Modulation of poly(ADP-ribose) polymerase during neutrophilic and monocytic differentiation of promyelocytic (NB4) and myelocytic (HL-60) leukaemia cells

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Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme which has been shown to play a role in the differentiation of haematopoietic cells. We report here that neutrophils are the first nucleated mammalian cell type demonstrated to be devoid of immunoreactive PARP. Both NB4 acute promyelocytic leukaemia and HL-60 (acute myelocytic leukaemia) cells were differentiated into non-malignant neutrophils with all-*trans*-retinoic acid (ATRA). Western blot analysis demonstrated that ATRA had no effect on PARP expression in HL-60 cells. However, PARP was completely down-regulated in NB4 cells within 36 h of treatment initiation. This decrease in PARP polypeptide coincided with growth arrest and preceded the appearance of neutrophilic differentiation features. NB4 cells require a combination of 1,25-dihydroxyvitamin D_3 (1,25-D_3) and phorbol 12-myristate 13-acetate (PMA) to differentiate completely into monocyte/macrophages, whereas HL-60 cells can be made to differentiate by combined or single agents. PARP expression was up-regulated 90-fold when NB4 cells were treated with PMA and 1,25-D_3 together, and this increase accompanied expression of the monocyte/macrophage phenotype. Only modest changes in PARP expression were observed when each agent was used alone in NB4 cells or when HL-60 cells were differentiated along the monocyte/macrophage pathway. In addition, PARP activity was modulated in a pattern similar to protein levels when NB4 cells were induced to differentiate along the neutrophilic and monocyte/macrophage pathways. This suggests that the activity of PARP may be controlled through regulation of protein levels during NB4 cell differentiation. We conclude that PARP levels are dramatically modulated during monocyte/macrophage and neutrophilic differentiation. On the basis of the tremendous changes in PARP polypeptide and total activity during myeloid differentiation, we propose that modulation of PARP gene expression is required for cellular maturation in both lineages.

INTRODUCTION

Poly(ADP-ribosyl)ation of nuclear proteins by poly(ADP-ribose) polymerase (PARP) has been implicated as a regulatory event during cellular differentiation [1,2]. These reactions, which transfer ADP-ribose units from NAD^+ to chromatin-associated proteins, are known to modulate DNA metabolism and architecture [3–6]. PARP binds to and is catalytically activated by single and double strand breaks in DNA [7]. This binding takes place via Zn^+ fingers [8], and PARP has been implicated in processes that involve the nicking and rejoining of DNA [9,10]. These include a variety of physiological events such as DNA repair, DNA replication during cellular proliferation and changes in DNA topology and gene expression during differentiation [11–13]. The activity of PARP and consequent formation of poly(ADP-ribose) can vary significantly during these events [14], although many of these studies do not differentiate between changes in specific activity and quantity of PARP polypeptide. A potential role for PARP in the process of differentiation is supported by experiments utilizing inhibitors and measurements of PARP activity. In K562 cells, PARP activity is lowered during erythrocytic differentiation [15]. Damji et al. [16] have shown that inhibitors of PARP, such as 3-aminobenzamide, synergize with neutrophil differentiation agents to induce the maturation of HL-60 cells in the granulocytic lineage. Alterations in polymer synthesis during differentiation of Friend leukaemic cells has been speculated to be a requirement for the differentiation process [17,18]. Nevertheless, data on the role of PARP in differentiation are conflicting, with some studies showing that increases in PARP activity precede differentiation and others showing a transient decrease in activity [19–21]. One consistent finding is that changes in PARP activity appear to be early events in differentiation processes, and this may be due to DNA strand breaks that accumulate during initiation of maturation [22,23].

Some studies have measured PARP transcript regulation during differentiation. Data available to date suggest that, although DNA damage may alter the specific activity of PARP, it does not directly alter transcriptional regulation PARP [23]. Therefore, cellular signals which cause modulation of PARP mRNA likely occur via a mechanism distinct from DNA strand breaks.

Several groups have suggested that the role of PARP during the differentiation process may be to regulate DNA recombination and gene rearrangement required for the expression of the mature cell phenotype [24,25]. Many leukaemia cell culture models have been developed to study cellular differentiation. HL-60 and NB4 cells both differentiate along the neutrophilic pathway in response to all-*trans*-retinoic acid (ATRA) [26,14]. In addition, the HL-60 cells differentiate in the monocytic pathway in response to phorbol 12-myristate 13-acetate (PMA) or 1,25-dihydroxyvitamin D_3 (1,25-D_3) [27,28]. Recently our group has shown that NB4 cells are also capable of monocyte/macrophage differentiation in response to 1,25-D_3 and PMA in combination [29].

HL-60 cells, although originally considered to be a promyelocytic leukaemic cell, have since been shown to be an acute

Abbreviations used: APL, acute promyelocytic leukaemia; ATRA, all-*trans*-retinoic acid; NBT, Nitro Blue Tetrazolium; PARP, poly(ADP-ribose) polymerase; PMA, phorbol 12-myristate 13-acetate ('TPA'); 1,25-D_3, 1,25-dihydroxyvitamin D_3.

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myelocytic cell line. NB4 cells are the first described acute promyelocytic leukaemia (APL) cell line and carry the classic t(15;17) translocation which is the currently used diagnostic indicator of APL [14]. NB4 cells are considered an important model for differentiation therapy in APL. An understanding of the molecular mechanisms of myeloid differentiation could provide valuable insights into both normal differentiation processes and the design of new differentiation agents that could be used in therapy.

Although all eukaryotic cells are thought to contain PARP (with the exception of yeast) [29], it has been reported that neutrophils contain no poly(ADP-ribose) [29]. In the present study we have investigated the reason for the absence of poly(ADP-ribose) from neutrophils and postulated that myeloid progenitors would modulate PARP metabolism during neutrophilic differentiation as they mature to functional neutrophils. This study shows that neutrophils are the first nucleated mammalian cell type from which immunoreactive PARP is absent. In addition, the PARP polypeptide is modulated differently in both the neutrophilic and monocyte/macrophage pathways and in response to different inducers of differentiation. Dramatic differences between the acute promyelocytic (NB4) and myelocytic (HL-60) leukaemic cells were also observed. Determination of PARP activity in NB4 cells during neutrophilic and monocyte/macrophage differentiation also suggests that activity of the enzyme is regulated by alteration in total PARP protein levels. The biological significance of these differing patterns of PARP regulation is discussed in terms of differentiation programmes.

MATERIALS AND METHODS

Cell culture

NB4 cells were obtained from Dr. M. Minden (Princess Margaret Hospital, Toronto, Ont., Canada). These cells were originally isolated and characterized from a human patient with APL [14]. The human HL-60 cell line was purchased from the American Tissue Culture Collection. Both cell lines were cultured in Iscove's modified Dulbecco's medium (IMDM) with 10% fetal-calf serum, supplemented with penicillin and streptomycin (50 units/ml) at 37 °C in an atmosphere of 5% CO_2 in air. Exposure of cultures to light was minimized, to prevent inactivation of light-sensitive 1,25-D_3 and ATRA. Cells were cultured in 22 mm- and 60 mm-diam. tissue-culture dishes (Corning or Falcon) and were routinely passaged in tissue-culture flasks (8 ml) as suspension cultures. Cell growth and viability were assessed by using a Coulter Counter (model ZM) and Trypan Blue dye exclusion, respectively. Cell cultures had a viability of greater than 78% in all experimental treatment groups.

Differentiation inducers

1,25-D_3 was purchased from Interscience (Calbiochem) and dissolved in ethanol to produce a stock of 1,25-D_3 at a concentration of 0.9 mM. ATRA was purchased from Sigma and dissolved in ethanol to produce a stock of 10 mM. Both stocks of 1,25-D_3 and ATRA were protected from light and were stored at −20 °C under nitrogen. PMA was purchased from Sigma and was dissolved first in DMSO and then diluted to a concentration of 170 μM in IMDM with 10% fetal-calf serum and antibiotics.

Treatment of cultures

All stocks were diluted in culture medium to give final working concentrations that have been shown to maximize the differentiation response. For neutrophilic differentiation, HL-60 and NB4 cells were treated with ATRA to give a final concentration of 2 μM and 1 μM respectively [26,14]. For monocyte/macrophage differentiation, HL-60 cells were treated with 1,25-D_3, PMA, and 1,25-D_3 and PMA, in combination at concentrations of 0.1 μM 1,25-D_3 and 7.0 nM PMA respectively. NB4 cells were treated similarly for monocyte/macrophage differentiation with 0.4 μM 1,25-D_3 and 0.4 μM PMA.

Markers of differentiation

Neutrophilic differentiation was assessed by Nitro Blue Tetrazolium (NBT) dye reduction as previously described [30]. Briefly, a kit from Sigma Chemical Co. was used: equal volumes of cells in media were combined with 0.1% solution of NBT in PBS with addition of stimulant for 20 min at 37 °C in 5% CO_2. Cytospins were prepared and cells examined for formazan deposits. A total of 200 cells were counted and the number of positive cells was expressed as a percentage. Characterization of neutrophil polynuclear morphology was accomplished by Giemsa–Wright staining of cytospin preparations. Monocyte/macrophage differentiation was assessed by measuring the adherent fraction, α-naphthyl acetate esterase activity by using a kit from Sigma, and phagocytosis of latex beads as described by Bhatia et al. [29]. In addition, the maturation response was also monitored in both pathways by a decrease in proliferative capacity.

Sample collection

Both NB4 and HL-60 cells were harvested at specified time intervals after exposure to differentiation-inducing agents. Cells were washed twice in PBS containing a mixture of protease inhibitors (Boehringer Mannheim, cat no. 1206893) and resuspended in a urea-based lysis buffer (6 M urea, 6% β-mercaptoethanol, 3% SDS, 0.003% Bromophenol Blue) to obtain a final cell concentration of 3.2×10^4 cells/μl. Lysates were then heated to 60 °C for 2 min, sonicated for 25 s and stored at −20 °C until analysis by SDS/PAGE.

Neutrophil isolation

Neutrophils were isolated by a centrifugal separation method using a kit (Sigma) with some modifications. In order to obtain inactive neutrophils which did not undergo proteolytic degradation of high-molecular-mass proteins, protease inhibitors were utilized at the maximum recommended concentrations (Boehringer Mannheim). Protease inhibitors were added to wash solutions, and cells were maintained at 4 °C during their manipulation. Neutrophils were then prepared for SDS/PAGE as described for NB4 and HL-60 cells. In addition, polyacrylamide gels were stained with Commassie Blue to verify that a full protein profile could be obtained without degradation of high-molecular-mass proteins. Purity of neutrophil isolates was greater than 98%, as assessed by Giemsa–Wright staining of cytospin preparations.

Western blotting

Proteins were separated by SDS/PAGE on 7.5%-acrylamide gels and transferred on to a polyvinylidene difluoride membrane in a semi-dry blotting apparatus for 2 h at 100 mA. Molecular masses of proteins were estimated by comparison with migration of standard molecular-mass markers (Sigma Chemical Co., St. Louis). Membranes were first blocked with 5% milk powder in Tris-buffered saline (TBS), washed in TBS with 0.05% Tween 20 and then incubated with primary antibody. A rabbit polyclonal

antibody (FII) directed against the human Zn-finger domain of PARP was used as the primary antibody (generously given by Dr. G. de Murcia, Pasteur Institute, Paris, France). Membranes were then washed and incubated with anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase. PARP polypeptide was detected with the ECL kit (Amersham) and quantified by autoradiography and densitometry. Signal intensity and purified PARP protein from 11 to 124 μg gave a linear response ($r = 0.998$, $P < 0.001$), and quantification of all image densities recorded fell within this linear portion. Equal quantities of lysed cells were loaded on each lane in each experiment. Quantification of PARP is represented as an average of at least three separate experiments.

PARP activity

PARP activity was determined as described previously with modifications which allowed optimization of conditions for our system [31]. Briefly, 10^6 NB4 cells exposed to ATRA, or PMA and 1,25-D_3 in combination, at concentrations which induced neutrophilic and monocyte/macrophage differentiation were collected and washed with 0.9% NaCl at various times indicated. Cells were then incubated in 1 ml of hypotonic permeabilization buffer (1 mM EDTA, 10 mM Tris/HCl, pH 7.8, 4 mM $MgCl_2$, 30 mM β-mercaptoethanol) for 15 min on ice. Cells were then pelleted at 200 *g* (0 °C, 10 min) and then resuspended in 53 μl of permeabilization buffer. On ice, 5 μg of palindromic decameric deoxynucleotide (CGGAATTCCTG) in 15 mM NaCl, 5 μl of [^{3}H]NAD$^+$ [18.4 kBq (= 0.5 μCi)] and 33 μl of reaction mixture (100 mM Tris/HCl, pH 7.8, 1 mM NAD$^+$, 120 mM $MgCl_2$) were added to permeabilized cells. Reactions were carried out for 25 s at room temperature and stopped by addition of ice-cold 10% trichloroacetic acid containing 2% sodium pyprophosphate. Samples were incubated overnight at 4 °C, and precipitates were then collected on GF/C filters, washed with 3 × 5 ml of 10% trichloroacetic acid, then 2 × 5 ml of 10% HCO_4 and finally 2 × 5 ml of 96% ethanol. Filters were air-dried and liquid-scintillation counting was carried out with 5 ml of Ecolite (Canlab, Missisauga, Ont., Canada). Counts were corrected for the amount of labelled NAD$^+$ which was associated non-enzymically with cellular material by subtracting counts determined from samples in which trichloroacetic acid was added before addition of radiolabel.

Statistics

Experimental data were analysed by ANOVA, followed by Dunnett's test using the general linear modelling procedure (SAS/PC V6.04; SAS, Cary, NC, U.S.A.). Differences were considered significant at $P < 0.05$.

RESULTS

Identification of PARP in leukaemic cells and human neutrophils

Previous reports had shown that neutrophils had little or no poly(ADP-ribose), but had not directly assessed the quantity of the enzyme [29]. Figure 1 shows the migration of purified PARP polypeptide at 116 kDa, which served as a control for identification. Both NB4 and HL-60 cells expressed PARP with the same apparent molecular mass as the purified enzyme. Neutrophils from healthy volunteers (four separate subjects) contained no detectable PARP. Thus the explanation for a lack of PARP in neutrophils can be attributed to an absence of the enzyme.

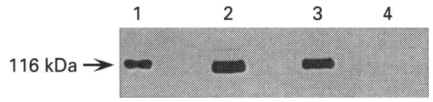

Figure 1 Expression of PARP polypeptide from cell lysates

Western-blot analysis of PARP protein levels: purified PARP from calf thymus (lane 1), untreated NB4 cells (lane 2), HL-60 cells (lane 3) and neutrophils from peripheral blood of a healthy volunteer (lane 4). Each lane contained protein from 5×10^5 cells. The autoradiogram was developed after 2 min of exposure by using ECL detection (see the Materials and methods section). The Figure is representative of four similar experiments.

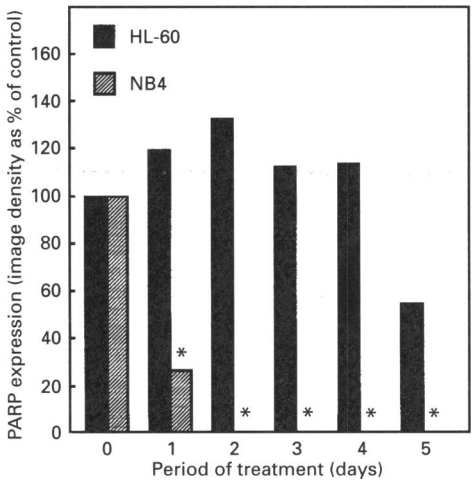

Figure 2 Expression of PARP protein in ATRA-treated HL-60 and NB4 cells

PARP protein was quantified by densitometric scanning of autoradiograms (prepared as described in Figure 1) and is expressed as a percentage of the image density of control cells: *indicates significant difference compared with control ($P < 0.05$, $n = 3$).

Down-regulation of PARP during neutrophilic differentiation occurs in NB4 cells, but not HL-60 cells

We hypothesized that, since normal circulating neutrophils lack PARP polypeptide, precursors may down-regulate PARP expression when induced to differentiate into mature neutrophils. We studied the leukaemic model cells NB4 and HL-60, which respond to ATRA and differentiate to morphological and functional neutrophils [26,14]. There is no significant regulation of PARP in HL-60 cells even after morphological and functional features of neutrophils can be identified (Figure 2). In contrast, NB4 cells completely down-regulate PARP levels by day 2 of exposure to the same differentiation agent (Figure 2). The absence of PARP polypeptide in differentiating neutrophils from NB4 cells is consistent with data obtained from purified neutrophils from healthy volunteers.

Down-regulation of PARP in NB4 cells coincides with the loss of proliferative capacity and precedes the appearance of neutrophilic differentiation features

We questioned whether changes in PARP protein levels were coincidental with, or preceded, other markers of cellular differ-

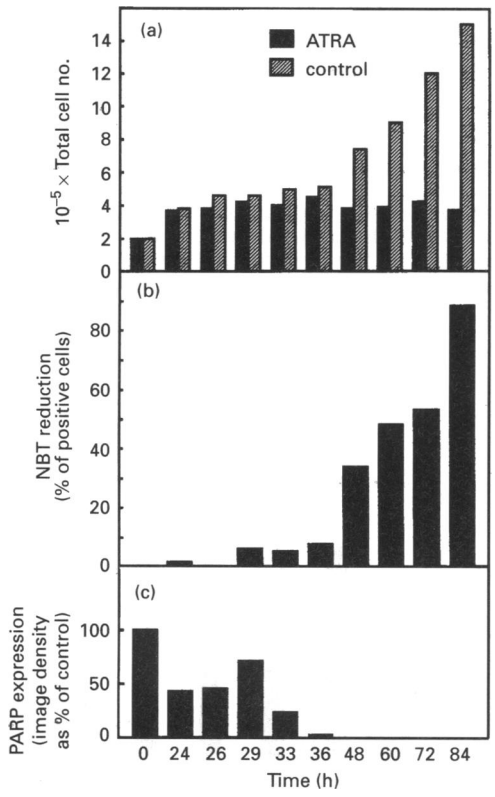

Figure 3 Cell growth, NBT reduction and PARP expression in NB4 cells treated with 1 μM ATRA

(**a**) Cell growth expressed as the total cell number of ATRA-treated and untreated (vehicle alone) NB4 cells. (**b**) Percentage of NBT-positive cells was based on the number of cells containing formazan deposits from a total of 200 cells examined at each time point. (**c**) PARP expression was quantified by densitometric scanning of autoradiograms. A total of 2.0×10^5 cells were plated in each 22 mm culture dish in duplicate for all experiments. Data are averages of two similar experiments.

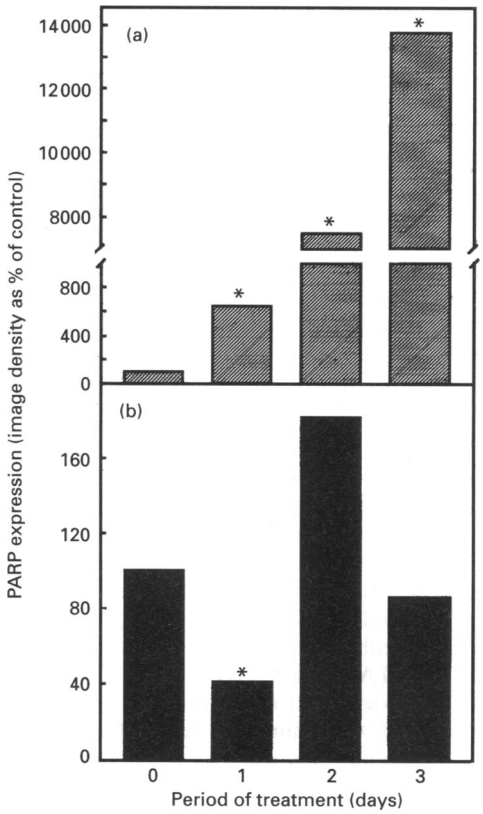

Figure 4 Expression of PARP in HL-60 and NB4 cells treated with 1,25-D_3 and PMA in combination

(**a**) NB4 cells treated with 0.4 μM PMA and 0.4 μM 1,25-D_3; (**b**) HL-60 cells treated with 7 nm PMA and 0.1 μM 1,25-D_3. PARP protein was quantified by densitometric scanning of autoradiograms and is expressed as a percentage of the image density of control cells: * indicates significant difference compared with control ($P < 0.05$, $n = 3$).

entiation. In Figure 3(a) we assessed the proliferation of untreated (vehicle alone) and ATRA-treated NB4 cells. Decreased proliferative capacity was evident within 24 h of treatment. The functional marker of neutrophil maturation, NBT reduction, appeared between 24 and 48 h after treatment began and was maximal at 84 h (Figure 3b). However, down-regulation of PARP protein occurred before significant numbers of NBT-positive cells appeared (Figure 3c). Thus down-regulation of PARP appeared to be a very early marker of neutrophilic differentiation in NB4 cells.

Up-regulation of PARP during monocyte/macrophage differentiation

NB4 cells require both PMA and 1,25-D_3 for complete maturation to monocyte/macrophages [29]. PARP levels increase dramatically in response to these differentiation inducers (Figure 4a). By day 3, NB4 cells apparently expressed 140 times as much PARP polypeptide as on day 0. We then used purified PARP standard to determine the linearity of the autoradiographic response ($r = 0.998$, $P < 0.001$). We diluted samples of day-1, -2 and -3 treated NB4 cells to obtain bands of similar density which fell within the linear portion of the standard curve. Using this method, we determined that on day 3 PARP levels in NB4 cells treated with PMA and 1,25-D_3 were increased 90-fold, rather than the apparent 140-fold increase suggested by Figure 4 (results not shown). The band intensity from these treated NB4 cells corresponds to 2.2×10^8 PARP molecules per cell (equivalent to 1 PARP molecule for every 10 base-pairs of DNA), compared with 2.3×10^6 PARP molecules found in untreated cells. In contrast, HL-60 cells, which also differentiate into monocyte/macrophages after the combination treatment, showed an initial decrease in PARP polypeptide on day 1 and a return to basal levels by day 2 (Figure 4b).

When NB4 and HL-60 cells were treated with PMA or 1,25-D_3 alone, a different pattern of modulation was observed. As described previously [29], PMA caused the NB4 cells to adhere, but they did not display other features of monocyte/macrophage differentiation (results not shown). The level of PARP increased modestly (2–3 times) in response to PMA compared with control, and was maintained at this level over the 3-day incubation period (Figure 5). Complete monocyte/macrophage differentiation in response to PMA alone is well documented in HL-60 cells [26]. Similarly to the response seen in NB4 cells, the PARP level in HL-60 cells was elevated 3–5-fold after PMA treatment (Figure 5). In response to 1,25-D_3, NB4 cells show a decreased rate of proliferation, but do not show any other signs of monocyte/macrophage differentiation [29]. In contrast, 1,25-D_3 caused HL-60 cells to develop into differentiated monocytes within 48 h of exposure [32]. PARP levels initially decreased in NB4 cells in response to 1,25-D_3 (day 1), and then returned to basal levels by

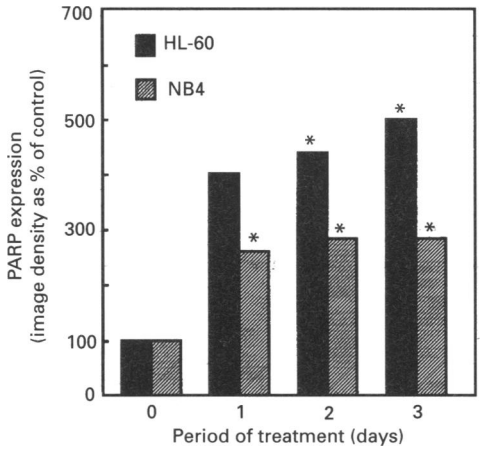

Figure 5 Expression of PARP in HL-60 and NB4 cells treated with PMA

HL-60 cells were treated with 7 nM PMA, and NB4 cells with 0.4 μM PMA. PARP protein was quantified by densitometric scanning of autoradiograms and is expressed as a percentage of the image density of control cells: * indicated significant difference compared with control ($P < 0.05$, $n = 3$).

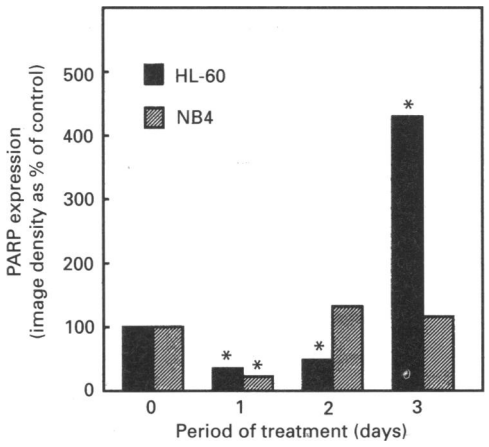

Figure 6 Expression of PARP in HL-60 and NB4 cells treated with 1,25-D_3

HL-60 cells were treated with 0.1 μM 1,25-D_3, and NB4 cells with 0.4 μM 1,25-D_3. PARP protein was quantified by densitometric scanning of autoradiograms and is expressed as a percentage of the image density of control cells: * indicated significant difference compared with control ($P < 0.05$, $n = 3$).

day 2 (Figure 6). HL-60 cells, however, showed a response distinct from that of NB4 cells. PARP levels initially declined to about 25% of control (day 0) and then substantially increased (4–5-fold increase over basal levels) by day 3. Thus NB4 and HL-60 cells clearly show differential regulation of PARP polypeptide in response to the same extracellular signals.

Regulation of PARP enzyme activity parallels polypeptide levels during neutrophilic and monocyte/macrophage differentiation in NB4 cells

Due to the importance of poly(ADP-ribosyl)ation reactions catalysed by PARP during cellular processes, it was essential to determine the potential activity of the enzyme during differentiation of NB4 cells. It has been previously demonstrated that PARP activity decreases during neutrophilic differentiation of HL-60 cells [33]. NB4 cells were treated with agents which induced maximal differentiation in both the neutrophil and monocyte/macrophage pathways. Figure 7(a) shows a decrease in PARP activity in NB4 cells in response to ATRA, whereas cells treated with vehicle alone were unaffected. By 48 h there was minimal incorporation of radioactive NAD^+, which was unaffected by the PARP enzyme inhibitor isoquinolinediol (results not shown). This decrease in PARP activity reflects similar changes in polypeptide levels observed in Western-blot analysis (Figure 2a). When NB4 cells were treated with TPA and 1,25-D_3 to induce monocyte/macrophage differentiation, there was a substantial increase in PARP activity (Figure 7b). Once again, NB4 cells treated with vehicle alone displayed no change in PARP activity. The pattern of the increase in PARP activity seen in Figure 7(b) is reminiscent of the increases seen in PARP polypeptide levels (Figure 4a). Together, these data suggest that changes in total PARP activity during differentiation of NB4 cells in both neutrophilic and monocyte/macrophage pathways are similar to the changes observed in total PARP protein levels. We hypothesize that the mechanism by which NB4 cells modulate PARP activity and necessary ADP-ribosylation reactions during induction of differentiation and maturation is directly related to changes in PARP polypeptide expression.

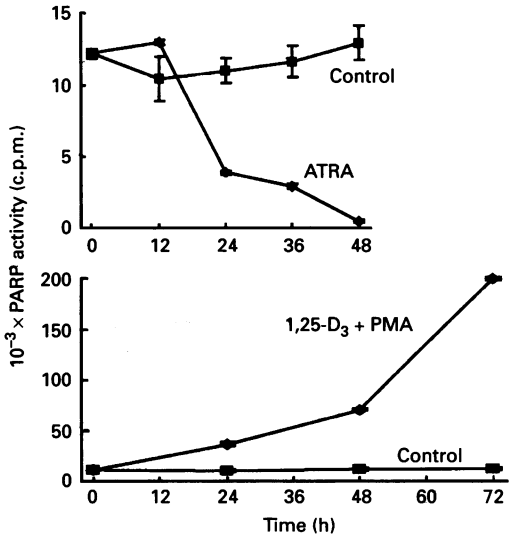

Figure 7 PARP activity during neutrophilic and monocyte/macrophage differentiation of NB4 cells

PARP activity in permeabilized cells was measured as a function of time exposed to inducers of differentiation. (**a**) NB4 cells were treated with ATRA to induce neutrophilic differentiation or treated with vehicle alone. (**b**) NB4 cells were treated with 0.4 μM PMA and 0.4 μM 1,25-D_3 in combination to induce monocyte/macrophage differentiation or treated with vehicle alone. See the Materials and methods section for details of assay.

DISCUSSION

This study reports that neutrophils are the first nucleated mammalian cell type that has been shown to contain no immunodetectable PARP. During neutrophilic differentiation of NB4 cells, PARP polypeptide and enzymic activity decline dramatically, whereas PARP levels are unchanged in differentiating HL-

60 cells. Thus ATRA-treated NB4 cells, which lack PARP, appear more like normal human neutrophils than do ATRA-treated HL-60 cells, which maintain PARP expression. In addition, as further characterized, the down-regulation of PARP in NB4 cells precedes the appearance of features of neutrophil differentiation. If PARP down-regulation in NB4 cells were to play a causative role in neutrophilic differentiation, this would be a necessary event.

It appears that the mechanism modulating PARP activity during neutrophil maturation is distinct in these two differentiation models. In HL-60 cells decreased polymerase activity is achieved through changes in the specific activity of PARP and increases in the enzyme's abortive NAD^+ glycohydrolase activity [34]. In NB4 cells and normal human neutrophils, decreased PARP activity is achieved by decreasing the amount of enzyme. A decrease in PARP enzyme could be achieved by increasing the degradation rate of mature polypeptide or through regulation at the levels of translation or transcription. The latter hypothesis would be consistent with results seen in cultured tumours, where measurements of PARP gene expression and amount of protein indicate that lower enzyme activity is achieved by decreases in PARP mRNA and consequently total polypeptide [35]. Experiments to test these possibilities are currently under way in our laboratory. During neutrophilic differentiation of HL-60 cells, down-regulation of PARP activity and PARP mRNA levels have been reported [23,13]. In addition, others have reported that PARP activity is down-regulated during differentiation of Friend leukaemic cells and in the erythroid differentiation of K562 human leukaemic cells [15,19].

We also investigated the temporal relationship between PARP protein expression and the differentiation programme of NB4 cells in the neutrophilic pathway. During neutrophil differentiation, both considerable chromatin restructuring and gross changes in the patterns of gene expression occur [36,37]. Changes in PARP levels could be pivotal in assisting this process. The absence of PARP in mature neutrophils may also be important to ensure that no attempt is made to repair DNA damage that results from oxidant stress during neutrophil activation. This would avoid NAD^+ depletion during neutrophil activation and allow the cell to function maximally throughout its short life span. It has been shown that normal circulating neutrophils undergo apoptosis [38], and the absence or decreased enzymic activity of PARP could also play a role in this process. Specific cleavage of PARP polypeptide has been shown to occur during induced apoptosis in leukaemic cells [39], thereby disrupting PARP protein and physical association with chromatin. However, decreases in PARP activity by substrate depletion or use of PARP inhibitors did not affect apoptosis in U937 cells [40]. This suggests either that PARP is important in the apoptotic process and degradation is a by-product of the process, or that it is PARP protein itself rather than its enzyme activity that plays a role during cell death. The NB4-cell model of neutrophilic differentiation should provide an excellent model to study the role of PARP in apoptosis.

We have shown that PARP is up-regulated in response to 1,25-D_3 and PMA in Acute Promyelocytic Leukemic cells induced to differentiate into monocyte/macrophages. The large increase in PARP expression coincided with acquisition of a mature cell phenotype. PMA alone, however, caused only a small increase in PARP expression and only one feature of monocyte/macrophage differentiation (adherence). 1,25-D_3 alone actually produced a decrease in PARP expression initially, with no subsequent differentiation response. Thus, only when PARP levels and activity were substantially up-regulated was complete monocyte/macrophage differentiation of NB4 cells achieved, although the functional importance of this dramatic increase in copy number of PARP molecules remains to be elucidated. It also remains to be determined whether these PARP molecules are associated with DNA and/or nuclear matrix, and whether they are active *in vivo*.

In HL-60 cells the correlation between PARP expression and monocyte/macrophage differentiation is not as clear. Either PMA or 1,25-D_3 alone can produce monocyte/macrophage differentiation [27,28]. PMA produced a steady increase in PARP protein level over the 3-day treatment period. Combinations of PMA and 1,25-D_3, or 1,25-D_3 alone on the other hand, initially caused a decrease in PARP protein levels, and by day 3 levels had come back to baseline (PMA and 1,25-D_3) or had risen to 4–5-fold above basal levels (1,25-D_3 alone). Changes in transcription rates, mRNA stability or protein turnover could account for the changes seen in PARP protein levels, but whether these changes are critical in the differentiation programme is unclear.

What role does PARP play during progression in the monocyte/macrophage lineage? DNA strand breaks certainly accompany differentiation along this pathway, but there is no evidence that PARP expression is regulated by DNA damage [22,23]. It is possible that the changes in DNA architecture or gene expression that accompany cellular differentiation require high levels of PARP protein, or that high levels of PARP ensure rapid DNA repair when damage to the monocyte/macrophage genome occurs as a consequence of carrying out mature cell function. Other studies have shown that activation of macrophages and lymphocytes modulates PARP transcription [41,42]. Menegazzi et al. [43] have shown that phorbol esters increase PARP mRNA stability in normal mature monocytes, and Berton et al. [44] have shown that activation of monocytes is accompanied by an increase in PARP activity.

Since changes in PARP activity reflected protein levels, it can be suggested that NB4 cells modulate ADP-ribosylation reactions during differentiation by altering enzyme levels. However, this does not prove that the relationship between PARP protein level and activity is linear in either the neutrophilic or monocyte/macrophage pathway. It is thus conceivable that some pools of PARP may be regulated differently in terms of their specific contribution to total ADP-ribosylation reactions within the nucleus. Also, the nature of the assay *in vitro* for PARP activity does not indicate whether this regulation truly reflects the ADP-ribosylation activity *in vivo* of PARP in its nascent environment.

Differences in supercoiling, DNA strand breaks and response to PARP inhibitors have been well documented in the differentiation of monocyte/macrophage and neutrophilic lineages. It has been shown that inhibitors of PARP enhance the differentiation response in the neutrophil pathway, whereas the response seems to be blocked in the monocyte/macrophage pathway [23]. Patterns of supercoiling and DNA condensation have also been shown to be quite different between the monocytic and neutrophilic lineages, suggesting that structural changes in DNa may be involved in neutrophil–monocyte switching [22], which may require modulation of PARP activity or quantity. Experiments are currently under way to determine the mechanism by which PARP is regulated in our model systems, and whether PARP modulation is coincidental or plays an instrumental role in the differentiation programme.

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REFERENCES

1 Ebisuzaki, K., Casley, W. H., Griffiths, A. and Wheaton, L. (1991) Cancer Res. **51**, 1668–1673
2 Francis, G. E., Gray, D. A., Berney, J. J., Wing, M. A., Guimaraes, J. E. T. and Hoffbrand, A. V. (1983) Blood **62**, 1055–1062
3 Sastry, S. S. and Kun, E. (1988) J. Biol. Chem. **263**, 1505–1512
4 Ferro, A. M. and Olivera, M.(1984) J. Biol. Chem. **259**, 547–554
5 Simbulan, C. M., Suzuki, M., Izuta, S., Sakura, T., Savoysky, E., Kojima, K., Miyahara, K., Shizuta, Y. and Yoshida, S. (1993) J. Biol. Chem. **268**, 93–99
6 Molinette, M. W., Vermeulen, W., Burkle, A., Menissier-de Murcia, J., Kupper, J. H., Hoeijmakers, J. H. J. and de Murcia, G. (1993) EMBO J. **12**, 2109–2117
7 Lautier, D., Lagueux, J., Thibodeau, J., Menard, L. and Poirier, G. (1993) Mol. Cell. Biochem. **122**, 171–193
8 Mazen, A., Mennissier-de Murcia, J., Molinette, M., Simonin, F., Gradwohl, G., Poirier, G. and de Murcia, G. (1989) Nucleic Acids Res. **17**, 4689–4698
9 Menissier-de Murcia, J., Molinette, M., Gradwohl, G., Simonin, F. and de Murcia, G. (1989) J. Mol. Biol. **210**, 229–233
10 Gradwohl, G., Menissier-de Murcia, J., Molinette, M., Simonin, F., Koken, M., Hoejmakers, J. H. J. and de Murcia, G. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 2990–2994
11 Mathis, G. and Athanus, F. R. (1990) Carcinogenesis **11**, 1237–1239
12 Tanuma, S. and Kanai, Y. (1982) J. Biol. Chem. **257**, 6565–6570
13 Suzuki, H., Uchida, K., Shima, H., Sato, T., Okamoto, T., Kimura, T. and Miwa, M. (1987) Biochem. Biophys. Res. Commun. **146**, 403–409
14 Lanotte, M., Martin-Thouvenin, V., Najman, S., Balerini, P., Valensi, F. and Berger, R. (1991) Blood **77**, 1080–1088
15 Tomoda, T., Kurashige, T., Yomamoto, H., Fujimoto, S. and Taniguchi, T. (1991) Biochim. Biophys. Acta **1088**, 359–364
16 Damji, N., Khoo, K. E., Booker, L. and Browman, G. P. (1986) Am. J. Hematol. **21**, 67–78
17 Scher, W. and Friend, C. (1978) Cancer Res. **38**, 841–849
18 Terada, M., Nudel, U., Fibach, E., Rifkind, R. A. and Marks, P. A. (1978) Cancer Res. **38**, 835–839
19 Morioka, K., Tanaka, K., Nokuo, T., Ischizawa, M. and Ono, T. (1979) Gann **70**, 37–46
20 Ohaski, Y., Ueda, K., Hayaishi, O., Ikai, K. and Niwa, O. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 7133–7136
21 Francis, G. E., Ho, A. D., Gray, D. A., Berney, J. J., Wing, M. A., Yaxley, J. J., Ma, D. D. F. and Hoffbrand, A. V. (1984) Leuk. Res. **8**, 407–415
22 Khan, A. and Francis, G. E. (1987) Blood **54**, 1114–1119
23 Bhatia, K., Pommier, Y., Giri, C., Fornace, A. J., Imaizumi, M., Breitman, T. R., Cherney, B. W. and Smulson, M. E. (1990) Carcinogenesis **11**, 123–129
24 Farzaneh, F., Zalin, R., Brill, D. and Shall, S. (1982) Nature (London) **310**, 362–366
25 Johnstone, A. P. and Williams, G. T. (1982) Nature (London) **310**, 368–370
26 Abraham, J. and Rovera, J. (1981) Handb. Exp. Pharmacol. **57**, 405–425
27 Collins, S. J., Ruscelli, F. W., Gallagher, R. E. and Gallo, R. C. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 2458–2467
28 Lotem, J. and Sachs, L. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 5158–5165
29 Bhatia, M., Kirkland, J. B. and Meckling-Gill, K. A. (1994) Leukemia **8**, 1744–1749
30 Yam, L. T., Li, C. Y. and Crosby, W. H. (1971) Am. J. Clin. Pathol. **55**, 283–294
31 Grube, K., Kupper, J. H. and Burkle, A. (1991) Anal. Biochem. **193**, 236–239
32 Koeffler, H. P., Amatruda, T., Ikekawa, N., Kobayashi, Y. and DeLuca, H. F. (1984) Cancer Res. **44**, 5624–5633
33 Kanai, M., Miwa, M., Kondo, T., Tanaka, Y., Nakayasu, M. and Sugimura, T. (1982) Biochem. Biophys. Res. Commun. **105**, 404–411
34 Kirsten, E., Bauer, P. I. and Kun, E. (1991) Exp. Cell Res. **194**, 1–8
35 Chabert, M. G., Kopp, P. C., Bischoff, P. L. and Mandel, P. (1993) Int. J. Cancer **53**, 837–842
36 Mollinedo, F., Gajate, C., Tugores, A., Flores, I. and Naranjo, J. R. (1993) Biochem. J. **294**, 137–144
37 Farzaneh, F., Meldrum, R. and Shall, S. (1987) Nucleic Acids Res. **15**, 3493–3502
38 Payne, C. M., Glasser, L., Tischler, M. E., Wyckoff, D., Cromey, D., Fielderlein, R. and Bohnert, O. (1994) Microsc. Res. Tech. **28**, 327–344
39 Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E. and Poirier, G. G. (1993) Cancer Res. **53**, 3976–3985
40 Nosseri, C., Coppola, S. and Ghibelli, L. (1994) Exp. Cell Res. **212**, 367–373
41 Taniguchi, T., Yamauchi, K., Yamamoto, T., Toyoshima, K., Harada, N., Tanaka, S., Takahashi, S., Yamamoto, H. and Fujimoto, S. (1988) FEBS Lett. **201**, 571–575
42 Menegazzi, M., Suzuki, H., Carcereri de Prati, A., Tommasi, M., Miwa, M., Gandini, G. and Gerosa, F. (1992) FEBS Lett. **297**, 59–62
43 Menegazzi, M., Carcereri de Prati, A., Miwa, M., Suzuki, H. and Libonati, M. (1992) Biochem. Int. **26**, 69–77
44 Berton, G., Sorio, C., Laudana, C., Menegazzi, M., Carcereri de Prati, A. and Suzuki, H. (1991) Biochim. Biophys. Acta **1091**, 101–109

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