

CD44v6 cell surface expression is a common feature of macrophages and macrophage-like cells – implication for a natural macrophage extravasation mechanism mimicked by tumor cells

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Abstract Soluble CD44standard (sCD44s) and CD44v6 (sCD44v6) cannot only be detected in sera of patients with pancreatic carcinoma but also of healthy blood donors. To investigate whether sCD44s and sCD44v6 are derived from white blood cells, we stimulated whole blood with phytohemagglutinin and interleukin-2, which induced expression of CD44v6 only on monocytes. For further investigations, we used the promyelocytic leukemia cell line HL-60. Only HL-60 cells differentiating along the macrophage pathway showed increased expression of CD44s and CD44v6. Furthermore, only macrophages showed increased secretion of sCD44s and sCD44v6. Our data suggest that CD44s and CD44v6 are common adhesion molecules on macrophages and macrophage-like cells. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Peripheral mononuclear cell; HL-60; Differentiation; CD44; CD44v6

1. Introduction

CD44 is a cell surface proteoglycan expressed on a variety of cell types, including peripheral blood (PB) T and B lymphocytes, monocytes [1–4], Langerhans cells and dendritic cells [5,6]. CD44 has been shown to bind hyaluronan (HA) [3,4], fibronectin [5], MIP-1 β [6] and MHC class II invariant chain [7]. There is evidence for a role of CD44 as a receptor which mediates binding of mononuclear and epithelial cells to extracellular matrix ligands such as hyaluronic acid, fibronectin and collagen [3,7,8]. Following cell activation, the intracellular domain of surface CD44 undergoes phosphorylation [9], palmitoylation [10] and cytoskeletal binding [11,12], and possesses GTPase activity [13]. An *in vitro* role for CD44 has been demonstrated in T lymphocyte activation [10,14–20], NK target lysis [21], B cell lymphopoiesis [22] and cell–cell adhesion [15,23–26]. Human serum is able to induce binding of HA to PB monocytes, but not to T and B lymphocytes, and upregulate monocyte expression of high molecular weight CD44v isoforms [27].

The CD44 gene, located on chromosome 11, consists of at least 19 exons. Ten of which encode the standard form of CD44. Alternative splicing of the pre-mRNA leads to nine high molecular weight isoforms of CD44 (CD44v2–v10). In

humans, CD44v1 seems to be non-functional because of a stop codon [10]. The CD44v6 splice variant of the transmembrane receptor family of CD44 was first isolated and cloned from a rat pancreatic carcinoma cell line and has been shown to confer metastatic behavior to non-metastatic tumor cells [28]. CD44v6 is suggested to be a prognostic factor in many human malignancies including breast, gastric and colorectal cancer [29–31]. Furthermore, CD44v6 expression in non-small cell lung cancer [32] and gastric cancer [33] is supposed to contribute to a high metastatic potential and CD44v6 anti-sense inhibits metastases in the metastatic colorectal tumor cell line HT29 [34]. We could recently show that CD44v6 is expressed in both, normal pancreas and pancreatic cancer [35], and that, interestingly, soluble CD44standard (sCD44s) and CD44v6 (sCD44v6) are significantly reduced in pancreatic carcinoma patients compared to healthy volunteers [36]. There, immunoprecipitation of human sera with anti-CD44v6 revealed three CD44v6 isoforms ranging between 90 kDa and 160 kDa, while in the supernatant of pancreatic carcinoma cell lines, an isoform with 175 kDa could be detected, which was abandoned in human sera of pancreatic cancer patients which implies that PB leukocytes and not tumor cells could be the source of sCD44v6.

The aim of this study was to determine CD44 isoform expression on PB monocytes and lymphocytes and the ability of cultured mononuclear cells to upregulate CD44v6, under the hypothesis that expression of CD44v6 enables extravasation of cells into the tissue and that cancer cells mimic this natural occurring macrophage function.

2. Materials and methods

2.1. Reagents

DMEM media and fetal calf serum were purchased from Biochrom (Berlin, Germany). Phytohemagglutinin (PHA), interleukin-2 (IL-2), dimethylsulfoxide (DMSO), phorbol 12-myristate 13-acetate (PMA) and vitamin D3 were from Sigma (St. Louis, MO, USA). γ -Interferon was purchased from R+D System (Wiesbaden, Germany). FITC-conjugated F(ab')₂ goat anti-mouse Ig, PE-conjugated anti-CD3, anti-CD56, anti-CD19 and anti-CD14 were from Dako Corp. (Hamburg, Germany). Monoclonal control and mouse monoclonal antibody (mAb) recognizing CD44 standard and CD44v6 were from Bender-MedSystems (Vienna, Austria). Enzyme-linked immunosorbent assay (ELISA) kits for the detection of soluble human standard CD44 and CD44v6 were purchased from BenderMedSystems (Vienna, Austria).

2.2. Whole blood culture

Heparin-anticoagulated blood was cultured for 0 h, 24 h, 48 h and 72 h at 37°C in 5% CO₂ atmosphere in mitogen-containing lyophilized culture medium (PHA 1 μ g/ml, IL-2 1000 U/ml) in glass vessels. After

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incubation, blood cells were harvested, adherent cells were collected by vigorously shaking the vessel, red blood cells were lysed (Optilyse, Immunotech, Germany) and leukocytes were washed twice with phosphate-buffered saline (PBS). All experiments were replicated at least three times.

2.3. HI-60 cell culture

HI-60 cells, obtained from American Type Culture Collection, were cultured in DMEM media supplemented with 10% fetal calf serum. For experiments, cells were seeded at 5×10^5 cells/ml and treated with DMSO at a concentration of 1%, with PMA at doses of 1.5 nM, with vitamin D3 at doses of 1 nM and γ -interferon with 250 U/ml. After 12, 24, 36, 48 h of treatment, cells were harvested, adherent cells were harvested using Puck's EDTA, supernatants collected and immediately frozen at -20°C . Cells were washed twice with PBS. All experiments were replicated at least three times.

2.4. Determination of cell viability

For determination of cell viability, cells were stained by trypan blue (Sigma, St. Louis, MO, USA) and the numbers of viable and dead cells were determined by light microscopy.

2.5. ELISA

Commercial ELISAs were used to measure levels of soluble CD44 standard and CD44v6 in media from HI-60 cells exposed to DMSO, PMA, vitamin D3 and γ -interferon as provided by the manufacturers. Affinity-purified mAbs were used to capture and horseradish peroxidase-conjugated mAbs were directed against circulating adhesion molecules for detection. Results were obtained in ng/ml on the basis of four standards.

2.6. Immunofluorescence staining and flow cytometry

2.6.1. Whole blood cultures. Harvested cells were resuspended in PBS at 5×10^6 cells/ml, and 100 μl of each sample was incubated with a mixture of human immunoglobulins (Pentaglobin, Biotest, Dreieich, Germany) to block Fc-R. After 10 min of incubation, saturating concentrations of the following PE-labeled mAbs were added: CD3, CD56, CD19 and CD14, followed by FITC-labeled mAbs CD44s and CD44v6. PE-labeled and FITC-labeled mouse Ig served as negative control. After incubation for 30 min at 4°C , samples were then washed and immediately analyzed. Samples were examined in a fluorescence-activated cell sorter (FACScan) flow cytometer (Becton Dickinson, Heidelberg, Germany) equipped with CellQuest software. Cell surface expression of CD44s and CD44v6 on monocytes was assayed in double labeling protocols gating on CD14⁺ cells. Log forward light scatter and log 90° light scatter were used to gate out debris. 10 000 gated events were collected for each sample and plotted on a three decade fluorescence scale.

2.6.2. HI-60 cultures. After 12, 24, 36 and 48 h of treatment, cells were harvested, washed twice with PBS and resuspended in PBS at 5×10^6 cells/ml. 100 μl of each sample was incubated with a mixture of human immunoglobulins (Pentaglobin, Biotest, Dreieich, Germany) to block Fc-R. After 10 min of incubation, saturating concentrations of the unlabeled mAbs CD44s and CD44v6 or mouse Ig as negative control were added for 30 min at 4°C . Samples were then washed and incubated with fluoresceinated goat anti-mouse secondary antibody for 30 min, washed again and immediately analyzed. Cell viability was determined by flow cytometry using ethidium monoazide. Only viable cells were used for further flow analysis.

2.7. Western blot analysis

Cells were lysed by sonication and then boiled in sodium dodecyl sulfate (SDS) gel sample buffer for 5 min. 20 μg of protein was resolved electrophoretically on denaturing 10% polyacrylamide gels. Proteins were transferred to nitrocellulose membranes (Schleicher and Schüll, Dassel, Germany) using a transblot apparatus (Phase, Lübeck, Germany). Non-specific interactions were blocked by preincubation of the membranes with a milk powder suspension (10% dry milk in PBS). Membranes were incubated at room temperature with the following mAbs. Anti-CD44 standard and anti-CD44v6 (both Bender Medsystems, Vienna, Austria). After specific staining, membranes were incubated with biotinylated rabbit anti-mouse polyclonal serum for 1 h (Tropix, Bedford, MA, USA). After each antibody incubation, membranes were washed with PBS containing 0.3% Tween 20 (Sigma). Binding of antibodies was detected using Western-Light system (Tropix).

2.8. Reverse transcription (RT)-PCR amplification and exon specific analysis

Total RNA was prepared from 1×10^6 – 5×10^6 cells as described previously [32]. 5 μl total RNA was mixed with diethylpyrocarbonate-treated water, a CD44 specific oligonucleotide at the 3' side of constant exon 17 (C12A; 20 pmol) and 20 U RNasin (Stratagene, Heidelberg, Germany). The mixture was incubated at 70°C for 5 min; subsequently, dNTPs (final concentration 1 mM), RT buffer, dithiothreitol (5 mM) and 200 U Superscript Plus (Gibco BRL, Eggenstein, Germany) were added to make a volume of 20 μl . The mixture was incubated at 37°C for 1 h, followed by inactivation of the enzyme at 95°C for 5 min. For exon specific PCR amplification, primers were chosen at the 5' side of each variant exon using the recently published oligonucleotides [32]. PCR reactions were performed in a total volume of 20 μl containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl_2 , 50 μM each dNTP, 5 pmol each primer, 0.5 U Taq polymerase and 2 μl cDNA. The cDNA was amplified in 35 cycles: 0.5 min denaturation at 95°C ; 1.5 min annealing at 56°C ; and 1 min extension at 73°C , followed by a final extension at 73°C for 10 min. PCR products were analyzed on a 1.25% agarose gel in $1 \times$ Tris-borate EDTA. The amplification products were visualized under UV light at 590 nm after staining of the gel with ethidium bromide.

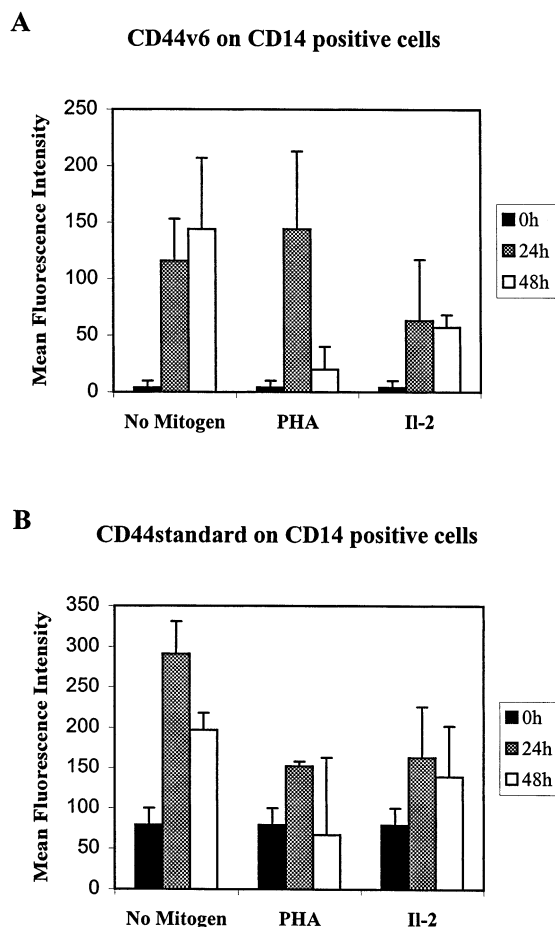


Fig. 1. Flow cytometric analysis of cell surface expression of CD44s and CD44v6 on PB monocytes. Whole blood was cultured for 0 h, 24 h, 48 h and 72 h with PHA (1 $\mu\text{g}/\text{ml}$), IL-2 (1000 U/ml) and without treatment. Cells were harvested, erythrocytes were lysed and cells were labeled with saturating amounts of FITC-conjugated anti-CD44 and anti-CD44v6 followed by PE-conjugated mAb anti-CD14. Cell surface expression of CD44s and CD44v6 on monocytes was assayed in double labeling protocols gating on CD14⁺ cells. Values shown are averaged from five experiments. Results from CD14⁺ monocytes labeled with CD44v6 mAbs are grouped (A) and monocytes labeled with CD44s mAbs are grouped (B).

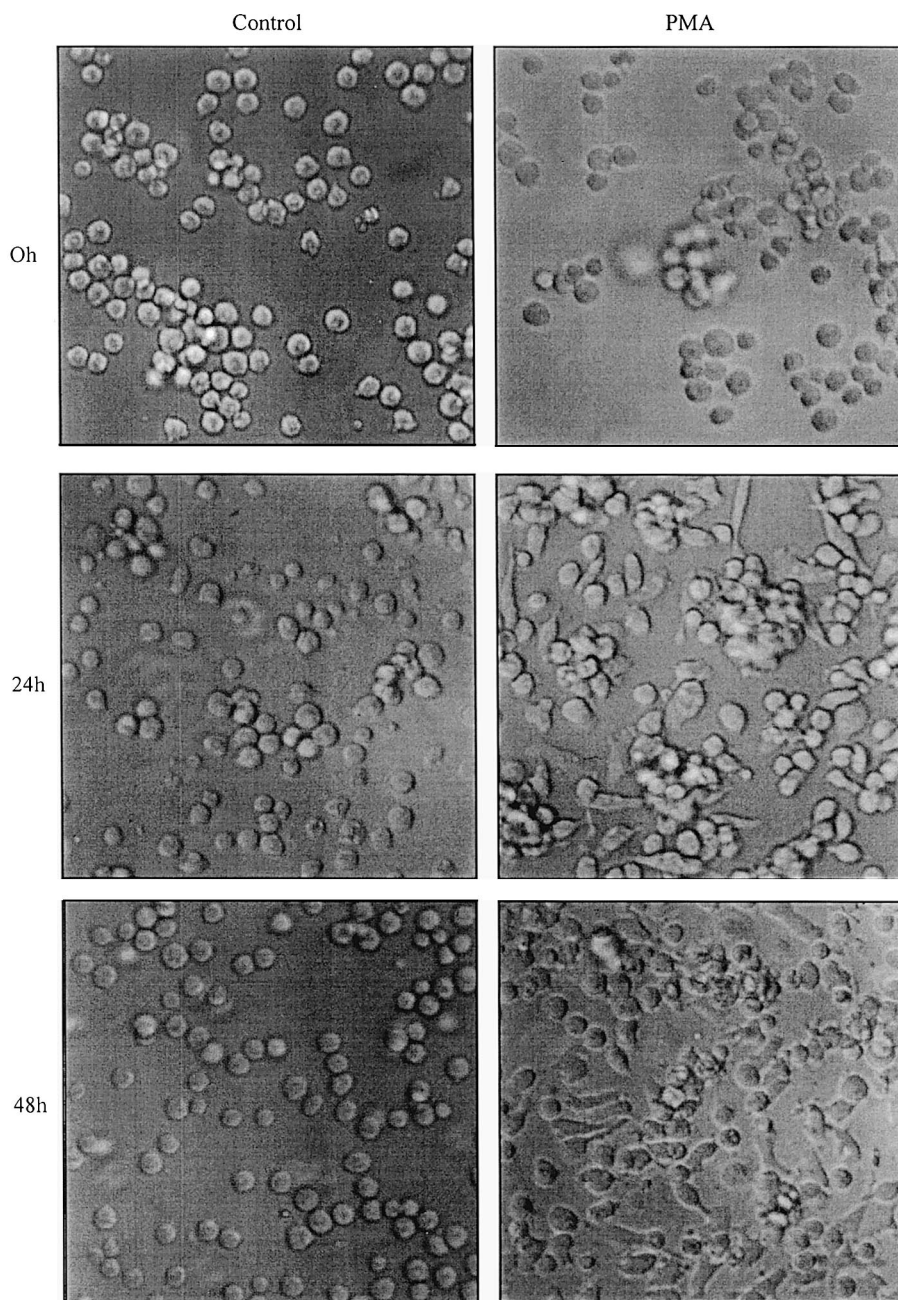


Fig. 2. Morphological changes in HI-60 cells exposed to PMA. PMA-treated cells changed from rounded to more spread compared to untreated control cells which remained round at any of the time points examined. 24 h after exposure to PMA, approximately 50% of the cells adhere to the dish, followed by more than 75% adhesion at 48 h.

2.9. Statistical analysis

The statistical significance was evaluated by Student's *t* test. Significant, when not specifically indicated, refers to $P < 0.05$.

3. Results

3.1. Effect of PHA and IL-2 on cell surface expression of CD44s and CD44v6 in PB lymphocytes and monocytes

Based upon previous findings that CD44v6 is transiently expressed on B and T lymphocytes following antigenic stimulation, we examined cell surface expression of CD44s and CD44v6 on peripheral lymphocytes and monocytes under cul-

ture conditions that stimulate T cells. Cell surface expression of CD44s and CD44v6 on PB monocytes was assayed in double labeling protocols gating on CD14⁺ cells. We could observe significantly increased cell surface expression of CD44s on PB monocytes cultured for 24 h and 48 h without stimulation ($P < 0.05$) and 24 h after stimulation with PHA ($P < 0.05$) (Fig. 1). CD44v6 on the cell surface of CD14⁺ monocytes increased significantly ($P < 0.05$) after 24 h and 48 h without treatment, after 24 h stimulation with PHA and after 24 h and 48 h stimulation with IL-2 compared with fresh blood monocytes (Fig. 1). 48 h after stimulation with PHA, there was a significant ($P < 0.05$) decrease of

CD44v6 compared to 24 h, whereas monocytes without mitogen showed a slight increase. It is well known that CD44v6 expression shows a peak 24–48 h following activation and a decline to baseline levels thereafter. It could be possible that monocytes without mitogen show the same kinetic later than

48 h of culture. Following in vitro culture of whole blood with PHA and IL-2, there was no increase in cell surface expression of CD44s and CD44v6 on T cells (CD3⁺), natural killer cells (CD56⁺) or B cells (CD19⁺) compared with fresh unstimulated lymphocytes (data not shown). To further investigate what

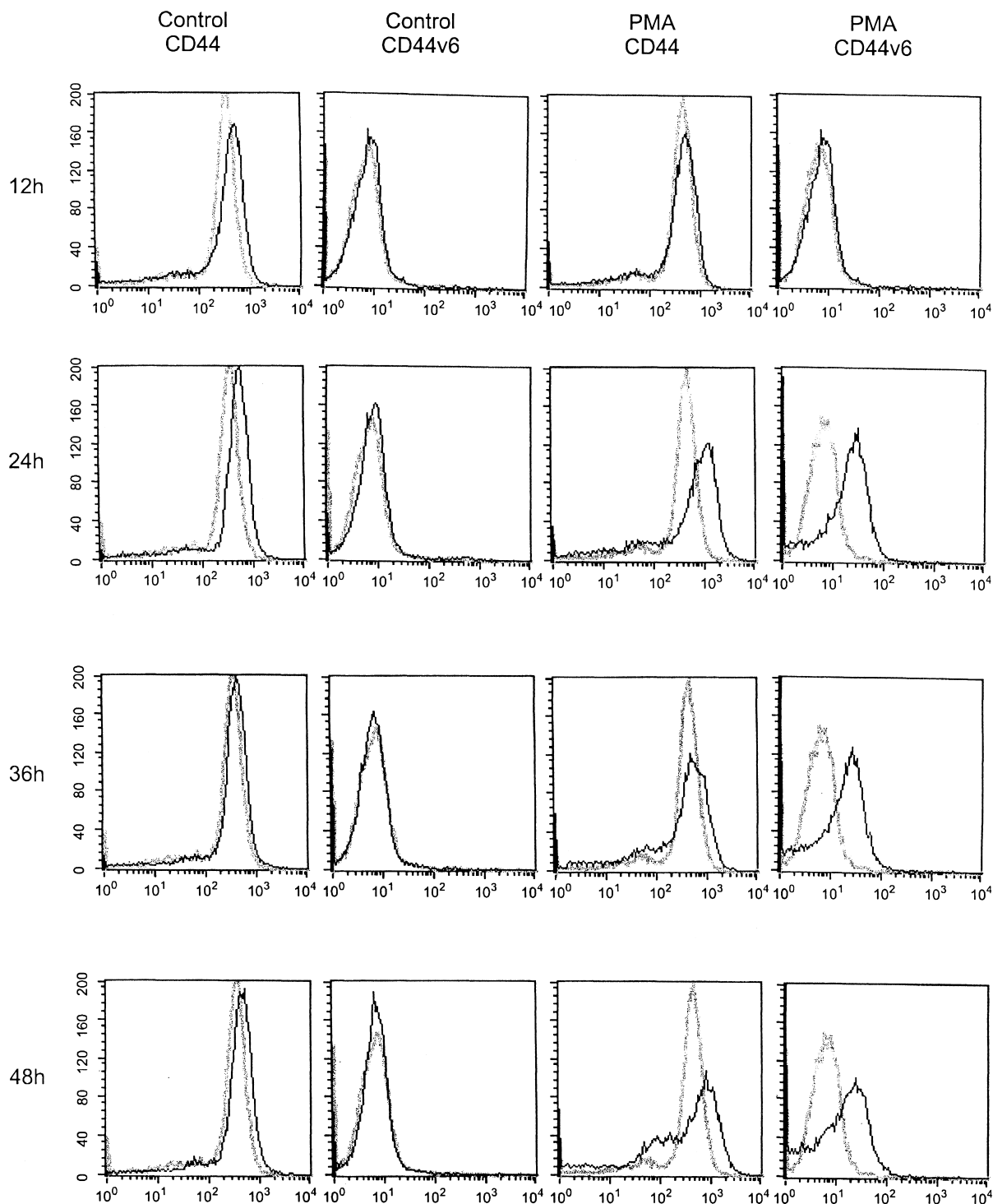


Fig. 3. Expression of CD44 and CD44v6 on the cell surface of HI-60 cells as measured by flow cytometry. HI-60 cells were cultured in the presence or absence (control) of PMA for 0, 12, 24, 36 and 48 h. Cells were stained with primary antibodies against the human CD44s and CD44v6 followed by FITC-labeled goat anti-mouse secondary antibody. The gray line shows fluorescence at 0 h, the black line shows fluorescence at 12, 24, 36 and 48 h. Cells were analyzed by flow cytometry. The y axis represents cell number, the x axis represents log fluorescence intensity. Results were from one representative experiment; the pattern shown here was reproduced in five separate experiments.

sort of white blood cells are able to express CD44s and CD44v6, we used the promyelocytic leukemia cell line HI-60 as a model of differentiation.

3.2. Effect of DMSO, PMA, vitamin D3 and γ -interferon on the morphology of HI-60 cells

HI-60 cells exposed to PMA exhibited a time course for adhesion, with changes in morphology from rounded to more spread. 12 h after exposure to PMA, 5–10% of the cells had changed morphology, followed by approximately 50% of the cells adhering to dishes by 24 h and more than 75% adhesion at 36 h and 48 h. In contrast, cells without treatment (control) or cells exposed to DMSO, vitamin D3 and γ -interferon did not exhibit adhesive properties at any time points examined, from 0 to 48 h. As shown in Fig. 2, cells treated with PMA for 24 h or 48 h adhere to the cell culture dish whereas untreated control cells remain rounded with no adherence.

3.3. Effect of DMSO, PMA, vitamin D3 and γ -interferon on cell surface expression of CD44s and CD44v6 in HI-60 cells

The effect of DMSO, PMA, vitamin D3 and γ -interferon treatment of HI-60 cells on expression of cell surface CD44s and CD44v6 was determined by flow cytometry. For differentiation, we used experimental protocols known to induce granulocyte, monocyte and macrophage differentiation in HI-60 cells. Surface expression showed that more than 90% of untreated HI-60 cells constitutively expressed the standard CD44 molecule. Intensity of fluorescence increased slightly between 24 h and 48 h in cells exposed to DMSO, vitamin D3 and γ -interferon. However, this increase did not reach statistical significance. A much stronger upregulation was detected upon treatment with PMA. Exposure of HI-60 cells to PMA resulted in an enhancement of CD44 standard protein on the cell surface, which was observed as early as 24 h (Fig. 3).

Untreated cells at the time point 0 h expressed the epitope encoded by exon v6 at low levels of about 8%. HI-60 cells without treatment and cells treated with DMSO, vitamin D3 and γ -interferon showed a slightly increased expression for CD44v6 between 24 h and 36 h (data not shown). However, HI-60 cells differentiating along the granulocyte and monocyte pathway exhibited the same upregulation of CD44v6 like control cells. In contrast, exposure to PMA resulted in a much stronger upregulation of CD44v6 much beyond that seen with untreated cells or cells treated with DMSO, vitamin D3 or γ -interferon. This strong increased expression for CD44v6 was observed at 24 h and maintained throughout the time period of this experiment. Compared to control levels, the addition of PMA leads to an about 3-fold increased CD44v6 expression (Fig. 3).

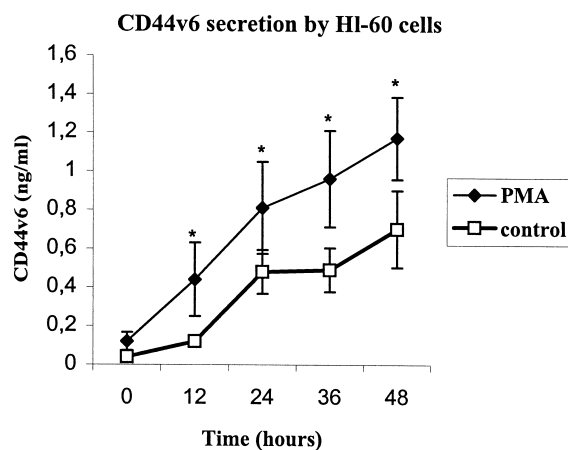
3.4. Effect of DMSO, PMA, vitamin D3 and γ -interferon on the amount of soluble CD44s and CD44v6 in cell culture supernatant

To determine whether cells expressing CD44 standard and CD44v6 were secreting these proteins, we determined the concentration of these proteins in media from HI-60 cells exposed to DMSO, PMA, vitamin D3 and γ -interferon at designated time points using an ELISA method. Untreated cells as well as cells treated with DMSO, PMA, vitamin D3 and interferon

induced the secretion of CD44 standard by HI-60 cells. Measurable levels of CD44 standard in media were noted by 0 h and increased steadily up to 48 h. There were no differences in the amount of soluble CD44 standard in media of untreated cells or HI-60 cells treated with DMSO, vitamin D3 or γ -interferon. As shown in Fig. 4, treatment with PMA induced significantly increased secretion of CD44 standard into the medium, compared to untreated control cells.

The same results were found with CD44v6. DMSO, vitamin D3 and γ -interferon could not increase the amount of soluble CD44v6 in the medium significantly, compared to untreated control cells, whereas exposure to PMA resulted in a significantly increased secretion of CD44v6 into the medium (Fig. 4).

A



B

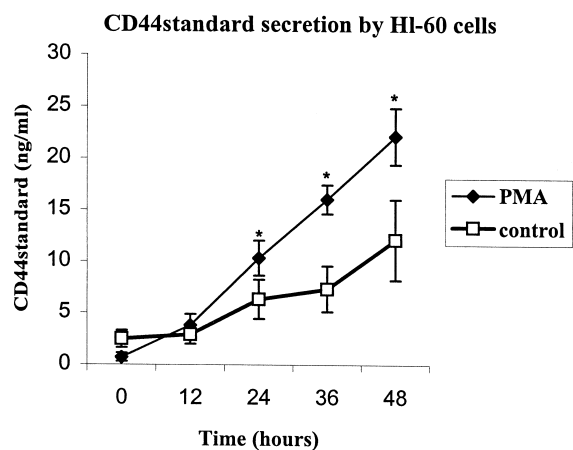


Fig. 4. CD44v6 and CD44s secretion in HI-60 cells treated with PMA and without treatment (control), as measured by ELISA. The concentration of CD44v6 and CD44s was measured 0, 12, 24, 36 and 48 h after exposure in the medium. Results are expressed as ng/ml. PMA led to an increased concentration of CD44v6 (group A) in medium at time points of 12 h, 24 h, 36 h and 48 h (* $P < 0.003$ as compared to control) and to an increased concentration of CD44 standard (group B) in medium at time points of 24 h, 36 h and 48 h (* $P < 0.03$ as compared to control).

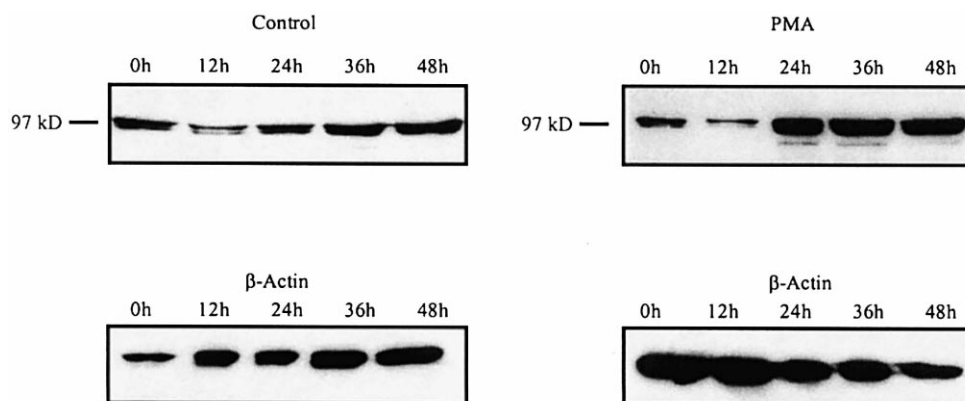


Fig. 5. Western blot analysis of CD44v6 in HI-60 cells in the absence (control) or presence of PMA for 0, 12, 24, 36 and 48 h. Cells were lysed and boiled in SDS gel sample buffer. 20 μ g of protein was run on 10% polyacrylamide gels and transferred to nitrocellulose. Blots incubated with anti-CD44v6 antibodies and developed with biotinylated rabbit anti-mouse polyclonal serum. Binding of antibodies was detected using Western-Light system (Tropix).

3.5. Effect of PMA on CD44 standard and CD44v6 measured by Western blotting

To confirm the results of flow cytometric analysis and ELISA, we performed Western blot analysis on HI-60 cells treated with PMA or without treatment. Cell lysates were analyzed by Western blotting using a CD44 antibody recognizing the standard form of CD44. A predominant CD44 protein of about 50 kDa was detected. CD44 standard was constitutively expressed on all cells independent of a given treatment. Western blotting using a CD44v6 specific antibody revealed a band at 97 kDa which was identified as CD44v6. As depicted in Fig. 5, untreated cells (control) expressed CD44v6 from 0 h up to 48 h. In vitro culture for 24 h in PMA induced increased expression of CD44v6.

3.6. Effect of PMA treatment on CD44v mRNA expression in HI-60 cells

To examine the patterns of splice variants in the presence or absence of PMA, total RNA was isolated, reverse-transcribed, and amplified using primers 5' and 3' of the site of insertion of variant exons. All samples showed a predominant PCR product of about 320 kb, the size of CD44 standard.

Exon specific analysis of HI-60 cells without PMA showed a rather constant expression of mRNA encoding CD44v6–CD44v10. HI-60 cells treated with PMA showed the same CD44v expression pattern. 0 h, 12 h, 24 h, 36 h and 48 h, this major variant chain consisting of CD44v6–10 was

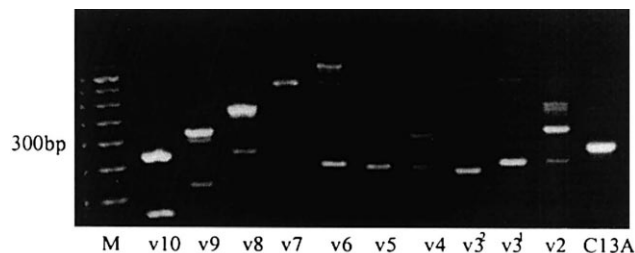


Fig. 6. Exon specific analysis of HI-60 cells stimulated with PMA for 36 h. After RT, exon specific PCR was performed using primers located on the 5' side of the constant region (C13A) or the variant exons (v2–v10). From the size and the presence of the splice products, the chain compositions can be concluded as described recently [32]. No splice product was overexpressed or missing, compared to untreated control cells.

present. There were no differences in the splice pattern between the two groups. No splice variant was missing or overexpressed. Fig. 6 shows the splice pattern of HI-60 cells treated with PMA for 36 h.

4. Discussion

CD44v6 seems to play an important role in tumor progression and is supposed to contribute to a high metastatic potential [29–34]. We could recently show that CD44v6 is expressed in both, normal pancreas and pancreatic cancer [35], and that, interestingly, sCD44s and sCD44v6 are significantly reduced in pancreatic carcinoma patients compared to healthy volunteers [36]. There, immunoprecipitation of human sera with anti-CD44v6 revealed three CD44v6 isoforms ranging between 90 kDa and 160 kDa, while in the supernatant of pancreatic carcinoma cell lines, an isoform of 175 kDa could be detected, which was abandoned in human sera. To determine whether sCD44s and sCD44v6 could be derived from peripheral leukocytes, we performed in vitro culture of whole blood. Based upon previous findings that CD44v6 is transiently expressed on B and T lymphocytes following antigenic stimulation, we used culture conditions that particularly stimulate T cells. We have shown that in vitro culture of monocytes is associated with increased cell surface expression of CD44s and CD44v6 independent of a given treatment. 48 h after stimulation with PHA, monocytes show a rapid decrease of CD44v6 expression, whereas monocytes without treatment or Il-2 show a slight increase. It is well known that CD44v6 expression shows a peak 24–48 h following activation and a decline to baseline levels thereafter [26]. It could therefore be possible that monocytes without mitogen or Il-2 show the same kinetic later than 48 h of culture. Stimulation with PHA and Il-2 could not upregulate cell surface expression on T cells, B cells or natural killer cells. It has been shown that in vitro culture of PB monocytes in the presence of human serum induces monocytes, but not T and B lymphocytes, to bind HA and upregulate monocyte expression of high molecular weight CD44v isoforms [27]. Interestingly, culture of whole blood without any stimulus served as a trigger to upregulate CD44v6 on monocytes. These results suggest that CD44v6 plays an important role in monocytes differentiating into macrophages. To further investigate which subset of leu-

kocytes is associated with increased levels of CD44v6, we used the promyelocytic cell line HL-60 as a model for leukocyte differentiation. Addition of DMSO increases spontaneous differentiation of HL-60 cells to granulocytes, with most cells acquiring morphological, functional, enzymatic and surface membrane antigen characteristics of mature granulocytes [37]. The phorbol ester PMA induces a macrophage-like phenotype. Several important phenotypic characteristics clearly distinguish the HL-60 macrophage-like differentiation from HL-60 monocytic differentiation induced by vitamin D3 and γ -interferon. Macrophage-like HL-60 cells are markedly more adherent than HL-60 monocytes, and do not have several specific surface antigens present on normal and HL-60 monocytes [38,39]. An important role of CD44v6 and CD44v9 in inflammation has been described, suggesting that CD44v expression is associated with differentiation of monocytes to tissue macrophages in vivo. It is possible, therefore, that changes in cell surface CD44v6 expression are not only important factors controlling differentiation of monocytes but also of granulocytes and macrophages. We have clearly demonstrated upregulation of CD44v6 isoform expression in HL-60 cells differentiating to macrophages but not in cells differentiating to monocytes or granulocytes. Our data raise the hypothesis that only macrophage differentiation of the lineage differentiation experiments shown here may be associated with increased expression of CD44v6 isoform. Interestingly, untreated cells and cells treated with DMSO, vitamin D3 or interferon showed the same basal increasing expression for CD44v6. CD44v6 on the cell surface was slightly upregulated between 12 and 24 h. After 36 h, CD44v6 was decreasing. One possible explanation for this effect is that about 5% of the HL-60 cells exhibit spontaneous differentiation to morphological mature cells. This percentage may be increased by addition of serum to media. Analysis of cultured monocytes has demonstrated that in vitro monocyte culture in the presence of serum promotes differentiation of monocytes into tissue macrophages [38]. Our results from Western blot and PCR indicate that high molecular CD44v isoforms are not only expressed after treatment with PMA but also in untreated control cells. PCR revealed that the mRNA is constantly transcribed independent of a given treatment. Furthermore, protein levels of CD44v6 in untreated control cells are markedly high, although we found hardly any expression of the protein on the cell surface. These results suggest that CD44 in our system is transcribed constantly, spliced to specific isoforms, including CD44v6, which might be stored intracellularly and only be translocated to the cell surface in cases of macrophage-like differentiation.

Langerhans cells and dendritic cells upregulate variant exons v4, v5, v6 and v9 upon antigen contact. These splice variants are obligatory for the migration and function of Langerhans cells and dendritic cells [5,6]. In the view of tissue migration, macrophages, dendritic cells, Langerhans cells and metastatic tumor cells share several similarities. All these cell types have to be able to migrate tissue and keep motility. CD44v6 was first described in metastasizing rat pancreatic carcinoma cell line, while non-metastasizing tumor cell line or most normal rat tissues show no expression. This metastatic potential could be conferred to non-metastasizing cells [28]. Our results of whole blood stimulation showed only macrophages to express CD44v6 on the cell surface. Therefore, one could think that CD44v6 surface expression is a

common feature of macrophages and macrophage-like differentiated cells. Based on this assumption, it could be possible that CD44v6 enables not only macrophages but also tumor cells to migrate and move in the tissue and therefore is a prognostic factor of tumor progression and metastasis at least in some tumors.

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