Expression of *c-jun*, *jun B* and *jun D* proto-oncogenes in human peripheral-blood granulocytes

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We have found that purified human peripheral-blood granulocytes express constitutively significant levels of proto-oncogenes *c-jun*, *jun B* and *jun D* mRNA. Upon functional activation of granulocytes by 4 β -phorbol 12-myristate 13-acetate (PMA), the levels of *c-jun*, *jun B* and *jun D* transcripts were increased. The three *jun* genes showed a similar time course in their induction by PMA, maximal mRNA levels being reached after 60 min of induction. These results suggest that expression of *c-jun*, *jun B* and *jun D* genes might be involved in terminal granulocyte differentiation or in regulating granulocyte functionality.

INTRODUCTION

The proto-oncogene *c-jun* encodes for the transcriptional factor AP-1 [1,2]. This c-Jun/AP-1 protein has been shown to be associated with the c-Fos oncoprotein in some transcriptional activators [3–6]. Two other *c-jun*-related cDNAs have been isolated (*jun B* and *jun D*), whose encoded proteins show extensive amino acid sequence identity with c-Jun/AP-1 and are able to form nuclear complexes with c-Fos or Fos-related proteins [7,8]. It has been found that human granulocytes express constitutively high levels of *c-fos* mRNA [9–12], which were increased upon cell activation by distinct stimuli [9–13]. Thus it was of importance to examine the expression of the *jun* family genes in granulocytes. In this context, the expression of *c-jun* mRNA in human myelomonocytic cells has been recently reported [14]. The present study was performed to analyse the expression of the three proto-oncogenes *c-jun*, *jun B* and *jun D* at the mRNA level in freshly isolated human peripheral-blood granulocytes.

MATERIALS AND METHODS

Cells

Granulocytes were obtained from fresh human peripheral blood after dextran-induced sedimentation of erythrocytes and density-gradient centrifugation of the leucocyte-rich supernatant on Lymphoprep, and hypo-osmotic lysis of contaminating erythrocytes as previously described [15]. Isolated granulocytes contained more than 98 % neutrophils and less than 1 % contaminating mononuclear cells, as assessed by Giemsa–Wright stain and flow-cytometric analysis. Cell viability was evaluated by Trypan Blue exclusion, and was always higher than 98 %.

The human promyelocytic cell line HL60 [16] and the human promonocytic cell line U937 were grown at 37 °C in a humidified atmosphere (CO₂/air, 1:19) in RPMI 1640 medium supplemented with 10 % (v/v) heat-inactivated fetal-calf serum, 2 mM-L-glutamine, penicillin (100 units/ml) and gentamycin (24 μ g/ml).

RNA analysis

Total RNA was isolated by the guanidine isothiocyanate/CsCl method [17] and analysed (10 μ g of RNA), either by electrophoresis through 1.1 % (w/v) agarose/formaldehyde gels, fol-

lowed by transfer to Hybond-N nylon membranes, or by dot-blot hybridization as previously described [18].

³²P-labelled cDNA probes were prepared using the random oligonucleotide priming method (Oligo-Labeling Kit; Pharmacia) to a specific radioactivity equal to, or higher than, 7 $\times 10^8$ c.p.m./ μ g of cDNA. The plasmid AH119 [19], containing *c-jun*, was generously provided by Dr. R. Bravo (Squibb Institute, Princeton, NJ, U.S.A.). cDNA probes for *jun B* [20] and *jun D* [21] were kindly provided by Dr. P. Sassone-Corsi (LGME, CNRS, Strasbourg, France). The plasmid pAc 18.1 [22] was used as a probe for β -actin. β -Actin mRNA was employed as a control probe.

Northern and dot-blots were prehybridized at 42 °C for 24 h in a buffer containing 50 % formamide, 0.75 M-NaCl, 0.2 % SDS, 25 mM-EDTA, 5 $\times$ Denhardt's solution (1 $\times$ Denhardt's is 0.02 % BSA/0.02 % Ficoll/0.02 % polyvinylpyrrolidone), 25 mM-Pipes (pH 6.8) and salmon sperm DNA (100 μ g/ml). Blots then were hybridized in the above buffer containing 1 $\times$ Denhardt's solution, 10 % (w/v) dextran sulphate and 10⁷ c.p.m. of probe at 42 °C for 16–24 h. After hybridization, the blots were washed twice in 2 $\times$ SSC (1 $\times$ SSC is 0.15 M-NaCl/0.015 M-sodium citrate)/0.1 % SDS for 30 min each wash at room temperature, once with the same buffer at 42 °C, twice in 0.1 % SSC/0.1 % SDS for 15 min at 42 °C, and once more with the same buffer for 15 min at 60 °C. Subsequently, blots were air-dried and exposed to Kodak X-Omat film with enhancing screens at –80 °C. Quantitative analysis of the autoradiograms was performed by integration of peak areas after scanning with a Molecular Dynamics 300A computing densitometer Fast Scan.

Labelled probes were removed from filters by immersion in boiling 2 % (v/v) glycerol for 2 min. Before hybridization to a second probe, removal of radioactivity was verified by autoradiography.

RESULTS AND DISCUSSION

Total RNA was extracted from highly purified granulocytes isolated from human peripheral blood and analysed by Northern and dot-blot techniques using specific probes for *c-jun*, *jun B* and *jun D*. We found that the average content of cell RNA in granulocytes ranged from 4.8 to 5.6 μ g of RNA/10⁷ cells. This value is very low when compared with that found in other cell

Abbreviations used: PMA, 4 β -phorbol 12-myristate 13-acetate.

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Fig. 1. Northern-blot analysis of the *c-jun*, *jun B* and *jun D* mRNA in human peripheral-blood granulocytes

Total RNA was isolated from unstimulated control granulocytes ('C') as well as from granulocytes stimulated with PMA (20 ng/ml) for 60 min at 37 °C ('PMA'), and Northern-blot analysis was carried out as described in the Materials and methods section. The sizes of the transcripts for *c-jun* (~2.7 kb and ~3.4 kb), *jun B* (~2.1 kb) and *jun D* (~2.0 kb) are indicated. β -Actin mRNA was used as a control. Autoradiograms were developed after 3 or 24 h of exposure as indicated.

types, such as the human promonocytic cell line U937 (about 60 μ g of RNA/ 10^7 cells) or the human promyelocytic cell line HL60 (about 40 μ g of RNA/ 10^7 cells). As shown in Fig. 1, high levels of *c-jun*, *jun B* and *jun D* mRNA could be detected constitutively in unstimulated granulocytes. However, unstimulated granulocytes expressed much higher levels of *jun B* and *jun D* transcripts than of *c-jun* transcripts (Fig. 1). Unlike granulocytes, uninduced U937 and HL60 cells did not express detectable levels of *c-jun*, *jun B* and *jun D* mRNA (results not shown). Treatment of granulocytes with the cell-activating agent 4 β -phorbol 12-myristate 13-acetate (PMA) enhanced the levels of *c-jun*, *jun B*

and *jun D* transcripts (Fig. 1). Densitometric analysis revealed a 3–4-fold increase in the mRNA levels of the three *jun* family members upon cell treatment with 20 ng of PMA/ml for 60 min. The size of the *c-jun* mRNA was about 2.7 kb, as already reported [1,2]. A minor band of about 3.4 kb, suggested to be a precursor molecule [1,2], was also evident, but only after over-exposure of the autoradiogram. The sizes of *jun B* and *jun D* transcripts were about 2.1 and 2.0 kb respectively, as already reported [10,21]. The level of expression found for *c-jun* mRNA was comparable with that found for the 2.2 kb *c-fos* mRNA ([9]; F. Mollinedo & J. R. Naranjo, unpublished work). Under the experimental conditions used in the Northern-blot analysis there was no cross-hybridization between the three *jun* probes. This lack of cross-hybridization was evidenced by: (a) differences in the sizes of the respective mRNAs for *c-jun* (~2.7 kb), *jun B* (~2.1 kb) and *jun D* (~2.0 kb) (Fig. 1); (b) a different basal and induced expression of the three *c-jun*, *jun B* and *jun D* transcripts in granulocytes (Fig. 1); (c) a distinct regulation in the expression of the three *c-jun*, *jun B* and *jun D* transcripts during macrophage-cell differentiation of U937 and HL60 cells induced by PMA, reaching maximal levels of *jun B*, *jun D* and *c-jun* transcripts after 30 min, 6 h and 24 h of induction respectively (F. Mollinedo & J. R. Naranjo, unpublished work).

Time-course experiments showed, by dot-blot analysis, that *jun B*, *jun D* and *c-jun* mRNA levels were augmented as early as 15–30 min after the addition of PMA, reaching their maximal levels (3–4-fold increase) at 60 min. Thereafter, *jun B*, *jun D* and *c-jun* mRNA levels decreased to values close to the preinduction levels within 2 h (Fig. 2).

The results herein reported demonstrate for the first time that *c-jun*, *jun B* and *jun D* proto-oncogenes are expressed constitutively in freshly isolated human peripheral-blood granulocytes and that the basal levels of the three *c-jun*, *jun B* and *jun D* mRNAs are increased by functional granulocyte activation with PMA. Furthermore, as mature granulocytes do not proliferate and are unable to undergo further differentiation, these results also demonstrate that the three *c-jun*, *jun B* and *jun D* genes are expressed in a terminally differentiated non-proliferating cell type. The three *jun* proto-oncogenes studied have been shown to be associated with c-Fos or Fos-related proteins to form transcriptional regulators [3–8]. Thus the data reported here suggest that expression of *jun* and *fos* proto-oncogenes might be involved in regulating gene expression related to granulocyte functionality or terminal cell differentiation.

We thank the Hospital Santiago Ramon y Cajal Blood Bank for providing us with fresh human peripheral blood. This work was supported by Grant PM 89-0003 from the Dirección General de Investigación Científica y Técnica (to F.M.) and by a Grant from the Fundación Ramón Areces (to J.R.N.).

REFERENCES

1. Bohman, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K. & Tjian, R. (1987) *Science* **238**, 1386–1392
2. Angel, P., Allegretto, E. A., Okino, S. T., Hattari, K., Boyle, W. J., Hunter, T. & Karin, M. (1988) *Nature (London)* **332**, 166–171
3. Sassone-Corsi, P., Ransome, L. J., Lamph, W. W. & Verma, I. M. (1988) *Nature (London)* **336**, 692–695
4. Rauscher, F. J., III, Sambucetti, L. C., Curran, T., Distel, R. J. & Spiegelman, B. M. (1988) *Cell (Cambridge, Mass.)* **52**, 471–480
5. Franza, B. R., Jr., Rauscher, F. J., III, Josephs, S. F. & Curran, T. (1988) *Nature (London)* **339**, 1150–1153
6. Halazonetis, T. D., Georgopoulos, K., Greenberg, M. E. & Leder, P. (1988) *Cell (Cambridge, Mass.)* **55**, 917–924
7. Ryder, K., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8464–8467

Fig. 2. Time course of *jun B*, *jun D* and *c-jun* mRNA induction by PMA in human peripheral-blood granulocytes

Cells were exposed for the indicated times to PMA (20 ng/ml) at 37 °C. Total RNA was isolated and dot-blot analysis was carried out as described in the Materials and methods section. Specific mRNA contents were quantified by densitometric scanning of the autoradiograms. Values were corrected for variations in β -actin mRNA content. Data are means for densitometric scans from three independent dot-blots. Standard errors were between 5 and 7%. Autoradiograms were developed after 24 h of exposure. The upper part of the Figure shows a representative dot-blot of cells treated with PMA (20 ng/ml).

8. Nakabeppu, Y., Ryder, K. & Nathans, D. (1988) *Cell* (Cambridge, Mass.) **55**, 907–915
9. Colotta, F., Wang, J. M., Polentarutti, N. & Mantovani, A. (1987) *J. Exp. Med.* **165**, 1224–1229
10. Heidorn, K., Kreipe, H., Radzun, H. J., Müller, R. & Parwaresch, M. R. (1987) *Blood* **70**, 456–459
11. Kreipe, H., Radzun, H. J., Heidorn, K., Mäder, C. & Parwaresch, M. R. (1987) *J. Histochem. Cytochem.* **35**, 837–842
12. Sariban, E., Mitchell, T., Rambaldi, A. & Kufe, D. W. (1988) *Blood* **71**, 488–493
13. Itami, M., Kuroki, T. & Nose, K. (1987) *FEBS Lett.* **222**, 289–292
14. Bertani, A., Bolentarutti, N., Sica, A., Rambaldi, A., Mantovani, A. & Colotta, F. (1989) *Blood* **74**, 1811–1816
15. Mollinedo, F., Nieto, J. M. & Andreu, J. M. (1989) *Mol. Pharmacol.* **36**, 547–555
16. Collins, S. J., Gallo, R. C. & Gallagher, R. E. (1977) *Nature* (London) **270**, 347–349
17. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Ryseck, R.-P., Hirai, S. I., Yaniv, M. & Bravo, R. (1988) *Nature* (London) **334**, 535–537
20. Ryder, K., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1487–1491
21. Hirai, S.-I., Ryseck, R.-P., Mechta, F., Bravo, R. & Yaniv, M. (1989) *EMBO J.* **8**, 1433–1439
22. Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, Z. & Yaffe, D. (1983) *Nucleic Acids Res.* **11**, 1759–1771

Received 24 July 1990/31 October 1990; accepted 12 November 1990