Characterization of Nuclear Retinoic Acid Binding Activity in Sensitive Leukemic Cell Lines : Cell Specific Uptake of ATRA and RARα Protein Modulation

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SUMMARY. The diverse effects of all-*trans* retinoic acid (ATRA) on growth, differentiation and homeostasis of vertebrate organisms are mediated by three distinct isoforms of retinoic acid receptors (RARs). Although it is not known to what extent each RAR contributes to the different effects of ATRA, several studies have demonstrated that ATRA induced granulocytic differentiation in human myeloid leukemic cell lines is mediated by RARα. In this study, we investigated ATRA binding affinity of the endogenous nuclear receptors of HL-60 and NB4 leukemic cells. Scatchard plot analysis yielded an apparent dissociation constant of 5 ± 0.3 nM and 1400 ± 80 receptor sites per cell in HL-60 cells, whereas the NB4 promyelocytic leukemic cell line showed a lower affinity (8.5 ± 0.5 nM and 900 ± 30 receptor sites per cell). Modulation of RARα protein (5 fold excess) was found in NB4 cells after 24 hours ATRA exposure, whereas HL-60 cells required a 72-hour culture period to weakly increase the RARα protein level. These data were closely related to the ATRA intracellular concentration and kinetics of terminal differentiation of the cells.

Retinoids are a class of compounds structurally related to vitamin A which have marked activities, experimentally as well as clinically. Retinoic acid (RA), has been shown to induce differentiation and inhibit *in vitro* proliferation of human myeloid leukemic cells

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(1,2). Several clinical studies have reported the induction of complete remission in patients with acute promyelocytic leukemia (APL) treated with all-*trans* retinoic acid (ATRA) (3-5).

The molecular mechanisms which control cell proliferation and differentiation are still largely unknown. In some cases a definite stimulus is required to initiate proliferation (e.g. antigen binding to lymphoid cells). However, even in these situations the chain of events which link the event of binding of a ligand to its receptor resulting in either initiation of cell division or differentiation remain poorly understood. Cytoplasmic retinoic acid binding protein (CRABP) was hypothesized as a biologic mediator of RA mechanism action (6). However, CRABP is undetectable in HL-60 cells or leukemic blasts from patients (7,8). It is now well established that the ultimate targets of retinoids are the nuclear receptors. RA modulates the transcription of a variety of genes associated with cellular proliferation and differentiation by binding retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which are the members of the steroid/thyroid hormone receptor superfamily of nuclear transcription factors (9-12). To date, of the natural retinoids, ATRA and 9-cis RA are considered as the most potent modulators of hematopoiesis. Whereas RXRs binds with high affinity the 9-cis RA isomer only, RARs bind both ATRA and 9-cis RA (13). The three types of RARs, α, β, and γ (for review see 14-16), and the three types of RXRs, α, β, and γ (11,12,17,18), are ligand-inducible *trans* regulators that modulate the transcription of target genes by forming dimers and interacting with cis-acting DNA retinoic acid response elements (RAREs) . Clearly, the existence of distinct dimer combinations with differential ligand specificity and affinity creates a very specific pathway for retinoid action.

Efforts to understand RA action in myeloid leukemic cells have shown it to be a multistep procedure amongst which uptake, biodisposition of ATRA and its binding to endogenous receptors have not been tackled. In the present investigation, the human myeloid leukemic cell lines NB4 and HL-60 [respectively AML_3 and AML_2 subtypes in the FAB cytological classification (19)], were used as model systems to examine the relationship between ATRA binding affinity of the endogenous nuclear receptors, intracellular concentration achieved, and RARα protein modulation.

MATERIALS AND METHODS

Retinoids. ATRA was kindly provided by Hoffman-La Roche Laboratories, Basel, Switzerland. The powdered retinoid was dissolved in dimethyl-sulfoxide at an initial stock concentration of 10^{-2} M, stored at -20°C and further diluted in RPMI 1640 medium. [^{3}H] ATRA (50 Ci/m mol) was obtained from DuPont/NEN.

Cell culture and differentiation assay. HL60 cells were provided by Dr. T. Breitman (National Cancer Institute, Bethesda) and NB4 cells was a gift from Dr. M. Lanotte (INSERM U301, Hôpital Saint-Louis, Paris). Cells were seeded at an initial concentration of $2x10^5$ cells/ml in RPMI 1640 medium supplemented with 15% heat inactivated foetal calf serum, penicillin (100 units/ml), streptomycine (100 μg/ml) and L-Glutamine (2 mM) and maintained in a 95% air/5% CO_2 atmosphere at 37°C.

Plasmid preparation and transient transfection. For transient transfection, COS-1 cells were grown in DMEM medium containing 10% fetal calf serum penicillin and glutamine and plated at 50% confluence the day before transfection. COS-1 cells were transiently transfected with the expression vector pSG5/RARα or pSG5/PML-RARα (gift from Hugues de The, Hôpital Saint-Louis, Paris) (10 μg DNA/dish) by the calcium phosphate coprecipitation protocol (20).

Preparation of nuclear extracts. The preparation of nuclear extracts was based on a procedure described by Nervi et al. (21), with several modifications. Briefly, approximately 10^8 HL-60 or NB4 cells, or 3-5 10^7 COS-1 transfected cells were collected by centrifugation at 1 000 x g for 10 minutes and washed twice with cold PBS buffer containing 2 mM EDTA. The cells were then washed gently at 4°C in 10 ml of buffer A (20 mM Hepes pH 8 / 5 mM potassium chloride / 5 mM magnesium chloride / 0.1% nonidet P-40 / 10 mM sodium fluoride / 1 mM levamisole / 1 mM benzamidine / 1 mM sodium metabisulfite / 10 units/ml aprotinin and 0.1 mM phenylmethylsulfonyl fluoride [PMSF]). Cells were homogeneised in 5 ml of buffer A with a dounce homogeneiser (pestle B, 60-80 strokes). The homogenate was centrifuged at 4°C for 15 minutes at 1 000 x g. The pellet containing the nuclei, was washed once with buffer A and extracted in 3-6 ml of buffer B (10 mM Hepes pH 8 / 25% glycerol / 5 mM magnesium chloride / 0.1 mM calcium chloride/0.1 mM EDTA / 0.1 mM EGTA / 0.6 M sodium chloride / 0.5 mM spermidine / 0.15 mM spermine / 7 mM β-mercaptoethanol / levamisole, benzamidine, sodium metabisulfite, aprotinin, and PMSF at the concentrations indicated above). The suspension was incubated for 1 hour on ice with repeated resuspension every 10 minutes and then centrifuged at 130 000 x g for 30 minutes. The resulting supernatant was referred to as the nuclear extract. Protein concentration was determined with a Pierce protein assay kit with bovine albumine as standard. Extracts were used either immediately or after storage at -70°C.

Retinoic acid binding assay and FPLC analysis. In routine assays, nuclear extracts (200-800 μg proteins) were incubated with increasing [^{3}H] ATRA concentrations (0.5, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50 and 100 nM), in binding buffer (50 mM Tris-HCl, pH 8 / 150 mM NaCl / 1 mM EDTA / 1 mM DDT), in the presence or absence of 200-fold excess of unlabeled ATRA. After 18 hours incubation at 4°C, 0.1 ml of chilled charcoal-dextran suspension (3% Norit A / 0.3% dextran T70 in 50 mM Tris-HCl, pH 8 / 10 mM KCl / 1 mM DTT) was added to 0.2 ml of incubates, mixed vigorously, and left for 15 minutes at 4°C. The tubes were then centrifuged at 5 000 x g for 10 minutes, and 0.15 ml supernatant samples were counted for radioactivity. At each retinoid concentration, the number of molecules bound was determined and Scatchard plot analysis performed. For FPLC a ıalysis (Pharmacia system), extracts were fractionated over a Superose 12-HR 10/30 size exclusion column (Pharmacia) at a flow rate of 0.5 ml/minute; the eluent buffer wa a mixture of sodium phosphate 5 mM (pH 6.8), thioglycerol 10 mM, glygerol 10% (w/v), and KCl 0.4 M. Fractions of 0.5 ml were collected and radioactivity was measured. Molecular weight calibration was achieved using calibrated markers (β-amylase : 200 kiloDaltons (kD), alcohol dehydrogenase : 150 kD, bovine serum albumine : 66 kD, carbonic anhydrase : 29 kD and cytochrome C : 14 kD).

Antibodies. Monoclonal and polyclonal anti-RARα antibodies, directed against the F region of the RARα1 isoform receptor were generously provided by M.P. Gaub and P. Chambon (Institut de Génétique Moléculaire, Strasbourg, France). IgG1, irrelevant control antibody (used at a 1:40 dilution) and FITC-goat anti-mouse antibody (used at a 1:200 dilution) were purchased from Coulter, France.

Flow cytometric assay. Flow cytometric analysis was carried out as previously described (22). HL-60 and NB4 Cells (1 x 10^6) were washed twice in PBS, fixed and permeabilized by addition of 1 ml of a 1% paraformaldehyde solution containing 20 μg/ml lysolecithin and incubated for 2 minutes at room temperature. The cells were then centrifuged for 5 minutes at 1500 r.p.m. at 4°C and kept for 10 minutes on ice in 2 ml (-20°C) absolute methanol. After another centrifugation, cells were resuspended in 1 ml of 1% Triton X-100 and placed on ice for 5 minutes. Cells were then resuspended in PBS solution containing 0.5% BSA and incubated for 20 minutes at 4°C with the primary antibody. Cells were then washed and incubated with goat anti-mouse-FITC at the same conditions as before. After another wash, cells were stained by a solution of propidium iodide (50 μg/ml) for cell cycle analysis. Flow cytometric studies were performed on an EPICS Profile II flow cytometer (Coulter) and percentage of cells in each phase of the cell cycle was analyzed by the

Multicycle software (Coulter). Relative RARα protein levels were determined by calculation of a corrected mean fluorescence intensity (difference in fluorescence intensity of Immunoglobulin control and anti-RARα antibody).

SDS-gel electrophoresis and Western immunoblotting. This technique was performed using Boehringer Western blotting kit assay. Discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), was performed using a 7% separating gel and a 5% stacking gel. Prestained molecular weight markers (Rainbow^{TM}, Amersham) were loaded in adjacent lanes. Nuclear extracts corresponding to approximately 100 μg proteins prepared from untreated or ATRA 10^{-6} M HL-60 or NB4 treated cells, were diluted 1:1 with 2X sample buffer [2% SDS, 0.125 M Tris-HCl (pH 6.8), 20% glycerol, 0.02% bromophenol blue, and 10% 2-mercaptoethanol], fractionated by SDS polyacrylamide gel as described above, and then elecrobloted to nitrocellulose membrane (Hybond-ECL, Amersham). The filters were then tested with a blocking buffer and immunoprobed over night with polyclonal antibody directed against the F region of the hRARα1 isoform receptor. Proteins that reacted with RARα were detected with a peroxydase conjugated goat-anti-rabbit antibody (Boehringer). Finally, the blot is washed autoradiographed and developped according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Endogenous nuclear retinoic acid receptors binding activity in RA-sensitive myeloid cells. Purification of nuclear retinoic acid receptors from primary tissues is difficult to achieve because of their low natural abundance (23). Therefore, only limited number of studies on myeloid leukmic cells, or transfected cell lines have been reported (14, 21, 23-27). To analyse the ATRA binding properties of the nuclear receptors present in RA-sensitive myeloid leukemic cells, nuclear extracts were prepared from HL-60 and NB4 cells and compared to those of COS-1 cells transiently transfected with either pSG5/RARα or pSG5/PML-RARα expression vectors. The extracts were labeled with 100 nM [^{3}H] ATRA in the absence or presence of 200-fold excess of unlabeled ATRA, and the binding activity was analysed by size exclusion FPLC. The FPLC profile of HL-60 and NB4 cells (Fig. 1a, b) showed the presence of 3 main peaks of specific ATRA binding activity eluting at 5, 16 and 19 ml elution volume respectively. The specific activity found in the first peak corresponded to the formation of high molecular weight complexes between the RA receptors and probably other nuclear proteins (21, 23, 26). The peak eluting at 16 ml corresponded to a molecular weight of 100 kiloDaltons (kD), and suggested the formation of homo and/or heterodimers proteins (RAR/RAR or RAR/RXR). This peak may also correspond in NB4 cells, which have been reported to contain the t(15;17) translocation, to PML-RARα protein (21). The specific ATRA binding eluting at 19 ml corresponded to a molecular weight of 50 kD, probably representing endogenous monomer RA receptor(s) and may include RAR_{α} or RXRα, which are the preferentially retinoid receptors expressed in myeloid cells (21, 26). The elution profile of nuclear extracts prepared from COS-1 cells transiently transfected with pSG5/RAR_{α}, displayed 2 peaks with specific ATRA binding activity (Fig. 1c). The first peak eluted at 6 ml corresponded to high molecular weight proteins complexed complexes with RARα (> 300 kD), and the second major peak eluted at 19 ml corresponded to a molecular weight of 50 kD which is the established molecular weight of RARs (10, 23, 24, 28, 29). Nuclear extracts from COS-1 cells transfected with pSG5/PML-RARα expression

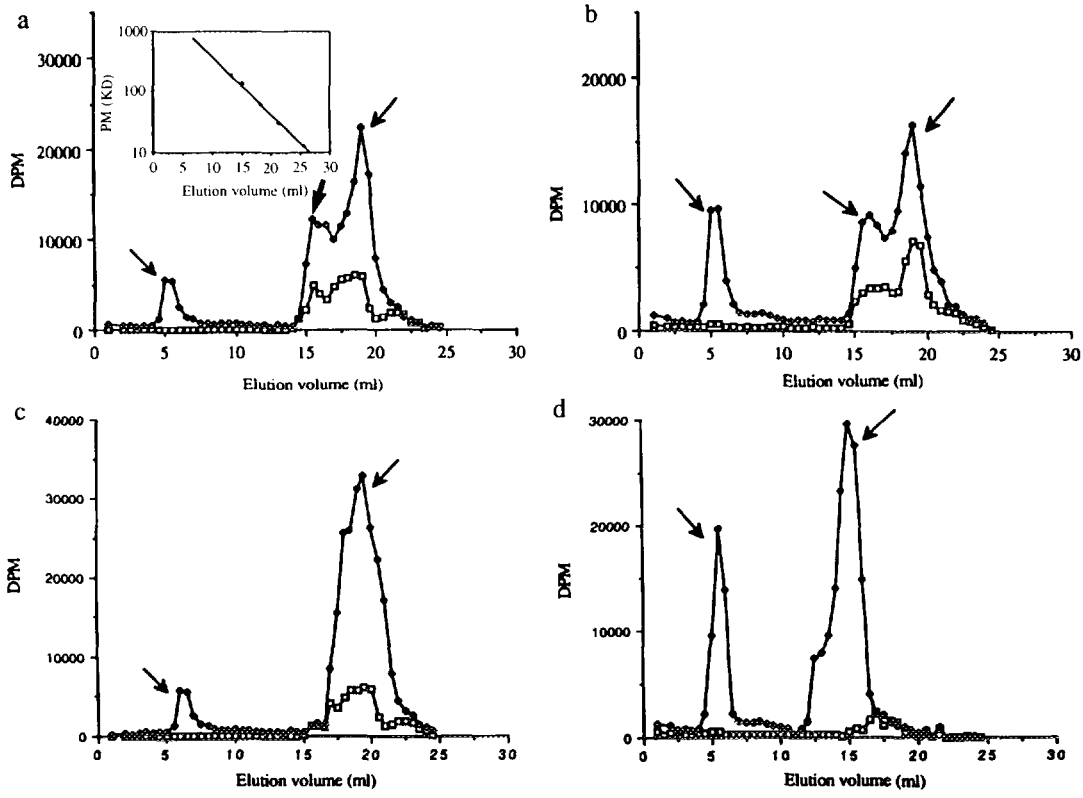

Fig. 1. Size exclusion FPLC analysis of nuclear extracts from myeloid leukemic cells HL-60 (a); NB4 (b) or from COS-1 cells, transiently transfected with pSG5-RARα (c) or pSG5/PML-RARα (d) expression vectors. The size exclusion chromatography system was calibrated with a series of marker proteins (β-amylase:200 kiloDaltons [kD], alcohol dehydrogenase:150 kD, bovine serum albumin : 66 kD, carbonic anhydrase:29 kD and cytochrome C : 14 kD). Peaks corresponding to specific ATRA binding activity are shown with arrows.

vector (Fig. 1d), showed also 2 specific peaks, one eluting at 6 ml corresponding to PML-RARα in complex with itself or other proteins (21) and a second peak with a 110 kD representing the monomeric form of PML-RARα protein (21). These data obtained in COS-1 cells are with those published by others (21, 24, 28, 29).

We also analysed ATRA protein binding affinity, by Scatchard plot analysis (Fig. 2). Extracts from HL-60, NB4 cell lines and COS-1 cells transfected with either RARα or PML-RARα expression vectors, were incubated for 18 hours with increasing [^{3}H] ATRA concentrations, as described in Material and Methods. Scatchard analysis, yielded a linear plot consistent with the presence of a single class of binding sites for HL-60 and NB4 cells (Fig. 2a,b) and for both transfected RARα and PML-RARα receptors (Fig. 2c,d). These data were consistent and reproductible in three independent experiments. The apparent equilibrium dissociation constants calculated were of 5 ± 0.3 nM and 1400 ± 80 receptor sites per cell for HL-60 cells (Fig. 2a), whereas NB4 cells showed a lower affinity (8.5 ± 0.5

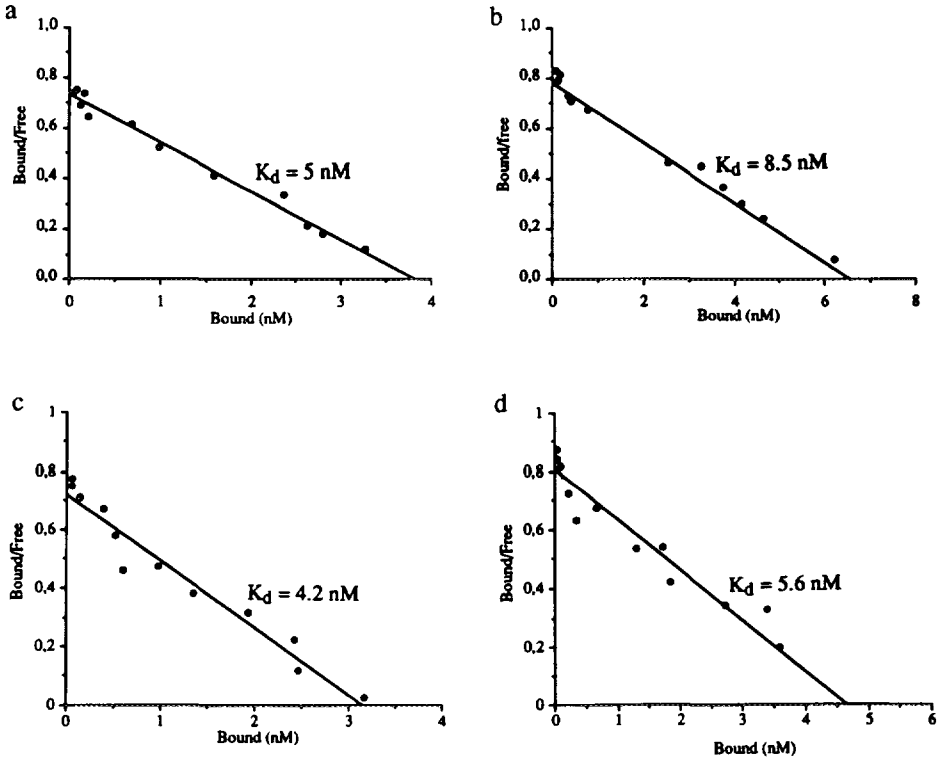

Fig. 2. Scatchard plot analysis of HL-60 cells (a); NB4 cells (b); or COS-1 cells transiently transfected with pSG5-RARα (c) or pSG5/PML-RARα (d). Dissociation constants (K_d) are displayed.

nM and 900 ± 30 receptor sites per cell) (Fig. 2b) probably due to changes in the conformation of the fusion protein induced by the native PML protein and/or the low abundance of RARα in the promyelocytic leukemic cell line (30 and data herein). In COS-1 transfected cells with either RARα or PML/RARα expression vectors, the apparent equilibrium dissociation constants calculated were of 4.2 ± 0.5 nM (Fig. 2c) and 5.6 ± 0.2 nM (Fig. 2c,d) respectively.

All-trans retinoic acid uptake by leukemic cells. We have investigated the coupling of differentiation, ATRA uptake and cell cycle analysis in HL-60 and NB4 leukemic cell lines. Fundamental to understanding the mechanisms of ATRA action is elucidating the effective intracellular concentration achieved. We have previously demonstrated, that the rate of ATRA uptake and maximal intracellular concentration (Cmax) is related to the extent and rapidity of differentiation of leukemic cells in suspension culture (31). We showed that, incubation of HL-60 and NB4 myeloid leukemic cell lines with 10^{-6} M ATRA results in a time-dependent increase of the intracellular concentration : NB4 cells achieved Cmax of 266 pmol/10^6 cells after only 24 hours of incubation, whereas HL-60 cells reached their Cmax (192 pmol/10^6 cells) after 48 hours exposure. To determine whether ATRA uptake is related to cell cycle, NB4 and HL-60 cells, were taken after 24 hours of

incubation with or without ATRA 10^{-6} M and analysed by flow cytometry. Figure 3 shows, a typical pattern of a dividing cell population with a prominent peak G0/G1 and a later S phase. In the control, the percentage of cells in the S phase of cell cycle was of 37% for HL-60 and 46% for NB4 (Fig. 3a, c) versus 31% and 44% respectively in the treated cell population (Fig. 3b, d). Furthermore, in standard liquid suspension culture conditions, HL-60 cells presented a specific exponential cell growth with a doubling time of 1.5 to 2 days, whereas NB4 cells were less proliferative with a doubling time of 3 days, both experiments implying a longer S phase in NB4 cells. The difference in the cell cycle disposition of these two cell populations, may explain the five fold higher ATRA concentration obtained in NB4 cells after 24 hours incubation period correlating with the highest percentage of differentiation achieved in these cells (31). These data indicate that induction of differentiation is closely related to a cell cycle dependent ATRA uptake and its intracellular concentration status in the leukemic cells. Our results are thus consistent with the interpretation, that cells transiting the cell cycle do not become susceptible to ATRA until S phase and that this susceptibility is mediated by drug uptake, as previously reported (32).

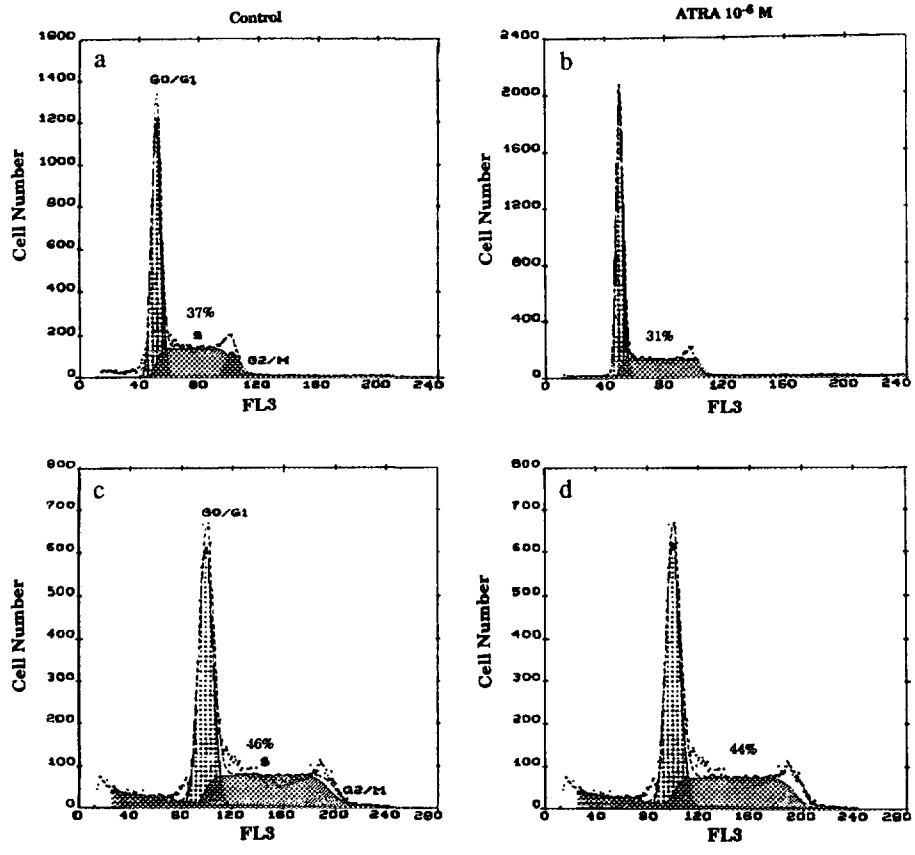

Fig. 3. Flow cytometry of S phase cell cycle analysis at day 1 of culture, in control HL-60 (a), NB4 (c) or 10^{-6} M treated HL-60 (b), NB4 (d) cells. FL3 represents the cell fluoresecence.

Retinoic acid receptor α expression. Terminal differentiation of acute myeloid leukemic cells has opened both new perspectives on the understanding of leukemogenesis, and new possibilities of therapies in malignancies. To date, *in vitro* and *in vivo* differentiation of APL cells with ATRA represents the first model of differentiation therapy (3, 4, 33, 34). Keeping in mind the strong implication of ATRA and RARα in normal (33, 35, 36) and leukemic (27) granulocytic differentiation, expression of RARα protein was analysed in HL-60 and NB4 cells. Flow cytometry analysis, using monoclonal antibody directed against the F region of the RARα1 receptor, revealed the presence of RARα protein in 74% ± 3 of HL-60 cells and in 92% ± 5 of NB4 cells (Fig. 4). As expected, the polyclonal anti-RARα1 antibody reacted specifically with nuclear extracts from NB4 cells (Fig. 5, lines a-b) and HL-60 cells (Fig. 5, lines e-f) and revealed a protein with an apparent molecular weight of ~48 kD with additional slower migrating species of ~54 and ~58 kD. Modulation of RARα protein was found in NB4 cells after 24 hours ATRA exposure, and was stable at 72 hours (Fig. 5, lines b,d). On the otherhand HL-60 cells required a 72 hours culture period to see a small increase in the RARα protein level (Fig. 5, lines e-h). In addition, a reactive protein with a molecular weight of ~100 kD corresponding to protein dimerization is sometimes detected in both NB4 and HL-60 cells (Fig. 5, lines d,g,h). These data confirm RARα gene expression studies in NB4 and HL-60 cells, showing a significant increase in RARα transcripts in only NB4 cells (30 and data not shown). The results are closely related to the ATRA intracellular concentration and terminal differentiation achieved (31). In F9 and

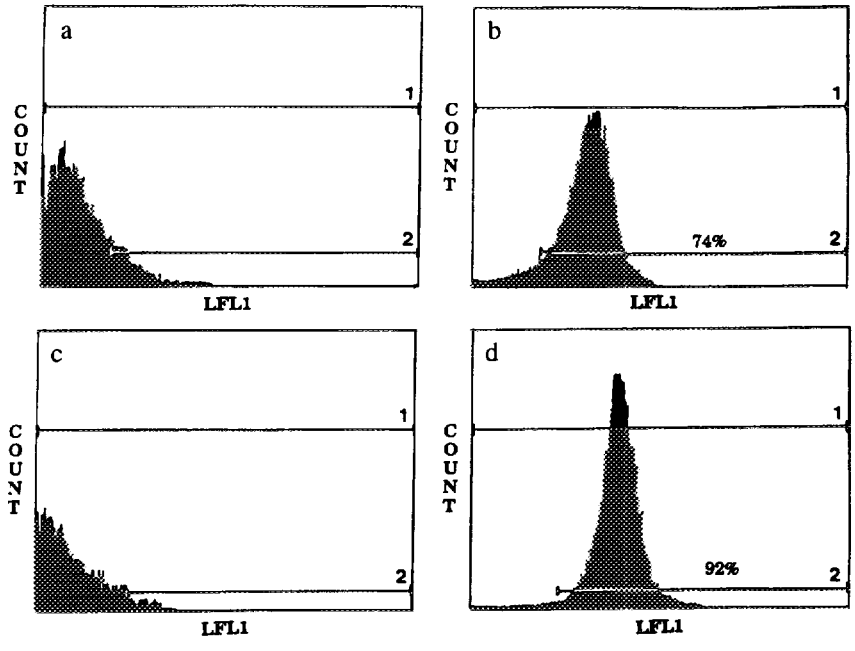

Fig. 4. Detection of RARα protein by flow cytometry. Control antibody : HL-60 (a), NB4 (c). RARα antibody : HL-60 (b), NB4 (d). The count indicated the number of cells and LFL1 the fluoresecence. RARα protein levels were determined by calculation of a corrected mean fluorescence intensity (difference in fluorescence intensity of Immunoglobulin control and anti-RARα antibody).

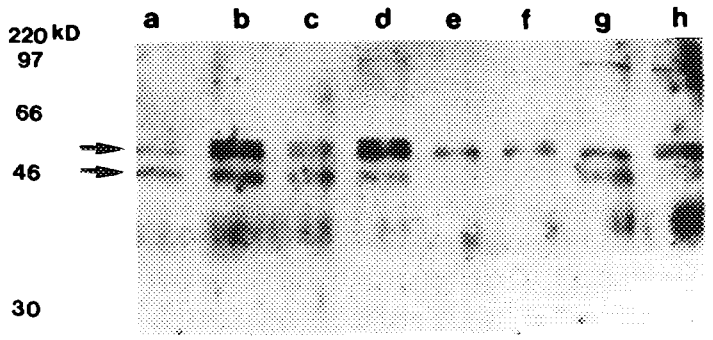

Fig. 5. Detection of RARα protein in nuclear extracts from NB4 (a-d) and HL-60 (e-h) cells. Control cells : day 1 (a, e); day 3 (c,g). 10^{-6} M treated cells : day 1 (b,f); day 3 (d,h). The position of prestained molecular weight standards (Rainbow, Amersham) is indicated in kiloDaltons (kD).

P19 EC cell lines, using the same antibody Gaub et al. (37) have equally detected similar RARα expression pattern. Our results are in agreement with those of Gaub et al. (37) and strongly suggest that the ~48 kD protein corresponded to unphosphorylated RARα1, and that the additional slower migrating species (~54, ~58 kD) may be generated by other modifications such as phosphorylation on thyrosine residues (38) or glycosylation (39). Phosphorylation sites have not yet been mapped, but putative phosphorylation sites for several kinases (40) are present in RARα protein, especially in the A/B, D and F regions.

Our results demonstrate that, the RARα protein is induced *in vitro* by ATRA in the NB4 APL cell line. In HL-60 or U-937 myelomonocytic leukemic cells, which have no rearranged RARα gene, the steady-state levels of the RARα transcripts are not affected by induction of terminal differentiation either to granulocytic or monocytic cells (28, 41). APL cells bear abnormal transcripts resulting from the fusion of the RARα and PML genes through the t(15;17) translocation (15, 37, 42, 43). The presence of chimeric PML/RARα fusion proteins in these cells may repress (directly or indirectly) the retinoic acid target genes. This repression might be overcome by pharmacological concentrations of ATRA (44) that restore functional levels of RARα, target gene activation, and granulocytic differentiation. These data suggest that, despite a spontaneous overall lower affinity of its endogenous receptors to ATRA, NB4 cells through a prolonged S phase, a high ATRA Cmax concentration and restored levels of RARα protein, triggers RA mediated granulocytic differentiation. Thus, an effective intracellular ATRA concentration and induction of a normal level of expression of the RARα protein in APL cells by ATRA, might be one of the intial steps restoring the granulocytic differentiation process.

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REFERENCES

1. Breitman, T.R., Selonick, S.E., and Collins, S.J. (1980) Proc. Natl. Acad. Sci. USA. 77, 2936-2940.

2. Koeffler, H.P. (1983) Blood 62, 709-721.

3. Huang, M.E., Ye, Yi., Chen, S.R., Lu, J.X., Zhoa, L., Gu, L.J., and Wang, Z.Y. (1988) Blood 72, 567-572.

4. Castaigne. S., Chomienne, C., Daniel, M.T., Berger, R., Fenaux, P., and Degos, L. (1990) Blood 76, 1704-1709.

5. Warrell, R.P. Jr., Frankel, S.R., Miller, W.H., Scheinberg, D.A., Itri, L.M., Hittelman, W.M., Vyas, R., Andreeff, M., Tafuri, A., Jakubowski, A., Grabrilove, J., Gordon, M.S., and Dmitrovsky, E. (1991) N. Engl. J. Med. 324, 1385-1393.

6. Chytil, F., and Ong, D. (1984) In Sporn MB, Roberts AB, Goodman DS (eds). The retinoids. Orlando : Academic Press, vol 2 : 89-123.

7. Douer, D., and Koeffler, H.P. (1982) J. Clin. Invest. 69, 277-283.

8. Cornic, M., Delva, L., Guidez, F., Balitrand, N., Degos, L., and Chomienne, C. (1992) Cancer Res. 52, 3329-3334.

9. Petkovich, M., Brand, J.N., Krust, A., and Chambon, P. (1987) Nature 330, 444-450.

10. Evans, R.M. (1988) Science 240, 889-895.

11. Mangelsdorf, D.J., Ong, E.S., Dyck, A., and Evans, R.M. (1990) Nature 345, 224-229.

12. Mangelsdorf, D.J., Borgmeyer, U., Heyman, R.A., Zhou, J.Y., Ong, E.S., Oro, A.E., Kakizuka, A., ans Evans, R.M. (1992) Genes Dev. 6, 329-344.

13. Allenby, G., Boquel, M.T., Saunders, M., Kazmer, S., Speck, J., Rosenberg, M., Leroy, A., Kastner, P., Grippo, J.F., Chambon, P., and Levin, A.A. (1993) Proc. Natl. Acad. Sci. USA. 90, 30-34.

14. De Luca, L.M. (1991) FASEB J. 5, 2524-2529.

15. Kastner, P., Perez, A., Lutz, Y., Rochette-Egly, C., Gaub, M.P., Durand, B., Lanotte, M., Berger, R., and Chambon, P. (1992) EMBO J. 11, 629-642.

16. Agadir, A., Cornic, M., Gourmel, B., Lefebvre, P., Degos, L., and Chomienne, C. (1994) Cell. Mol. Biol. 40, 263-274.

17. Hamada, K., Gleason, S.L., Levi, B.Z., Hirschfeld, S., Appella, E., and Ozato, K. (1989) Proc. Natl. Acad. Sci. USA. 86, 8289-8293.

18. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Sauders, M., Zacharewski, T., Chen, J.Y., Staub, A., Garnier, J.M., Mader, S., and Chambon, P. (1992) Cell 68, 377-395.

19. Bennet, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galdon, D.A.G., Gralnick, H.R., and Sultan, C. (1985) Ann. Internal. Med. 103 , 626-629.

20. Wigler, M., Sweet, R., Sim, G., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S., and Axel, R. (1979) Cell 16, 777-785.

21. Nervi, C., Poindexter, E.C., Grignani F., Pandolfi, P.P., Lo Coco, F., Avvisati, G., Pelicci, P.G., and Jetten A.M. (1992) Cancer Res. 52, 3687-3692.

22. Calabresse, C., Venturini, L., Ronco, G., Villa, P., Degos, L., Belpomme, P., and Chomienne, C. (1994) Biochem. Biophys. Res. Comm. 201, 266-283.

23. Nervi, C., Grippo, J.F., Sherman, M.I., Gerorge, M.D., and Jetten, A.M. (1989) Proc. Natl. Acad. Sci. USA. 86, 5854-5858.

24. Kagechika, H., Hashimoto, Y., Kawachi, E., and Shudo, K. (1988) Biochem. Biophys. Res. Comm. 155, 503-508.

25. Sani, B.P., and Banerjee, C.K. (1990) Arch. Biochem. Biophys. 283, 107-113.

26. Dallery, N., Sablonnière, B., Grillier, I., Formstecher, P., and Dautrevaux, M. (1993) Biochem. 32, 12428-12436.

27. Collins, S. J., Robertson, K.A., and Le Moyne, M. (1990) Mol. Cell. Biol. 10, 2154-2163.

28. Largman, C., Dtemer, K., Corral, J.C., Hack, F.M., and Lawrence H.J. (1989) Blood 74, 99-102.

29. Thé de, H., Lavau, C., Marchio, A., Chomienne, C., Degos, L., and Djean, A. (1991) Cell 66, 675-684.

30. Chomienne, C., Balitrand, N., Ballerini, P., Castaigne, S., Thé de, H., and Degos, L. (1991) J. Clin. Invest. 88, 2150-2154.

31. Agadir, A., Cornic, M., Lefebvre, P., Gourmel B., Balitrand, N., Degos, L., and Chomienne, C. (1995) Leukemia 9, 139-145.

32. Yen, A., and Albright, K.L. (1984) Cancer Res. 44, 2511-2515.

33. Chomienne, C., Ballerini, P., Balitrand, N., Daniel, M.T., Fenaux, P., Castaigne, S., and Degos, L. (1990) Blood 76, 1710-1717.

34. Degos, L., Chomienne, C., and Daniel, M.T. (1990) Lancet 336, 1440-1441.

35. Gratas, C., Menot, M.L., Dresh, C., and Chomienne, C. (1993) Leukemia 7, 1156-1162.

36. Micléa, J.M., and Chomienne, C. (1994) Leukemia 8, 214-215.

37. Gaub, M.P., Rochette-Egly, C., Lutz, Y., Ali, S., Matthes, H., Scheuer, I., and Chambon, P. (1992) Exp. Cell Res. 201, 335-346.

38. Tonks, N.K. (1990) Curr. opinion Cell. Biol. 2, 1114-1124.

39. Jackson, S.P., and Tjian, R. (1988) Cell 55, 125-133.

40. Kemp, B.E., and Pearson R.B. (1990) TIBS 15, 342-346.

41. Kizaki, M., Koeffler, H.P., Lin, C.W., and Miller, C.W. (1990) Leuk. Res. 14, 645-655.

42. Kakizuka, A., Miller, W.H., Umesono, K., Warrell, R.P., Frankel, S.R., Murty, S., Dmitrovsky, E., and Evans, R.M. (1991) Cell 66, 663-674.

43. Pandolfi, P.P., Alcalay, M., Fagioli, M., Zangrilli, D., Mencarelli, A., Diverio, D., Biondi, A., LoCoco, F., Rambaldi, A., Grignani, F., Rochette-Egly, C., Gaub, M.P., Chambon, P., and Pelicci, P.G. (1992) EMBO J. 11, 1397-1407.

44. Rousselot, P., Hardas, B., Patel, A., Guidez, F., Gäken, J., Castaigne, S., Dejean, A., Thé, de H., Degos, L., Farzaneh, F., and Chomienne, C. (1994) Oncogene 9, 545-551.