset, Tissue Typing Laboratory, Hôpital Saint Louis, Paris). Mononuclear cells isolated from the peripheral blood by Ficoll-Isopaque centrifugation (density, 1.077) were washed in phosphate-buffered saline and viability tested by trypan blue exclusion. Biochemical studies were performed before and after culture. Some 3 x 10^5 cells, resuspended at a concentration of 10^6 cells/mL in RPMI 1640 (GIBCO, Grand Island, NY), supplemented with 10% heat-inactivated fetal calf serum, were cultured for 5 days at 37°C in a 5% CO2 humidified incubator with or without (A) TPA (1.6 x 10^-3 mol/L); (B) sodium butyrate (5 x 10^-4 mol/L); (C) 5-azacytidine (5 μmol/L); (D) PHA-P (1%). Control cells consisted of either 3 x 10^5 peripheral blood B lymphocyte-enriched population (E rosette-positive depleted sample) or 3 x 10^6 whole blood cells stimulated with PHA-P for 5 days from a normal subject matched at the DR locus (DR2/DR7).

To quantitate accurately the biochemical expression of MHC class II antigens, cells were precisely counted before and after culture to be sure to immunoprecipitate the same number of cells (3 x 10^7 cells).

Monoclonal Antibodies

The two monoclonal antibodies used were two BALB/c mouse anti-human class II antigens. VI 15 C (provided by Dr. D. Pious) is an IgG2b, precipitation DR antigens of every allotype. CA 2.06 precipitates DR antigens of all allotypes plus DC/DS antigens in DR7 allotypes (and D. Charron, unpublished observations). Moreover, a polyclonal rabbit anti-DS specific antibody, RBO 3 (provided by Dr Silver), was also used.

Radiolabeling of Cells and Membrane Extracts

A quantity of 3 x 10^6 hairy cells or normal lymphocytes were incubated for 5 hours at 37°C in a 5% CO2 incubator in 3 mL of methionine-free RPMI 1640, containing 5% dialyzed fetal calf serum and 500 μCi of [35S-methionine (Amersham, Arlington Heights, IL; 500-800 Ci/mmol). Washed radiolabeled cells were extracted with 0.5% nonidet P 40 (NP 40) for 30 minutes at 4°C and extracts were then centrifuged at 11,000 g for 5 minutes.

Immunoprecipitation

One hundred microliters of NP 40 extracts was precleared using 100 μL of Staphylococcus aureus Cowan I strain protein A (STAPH-A) for 30 minutes at 4°C. Specific radiolabeled cell proteins were immunoprecipitated by the addition of 10 μL of undiluted monoclonal antibody (ascites fluid) to 100 μL of STAPH-A precleared extracts for a 90-minute incubation period. Antigen–antibody complexes were eluted with 30 μL of electrofocusing lysis sample buffer. Supernatant fluid was stored at –70°C.

Two-dimensional Gel Electrophoresis (2D-PAGE)

Samples were prepared according to the technique described by Laemmli, as adapted by O’Farrell et al. In the first dimension, the proteins were separated according to their charges using nonequilibrium pH gradient electrophoresis (NEPHGE), which allows resolutions of more basic proteins (pH 7–9) than isoelectrofocusing (IEF). The second dimension was run in 10% acrylamide slab gel.

RESULTS

The two-dimensional gel electrophoresis pattern represents the classical profile of human DR (I-E-like) molecules, associating a 32–34 kd acidic chain, a more basic 26–29 kd set of spots for the β-chain, and an additional 31-kd polypeptide corresponding to the invariant Ii chain. The structural polymorphism of DR antigens was previously assigned to the β-chain, thus allowing a molecular genotyping based on 2D gel patterns. For any given DR allotype, the corresponding DR β-chain is composed of 3–4 spots previously characterized in homozygous cells. In a heterozygous cell, the β-chain displays a complex profile that represents the combined pattern of the two sets of spots characteristic of each allotype. Although there is some overlap between the 2D gel pattern of the DR2 and DR7 chains, they can be readily distinguished: the DR7 set of spots is slightly more acidic and higher than the DR2 set of spots, as shown in Fig 1.

Comparison of the 2D gel pattern of DR antigens from the DR2/DR7 hairy cells (Fig 1A) with the pattern from a normal subject of the same DR allotype (2/7) (Fig 1B) demonstrates complete identity of spots with the same intensity when immunoprecipitated by the anti-DR mAb VI 15 C, indicating a normal biochemical structure of DR antigens in hairy cells.

In addition to DR antigens, mAb CA 2.06 precipitates DC/DS molecules corresponding to the DR7 haplotype in the normal control (Fig 1D). The DC/DS molecule is readily characterized as a set of more basic β-chain spots. In contrast, in hairy cells, no spot can be detected in the DC/DS β-chain area (Fig 1C). Moreover, a polyclonal rabbit anti-DS specific antibody, RB03, fails to precipitate any molecule from hairy cells.

After culture with TPA (Fig 2A), sodium butyrate (Fig 2B), and 5 azacytidine (Fig 2C), two modifications in the expression of MHC class II antigens were studied in a homogeneous hairy cell population that was 98% pure, as verified by phase contrast microscopy and TRAP. Biosynthetic studies were feasible because of the unusual availability of a large number of pure hairy cells. Moreover, the DR type of cells (DR2/DR7) allowed us to overcome the lack of monomorphic monoclonal anti-DC/DS reagents by using the differential reactivity of two mAb to characterize both DR and DC/DS molecules.

DISCUSSION

The expression and molecular structure of MHC class II antigens were studied in a homogeneous hairy cell population that was 98% pure, as verified by phase contrast microscopy and TRAP. Biosynthetic studies were feasible because of the unusual availability of a large number of pure hairy cells. Moreover, the DR type of cells (DR2/DR7) allowed us to overcome the lack of monomorphic monoclonal anti-DC/DS reagents by using the differential reactivity of two mAb to characterize both DR and DC/DS molecules.
Although several studies on expression of Ia antigens in CLL and acute leukemia of lymphoid and nonlymphoid origin have been reported, most work was done by serology, immunofluorescence, and radioimmunoassays.

Because cell-surface phenotyping with mAb has been shown that some MHC class II epitopes may not be expressed at the cell surface of leukemic cells, it was of importance to assess MHC class II antigens in molecular terms to rule out the altered display of epitopes at the membranes of abnormal cells. Our data demonstrate the absence of biosynthesis of HLA-DC/DS molecules in hairy cells, whereas HLA-DR molecules are readily detectable in normal amounts and appear to be qualitatively identical to normal.

A lack of DC surface expression has been recently reported in some CLL by serology, using a polymorphic monoclonal antibody to DC with a restricted allotype distribution, confirming previous data obtained in AML with alloanthesera.

We are unaware of any biosynthetic studies of MHC class II antigens in HCL. Our data suggest that HCL, like some cases of CLL, represents an abnormal cell population with a dissociation of DR, DC/DS expression. With regard to the ambiguous origin of HCL, one should mention that a normal counterpart DR⁺, DC/DS⁻ cell has been identified in the monocytic lineage while a DR⁺, DC/DS⁻ normal B cell population has yet to be described. Thus, DR and DR-linked DC/DS molecules represent antigenic determinants useful as markers of differentiation in at least the hematopoietic cell lineages.

Fig 1. Two-dimensional gel pattern of HLA-DR antigens from hairy cells and normal lymphocytes. Immunoprecipitates with VI 15C from DR2/DR7 hairy cells (A) and normal DR2/DR7 lymphocytes (B) show the α-chain (34 kD) on the right (acidic area). Ii designates the 31-kd invariant chain and α designates actin. The β-chain area of the gel displays an overlap of DR2 plus DR7-specific spots (graph). Immunoprecipitates with CA 2.06 of hairy cells (C) and normal lymphocytes (D) show the absence of DC/DS β spots in C, in contrast to their presence in D (arrows).

Fig 2. Two-dimensional gel pattern of hairy cells immunoprecipitated with CA 2.06 after stimulation by TPA (A), sodium butyrate (B), and 5 azacytidine (C). Beta DC/DS spots are identified by arrows.
CLL has been studied using radioimmunoassays. A three- to sixfold increase was found in the amount of Ia molecules detectable at the surface of CLL cells after TPA treatment, whereas there was a concomitant conversion of surface immunoglobulin-positive cells into cytoplasmic Ig-positive cells. The capacity of leukemic cells to stimulate in the mixed lymphocyte reaction (MLR), known to be poor, was greatly increased after culture with TPA. Moreover, other data reported induction of DC expression after TPA treatment. The restoration of DC/DS expression, as well as the increased expression of DR, could provide a possible mechanism for the observed increase in capacity to stimulate in MLR.

Precise evaluation of DR, DC, and other class II antigen expression is of importance to the study of every discrete step of differentiation of lymphoid and nonlymphoid lineages. Leukemic cell populations may reflect some, if not all, of these discrete stages of maturation, and DR and DR-linked DC/DS should be precious in identifying these. Although it cannot be formally ruled out that the DC/DS lack of expression in hairy cells is related to the malignant origin of the cell, the possibility of using differentiating agents to drive these malignant cells of B cell origin to express DC/DS antigen is more in favor of a phenotypic linkage between DC/DS expression and the stage of differentiation. Whether the absence of detection of DC/DS molecules is the result of a lack of transcription of the gene coding for the determinant recognized by CA 2.06 or of an insufficient number of molecules present at the cell surface is presently unknown. Messenger RNA studies are in progress to answer this question. Thus, together with the use of modulation agents, leukemic cells provide unique material to assess the molecular biology of MHC class II antigens at the DNA, RNA, and protein levels as well as their correlation with immune functions.

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