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Induction of phagocytic activity of M1 cells by an inhibitor of vacuolar H^+-ATPase, bafilomycin A_1

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Abstract

Bafilomycin A_1, a selective inhibitor of vacuolar H^+-ATPase, time- and dose-dependently induced the differentiation of M1 cells, a murine myeloid leukemic cell line, into macrophage-like cells as revealed by the phagocytosis of polystyrene latex particles. This differentiation was inhibited not only by actinomycin D and cycloheximide but also by ST-638 (an inhibitor of tyrosine kinase). However, it was affected neither by K-252a (an inhibitor of C-kinase) nor by H-89 (an inhibitor of A-kinase), in contrast to the M1 cell differentiation induced by leukemia inhibitory factor (LIF). Okadaic acid inhibited both the bafilomycin A_1-induced and LIF-induced differentiation of M1 cells.

Key words: Bafilomycin A_1; Phagocytic activity; Macrophage; Leukemia inhibitory factor; Vacuolar H^+-ATPase

1. Introduction

Vacuolar H^+-ATPases, a class of proton pump found on the membranes of acidic vacuolar compartments of eukaryotic cells such as lysosomes, endosomes, the Golgi complex, and secretory granules, including synaptic vesicles, acidify the interior of these compartments [1]. They generate an electrochemical gradient of protons across vacuolar membranes, thus providing the driving force required to support the functions of endocytic and exocytic pathways, including the function of active transport [2]. However, it is not yet clear whether this vacuolar acidification regulates other cellular responses, such as cell proliferation and cell differentiation.

Recently, we reported that bafilomycin A_1, a potent selective inhibitor of the vacuolar H^+-ATPases isolated from *Streptomyces* spp. [3,4], induced the differentiation of a cloned pheochromocytoma cell line, PC12 cells, into sympathetic neuron-like cells [5]. To elucidate key reactions in cell differentiation, we investigated whether bafilomycin A_1 induced differentiation in types of cells other than PC12, basing our study on these findings.

In this study, we showed that bafilomycin A_1 also induced M1 cells, a murine myeloid leukemic cell line [6–8], to differentiate into macrophage-like phagocytic cells; we also characterized, and, in the discussion, explored possible cascade(s) of information transmission common in M1 cell differentiation (for phagocytic activity) and PC12 cell differentiation (for neurite outgrowth). This work was presented in abstract form at the forty-fifth Annual Meeting of the Japan Society for Cell Biology [1992, Cell Struct. Funct. 17, 499a].

2. Materials and methods

2.1. Materials

Bafilomycin A_1 was kindly provided by Prof. K. Altendorf (University of Osnabrück, Germany). Leukemia inhibitory factor (LIF, ESGRO) was obtained from Wako Pure Chemical Industries, Inc. (Tokyo). ST-638 was kindly provided by Kanegafuchi Chemical Industry Co., Ltd. (Takasago, Japan). Polystyrene latex particles (average diameter 0.905 μm) were obtained from Magsphere Inc. (MA, USA). The majority of the other reagents used were obtained from Sigma.

2.2. Cells and cell culture

M1 cells (clone T22-3), kindly provided by Prof. K. Nagata (Kyoto University, Japan) [9], were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (200 U/ml), streptomycin (100 μg/ml), and 10% heat-inactivated horse serum at 37°C under 5% CO_2-air.

2.3. Determination of phagocytic activity

Cells (2×10^5/ml), incubated in the presence or absence of either bafilomycin A_1 or LIF, were harvested, resuspended in serum-free medium containing 0.2% polystyrene latex particles, and incubated for another 4 h. After being washed three times with PBS(−), cells containing more than 5 latex particles were scored as phagocytic. More than 200 cells per well were counted; the percentage of phagocytic cells was expressed as percent phagocytosis [6,10]. All the data shown are the averaged results (mean ± S.D.) from duplicate experiments. Cell viability was determined by the eosine dye exclusion test.

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3. Results

3.1. Bafilomycin A_1 induced phagocytosis of M1 cells

When M1 cells were incubated in the presence of LIF, they acquired the phagocytic activity of differentiated phagocytic cells (macrophages), as shown in Fig. 1C. Similar phagocytic activity was induced when cells were incubated with bafilomycin A_1, as shown in Fig. 1B. The number of phagocytized latex particles per phagocytic cell was similar in bafilomycin A_1- and LIF-treated cells (Fig. 1).

The bafilomycin A_1-induced differentiation of M1 cells into phagocytic cells occurred time- and dose-dependently, as shown in Fig. 2. Induction of phagocytic activity required an 18 h incubation, the activity reaching a plateau in 24 h, when more than 50% of the cells were phagocytic.

3.2. Induction of phagocytic activity by bafilomycin A_1 required de novo synthesis of RNA and protein

Actinomycin D inhibits the LIF-induced differentiation of M1 cells into macrophage-like phagocytic cells, suggesting its requirement for the de novo synthesis of RNA and, probably, proteins [6]. As shown in Fig. 3, both Actinomycin D and cycloheximide inhibited the induction of phagocytic activity evoked not only by LIF but also by bafilomycin A_1, suggesting that such induction of phagocytic activity is an active process requiring the de novo synthesis of both RNA and protein.

3.3. Inhibitors of protein kinases and protein phosphatases affected the induction of phagocytic activity by bafilomycin A_1

Recent studies, in which various protein kinase and protein phosphatase inhibitors have been used, have suggested that cellular differentiation depends on information transmission cascade(s) involving protein phosphorylation [11–15]. The possible involvement of protein phosphorylation in the induction of phagocytic activity by bafilomycin A_1 was pursued in this study. As shown in Fig. 4, a protein tyrosine kinase inhibitor, ST-638, inhibited the induction of phagocytic activity evoked by both bafilomycin A_1 and LIF equally well. However, K-252a, a protein kinase C inhibitor, did not block the induction of phagocytic activity evoked by bafilomycin A_1, but completely blocked that evoked by LIF at 200 nM (Fig. 5A). Similarly, H-89, an inhibitor of protein kinase A, at concentrations (10 μM) of H-89 that strongly inhibited the effect of LIF, marginally affected the induction of phagocytic activity evoked by bafilomycin A_1 (Fig. 5B). Further, dibutyryl cyclic AMP did not induce phagocytic activity in M1 cells at all (data not shown). These results suggest the involvement of protein tyrosine kinase, but not A- or C-kinases, in the induction of phagocytic activity by bafilomycin A_1. Interestingly, okadaic acid, a protein serine/threonine phosphatase inhibitor, also inhibited the induction of phagocytic activity evoked by both LIF and bafilomycin A_1 (Fig. 6), suggesting either that the protein phosphorylation could be transient, or that a cyclic reaction of phosphorylation and dephosphorylation was required for the induction of phagocytic activity.

4. Discussion

The mechanism responsible for myelomonocytic differentiation has been studied with various inducers (e.g. LIF, interleukin-6) in myeloid leukemia cells of human (HL-60, K562) and mouse (M1) origin. Accumulating

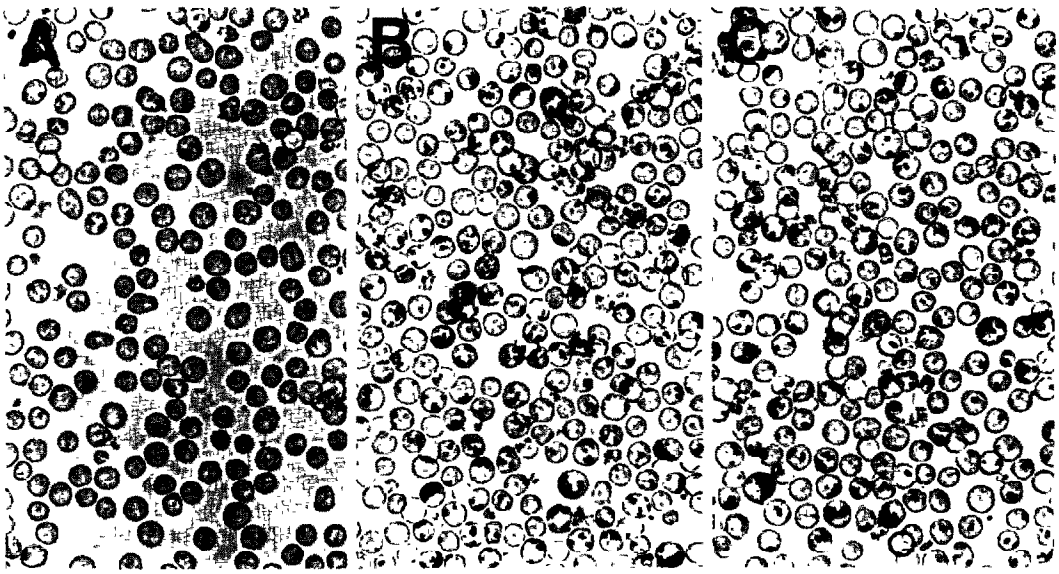

Fig. 1. Phagocytic activity of M1 cells induced by bafilomycin A_1 and LIF. (A) control; (B) bafilomycin A_1 (50 nM); (C) LIF (150 units/ml). Cells were treated for 48 h. ×225.

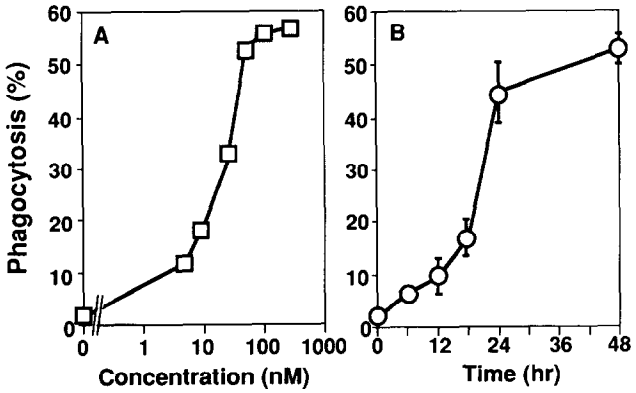

Fig. 2. Dose-response (A) and time-course (B) of bafilomycin A_1-induced phagocytic activity in M1 cells. (A) Cells were scored for phagocytic activity, as described in Section 2, after 48 h incubation with bafilomycin A_1. Phagocytosis of control cells (without drugs) was less than 3%. DMSO (solvent for bafilomycin A_1) did not induce phagocytosis, at a final concentration of 0.1% (v/v). (B) Cells were scored for phagocytic activity at the indicated times after the addition of bafilomycin A_1 (50 nM).

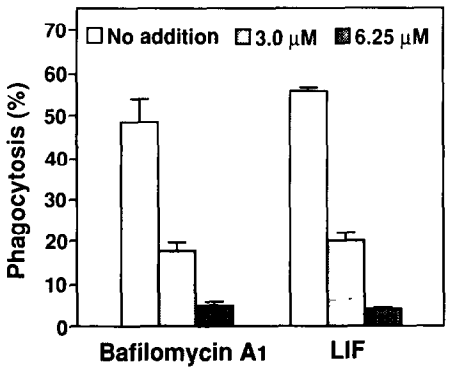

Fig. 4. Effects of ST-638 on the induction of phagocytic activity by bafilomycin A_1 and LIF. M1 cells were incubated for 48 h with bafilomycin A_1 (50 nM) or LIF (150 units/ml) in the presence or absence of the indicated concentrations of ST-638.

evidence suggests that protein kinase C is involved in the differentiation induced by these agents [16–21]. However, the precise requirements of intracellular signaling pathway(s) for the differentiation of myeloid into phagocytic cells have not yet been established.

In this study, we showed that: (i) bafilomycin A_1 time- and dose-dependently induced M1 cells to differentiate into macrophage-like phagocytic cells; (ii) this differentiation was an active process requiring the de novo synthesis of RNA and protein, (iii) the process was inhibited by an inhibitor of tyrosine kinase (ST-638), but not by an inhibitor of C-kinase (K-252a) or A-kinase (H-89), in contrast to the LIF-induced differentiation and (iv) the process was also inhibited by a protein phosphatase inhibitor (okadaic acid).

The small inhibitory effect of H-89 on bafilomycin A_1-induced phagocytic activity (Fig. 5B) might be explained in terms of its possible effect on protein tyrosine kinase, since, at high concentrations, H-89 also inhibits various other protein kinases besides C-kinase [22]. Therefore, the results as a whole suggest that K-252a-insensitive protein tyrosine kinase, but not A-, C-kinase, or K-252a-sensitive protein tyrosine kinase(s), participates in the induction of phagocytic activity evoked by bafilomycin A_1. On the other hand, okadaic acid would be expected to increase the level of protein phosphorylation by suppressing protein phosphatase activity [23]. Therefore, the inhibitory effect of okadaic acid suggests that M1 cell differentiation requires either transient proteins phosphorylation, or a continuous cycle of protein phosphorylation and dephosphorylation.

These effects are quite similar to those seen with bafilomycin A_1-induced neurite outgrowth in PC12 cells ([15] and manuscript in preparation), suggesting a common information transmission pathway in PC12 and M1 cells. The results also suggest the possible participation, with regard to the effect of LIF, of an unknown K-252a-sensitive protein tyrosine kinase similar to the Trk-tyrosine kinase that is required for nerve growth factor-

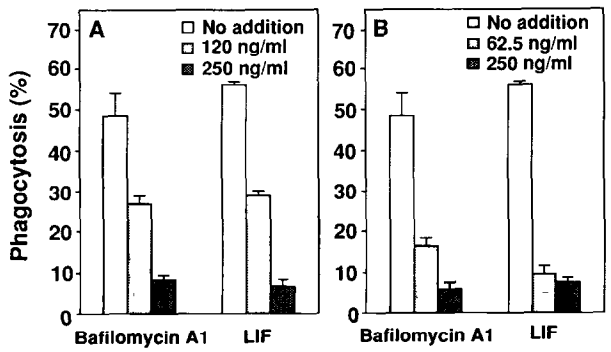

Fig. 3. Effects of actinomycin D (A) and cycloheximide (B) on the induction of phagocytic activity by bafilomycin A_1 and LIF. Cells were scored for phagocytic activity after 48 h incubation with bafilomycin A_1 (50 nM) or LIF (150 units/ml) in the presence or absence of the indicated concentrations of actinomycin D and cycloheximide.

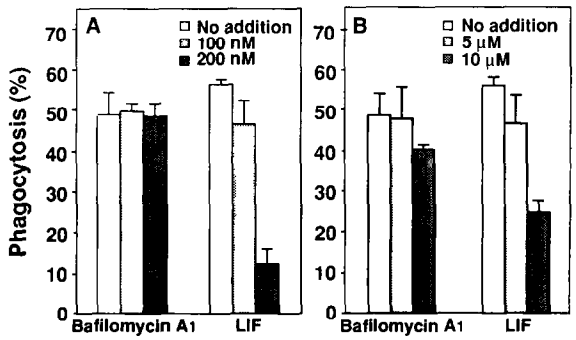

Fig. 5. Effects of K-252a (A) and H-89 (B) on the induction of phagocytic activity by bafilomycin A_1 and LIF. M1 cells were incubated for 48 h with bafilomycin A_1 (50 nM) or LIF (150 units/ml) in the presence or absence of the indicated concentrations of K-252a and H-89.

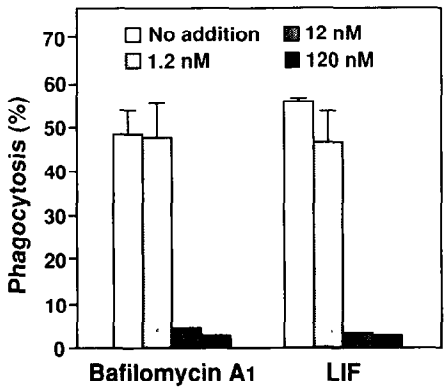

Fig. 6. Effects of okadaic acid on the induction of phagocytic activity by bafilomycin A_1 and LIF. M1 cells were incubated for 48 h with bafilomycin A_1 (50 nM) or LIF (150 units/ml) in the presence or absence of the indicated concentrations of okadaic acid.

induced neurite outgrowth. A similar conclusion has been reached for both LIF- and interleukin-6-induced myeloid leukemia differentiation, where the tyrosine phosphorylation of a 160 kDa protein has been reported as an essential step in mediating the early activation of MyD gene expression [13]. Gp-130, a probable signal transducing component of high affinity LIF receptors [24,25], may be a mediator of such a protein tyrosine kinase reaction.

Bafilomycin A_1 is a potent selective inhibitor of vacuolar H^+-ATPase [4]. The suppression of H^+-ATPase activity results in an increase in lysosomal pH [5,26], the degree of this increase being similar to that evoked with NH_4Cl, an agent that increases intra-vacuolar pH [27,28]. However, NH_4Cl, even at 20 mM, neither induced phagocytic activity nor inhibited the induction of phagocytic activity by bafilomycin A_1 (our unpublished observation). This result shows that the increase in lysosomal pH has nothing to do with the differentiation of M1 cells into phagocytic cells. This was also the case with the induction of neurite outgrowth in PC12 cells [5]. Furthermore, the results with PC12 cells (manuscript in preparation) indicate that cytoplasmic pH may also be unrelated to M1 cell differentiation. This raises a problem regarding the primary action site of bafilomycin A_1 in cell differentiation: Namely, a cellular component other than vacuolar H^+-ATPase might be a target of bafilomycin A_1, suggesting that this agent is *not* specific for vacuolar H^+-ATPase. In this case, protein tyrosine kinase (Src, MAP-kinase-kinase, and others) or related structures may be the target. Alternatively, it is conceivable that subunits of vacuolar H^+-ATPases have functions other than H^+-pumping, such as involvement in the regulation of the induction of cell differentiation.

Irrespective of its primary action site, bafilomycin A_1, although it does not induce differentiation in cells that require proliferation (e.g. the erythroid cell differentiation of Friend leukemia cells; unpublished observation), due to its growth inhibitory activity [29], should help us to elucidate the general cascade of information transmission in cell differentiation.

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