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Journal of Cellular Biochemistry 114:551–557 (2013)

Journal of Cellular Biochemistry

Annexin A1 Mediates the Anti-Adhesive Effects of the Dexamethasone-Treated Promyelocytic Leukemic Cells

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ABSTRACT

Annexin A1 (AnxA1) is an important anti-inflammatory mediator during granulocytic differentiation in all trans-retinoic acid (ATRA) treated acute promyelocytic leukemic (APL) cells. Dexamethasone has been used successfully to prevent complications in ATRA-treated APL patients, although its mechanism of action is still not clear. In the present study, we have examined the effect of dexamethasone on the modulation of AnxA1 in ATRA-APL NB4 (ATRA-NB4) cells, ATRA-NB4 cells-derived microparticles (MPs) and its role during cell–cell interaction between ATRA-NB4 cells and endothelial cells. Our results have shown that dexamethasone can inhibit the percentage of ATRA-NB4 cells expressing surface AnxA1 and its receptor FPR2/ALX in a time-dependent manner based on flow cytometric analysis. However, dexamethasone treatment of ATRA-NB4 cells has no significant effect on the level of AnxA1 mRNA, the total cellular level of AnxA1 protein or the release of AnxA1 from these cells, as determined by RT-PCR, Western blotting, and ELISA, respectively. Further studies demonstrate that dexamethasone is able to significantly inhibit the adhesion of ATRA-NB4 cells to endothelial cells, and this anti-adhesive effect can be inhibited if the cells were pre-treated with a neutralizing antibody specific for AnxA1. Finally, dexamethasone also enhances the release of AnxA1-containing MPs from ATRA-NB4 cells which can in turn prevent the adhesion of the ATRA-NB4 cells to endothelial cells. We conclude that biologically active AnxA1 originating from dexamethasone-treated ATRA-APL cells and their MPs plays an anti-adhesive effect and this contributes to inhibit the adhesion of ATRA-APL cell to endothelial cells. J. Cell. Biochem. 114: 551–557, 2013.

KEY WORDS: ACUTE PROMYELOCYTIC LEUKEMIA; ALL-TRANS RETINOIC ACID; ANNEXIN A1; DEXAMETHASONE; DIFFERENTIATION SYNDROME; MICROPARTICLES

Mature neutrophils contain high levels of annexin A1 (AnxA1; 2–4% of total cytosolic proteins) [Perretti and Flower, 2004] and there is ample experimental evidence to suggest that endogenous AnxA1 is an important anti-inflammatory mediator during the resolution phase of an inflammation. These include the inhibition of neutrophil chemotaxis as well as their

Abbreviations used: AnxA1, annexin A1; AnxV, annexin V; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; ATRA-APL cells, all-trans retinoic acid-treated acute promyelocytic leukemic; ATRA-NB4 cells, all-trans retinoic acid-treated NB4 cells; ATRA-NB4-derived MPs, All-trans retinoic acid-treated NB4 cells-derived microparticles; (dexa + ATRA)-NB4 cells, (dexamethasone + all-trans retinoic acid)-treated NB4 cells; (dexa + ATRA)-NB4-derived MPs cells, (dexamethasone + all-trans retinoic acid)-treated NB4 cells-derived microparticles; DS, differentiation syndrome; MPs, microparticles.All authors declare that there is no conflict of interest.

Additional supporting information may be found in the online version of this article.

Grant sponsor: National Science Council, Taiwan; Grant number: NSC 98-2314-B-010-026-MY3.

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Manuscript Received: 24 July 2012; Manuscript Accepted: 6 September 2012

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 18 September 2012

DOI 10.1002/jcb.24394 • © 2012 Wiley Periodicals, Inc.

activation and migration [Perretti et al., 1996; Perretti and Flower, 2004; Chatterjee et al., 2005]. AnxA1 can be rapidly mobilized to the cell surface when neutrophils are activated or when they adhere to endothelial cells [Francis et al., 1992; Perretti et al., 2000; Hayhoe et al., 2006]. Once expressed on the plasma membrane, AnxA1 binds to its cognate receptor FPR2/ALX and exerts its anti-inflammatory effects [Perretti et al., 2000; Hayhoe et al., 2006]. AnxA1 can also act as a downstream mediator of corticosteroids which are commonly known to be able to regulate the expression and functioning activities of AnxA1 in innate and adaptive immune cells in order to mediate the resolution of inflammation [Perretti and D'Acquisto, 2009]. Previous studies have demonstrated that corticosteroids are able to ameliorate pulmonary inflammation; these findings have provided an important rationale for studying their use in the treatment of acute lung injury [Meduri et al., 1995], although their clinical value remains controversial [Bernard et al., 1987; Tang et al., 2009].

All-trans retinoic acid (ATRA) has been used successfully in the treatment of acute promyelocytic leukemia (APL) by inducing the APL cells to undergo granulocytic differentiation [Huang et al., 1988; Frankel et al., 1992; de la Serna et al., 2008]. However, this treatment is often complicated by the occurrence of differentiation syndrome (DS) in 6–31% of APL patients, which is characterized by the enhanced transmigration of ATRA-treated APL (ATRA-APL) cells into the alveolar spaces causing the manifestation of acute respiratory distress syndrome [Huang et al., 1988; Frankel et al., 1992; Luesink and Jansen, 2010]. Dexamethasone therapy has been used successfully to prevent the development of DS although its exact mechanism is still not clear [Frankel et al., 1992; Wiley and Firkin, 1995; Ninomiya et al., 2004]. ATRA is able to induce the pro-inflammatory properties of APL cells by enhancing the release of chemokines such as IL-8, MCP-1, and IL-1β and the expression of receptors for chemokines, such as CXCR2 and CCRs, which may thereafter contribute to the functional activation of ATRA-APL cells [Marchetti et al., 1996; Hsu et al., 1999; Zang et al., 2000; Ninomiya et al., 2004; Tsai et al., 2007, 2008; Luesink et al., 2009]. Previous studies have demonstrated that dexamethasone does not affect the induction of terminal differentiation of APL cells because it neither abrogates the induction of chemokine release nor inhibits the transmigratory activity of the differentiating ATRA-APL cells [de Ridder et al., 1999; Tsai et al., 2007, 2008; Luesink et al., 2009]. On the other hand, ATRA is able to induce the anti-inflammatory properties of APL cells in order to keep them under homeostatically inhibitory control. With respect to the above, we have reported recently that endogenous AnxA1 mediates the anti-inflammatory effect of ATRA-APL cells and contributes to the inhibition of recipient cells in terms of their transmigratory activity and adhesion to endothelial cells [Tsai et al., 2012b]. Moreover, AnxA1 would also be found on the surface of ATRA-APL cells-derived microparticles (MPs) which are known to have anti-inflammatory effects during cell–cell interactions between ATRA-APL cells and endothelial cells [Tsai et al., 2012b].

Based on the above findings, it is logical to examine the effect of dexamethasone on the modulation of AnxA1 with respect to ATRA-APL cells and their MPs, as well as the role of dexamethasone in the cell–cell interaction between ATRA-APL cells and endothelial cells.

MATERIALS AND METHODS

CELL CULTURES AND PREPARATION OF CONDITIONED MEDIUM (CM)

Human APL NB4 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (GIBCO, Grand Island, NY) at 37°C in a humidified incubator with 5% CO_2. Human umbilical vein endothelial cells (HUVEC) were cultured on 0.2% gelatin coated tissue culture plates using M199 endothelial growth medium supplemented with 20% fetal calf serum, 10 mg/ml heparin and 50 mg/ml ECGF as previously described [Secchiero et al., 2005]. To prepare CM from ATRA-NB4 cell cultures, NB4 cells(1×10^5 cells/ml) were cultured with ATRA (1 μM; Sigma, St. Louis, MO) for 3 days. The supernatants were harvested, centrifuged at 250*g* for 5 min to remove all cellular components, and then stored as aliquots at −20°C.

MICROPARTICLE PREPARATION AND FLOW-CYTOMETRIC ANALYSIS OF MPs

MPs were prepared as reported by Gasser et al. [2003]. The CM of ATRA-NB4 cell culture (ATRA 1 μM for 3 days) was collected and centrifuged at 1,500*g* for 5 min prior to further ultracentrifugation at 100,000*g* for 1 h (Optima™ L-100XP Ultracentrifuge; Beckman Coulter, Fullerton, CA). The MP pellet was washed once and re-suspended in PBS. For flow-cytometry, the MPs were stained with the following monoclonal antibodies:anti-AnxA1 (Ab33061; Abcam, Cambridge, UK); anti-AnxV or isotype control (R&D Systems, Minneapolis, USA) for 1 h at 4°C; a final staining with a rabbit anti-mouse IgG (Ab6717; Abcam, Cambridge, UK) was then conducted before analysis by FAC Scan. FITC-labeled annexin V was also added (NXPE; R&D Systems) to monitor the exposure of phosphatidylserine on the outer side of the MPs. The flow-cytometric analysis of the MP preparations showed the expected heterogonous populations, with sizes varying approximately between 0.1 and 2 μm as verified by control beads [2 μm each; ACBP-20-10; Becton Dickson; Dalli et al., 2008]. The number of MPs within the same gated area of MPs in the dot plots of flow cytometry was calculated.

FLOW CYTOMETRIC ANALYSIS OF THE ATRA-NB4 CELLS

Expression of various markers including AnxA1, FPR2/ALX, and AnxV on the NB4 cells were determined by flow-cytometry [Tsai et al., 2007]. Briefly, NB4 cells were pretreated with ATRA (1 μM) for 3 days and adjusted to a cell concentration of 1×10^6 cells/ml. The washed cells were sequentially incubated with rabbit polyclonal antibody and goat FITC-conjugated IgG (Abcam, Cambridge, MA) before analysis by FAC Scan.

ASSAY FOR AnxA1

The level of AnxA1 in the conditioning medium was determined by enzyme-linked immunosorbent assay (ELISA) as reported by Goulding et al. [1990].

ADHESION TEST BY COLORIMETRIC ASSAY

HUVECs were seeded into 24-well culture dishes at density of 5×10^4 cells/well and were incubated at 37°C with 5% CO_2 and 100% humidity until complete confluence was observed. NB4 cells were firstly treated with ATRA for 3 days before testing the effect of either dexamethasone or purified MPs [Tsai et al., 2012a]. The adhesion index is defined as the ratio of adhesive activity of the tested samples as compared to that of ATRA-NB4 cells incubated with culture medium (vehicle) alone.

QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

Total RNA was isolated as reported by Tsai et al. [2012a]. Total RNA was isolated using an RNeasy Plus mini kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions. Intact RNA was verified by 1% agarose gel electrophoresis and cDNA was prepared using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Ont., Canada) and random-hexamer priming. Gene expression was measured on LightCycler® 480 Real-Time PCR System (Roche Applied Science, Indianapolis, USA) and fluorescent primers obtained from LightCycler® 480 SYBR Green I Master (Roche). All genes were analyzed using the same PCR conditions, that is, 95°C for 10 min followed by 45 cycles of: 95°C for 15 s, 60°C for 1 min and 72°C for 10 s. The primer sequences for AnxA1were: forward primer, 5′AAGTTCTGGACCTG GAGTTGAAAG3′ and reverse primer, 5′TGTGGCGCACTTCACGAT3′ [Kovacic et al., 1991]. The primer sequences for the control gene, β-2 microglobulin were: forward primer, 5′GGCTATCCAGCGTACTCCA AAG3′ and reverse primer, 5′CACAGGCAGG CATACTCAT3′ [Gattoni-Celli et al., 1992]. The results were first normalized against the housekeeping gene β2-microglobulin and were expressed as a fold increase compared to the ATRA-untreated NB4 cells.

WESTERN BLOTTING, TOTAL CELLULAR FRACTION AND CYTOPLASMIC FRACTION

Cells for protein extraction were washed with phosphate buffered saline and the cell pellets stored at −80°C. Whole-cell protein lysates were prepared using a CelLytic™ M Cell Lysis Reagent (Sigma) according to the manufacturer's instructions, followed by sonication for 15 s and centrifugation at 12,000 rpm for 15 min. Protein concentrations were measured with a Coomassie (Bradford) Protein Assay Kit (Pierce, IL) using a Spectra Max M5/M5e multi-detection reader (Molecular Devices, CA). We also isolated cytoplasmic proteins using a Proteo JET™ Cytoplasmic and Nuclear Protein Extraction Kit (Fermentas) according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. Rabbit polyclonal antibodies against human AxnA1 (Abcam; Cambridge, MA) and monoclonal anti-GAPDH antibody (Sigma) were used for the Western blotting. The results were firstly normalized against GAPDH present in the same cells and are expressed as a fold increase relative to the ATRA-untreated control NB4 cells.

STATISTICAL ANALYSIS

The results were evaluated by ANOVA using SPSS software. A value of $P < 0.05$ was considered significant. All results are presented as mean ± SD.

RESULTS

DEXAMETHASONE INHIBITED THE EXPRESSION OF AnxA1 AND FPR2/ALX ON THE SURFACE OF ATRA-NB4 CELLS

Our previous study [Tsai et al., 2012a] demonstrated that AnxA1 was constitutively expressed on the surface of untreated NB4 cells and ATRA treatment significantly enhanced its expression on the surface of ATRA-NB4 cells. In this study, we had demonstrated that there was no significant change in the percentage of ATRA-NB4 cells expressing surface AnxA1 when ATRA-NB4 cells were treated with dexamethasone for a short period (2–6 h; Supplementary data). However, the percentage of ATRA-NB4 cells expressing surface AnxA1 and/or FPR2/ALX was significantly inhibited in a time-dependent manner when ATRA-NB4 cells were treated with dexamethasone for longer periods of time between 1 and 3 days (Fig. 1 and Table I). In order to study the effect of dexamethasone on the regulation of AnxA1 in ATRA-NB4 cells, we firstly determined their mRNA levels by RT-PCR. Figure 2A demonstrated that ATRA was able to up-regulate AnxA1 mRNA expression in NB4 cells after treatment with ATRA for 1 day ($P < 0.05$); however, the level of AnxA1 mRNA decreased thereafter when the cells were treated with ATRA for 3 days ($P < 0.05$). Furthermore, concurrent

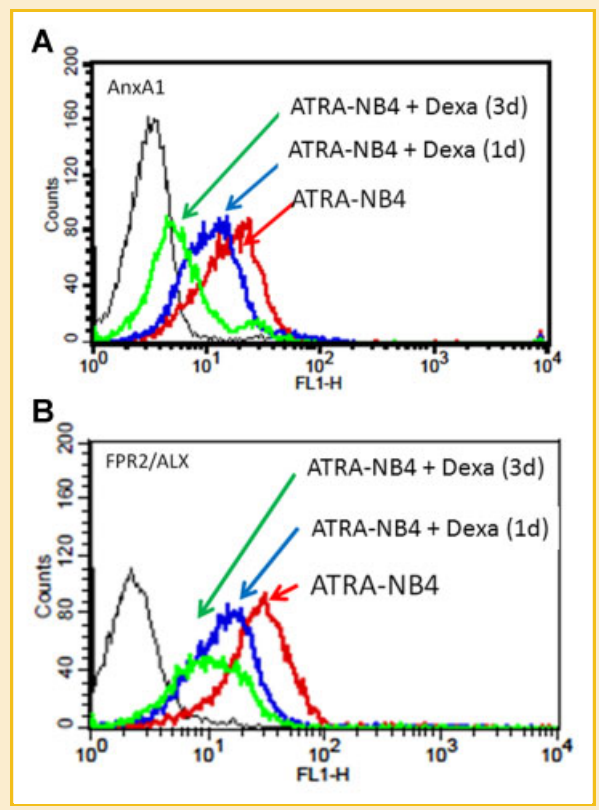

Fig. 1. Characterization of the expression of AnxA1 and its receptor (FPR2-ALX) in the ATRA-treated NB4 cells by flow cytometric analysis. NB4 cells were treated with ATRA (1 μM) for 3 days (ATRA-NB4) and dexamethasone (Dexa; 1 μM) for 1–3 days (ATRA-NB4 + Dexa) before flow cytometric analysis. This is a representative figure out of four experiments.

TABLE I. Effect of Dexamethasone on the Expression of Annexin A1 and Its Receptor (FPR2/ALX) in ATRA-NB4 Cells

Dexamethasone treatment	% of ATRA-NB4 cells expressing[a]	
	AnxA1	FPR2/ALX
0	39.9 ± 9.8	87.4 ± 5.9
1	16.8 ± 5.8	60.3 ± 10.5
3	10.5 ± 1.8	42.2 ± 9.5
P value[b]	0.05	0.05

[a]Mean ± SD of four experiments.
[b]By one-way ANOVA method.

dexamethasone treatment had no significant effect on the level of AnxA1 mRNA in these cells (Fig. 2A). We next performed Western blotting analysis to determine the level of total AnxA1 protein level in ATRA-NB4 cells. Figure 2B demonstrated that the level of AnxA1 protein was significantly elevated in ATRA-NB4 cells after treatment with ATRA for 1 day, but this level underwent no further change when the cells were treated with ATRA for a longer period of time. Similarly, concurrent dexamethasone treatment did not significantly change the level of total AnxA1 proteins in ATRA-NB4 cells.

DEXAMETHASONE ENHANCED THE RELEASE OF AnxA1(+) MPs FROM ATRA-NB4 CELLS

In order to study whether or not dexamethasone treatment is able to induce the release of AnxA1 byATRA-NB4 cells, we firstly harvested CM from ATRA-NB4 cells and then determined the level of AnxA1 protein by ELISA. The levels of AnxA1 underwent no significant change when ATRA-NB4 cells were treated with dexamethasone for 1–3 days (Supplementary data). We further purified MPs from the CM of ATRA-NB4 cells in order to determine the levels of surface expression of Annexin V and AnxA1 by flow cytometry. Figure 3A demonstrated that the amount of MPs in the CM harvested from ATRA-NB4 cells was higher than that for the same volume of CM harvested from the untreated NB4 cells; moreover, it was found that dexamethasone was able to significantly induce the ATRA-NB4 cells to release MPs into the CM ($P < 0.05$). Further investigations demonstrated that concurrent dexamethasone treatment was also able to significantly induce ATRA-NB4 cells to release AnxA1(+) MPs ($P < 0.05$; Fig. 3B).

DEXAMETHASONE INHIBITED THE ADHESION OF ATRA-NB4 CELLS TO ENDOTHELIAL CELLS

Recently, we have reported that AnxA1 originating from ATRA-NB4 cells and their MPs contributes to the anti-inflammatory properties of the cells by inhibiting cells adhesion to endothelial cells [Tsai et al., 2012]. In the present study, we further demonstrated that dexamethasone was able to significantly inhibit the adhesion of ATRA-NB4 cells to the HUVEC cells when the former cells were treated with dexamethasone for 1–3 days, and that this anti-adhesive effect could be abrogated by the use of an antibody specific for AnxA1 immediately before the adhesive assay (Fig. 4).

AnxA1 MEDIATED THE ANTI-ADHESIVE EFFECT OF ATRA-NB4-DERIVED MPs

Finally, we determined the role of ATRA-NB4-derived MPs on the adhesive activity of newly washed ATRA-NB4 cells. Figure 5 demonstrated that ATRA-NB4-derived MPs were able to significantly inhibit the adhesive activity of recipient ATRA-NB4 cells with

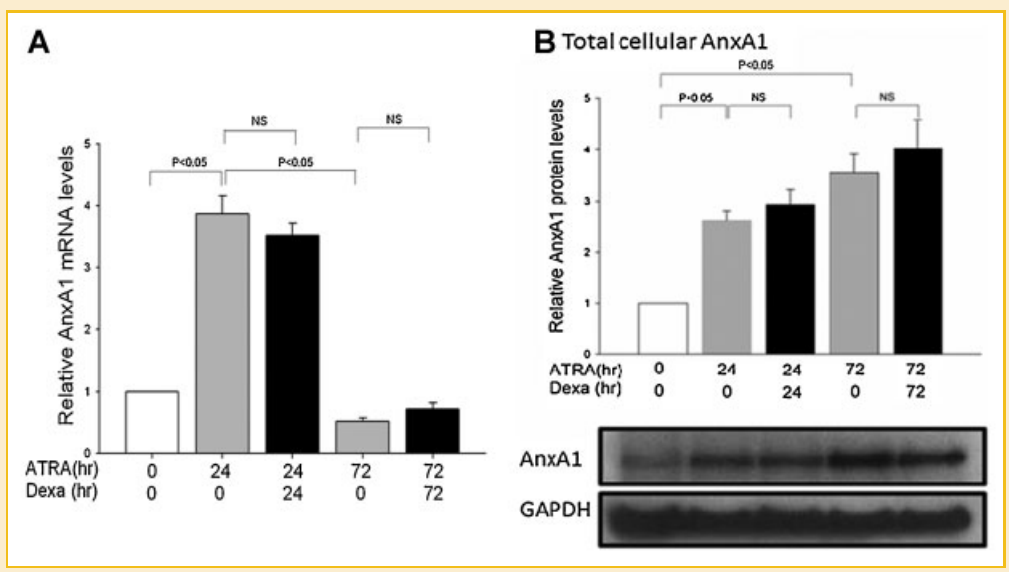

Fig. 2. The effect of dexamethasone on the expression of AnxA1 in the ATRA-NB4 cells as measured by real time-PCR and Western blotting. A: The levels of AnxA1 mRNA in dexamethasone (Dexa; 1 μM)-treated ATRA-NB4 cells were quantified by real-time PCR. The results were first normalized against the housekeeping gene β2-microglobulin from the same cells and subsequently expressed as a fold increase compared to ATRA-untreated NB4 cells. B: The levels of AnxA1 protein in dexamethasone-treated ATRA-NB4 cells (B) were determined by Western blotting assay. The results were firstly normalized against GAPDH from the same cells and subsequently expressed as a fold increase compared to ATRA-untreated NB4 cells. The results represent the mean ± SD from five independent experiments.

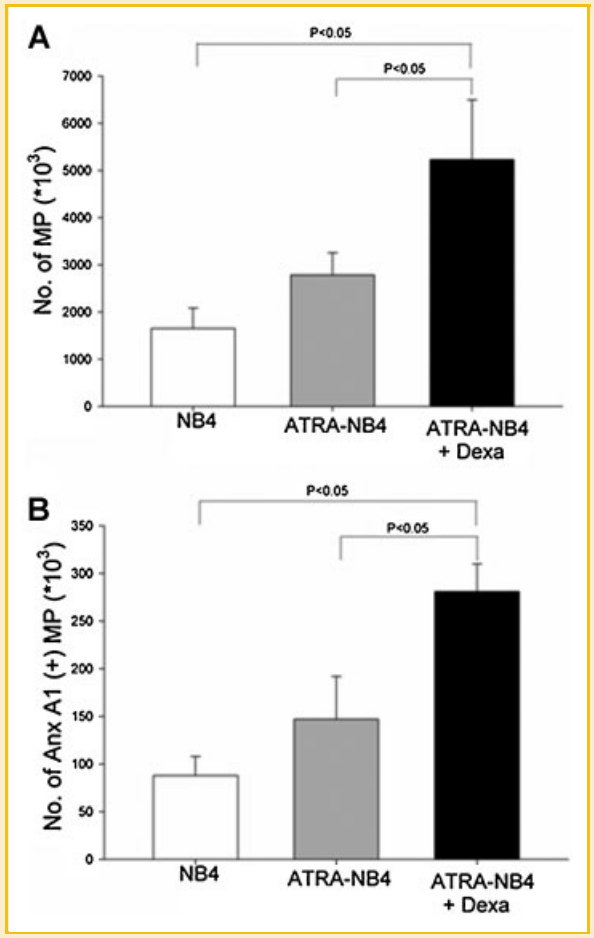

Fig. 3. The effect of dexamethasone treatment on the release of microparticles by ATRA-NB4 cells. NB4 cells (blank) were treated with ATRA (1 μM) in the absence (gray) and presence of dexamethasone (black; Dexa; 1 μM). A,B: The detection of MPs in the CM harvested from NB4 cell cultures were determined by flow cytometric analysis using antibodies specific for AnxV(A) and AnxA1(B). The total numbers of MPs (A) and AnxA1(+) MPs (B) in the same volume of CM harvested from cell cultures treated differently were calculated using a known concentration of flow count beads. This represents the mean ± SD from five independent experiments.

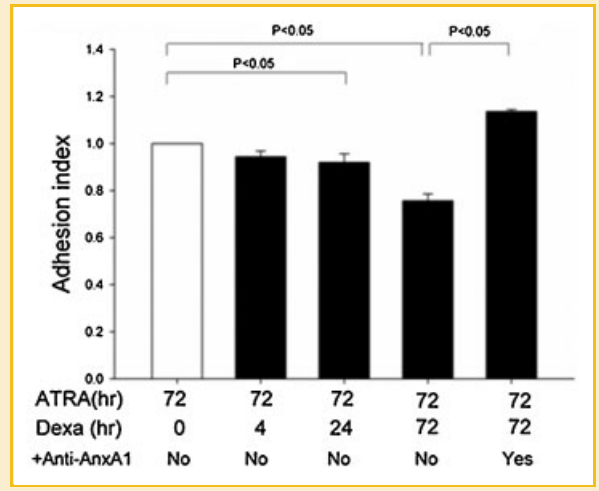

Fig. 4. The effect of dexamethasone treatment on the adhesion of ATRA-NB4 cells to endothelial cells. NB4 cells were previously cultured with ATRA (1 μM) for 3 days, in the absence or presence of dexamethasone (Dexa;1 μM for 4–72 h) and/or antibodies specific for AnxA1 (Anti-AnxA1; for 2 h) before the adhesion assay. A non-specific antibody has been used as control antibody, which had no effect on the adhesive activity of ATRA-NB4 cells. The results are the means ± SD from seven independent experiments.

respect to HUVEC ($P < 0.05$), as compared to vehicles alone. We further demonstrated that (Dexa + ATRA)-NB4-derived MPs had a significantly higher anti-adhesive activity. Moreover, the anti-adhesive effect of MPs, derived from either (Dexa + ATRA)-NB4 cells or ATRA-NB4 cells, was significantly inhibited when those MPs were pre-treated with a neutralizing antibody specific to the AnxA1 molecules.

DISCUSSION

Results of the present study have demonstrated that dexamethasone is able to inhibit the adhesion of ATRA-NB4 cells to endothelial cells and that AnxA1 played an important role in this cell–cell interaction.

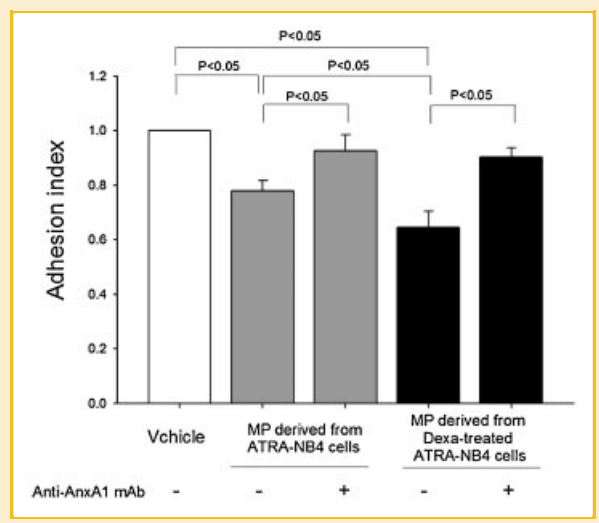

Fig. 5. The anti-adhesive effect of microparticles derived from dexamethasone-treated ATRA-NB4 cells. Microparticles (MPs) were harvested from NB4 cells which were treated with either ATRA (1 μM) in the absence (gray) or presence (black) of dexamethasone (Dexa; 1 μM). These MPs were further pre-treated with or without an antibody specific for AnxA1 immediately before incubation with newly washed ATRA-NB4 cells and then the adhesion assay was carried out. The adhesive activity of microparticles were compared with that of culture medium (vehicle). A non-specific antibody has been used as control antibody, which had no effect on the adhesive activity of ATRA-NB4 cells. The results are the means ± SD from five independent experiments.

When neutrophils are either chemotactically activated or following adhesion to endothelial cells during acute inflammation, cytoplasmic AnxA1 translocates onto the plasma membrane and interacts with its receptor FPR2/ALX to control the migration of the neutrophils into the inflammatory sites [Perretti et al., 1996, 2000; Hayhoe et al., 2006]. In addition to acting on mature neutrophils, AnxA1 also serves as an important anti-inflammatory mediator on immature myeloid cells during the process of granulocytic differentiation [Hsu et al., 1999; Tsai et al., 2012b]. In this study, we try to address the possible role of AnxA1 in dexamethasone-mediated cell–cell interaction between ATRA-NB4 cells and endothelial cells. Our results indicate that dexamethasone is able to inhibit the surface expression of AnxA1 in ATRA-NB4 cells (Fig. 1), but had no effect on the release of AnxA1 by the latter cells. At the same time, the surface expression of FPR2/ALX by ATRA-NB4 cells is also progressively inhibited. Recently, Maderna et al. [2010] have demonstrated unequivocally that there is a functional link between the internalization of surface AnxA1-FPR2/ALX complex and the clearance of apoptotic cells by macrophages. Along these lines, we postulate that dexamethasone is able to enhance the binding of surface AnxA1 to FPR2/ALX and thereby induce the internalization of the ligand complex, which may cause inhibition of ATRA-NB4 cell adhesion to endothelial cells. Our results tend to lend support to our hypothesis such that the anti-adhesive effect of dexamethasone on the ATRA-NB4 cells can be inhibited when the surface AnxA1 on the latter cells is neutralized with an anti-AnxA1 specific antibody. Taken together, we conclude that dexamethasone have a direct anti-inflammatory effect on ATRA-NB4 cells via the AnxA1-FPR2/ALX axis.

Our results demonstrate that dexamethasone is not able to upregulate the expression of AnxA1 mRNA or enhance the release of AnxA1 protein in ATRA-NB4 cells (Fig. 2), which is in different from previous studies. Carnuccio et al. [1981] has reported that glucocorticoid had a dual effect on macrophages by enhancing release of AnxA1 after short term treatment (up to 30–90 min), which was followed by the enhanced expression of AnxA1 mRNA and synthesis of new anxA1 protein after a longer duration of treatment (up to 18–24 h). On the contrary, glucocorticoid actually downregulates the AnxA1 mRNA expression and synthesis of new AnxA1 protein in T cells [D'Acquisto et al., 2008]. This indicates that dexamethasone has a differential effect on the disposition and synthesis of AnxA1 among the different cell types.

At the earliest stage of inflammation, activated neutrophils are able to release MPs, which serve as potent anti-inflammatory effectors that down-modulate the cellular activation in macrophages [Gasser and Schifferli, 2004]. Dalli et al. [2008] and Tsai et al. [2012b] have recently reported that AnxA1 can mediate the anti-inflammatory effects of MPs that are either derived from activated neutrophils or from differentiated myeloid cells. These AnxA1-containing MPs are able to inhibit the interaction between leukocytes and endothelial cells in vitro or in an animal model in vivo [Dalli et al., 2008; Tsai et al., 2012b]. This effect is able to potentially dampen neutrophils recruitment in the resolution phase of an inflammatory response. In this study, we have further demonstrated that dexamethasone is able to significantly induce ATRA-NB4 cells to release AnxA1(+) MPs which are also able to inhibit the adhesion of ATRA-NB4 cell to endothelial cells. The effect of dexamethasone on the release of MPs from neutrophils has been rarely studied. In contrast to our results, Pisetsky and Spencer [2011] reported that dexamethasone was able to inhibit the release of MPs by macrophages. The reason of this controversy is still not clear and needs further study. Taken together, our results indicate that dexamethasone is also able to exert the anti-adhesive effects on ATRA-NB4 cells indirectly via an induction of the release AnxA1(+) MPs from ATRA-NB4 cells.

Previous studies on the use of dexamethasone to prevent DS in APL patients undergoing ATRA treatment have no indication of the possible involvement of ATRA-APL cells specifically and studies by Tsai et al. [2007] and Luesink et al. [2009] have demonstrated that dexamethasone is not able to inhibit the induction of terminal granulocytic differentiation of ATRA-APL cells. Furthermore, dexamethasone is also unable to inhibit the production of pro-inflammatory cytokines or the transmigration activity of ATRA-APL cells. In the present study, we have clearly demonstrated that dexamethasone has a direct anti-adhesive effect on ATRA-NB4 cells via the AnxA1-FPR2/ALX axis. In addition, dexamethasone is also able to induce the ATRA-NB4 cells to release AnxA1(+)-MPs which results in an indirect anti-adhesive effect. These two effects are able to dampen the transmigratory activity of ATRA-APL cells. In parallel clinical studies have also demonstrated that prophylactic dexamethasone therapy is able to effectively prevent the development of DS during the early stage of ATRA treatment in APL patients [Frankel et al., 1992; Wiley and Firkin, 1995], suggesting that the anti-adhesive effect of dexamethasone may be responsible for the inhibition of the transmigration of ATRA-APL cells through the endothelial lining into the alveolar spaces. Furthermore, our previous study has also shown that dexamethasone is able to inhibit the release of chemokines such as IL-8 and MCP-1 by alveolar epithelial cells [Tsai et al., 2007, 2008], which may also contribute to the decreased chemotactic transmigration of ATRA-APL cells into alveolar spaces. Taken together, dexamethasone had anti-adhesive effect on the ATRA-APL cells, which may contribute to damper the transmigration of the latter cells into alveolar spaces. Therefore, it is worthwhile to conduct clinical studies on the application of AnxA1 in the APL patients during ATRA treatment.

ACKNOWLEDGMENTS

This study was conducted in the Clinical Research Core Laboratory of Taipei Veterans General Hospital. We are indebted to Dr. Chi-Kuan Ho and Dr. Ralph Kirby, Department of Life Sciences, National Yang-Ming University, for their help with the language editing.

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