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Down-regulation of monocytic VLA-4 leads to a decreased adhesion to VCAM-1

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The $\alpha_4\beta_1$ integrin VLA-4 is expressed on practically all leukocytes, except on mature granulocytes. Here we show that in vitro treatment of monocytic cells with phorbol-12-myristate-13-acetate (PMA) leads to a selective decrease in the VLA-4 α-chain expression, both at the RNA and protein level. Meanwhile the expression of β_1 and that of α_5, another α-chain associating with β_1, was seen to increase. The decrease of α_4 expression was restricted to monocytic cells, and was not observed on other VLA-4-positive cells tested (MOLT-4 T cells and HOS sarcoma cells). The down-regulation of the VLA-4 α-chain was followed by a decreased binding capacity of the cells to recombinant VCAM-1. This data indicates that while previous findings show that the integrin-dependent adhesion may rapidly be regulated by altering the avidity of the interacting molecules, their quantitative modulation also has a clear impact on adhesion.

Integrin; VLA-4; VCAM-1; Adhesion

1. INTRODUCTION

Leukocytes circulating in the blood extravasate through the endothelium after first adhering to endothelial cells. This adherence is mediated by the interaction between adhesion molecules on endothelial cells and their counterpart ligands on leukocytes. Several different leukocyte molecules, with both carbohydrate and protein recognition epitopes, are involved in extravasation [1–4]. VLA-4 is a leukocyte-adhesion molecule that, among a few other ligands for endothelial adhesion molecules, belongs to the family of integrins [5].

Although all members of the β_1 integrin family are involved in cell–extracellular matrix interactions, VLA-4 is the only member of the family participating in various cell–cell adhesion-dependent events [6]. VLA-4-expressing T and B lymphocytes, monocytes, eosinophils, basophils and natural killer cells (NK) are able to adhere to the endothelium through recognition of their counter receptor, the vascular cell adhesion molecule-1 (VCAM-1) [7–12].

Regulation of VLA-4 has mainly been studied in lymphocytes and very little is known of its regulation in monocytic cells. We have studied VLA-4 expression on THP-1 and HL-60 leukemia cell lines. Activation of these cells with a protein kinase C stimulator, phorbol-myristate acetate (PMA), led to a significant decrease in VLA-4 α-chain expression in these cells, both at the RNA and protein level. The decreased expression of the α_4-chain was linked to a decrease in the binding of THP-1 cells to recombinant soluble VCAM-1 fixed on microtiter plates. This finding might have implications in the extravasation (metastasis) of monocytic leukemias and initiation of inflammatory and arteriosclerotic lesions.

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Abbreviations: VLA-4, very late activation antigen; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule-1.

2. MATERIALS AND METHODS

2.1. *Materials*

The monocytic cell lines, THP-1 and HL-60, the human osteosarcoma, HOS, and a T cell line, MOLT-4, were obtained from ATCC and cultured in RPMI supplemented with 10% FCS and antibiotics. PMA was obtained from Sigma (St. Louis, MO). Recombinant IFN-γ (2.5×10^7 U/mg) and IL-1 (3×10^8 U/mg) were generous gifts from Dr. C.W. Reynolds, National Cancer Institute (Frederick, MD). Recombinant TNFα (2.0×10^7 U/mg) was purchased from Boehringer (Mannheim, Germany). Recombinant soluble (rs) VCAM-1 was a kind gift from Dr. Roy Lobb, Biogen Inc., Cambridge, MA, USA [13]. [^{32}P]dCTP was purchased from Amersham (England). The monoclonal antibody, anti-α_4 (clone HP1/2) was a kind gift from Dr. Roy Lobb, Biogen Inc. The monoclonal anti-VCAM-1 antibody (4B9) was a kind gift from Dr. John Harlan, Seattle. The anti-β_1 (clone K20), anti-CD18 (clone IOT18) and anti-ICAM-1 (clone 84H10) antibodies were obtained from Immunotech (Marseille, France). The secondary antibody for flow cytometry was the fluorescein-conjugated F(ab')2 fragment of rabbit immunoglobulins to mouse immunoglobulins purchased from Dakopatts a/s (Glostrup, Denmark).

2.2. *Flow cytometry*

1×10^6 cells were incubated with antibody diluted with washing buffer (0.2% FCS/PBS) at 4°C for 30 min. The cells were then washed twice with washing buffer and were incubated with the fluorescein-labeled secondary antibody as above. After two washings the cells were suspended in 3% PFA/PBS and analyzed in a Beckton Dickinson FACSCAN analyzer. The results are expressed as a mean fluorescence index (MFI). For negative controls the cells were treated with secondary antibody only.

2.3. *Northern blotting*

Total RNA was extracted according to standard protocols. 15 μg of RNA was transferred to a nylon filter and hybridized with [^{32}P]dCTP-labelled α_4 and β_1 probes. As a probe for detecting human VLA-4 α-chain mRNA, we used a PCR product including bases 2,237–2,609 in the VLA-4 α-chain gene. In the PCR reaction we used cDNA of THP-1 cells and the amplification was carried out in 1,000 μl *Taq* DNA polymerase buffer (Promega) in the presence of 1 μl of primers, ATGTGAGCTCACTCAGA and CAAAGTGG-CATTCTCCAG, 200 μM of each dNTP and 10 μl of *Taq* DNA polymerase (Promega). The reaction was carried out for 30 cycles (denaturation at 93°C for 1 min, annealing at 54°C for 1 min, polymerization at 72°C for 2 min) and it yielded a single 372-bp long amplified product. The DNA fragment was purified in-gel and was analyzed by cutting with *Sph*I, *Dra*I and *Kpn*I which revealed restriction sites corresponding to restriction sites inside bases between 2,237–2,609 of VLA-α_4 gene. The β_1 probe was purchased from Telios Co. (La Jolla, CA, USA). Actin was used as a loading control probe. After washings the filters were exposed to X-ray film. The films were scanned with a densitometer and the results are given as relative RNA levels (corrected for the amount of RNA loaded in the gels).

2.4. *Adhesion assay*

Microtiter plates were coated with recombinant soluble VCAM-1 (0.5 μg/well) overnight at 4°C. Plates were blocked with 1% BSA in PBS for 1 h at room temperature, the cells were added and allowed to adhere for 10 min. The number of adherent cells was quantified by determining the number of adhered cells in 10 high-power microscopic fields. For antibody inhibitions the cells were preincubated with anti-α_4 antibody (10 μg/ml) for 30 min at room temperature or the plates were incubated with anti-VCAM-1 antibody (10 μg/ml) for 30 min at room temperature and the adhesion assay was performed as above. For negative controls the cells were treated with anti-CD18 antibody or the plates with anti-ICAM-1 antibody. Background binding was determined from plates treated with BSA only.

3. RESULTS AND DISCUSSION

3.1. *PMA treatment selectively decreases the surface expression of the VLA-4 α-chain on monocytic cells*

Treatment with PMA differentiates the myelomonocytic THP-1 and HL-60 cell lines into macrophages. Concomitantly, a significant decrease in their VLA-4 α_4-chain expression was observed (Fig. 1). The down-regulation of the α_4-chain was dose dependent and was seen at PMA concentrations exceeding 0.

being optimal (not shown). Furthermore, the surface expression of the β_1-chain was simultaneously increased. The decrease in α_4 expression, as well as the increase in the β_1 expression, was already observed after a 24 h stimulation, but progressed steadily at least up to 72 h (data not shown). The effects were, however, more pronounced in THP-1 cells, where the initial MFI for anti-α_4 monoclonal antibody was higher than for HL-60 cells (from MFI 74 to 23, and from 38 to 22 for THP-1 and HL-60 cells, respectively). Further experiments were therefore performed using this cell line. The β_1-chain of VLA-4 associates with several other α-chains, e.g. α_5 forming an integrin that binds to fibronectin. The expression of the α_5-chain was seen to increase upon PMA treatment (MFI from 41 to 72, and 21 to 70 for THP-1 and HL-60, respectively) (Fig. 1) showing a very different regulation pattern for the two α-chains associating with the β_1-chain. We further assayed the ability of the cytokines, TNFα, IFN-γ and IL-1, to modify the VLA-4 expression. None of the cytokines was able to induce a similar effect as that observed with PMA (not shown).

Our findings are supported by a previous report by Ferreira et al. showing similar effects in another myelomonocytic cell line, U937 [14]. It is also worth noting that the observed down-regulation is unique for the VLA-4 molecule among the integrins involved in cell–cell adhesion. In addition to the up-regulation of the α_5-chain reported here, the surface expression of both the α- and β-chains of the β_2-integrins, LFA-1, MAC-1 and p150,95, are strongly up-regulated in myelomonocytic cells [15] upon PMA treatment.

3.2. *The decrease of α_4 surface expression by PMA treatment is not a common feature of all VLA-4-expressing cells*

We next analyzed whether the above-mentioned alterations in the VLA-4 expression pattern could also be

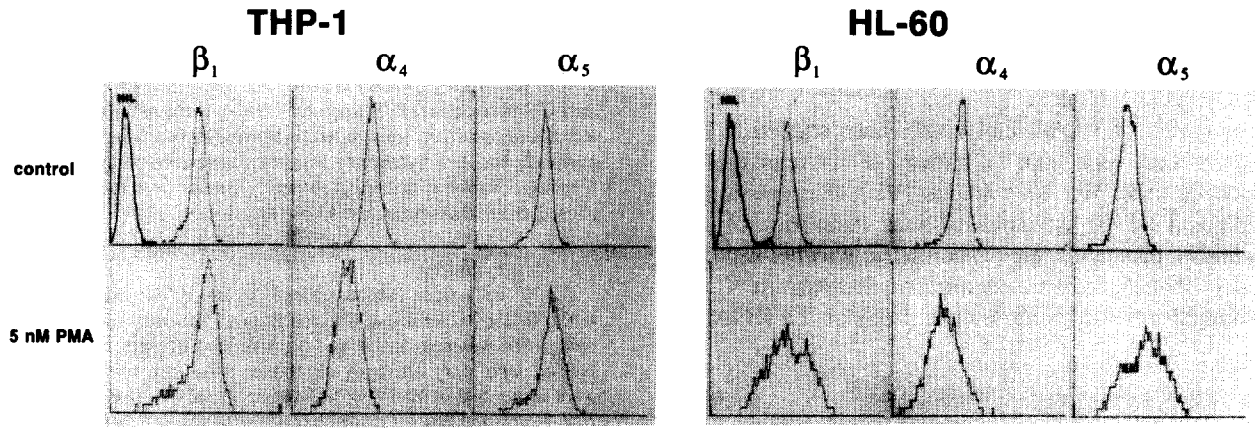

Fig. 1. PMA treatment modifies VLA-4 α- and β-chain expression of myelomonocytic THP-1 and HL-60 cells. Cells were treated with 5 nM PMA for 72 h and were assayed by flow cytometry using either no antibody (NIL) or specific anti-β_1, anti-α_4 or anti-α_5 antibodies and FITC-conjugated goat anti-mouse antibody as secondary antibody. One representative experiment out of three is presented.

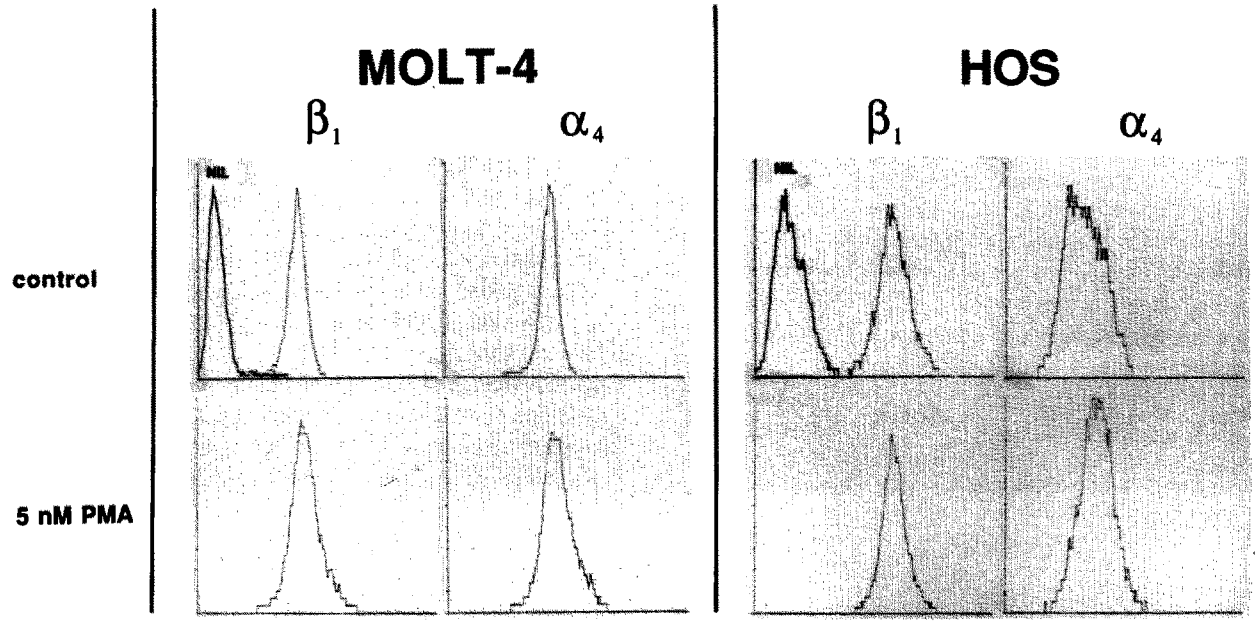

Fig. 2. VLA-4 regulation of an osteosarcoma cell line HOS and a T lymphocyte line MOLT-4. Cells were treated with 5 nM PMA for 72 h and analyzed by flow cytometry as described above. NIL = secondary antibody only. One representative experiment out of four is presented.

induced in other VLA-4-expressing cell lines. The expression of α_4- and β_1-chains on a T cell line, MOLT-4, slightly increased during PMA stimulation compared to untreated cells. HOS is a human osteosarcoma cell line expressing VLA-4, and PMA induction slightly increased the α_4 expression (from MFI 26 to 42). At the same time no alterations could be demonstrated in the β_1 expression of HOS cells (Fig. 2).

These data indicate that the down-regulation of VLA-4 α-chain upon activation described above is not a general phenomenon for all VLA-4-expressing cells. However, although peripheral B lymphocytes strongly express α_4, a decrease in their α_4 expression has been reported after 3 day treatment with PMA. Meanwhile tonsillar lymphocytes express only minor amounts of VLA-4, but after a 3 day PMA stimulation their VLA-4 expression is induced [15]. VLA-4 expression has been shown to increase during T cell development. Virgin T cells express only low amounts of VLA-4, whereas memory T cells are strongly VLA-4 positive [16,17]. Long-term activation with lectins has been shown to increase VLA-4 expression on T cells [18].

3.3. *Regulation of VLA-4 expression at the RNA level*

The changes in the expression pattern of both the α- and β-chains of VLA-4 require a relatively long-term treatment, suggesting that these modifications take place both at the RNA and protein synthesis level. To verify this we isolated total RNA from THP-1 cells treated with 5 nM PMA for 24 and 48 h and analyzed the amount of α_4 and β_1 transcripts by Northern blot hybridization. As shown in Fig. 3, the decrease in the level of α_4 transcripts can already be seen at 24 h of PMA stimulation. As previously demonstrated, the α_4 probing yields two distinct RNA bands [19], both of which decreased on PMA treatment. Concomitantly a clear increase in the amount of β_1 transcript was observed, correlating to the increased total protein levels.

3.4. *Down-regulation of α_4 expression leads to a decrease in the binding of the cells to VCAM-1*

To answer whether the decreased expression of VLA-4 correlates with VCAM-1 adhesion we assessed the binding of the cells to the recombinant soluble purified VCAM-1 protein. Non-stimulated THP-1 cells adhered strongly to the VCAM-1-coated surfaces (Fig. 4). This adhesion was selective since pretreatment of the cells with anti-α_4 or the pretreatment of rsVCAM-1-coated plates with anti-VCAM-1 monoclonal antibody blocked the cell adhesion, whereas non-relevant antibodies had no effect. Binding to BSA-treated control was maximally 5% of the VCAM-1 binding. Down-regulation of the α_4-chain by PMA treatment led to a nearly 10-fold decrease in the capacity of THP-1 cells to adhere to VCAM-1-coated surfaces (average number of cells adhered per one microscopic field decreased from 590 ± 50 to 63 ± 10, $P < 0.0001$ in Student's t-test, Fig. 3). The remaining binding appeared to be VLA-4/VCAM-1 specific, since it could be further diminished to basal level by anti-VCAM-1 and anti-VLA-4 antibody treatments. Again no effect was observed with irrelevant antibodies.

Classically the regulation of integrin-mediated leukocyte binding to endothelial cells is believed to be regu-

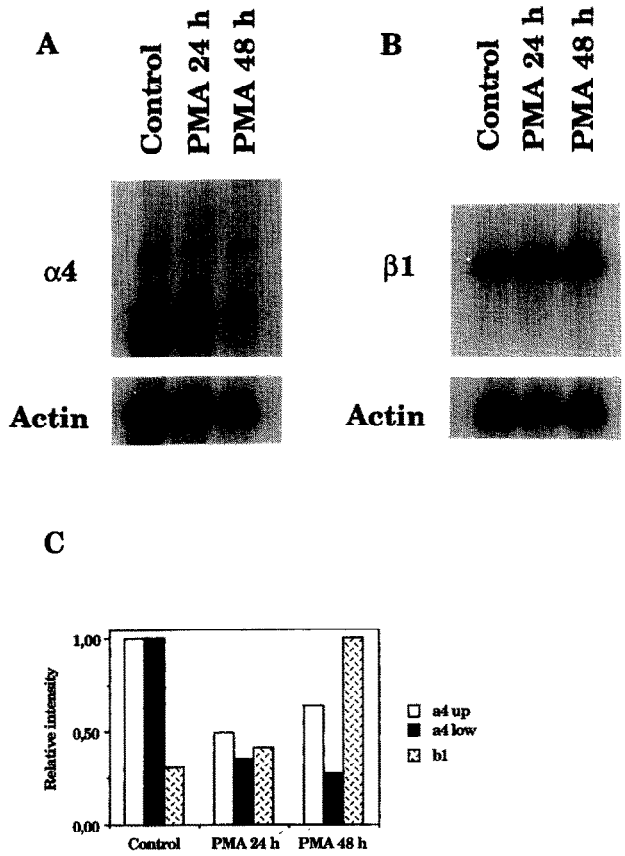

Fig. 3. The effect of PMA on THP-1 VLA-4 α- and β-chain RNA levels. Total RNA was isolated from untreated THP-1 cells and cells treated with 5 nM PMA for 24 and 48 h. The amount of α- and β-chain RNA was quantified by Northern blot analysis and the amount of RNA was normalized to actin RNA. (A) The α-chain probe detects two bands, both of which decrease during PMA treatment. (B) PMA treatment increased the RNA expression of the β-chain. (C) Relative scanning values for both α-chains (named a4 up and a4 low) and the β-chain.

lated by modifying (i) the avidity of the integrin, and (ii) the amount of its endothelial counterpart. This view is supported by studies showing that the amount of VLA-4 expressed on the cell surface is not directly linked to its ability to adhere. For instance, even though peripheral B cells are VLA-4 positive they do not adhere to fibronectin. However, the avidity of VLA-4 to bind fibronectin can be increased by a short-term PMA stimulation [15]. The avidity of leukocytic VLA-4 can also be increased by treating the cells with a monoclonal anti-β_1-integrin antibody (8A2) [20]. However, some reports linking quantitative up-regulation of VLA-4 to enhanced binding also exist. Although a short-term PMA stimulation increases the avidity of VLA-4 on T cells, their fibronectin binding is also enhanced when T cells are differentiated and concomitantly increase their VLA-4 expression [16]. Also, a 3 day PMA treatment of tonsillar lymphocytes has been shown to dramatically enhance their adherence to fibronectin due to an increase in their VLA-4 surface expression. Surprisingly, however, the same treatment of peripheral B lymphocytes led to a similar increase in binding, although simultaneously the VLA-4 expression was seen to decrease [15]. This is the first time that the down-regulation instead of up-regulation of VLA-4 has correlated to VCAM-1 binding. In the report of Ferreira et al., similar changes in U937 $\alpha_5\beta_1$ and $\alpha_4\beta_1$ were linked to an enhanced binding to fibronectin, whereas the effect on VCAM-1 binding was not studied [14].

Only very little information is available on the regulation of VLA-4 expression in vivo. Previous studies have shown that the VLA-4/VCAM-1 interaction is essential in inflammatory reactions as well as in embryonic development. Recently, the VLA-4/VCAM-1 interaction was shown to play a role in myogenesis [21], linking for the first time this interaction to the events of tissue development. Increased VLA-4 expression has, on the other hand, been shown to be associated with some diseases. T cells isolated from peripheral blood and affected synovias from patients with rheumatic arthritis expressed more VLA-4 than those isolated from healthy donors [22]. In addition to these hematopoetic cells, VLA-4 is also expressed on some non-hematopoetic malignant cells, like sarcomas and melanomas, but not on their benign counterparts [5,23–25]. These findings suggest that the regulation of VLA-4/VCAM-1 interaction is putatively important during metastatic spread of these cells. On the other hand in a rabbit model the VCAM-1/VLA-4-mediated binding has been shown to play a role in the formation of atherosclerotic plaques [26].

Being able to down-regulate the VLA-4/VCAM-1 interaction might have applications in treating or prevent-

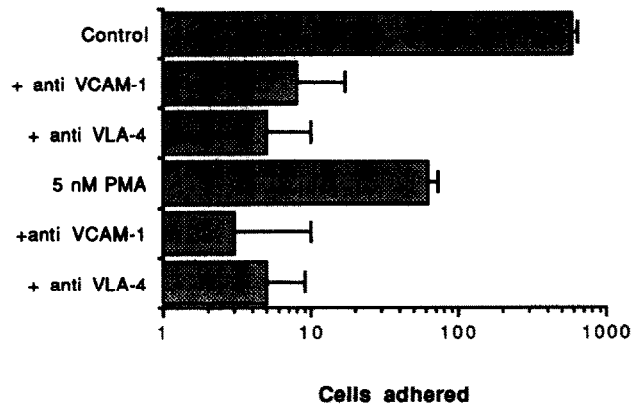

Fig. 4. The effect of PMA on THP-1 binding to rsVCAM-1 fixed on microtiter plates. Non-treated THP-1 cells or cells treated with anti-CD18 antibody bound avidly to the rsVCAM-1 coated plates, whereas there was practically no binding to BSA-coated control wells (data not shown). The observed binding was VLA-4 and VCAM-1 dependent, since both the anti-VLA-4 α-chain (CD49d) and anti-VCAM-1 monoclonal antibodies abolished it. PMA treatment (5 nM, 72 h) dramatically decreased the VLA-4/VCAM-1-dependent binding of THP-1 cells to rsVCAM-1. One representative experiment out of five is shown. Note the logarithmic horizontal axis.

ing diseases that involve cell-to-cell adhesion via this interaction, and this might have applications in anti-inflammatory, -arteriosclerosis and -metastatic approaches.

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REFERENCES

[1] Osborn, L. (1990) Cell 62, 3.
[2] Butcher, E.C. (1991) Cell 67, 1033.
[3] Springer, T.A. (1990) Nature 346, 425.
[4] Brandley, B.K., Swiedler, S.J. and Robbins, P.W. (1990) Cell 63, 861.
[5] Hemler, M.E., Huang, C., Takada, Y., Schwarz, L., Strominger, J.L. and Clabby, M.L. (1987) J. Biol. Chem. 262, 11478.
[6] Ruoslahti, E. (1991) J. Clin. Invest. 87, 1.
[7] Allavena, P., Paganin, C., Martin-Padura, I., Peri, G., Gaboli, M., Dejana, E., Marchisio, P.C. and Mantovani, A. (1991) J. Exp. Med. 173, 439.
[8] Osborn, L., Hession, C., Tizard, R., Vasallo, C., Luhowskyj, S., Chi-Rosso, G. and Lobb, R. (1989) Cell 59, 1203.
[9] Weller, P.F., Rand, T.H., Goelz, S.E., Chi-Rosso, G. and Lobb, R.R. (1991) Proc. Natl. Acad. Sci. USA 88, 7430.
[10] Bochner, B.S., Luscinskas, F.W., Gimbrone, M.A., Newman, W., Sterbinsky, S.A., Derse-Anthony, C.P., Klunk, D. and Schleimer, R.P. (1991) J. Exp. Med. 173, 1553.
[11] Carlos, T., Kovach, N., Schwartz, B., Rosa, M., Newman, B., Wayner, E., Benjamin, C., Osborn, L., Lobb, R. and Harlan, J. (1991) Blood 77, 2266.
[12] Dobrina, A., Menegazzi, R., Carlos, T.M., Nardon, E., Cramer, R., Zacchi, T., Harlan, J.M. and Patriarca, P. (1991) J. Clin. Invest. 88, 20.
[13] Lobb, R., Chi-Rosso, G., Leone, D., Rosa, M., Newman, B., Luhowsky, S., Osborn, L., Schiffer, S., Benjamin, C., Dougas, I., Hession, C. and Chow, P. (1991) Biochem. Biophys. Res. Commun. 178, 1498.
[14] Ferreira, J.O.C., Valinsky, J.E., Sheridan, K., Wayner, E.A., Bianco, C. and Garcia-Pardo, A. (1991) Exp. Cell. Res. 193, 20.
[15] Postigo, A.A., Pulido, R., Campanero, M.R., Acevedo, A., Garcia-Pardo, A., Corbi, A.L., Sanchez-Madrid, F. and de Landazuri, M.O. (1991) Eur. J. Immunol. 21, 2437.
[16] Shimizu, Y., van Seventer, G.A., Horgan, K.J. and Shaw, S. (1990) Nature 345, 250.
[17] Picker, L., Terstappen, L.W.M.M., Rott, L.S., Streeter, P.S., Stein, H. and Butcher, E.C. (1990) J. Immunol. 145, 3247.
[18] Hemler, M.E. (1990) Annu. Rev. Immunol. 8, 365.
[19] Neuhaus, H., Hu, M.C.-T., Hemler, M., Takada, Y., Holzmann, B. and Weissman, I. (1991) J. Cell. Biol. 115, 1149.
[20] Kovach, N.L., Carlos, T.M., Yee, E. and Harlan, J.M. (1992) J. Cell. Biol. 116, 499.
[21] Rosen, G.D., Sanes, J.R., LaChance, R., Cunningham, J.M., Roman, J. and Deand, D.C. (1992) Cell 69, 1107.
[22] Laffon, A., Garcia-Vinuca, R., Humbria, A., Postigo, A.A., Corbi, A.L., de Landazuri, M.O. and Sanchez-Madrid, F. (1991) J. Clin. Invest. 88, 546.
[23] Lauri, D., Martin-Padura, I., Biondelli, T., Rossi, G., Bernasconi, S., Giavazzi, R., Passerini, F., van Hinsbergh, V. and Dejana, E. (1991) Lab. Invest. 65, 525.
[24] Taichman, D.B., Cybulsky, M.I., Djaffar, I., Longenecker, B.M., Teixido, J., Rice, G.E., Aruffo, A. and Bevilacqua, M.P. (1991) Cell Regul. 2, 347.
[25] Mattila, P., Majuri, M.-L. and Renkonen, R. (1992) Int. J. Cancer 52, 1.
[26] Cybulsky, M.I. and Gimbrone, M.A. (1991) Science 251, 788.