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Regulation of α_1-antichymotrypsin synthesis in cells of epithelial origin

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Abstract Oncostatin M, interleukin-1 and the glucocorticoid analog, dexamethasone, have been identified as potent stimulators of α_1-antichymotrypsin production in various cells of epithelial origin. Although being able to act individually, these factors exerted a dramatic increase in α_1-antichymotrypsin synthesis when administrated in combination. Their stimulatory effect was independent of the levels of constitutive synthesis of this inhibitor, which was already high in lung- and breast- and low in skin-derived epithelial cells. Since α_1-antichymotrypsin controls chymotrypsin-like proteinases which are released during inflammation, these data support the concept that local synthesis of this inhibitor may be important in reducing tissue damage associated with this process.

Key words: Antichymotrypsin; Oncostatin M; Interleukin-1; Cytokine; Epithelial cell; Protein synthesis

1. Introduction

Human α_1-antichymotrypsin (Achy) is a plasma proteinase inhibitor which specifically inactivates chymotrypsin-like proteinases, including neutrophil cathepsin G and mast cell chymase [1]. Although its biological role is uncertain, this protein appears to be physiologically indispensable since no homozygous-deficient Achy individuals have been reported [2]. Achy heterozygote individuals suffer from disturbances in lung and liver function, and some of these abnormalities have been correlated with a protective function for this inhibitor against proteolytic attack [3]. Significantly, Achy is known to increase rapidly in concentration during tissue injury or malignant proliferation [4–6] when proteolytic enzymes are believed to be released [7]. Because of this response it is important to determine the factors which regulate the concentration of Achy in tissues and body fluids. While the major source of Achy is liver, this inhibitor has also been shown to be expressed in extrahepatic cell types, including cells of epithelial origin [8,9], and we have recently demonstrated that the human hepatoma HepG2 and human lung epithelial-like cell line HTB55 produce comparable amounts of Achy (manuscript submitted). A number of factors with hepatocyte stimulating activity have already been identified [4,5]; however, much less is known about the regulation of Achy expression in epithelial cells. Analysis of Achy synthesis by the HTB55 cell line indicates that two inflammatory cytokines, oncostatin M (OSM) and interleukin-1 (IL-1), may be the most potent stimulators, especially in combination with the glucocorticoid analog dexamethasone (DEX), and IL-1 has also been shown to efficiently stimulate another epithelial cell line, MCF-7, for Achy production [8]. However, interleukin-6 (IL-6), which has been recognized as the principal regulator of Achy synthesis in hepatocytes, appeared to have little or no effect on MCF-7 and HTB55 cells, respectively. In the current study we have evaluated the role of different factors associated with inflammation on Achy synthesis in various cells of epithelial origin. We chose to use breast adenocarcinoma MCF-7, lung adenocarcinoma HTB55, and squamous carcinoma Colo-16 as an epithelial cell model for Achy production, since these cells express different amounts of this inhibitor. Additive, stimulatory effects of OSM, IL-1 and DEX on Achy synthesis were observed in each cell line examined, raising the possibility that epithelial cells are a local source of this inhibitor during inflammation.

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Abbreviations: Achy, α_1-antichymotrypsin; OSM, oncostatin M; IL-1, interleukin-1; IL-6, interleukin-6; DEX, dexamethasone; APP, acute phase proteins; HTB55, human lung adenocarcinoma; MCF-7, human breast adenocarcinoma; Colo-16, human squamous cell carcinoma; FBS, fetal bovine serum; PAGE, polyacrylamide gel-electrophoresis.

2. Materials and methods

2.1. Factors

Human IL-1 was donated by Dr. D. Schenk (Athena Neuroscience Inc., San Francisco, CA). Human IL-6 and OSM were generously provided by Immunex (Seattle, WA). DEX was purchased from Sigma (St. Louis, MO).

2.2. Cell culture and stimulation

HTB55 (Calu-3) human lung adenocarcinoma and MCF-7 human breast adenocarcinoma cell lines were obtained from the American Type Culture Collection (Rockville, MD). The Colo-16 human squamous cell carcinoma cell line was a generous gift of Dr. H. Baumann (Buffalo, NY). Cells were cultured in Eagle's MEM supplemented with 0.1 mM non-essential amino acids solution, 1 mM sodium pyruvate, 100 U/ml penicillin G, 100 mg/ml streptomycin (all from Gibco, Grand Island, NY) and 10% FBS (Atlanta Biologicals, Norcross, GA). For the MCF-7 cell line the medium contained, in addition, 10 mg/ml bovine insulin (Gibco). Cells were plated, allowed to grow to 70–100% confluency before assay and then treated in serum-free medium with the indicated stimulating factors.

2.3. Northern blot analysis

Total RNA was isolated by the method of Scherrer and Darnell [10] with the modification of Rose-John [11]. RNA was subjected to formaldehyde-agarose gel-electrophoresis [12], transferred to a Hybond-N membrane (Amersham, Arlington Hts., IL) according to the manufacture's instruction, and then hybridized with ^{32}P-labeled probe overnight at 65°C in a mixture containing 1 M NaCl, 1% SDS and 10% dextran sulfate. The probe, a 1.4 kb *Eco*RI–*Eco*RI restriction fragment of human Achy cDNA [13], (a generous gift of Dr. H. Rubin, University of Pennsylvania) was labeled with the Megaprime Labeling Kit (Amersham).

2.4. Determination of Achy concentration

Culture medium was collected 24 or 48 h after factor(s) addition. In the latter case the culture medium was replaced at 24 h by fresh medium containing stimulating factors so that the quantitated amount of Achy

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represents a 24 h incubation period. The amount of accumulated Achy was detected by rocket immunoelectrophoresis using polyclonal rabbit antibodies against human Achy (Dako, Carpinteria, CA). Purified human plasma Achy (kindly provided by Miles Inc., Berkeley, CA) was used as a standard. The results were expressed in ng of Achy released into the medium by 1×10^6 cells within 24 h.

2.5. Biosynthetic labeling and immunoprecipitation

Confluent monolayers of Colo-16 cells were treated for 24 h with indicated factors. The cells were then rinsed and incubated for 3 h in methionine-free medium containing both stimulating factors and 200 mCi/ml [^{35}S]methionine/cysteine (Tran^{35}S-label) (ICN, Costa Mesa, CA). Aliquots of medium were pretreated with preimmune serum and Pansorbin (Calbiochem, La Jolla, CA), as previously described [20]. The supernatants were then incubated with excess anti-Achy antibody in 20 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1% Triton X-100 and 5 mM EDTA, overnight at 4°C. Immune complexes were precipitated with Pansorbin, washed, released by boiling in Laemmli sample buffer, and subjected to 9% SDS-PAGE [14]. Achy was then detected by fluorography as described in [15].

3. Results

We have previously shown that epithelial-like HTB55 cells respond to OSM but not to IL-6 in terms of Achy synthesis, and that DEX potentiates the effect of OSM (manuscript submitted). To find out whether Achy is similarly regulated in other epithelial cells, we tested the effect of these factors on MCF-7 and Colo-16 cells. MCF-7 cells constitutively express relatively high amounts of Achy mRNA, whereas in Colo-16 these levels were low (Table 1). As shown in Fig. 1, production of Achy in MCF-7 cells was affected by OSM and DEX individually, as well as by a combination of DEX with either OSM or IL-6. In Colo-16 cells, Achy mRNA levels were substantially induced by DEX, while IL-6 and OSM appeared to have no effect. However, OSM and to a lesser extent IL-6, in combination with DEX markedly up-regulated Achy expression in the Colo-16 cells (Fig. 1).

Table 1
Effect of OSM, IL-6, IL-1 or DEX on Achy synthesis in cells of epithelial origin

Treatment	Achy secretion (ng/ml × 10^6 cells)		
	MCF-7	HTB55	Colo-16
Control	84 ± 43	1170 ± 400	< 2.5
OSM	247 ± 52	1980 ± 700	< 2.5
IL-6	71 ± 18	1430 ± 430	< 2.5
IL-1	280 ± 72	1910 ± 490	< 2.5
OSM + IL-1	675 ± 163	2490 ± 680	< 2.5
DEX	198 ± 41	1400 ± 480	5 ± 2
DEX + OSM	556 ± 111	2340 ± 760	15 ± 6
DEX + IL-6	364 ± 72	1870 ± 570	7 ± 2
DEX + IL-1	523 ± 116	2380 ± 650	7 ± 2
DEX + OSM + IL-1	981 ± 217	3200 ± 860	19 ± 5

Cells were incubated in serum-free MEM containing 50 ng/ml OSM, 50 ng/ml IL-6, 100 U/ml IL-1 or 10^{-6} M DEX. Aliquots of cell culture media collected at 48 h (from 24–48 h incubation period) were analyzed by rocket immunoelectrophoresis in order to measure secreted Achy. In the case of Colo-16 cells, medium was dialyzed against 25 mM amonium bicarbonate, lyophilized, and then dissolved in 1/10 of the original volume before immunoelectrophoresis. Mean ± S.D. of three determinations of two or three separate experiments are shown.

Since previous studies had demonstrated that IL-1 was the most potent in inducing Achy synthesis in MCF-7 cells [8], and also significantly up-regulated Achy expression in HTB55 cells, we wished to compare the effect of OSM and IL-1. Northern blot analysis of RNA extracted from MCF-7 and HTB55 cells indicated that these cytokines produced comparable increases

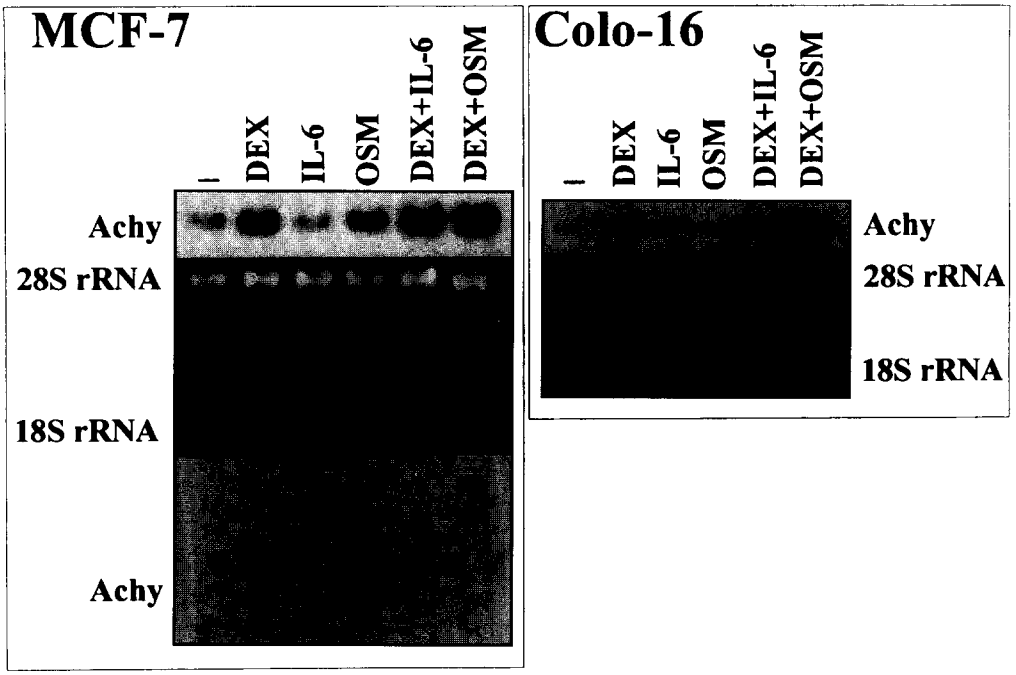

Fig. 1. Comparison of the effect of OSM and IL-6 on Achy synthesis in MCF-7 and Colo-16 cells. Cells were incubated in serum-free MEM containing 50 ng/ml IL-6, 50 ng/ml OSM or 10^{-6} M DEX. At 24 h total cellular RNA was isolated and subjected to Northern blot analysis. The blots were photographed to demonstrate equal loading (middle panel) and then hybridized with an Achy probe (upper panel). Samples of MCF-7 medium collected at 48 h (from 24–48 h incubation period) were subjected to rocket immunoelectrophoresis using antiserum to Achy. The gel was stained with Coomassie blue to demonstrate precipitin lines (bottom panel).

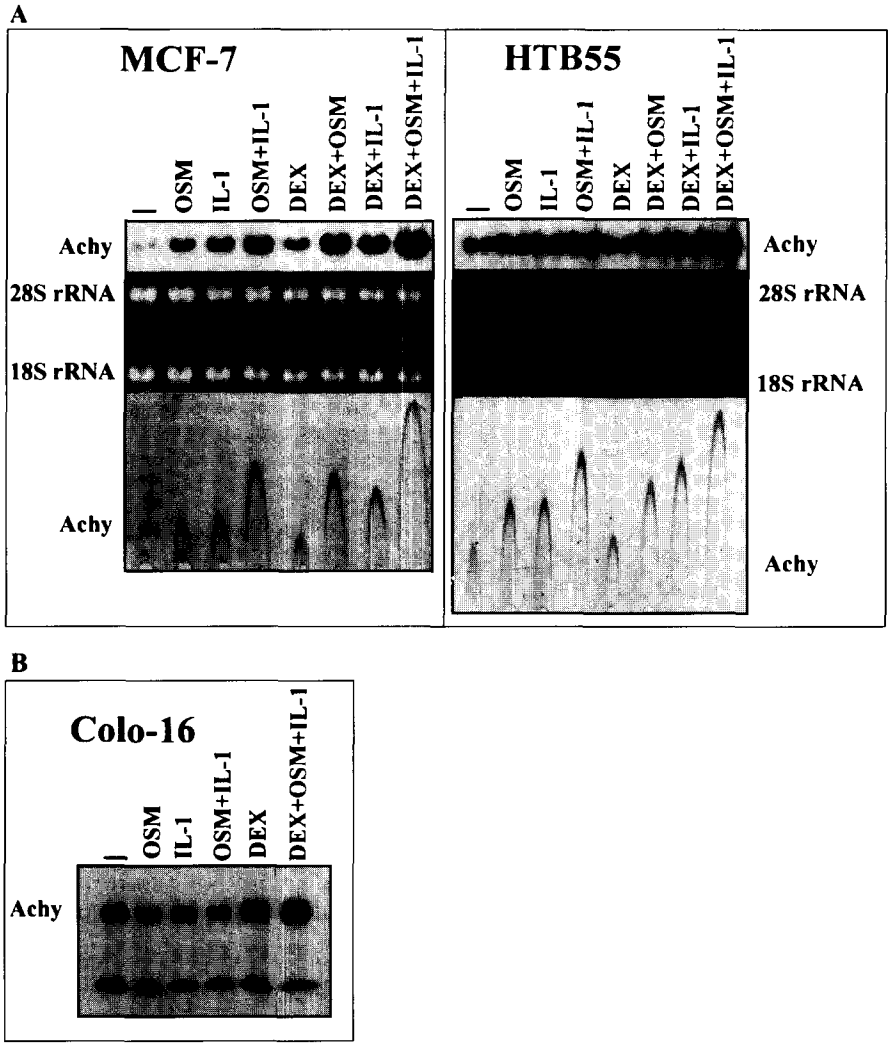

Fig. 2. Effect of OSM and IL-1 on Achy production in cells of epithelial origin. Cells were incubated in serum-free MEM supplemented with 50 ng/ml OSM, 100 U/ml IL-1 or 10^{-6} M DEX. (A) Total cellular RNA isolated at 24 h from MCF-7 and HTB55 cells was separated on gels and transferred to nylon membranes. The blots were photographed (middle panels) and then hybridized to an Achy probe (upper panels). Samples of MCF-7 and HTB55 media collected at 48 h (from 24–48 h incubation period) were subjected to rocket immunoelectrophoresis using antiserum to Achy (bottom panels). (B) At 21 h Colo-16 cells were subjected to radiolabeling for 3 h. Aliquots of culture medium were immunoprecipitated with human anti-Achy. Immunoprecipitates were then subjected to 9% SDS-PAGE followed by fluorography.

in Achy levels (Fig. 2A). The effect of OSM together with IL-1 was additive and strongly enhanced by DEX. These observations were confirmed by analysis of the amount of secreted Achy. In contrast to both of the adenocarcinoma cell lines tested, the additive effect of OSM and IL-1 in Colo-16 cells was observed only in the presence of DEX. This was demonstrated by an increase in accumulation of newly synthesized Achy (Fig. 2B). Synthesis of Achy in Colo-16 cells determined by rocket immunoelectrophoresis just reached the limits of detection in response to DEX and appeared to be slightly up-regulated when either IL-1 or IL-6 were added (Table 1). Neither OSM nor IL-1, alone or together, were able to significantly increase the amount of secreted Achy (Fig. 2B). The effect of the cytokines given together with DEX is summarized in Table 1. Note that the increase in levels of secreted Achy resulting from OSM and DEX addition paralleled the changes described in Fig. 1B at mRNA levels. The faster migrating bands shown in Fig. 2B most likely represent free [^{35}S]methionine/cysteine, since in contrast to slower migrating bands they were also detected when preimmune serum was used (data not shown).

Because the effect of tested cytokines was most evident in MCF-7 cells, this line was chosen to study Achy regulation by OSM and IL-1 in the absence of DEX in detail. As shown in Fig. 3, the up-regulating effect of these factors on Achy synthesis reached a maximum at 50 ng/ml OSM and 100 U/ml IL-1 and did not change significantly when higher concentrations were tested. As little as 1 U/ml IL-1 was able to stimulate Achy synthesis when acting in concert with OSM, whereas at least 10 ng/ml OSM appeared to be necessary to observe an additive effect with IL-1.

4. Discussion

During the host response to tissue injury or inflammation there is a dramatic increase in the plasma concentration of several proteins, including Achy, all of which are referred to as

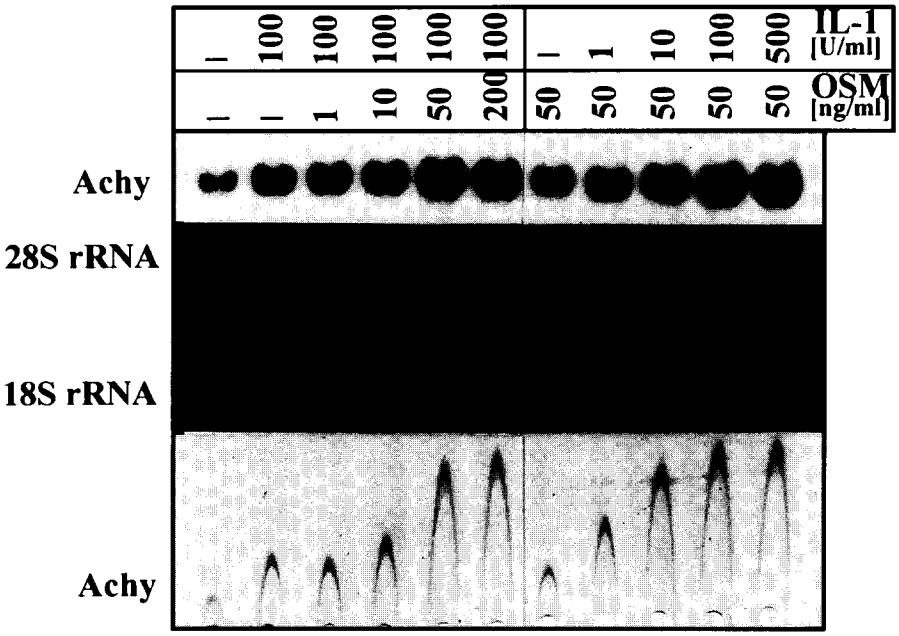

Fig. 3. Dose-dependent stimulation of Achy synthesis in MCF-7 cells treated with OSM and IL-1. Cells were incubated in serum-free MEM containing indicated amounts of OSM and IL-1. At 24 h, medium was collected and total cellular RNA extracted. RNA was then subjected to Northern blot analysis. The blots were photographed (middle panel) and hybridized to an Achy probe (upper panel). Achy accumulated in culture medium was demonstrated by rocket immunoelectrophoresis (bottom panel).

acute-phase proteins (APP). Over the past several years a number of factors capable of stimulating production of these APP in hepatocytes have been identified. These include IL-6, IL-6-related cytokines (OSM, leukemia inhibitory factor, interleukin-11, cilary neurotrophic factor), IL-1, tumor necrosis factor-α and glucocorticoids [4]. Most of these factors were examined as potential stimulators of Achy synthesis in lung-derived HTB55 cells, and OSM as well as IL-1 appeared to play the most important role in regulation of expression of this inhibitor (manuscript submitted). A few cytokines and steroid hormones were also tested for their effect on synthesis of Achy in breast- derived MCF-7 cells, of which IL-1 caused the most dramatic increase [8]. These observations led us to investigate the possibility that epithelial cells of different origin can respond to OSM and IL-1 in terms of Achy synthesis. Indeed, in all three epithelial cell lines tested production of Achy was significantly up-regulated by both cytokines, although in the case of keratinocyte-like Colo-16 cells DEX was required to observe an effect.

A number of cell lines originating from the epithelium have been shown to produce Achy [9,16]. This inhibitor has also been detected in normal and dysplastic epithelial tissue, including those from lung and breast [17]. Synthesis of Achy by epithelial cells indicates that control of the levels of this inhibitor in specific organs could be critical for reducing tissue proteolysis. In lung- and breast-derived epithelial cells the already relatively high levels of Achy were elevated several times by OSM, IL-1 and DEX, especially when they were used in combination. The reason why epithelial cells are particularly responsive to these factors is unknown, but other observations support their influence on this tissue. For instance, lung, breast, as well as several other cells of epithelial origin have been shown to bind significant amounts of OSM [18]. Since OSM and IL-1 are secreted by host defense effector cells [19–22], up-regulation of Achy expression in epithelium may occur during inflammation. Significantly, neutrophil cathepsin G and mast cell chymase, the enzymes controlled by Achy, have been suggested to be the major components of the inflammatory response in skin and lung [2]. For these reasons it seems likely that the local regulation of Achy synthesis by inflammatory factors is important in the maintenance of a proper proteinase–proteinase inhibitor equilibrium in tissues.

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References

[1] Travis, J. and Salvesen, G. (1983) Annu. Rev. Biochem. 52, 655–709.
[2] Rubin, H. (1992) Biol. Chem. Hoppe-Seyler 373, 497–502.
[3] Poller, W., Faber, J.P., Scholtz, S., Weidinger, S., Bartholome, K., Olek, K., Eriksson, S. (1992) Lancet 339, 1538.
[4] Baumann, H. and Gauldie, J. (1994) Immunol. Today 15, 74–80.
[5] Koj, A., Magielska-Zero, D., Bereta, J., Kurdowska, A., Rokita, H. and Gauldie, J. (1988) Tokai. J. Exp. Clin. Med. 6, 255–264.
[6] Richards, C.D., Brown, T.J., Shoyab, M., Baumann, H. and Gauldie, J. (1992) J. Immunol. 148, 1731–1736.
[7] Potempa, J., Korzus, E. and Travis, J. (1994) J. Biol. Chem. 269, 15957–15960.
[8] Finlay, T.H., Tamir, S., Kadner, S.S., Kruz, M.R., Yavelow, J. and Levitz, M. (1993) Endocrinology 133, 996–1002.
[9] Molmenti, E.P., Ziambaras, T. and Perlmutter, D.H. (1993) J. Biol. Chem. 268, 14116–14124.
[10] Scherrer, K. and Darnell, J.E. (1962) Biochem. Biophys. Res. Commun. 7, 486–490.
[11] Rose-John, S., Dietrich, A. and Marks, F. (1988) Gene 74, 465–471.
[12] Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular Clon-

ing: A Laboratory Manual, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
[13] Rubin, H., Wang, Z., Nickbarg, E.B., McLarney, S., Naidoo, N., Schoenberger, O.L., Johnson, J.L. and Cooperman, B.S. (1990) J. Biol. Chem. 265, 1199–1207
[14] Laemmli, U.K. (1970) Nature 227, 680–684.
[15] Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88.
[16] Tamir, S., Kadner, S.S., Katz, J. and Finlay, T.H. (1990) Endocrinology. 127, 1319–1328.
[17] Permanetter, W. and Meister, P. (1984) Acta Histochem. 74, 173–1797.
[18] Linsey, P.S., Bolton-Hanson, M., Horn, D., Malik, N., Kallestad, J.C., Ochs, V., Zarling, J.M. and Shoyab, M. (1989) J. Biol. Chem. 264, 4282–4289.
[19] Brown, T.J., Lioubin, M.N. and Marquardt, H. (1987) J. Immunol. 139, 2977–2983.
[20] Malik, N., et al. (1989) Mol. Cell. Biol. 9, 2847–2853.
[22] Diranallo, Ch.A. (1992) Immunol. Rev. 127, 119–146.