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EXPRESSION OF SURFACE ANTIGEN AND mRNA FOR THE CD11c (αX, p150) SUBUNIT OF THE HUMAN LEUKOCYTE ADHERENCE RECEPTOR FAMILY IN HEMATOPOIETIC CELLS

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SUMMARY: We characterized the surface antigen and mRNA expression for the CD11c (αX, p150) subunit of the human leukocyte adherence receptor family during hematopoietic cell differentiation. The CD11c subunit antigen and mRNA are constituitively expressed in undifferentiated HL-60 promyelocytic leukemia cells, and levels increase markedly with differentiation along the monocyte/macrophage pathway using phorbol myristate acetate. Human monocyte-derived macrophages and human alveolar macrophages express elevated levels of the CD11c subunit antigen and mRNA, indicating that the changes observed in vitro are present in vivo. Dot blot analysis of immature and mature lymphoid and myeloid cells and cell lines demonstrate equivalent levels of CD11c mRNA expression. We conclude that CD11c gene expression is selectively increased during hematopoietic cell differentiation along the monocyte/macrophage pathway.

Multifaceted adherance reactions involving human leukocytes appear to be mediated at least in part by a family of receptors located on the surface of these cells (1,2). The members of this family of receptors consist of one of three different α subunits, designated CD11a (LFA-1, αL), CD11b (Mac-1, αM), or CD11c (p150, αX), each of which is noncovalently associated with a single β subunit, designated CD18, in an α1β1 complex (1,2). The CD11a/CD18 complex appears in highest levels on the surface of T-lymphocytes where it mediates T-lymphocyte mediated killing (3,4). The CD11b/CD18 heterodimer is located primarily on human neutrophils (polymorphonuclear leukocytes or PMN) where it mediates diverse adherence-related events, including the attachment to C3bi-coated particles and adherence to human umbilical vein endothelial cells

Abbreviations used in this paper: CML, chronic myelogenous leukemia; EBV, Epstein-Barr virus; MAb, monoclonal antibody; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; PMN, polymorphonuclear leukocyte; mRNA, messenger ribo-nucleic acid.

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(HUVE) (5-8). The third member of this antigen family, the CD11c/CD18 complex, is located on activated T-lymphocytes, activated neutrophils, and monocytes (9-11). The CD11c/CD18 complex may mediate the uptake of C3bi-coated particles by monocyte/macrophages (12).

In previous studies we have investigated the developmentally related expression of this family of antigens during hematopoietic cell differentiation (13-15). Undifferentiated HL-60 promyelocytic leukemia cells express low levels of the common CD18 or β subunit mRNA and surface antigen (13,14). CD18 subunit mRNA increases 10-fold or greater when HL-60 cells are induced to differentiate along the granulocytic pathway with retinoic acid or along the monocyte/macrophage pathway with phorbol myristate acetate (PMA) (14). In retinoic acid-treated HL-60 cells the increase in CD18 subunit mRNA expression is transcriptionally regulated (14). In addition, CD18 subunit mRNA expression is increased in mature human PMN compared to myeloid precursors (14,).

Recently we have shown that surface antigen and mRNA for the CD11b subunit increase markedly in HL-60 cells differentiated along the granulocytic pathway, consistent with the high levels of expression of this subunit on human nuetrophils (15). In the present study we report that surface antigen and mRNA for the CD11c subunit are constituitively expressed in undifferentiated HL-60 promyelocytic leukemia cells and increase markedly with monocyte/macrophage differentiation. Human monocyte-derived macrophages and alveolar macrophages also express elevated levels of CD11c subunit antigen and mRNA.

METHODS

Cell Preparation

Human neutrophils, B-lymphocytes, and T-lymphocytes were obtained from the peripheral blood of normal human subjects according to institutional protocols. Neutrophils were separated from mononuclear cells using an isolymph (Gallard/Schleisinger, Carle Place, NY) gradient followed by dextran sedimentation (16). T-lymphocytes were collected from the mononuclear cell layer and separated by immune rosetting with SRBC (17). The T-lymphocytes were analyzed in the resting state, and after activation with PHA (17). B-lymphocytes were obtained from the peripheral blood and transformed with EBV (18). Human alveolar macrophages were obtained by bronchoalveolar lavage of normal human volunteers (19). Human leukemia lymphoblasts and myeloblasts were isolated from the peripheral blood of individuals with CML/lymphoblastic transformation or CML/myeloblastic transformation (20).

The cell lines K-562 (21), KCL-22 (22), EM-3 (23), HEL (24), Jurkat (25), and HL-60 (26) were maintained in RPMI 1640 (Irving Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, NT). HL-60 cells were induced to differentiate to granulocytes by 10^{-6}M

retinoic acid (Sigma) (27) or to monocyte/macrophage-like cells with 10ng/ml of phorbol myristate acetate (PMA) (28) (Consolidated Midland Corp., Brewster, NY).

Monoclonal Antibodies

mAb anti-LeuM5 (IgG2b-k), which binds to the 150-kDa (CD11c) (p150, αX) subunit, was purchased from Becton-Dickinson (Mountain View, CA) (9,10). mAb 9E8 (IgG_{2a}-k), which binds to the murine leukemia virus, was provided by Dr. Patrick Beatty, Fred Hutchinson Cancer Research Center, Seattle, WA (29).

Immunofluorescence Flow Cytometry

Indirect immunofluorescence with flow cytometry was used to estimate the expression of the CD11c subunit on HL-60 cells as previously described (13).

RNA Isolation, Northern Blotting and Dot Blotting

Total RNA was isolated from human cells and cell lines as described (20), and 25 μg of total RNA was electrophoresed on a formaldehyde-agarose gel. After transfer to nitrocellulose the Northern Blots were hybridized to a ^{32}P-end-labeled 26 base pair oligonucleotide corresponding to nucleotides 2882-2908 in the CD11c cDNA (30). Northern blots were washed at 65°C in 0.1X SSC, 0.1% SDS and autoradiographs were developed. Northern blots for HL-60 cells were also hybridized to a ^{32}P-labeled 2.0 Kb cDNA corresponding to β-actin (31).

For the dot blotting experiments serial dilutions of total cellular RNA were spotted onto nitrocellulose as described (14) and hybridized to the identical 26 mer corresponding to the CD11c subunit described above.

RESULTS

Surface expression of the CD11c subunit on HL-60 human promyelocytic leukemia cells- We analyzed CD11c surface antigen representation on undifferentiated HL-60 cells, HL-60 cells induced to differentiate along the granulocytic pathway with retinoic acid, HL-60 cells induced to differentiate along the monocyte/macrophage pathway with PMA, normal human PMN, and normal human monocyte-derived macrophages by using indirect immunofluorescence followed by flow cytometry. The CD11c antigen was present in low levels on undifferentiated HL-60 cells and did not increase upon exposure of HL-60 cells to retinoic acid, consistent with the low levels of this antigen on mature human PMN (Table I).

TABLE I

Expression of the CD11c subunit on HL-60 promyelocytic leukemia cells

	RELATIVE MEAN FLUORESCENCE[a]
Undifferentiated HL-60 cells	76
Retinoic acid-differentiated HL-60 cells (2 days)	76
Normal human PMN	81
PMA-differentiated HL-60 cells (2 days)	128
Normal human monocyte-derived macrophages	165

Each cell population was incubated with mAb anti-LeuM5 followed by a FITC-linked sheep anti-mouse antibody and analyzed by flow cytometry

[a] Results are expressed as the relative mean fluorescence on an arbitrary log scale from 0 to 200 where an increment of 18.5 U represents a doubling of fluorescence intensity. Data on 5000 cells were collected for each sample.

However, CD11c antigen expression increased more than 4-fold when HL-60 cells were induced to differentiate along the macrophage pathway with PMA, consistent with the high levels of this subunit on the surface of human monocyte-derived macrophages. These results support our previous studies in which differentiation of HL-60 cells along the granulocytic or monocyte/macrophage pathway with a variety of agents resulted in a surface phenotype characteristic of mature PMN or macrophage, respectively (13).

CD11c subunit mRNA expression in HL-60 cells, PMN, and alveolar macrophages- To determine whether leukocyte adherence protein CD11c subunit RNA expression paralleled antigen expression in HL-60 cells, we performed Northern blot hybridization studies using RNA extracted from undifferentiated HL-60 cells and HL-60 cells treated with retinoic acid or PMA. Low levels of the 4.7 Kb RNA transcript previously described (30) were present in undifferentiated HL-60 cells and increased only slightly following exposure to retinoic acid (Fig. 1A, lanes 2-8). Exposure of HL-60 cells to PMA resulted in the expression of higher levels of CD11c mRNA (Fig. 1A, lanes 9-11). These differences in expression of the CD11c subunit mRNA were not due to inadvertently loading different amounts of RNA in each lane because hybridization of the same Northern blot with a cDNA

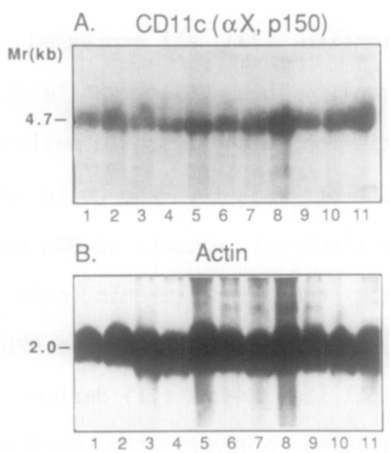

FIGURE 1. Northern bot analysis of total RNA (25μg) from undifferentiated and differentiated HL-60 cells. Total RNA was extracted from: undifferentiated HL-60 cells (lane 1); HL-60 cells exposed to retinoic acid for 3 hours, 6 hours, 1 day, 2 days, 3 days, 4 days, 5 days, (lanes 2-8); HL-60 cells exposed to PMA for 1 hour, 1 day, 3 days, (lanes 9-11). RNA samples from each cell population were separated on formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized to (A) the ^{32}P-end labeled 26 mer corresponding to the CD11c subunit or to (B) the 2.0-Kb β-actin cDNA.

corresponding to β-actin indicated that approximately the same amount of RNA was loaded in each lane (Figure 1B).

RNA dot blotting was used to quantitate CD11c subunit mRNA expression in HL-60 cells, and to compare these levels to those present in normal human PMN and normal human alveolar macrophages. mRNA for the CD11c subunit was present in undifferentiated HL-60 cells, and levels did not increase after 2 days of exposure to retinoic acid (Fig. 2, row B). Expression of CD11c mRNA increased markedly with differentiation of HL-60 cells along the monocyte/macrophage pathway with PMA (Fig.2, row C). mRNA for the CD11c subunit was present in low levels in human PMN and in high levels in human alveolar macrophages (Fig. 2 compare rows D and E). Reprobing the same blot with a cDNA for β-actin indicated that approximately equal amounts of RNA were loaded in each row (data not shown).

Expression of CD11c subunit mRNA on human leukocytes, leukemia cells, and leukemia cell lines- We extended our studies of CD11c mRNA expression to encompass a variety of lymphoid and myeloid cells and cell lines. These cells and cell lines were selected to include both immature and mature cells. RNA dot blotting of lymphoid cells indicated that EBV B-cells, Jurkat T-cells, PHA-activated T-lymphocytes, and lymphoblasts from an individual with CML/lymphoblastic transformation (33) all expressed similar levels of CD11c subunit mRNA (data not shown). Thus, CD11c mRNA levels did not vary with cell type or stage of differentiation in the lymphoid series.

Myeloid cell expression of mRNA for the CD11c subunit was also examined using RNA blotting. Nearly identical amounts of RNA for the CD11c subunit were present in: myeloblasts from the human leukemia cell line KCL-22 (22) derived from a patient with CML/myeloblastic transformation (Fig.3, row A), myeloblasts from the human leukemia cell line K-562 (21) derived from an individual with CML/blast crisis and possessing predominantly erythroid characteristics (32) (row B), and myeloblasts from the CML/blast crisis cell line EM-3 (23) (row D). Treatment of K-562 cells with PMA resulted in no change in CD11c subunit mRNA levels (row C). The HEL (24) human erythroleukemia cell line displayed a very similar amount of mRNA for the CD11c subunit (row E). CD11c subunit transcripts

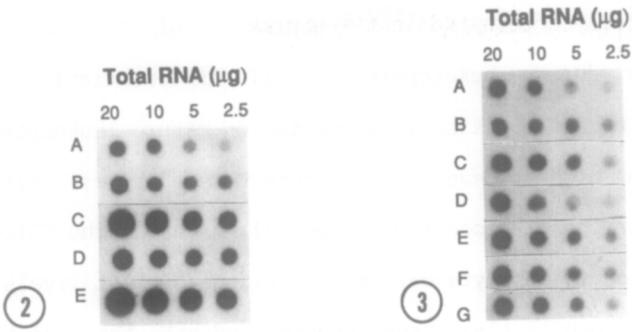

FIGURE 2. Dot blot analysis of RNA from undifferentiated and differentiated HL-60 cells, normal human PMN, and normal human alveolar macrophages. Total RNA was isolated from undifferentiated HL-60 cells (row A); HL-60 cells treated with retinoic acid for 2 days (row B); HL-60 cells treated with PMA for one day (row C), normal human PMN (row D), normal human alveolar macrophages (row E). The indicated amounts of RNA were loaded, and the dot blot was hybridized to the 32P-labeled 26-base pair oligonucleotide probe corresponding to the CD11c subunit.

FIGURE 3. Dot blotting of RNA from human myeloid cells and cell lines. RNA was dot-blotted onto nitrocellulose and hybridized to the 32P-labeled 26 mer corresponding to the CD11c subunit. Total RNA was isolated from: (A) KCL-22 cells, (B) K562 cells, (C) K562 cells treated with PMA, (D) EM-3 cells, (E) HEL cells , (F) myeloblasts from CML/myeloblastic transformation, (G) normal human PMN.

were present in nearly identical levels in fresh human leukemia cells from an individual with CML/myeloblastic transformation (row F) and in normal human PMN (row G). When the same dot blot was probed with a cDNA for β-actin, no significant differences were present in the intensity of hybridization of the rows (data not shown).

DISCUSSION

We investigated the surface antigen and mRNA expression for the CD11c subunit of the human leukocyte adherence receptor family during hematopoietic cell differentiation. The CD11c subunit is constituitively expressed in low levels on undifferentiated HL-60 promyelocytic leukemia cells and levels of surface antigen and mRNA increase markedly when these cells are induced to differentiate along the monocyte/macrophage pathway with PMA. Similarly, human monocyte-derived macrophages from the peripheral blood and alveolar macrophages obtained by lung lavage express high levels of CD11c antigen and mRNA. A variety of additional human leukocytes, leukemia cells, and leukemia cell lines expressed similar, low levels of CD11c mRNA, indicating the selective nature of the increase in CD11c subunit mRNA in monocyte/macrophages.

We are particularly interested in the expression of this family of leukocyte adherence proteins during hematopoietic cell differentiation since the α subunits appear to be differentially expressed on mature leukocytes. The CD11a subunit appears in highest levels on mature T-lymphocytes, although it is present on other peripheral blood leukocytes (2). Although the CD11b subunit is expressed on granulocyte-monocyte derived cells, the highest levels are present on mature human neutrophils (25). These studies indicate that the highest levels of CD11c surface antigen and mRNA are present on human macrophages. Thus, although these three α subunits share significant sequence homology (34), are located in close proximity to each other on chromosome 16 (35), and most likely arose by gene duplication, their expression is differentially regulated with hematopoietic cell differentiation. The specific factors responsible for this differential gene expression have not been characterized.

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