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Differential activation of MAP kinase family members triggered by CD99 engagement

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Abstract The molecular basis for the modulatory properties of CD99 is not well understood. Treatment of human Jurkat T lymphocytes with anti-CD99 antibody led to activation of three mitogen-activated protein kinase (MAPK) members, ERK, JNK, and p38 MAPK, along with homotypic aggregation. While phosphorylation of ERK and JNK was inhibited by the pretreatment of a PKC inhibitor, bisindolylmaleimide I, activation of p38 MAPK was upregulated by the same pretreatment. The signaling pathways to MAPKs by CD99 engagement were independent of PI-3 kinase, distinguishing from those by CD3 engagement. Among MAPKs, ERK pathway was essential for homotypic aggregation together with intracytoplasmic Ca^{2+}.

Key words: T lymphocyte; Cellular activation; Signal transduction; Cell surface molecule; Protein kinase/phosphatase

1. Introduction

The CD99 protein, a monomeric 32-kDa transmembrane glycoprotein, has been implicated in various cellular processes; homotypic aggregation, apoptosis [1,2], vesicular protein transport [3], and Th1 cell differentiation [4]. The ligand(s) for CD99 is not yet known, but ligation of CD99 with anti-CD99 monoclonal antibody (mAb) was shown to induce a prominent homotypic aggregation of $CD4^+CD8^+$ thymocytes [5] and cell lines such as IM9 B and Jurkat T cells [6]. So far, signaling pathways taken downstream of CD99 are poorly understood, although CD99 ligation was shown to elevate intracellular Ca^{2+} and enhance TCR-dependent cellular activation [4,7].

In this report, we investigated whether CD99 ligation activates mitogen-activated protein kinases (MAPKs) in Jurkat T cells, and which pathway regulates homotypic aggregation. MAPKs are divided into subfamilies based on specific conserved residues, particularly a TXY motif in the activation loop [8]. The well-characterized MAPK subfamilies are the extracellular signal-related kinase (ERK) subfamily, the c-jun N-terminal kinase (JNK) subfamily, and p38 MAPK subfamily. In general, MAPKs of different subfamilies are activated by distinct upstream regulators [9], but it seems to be a rule that redundant pathways are responsible for each MAPK activation in different situations. In the case of ERK, this MAPK is activated strongly by receptor tyrosine kinases or by T and B cell antigenic receptors which are associated with non-receptor tyrosine kinases [10,11]. However, ERK was shown to be activated also by other stimuli including protein kinase C (PKC) activation, increased intracytoplasmic Ca^{2+}, and heterotrimeric G proteins [12,13]. As well known, ERK is central to cell growth, supporting the transition from the quiescent state to cell cycle [14]. However, the activity of ERK is found not only in the nucleus but also in the cytoplasm, and it is thought to be responsible for various cell functions such as cell differentiation and cytoskeletal rearrangements [15,16]. In this study, we showed that CD99 triggered all three members of MAPK subfamily which were regulated differentially. We also showed that CD99 activated MAPKs independently of PI-3K and among them, ERK pathway was essential for CD99-induced homotypic aggregation along with intracytoplasmic Ca^{2+}.

2. Materials and methods

2.1. Cells and reagents

Jurkat or Jurkat TAg T cells were maintained at 37°C and 5% CO_2 in RPMI 1640 (Gibco, Grand Island, NY, USA), supplemented with 10% fetal calf serum (FCS) (HyClone, Logan, UT, USA), penicillin at 400 units/ml, and streptomycin at 150 μg/ml. For CD99 ligation, cells were washed with serum-free RPMI medium or PBS and stimulated with anti-CD99 (DN16) mAb at 10 μg/ml and rabbit or goat anti-mouse IgG at 10 μg/ml for crosslinking.

Antibodies to phospho-ERK, phospho-JNK, and phospho-p38 were purchased from New England Biolabs (Beverly, MA, USA) and antibodies against ERK, p38 MAPK and JNK were from Santa Cruz (Santa Cruz, CA, USA). The mAbs against CD99, DN16 and YG32, were obtained from DiNonA (Suwon, Korea). Bisindolylmaleimide I, chelerythrine chloride, SB203580, PD98059, herbimycin A and 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetra-(acetoxymethyl) ester (BAPTA-AM) were purchased from Calbiochem (La Jolla, CA, USA). Phorbol 12-myristate 13-acetate (PMA) and wortmannin were from Sigma (St. Louis, MO, USA).

2.2. Cell lysis and immunoblotting

Cells were washed twice in cold PBS and solubilized in lysis buffer (10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 2 mM EDTA, 1% Nonidet

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Abbreviations: MAPK, mitogen-activated protein kinase; PI-3K, phosphatidylinositol-3 kinase; JNK, c-jun N-terminal kinase; ERK, extracellular signal-regulated kinase; PKC, protein kinase C; MEK, MAPK kinase; BAPTA-AM, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetra(acetoxymethyl) ester; mAb, monoclonal antibody

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P-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, and 10 μg/ml leupeptin for 15 min on ice. The lysates were clarified by centrifugation at 16000×*g* for 15 min at 4°C, and the protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Immunoblotting of soluble cell lysates was performed. Briefly, proteins separated by SDS–polyacrylamide gels were transferred to PVDF membrane, which was blocked for 60 min in 5% non-fat milk. The membranes were incubated for 60–120 min in primary antibody, washed three times in TBST, incubated for 60 min in HRP-conjugated rabbit anti-mouse or goat anti-rabbit immunoglobulin, and then washed three times. Finally, a chemiluminescence detection system (Amersham, Arlington Heights, IL, USA) was used for visualization of relevant proteins. Individual blots were exposed for 30 s to 20 min to gain an optimal signal to noise ratio.

2.3. Cell aggregation assay

Jurkat cells which were growing exponentially in RPMI 1640/10% FCS were washed twice with RPMI 1640 without serum, adjusted to 10^6 cells/ml, and 100 μl (1×10^5 cells) were placed into wells of flat-bottom 96-well tissue-culture plates (Corning, Corning, NY, USA). Cells were stimulated with 1 μg/ml anti-CD99 mAb (DN16 or YG32). The primary antibodies were crosslinked by adding goat anti-mouse IgG (1 μg/ml). 100 μl of serum-free RPMI 1640 was added to the wells and the contents of the wells were mixed by gentle pipetting. Plates were incubated for 3–4 h at 37°C before photographing the wells or determination of the degree of aggregation. Semiquantitative scoring of the index of adhesion was carried out as described [6].

3. Results

3.1. Phosphorylation of three different MAPK family members, ERK, JNK, and p38 MAPK following CD99 crosslinking

To understand how engagement of CD99 triggers various kinds of modulatory effects, we first tested whether CD99 crosslinking in Jurkat T cells induces activation of members of the MAPK family; ERK, JNK, and p38 MAPK. Cell lysates were prepared from Jurkat T cells stimulated with anti-CD99 (DN16) mAb and second crosslinking antibody for given durations. As an assay for MAPK activation, we measured the phosphorylation of MAPKs by immunoblotting

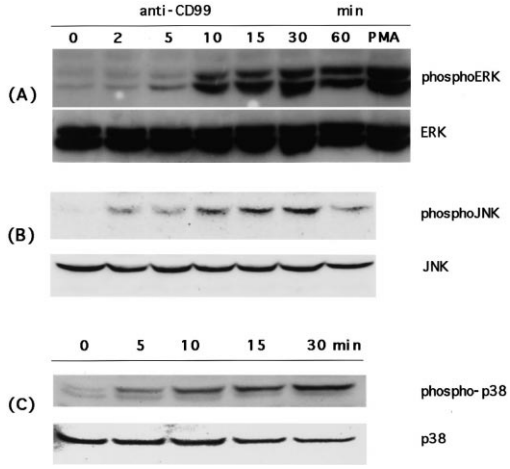

Fig. 1. Phosphorylation of MAPKs by CD99 ligation. Jurkat T cells were stimulated with anti-CD99 mAb or PMA. After indicated time, cell lysates were immunoblotted by anti-phospho-ERK and anti-ERK antibody (A); anti-phospho-JNK and anti-JNK (B); and anti-phospho-p38 MAPK and anti-p38 MAPK (C).

using antibodies against ERK and phospho-ERK; JNK and phospho-JNK; and p38 MAPK and phospho-p38 MAPK. Both phosphorylated ERK1 and ERK2 were detected between 5 and 15 min after anti-CD99 stimulation (Fig. 1A). Phosphorylation of JNK and p38 MAPK was also evident at 5 min (Fig. 1B,C). Maximal phosphorylation of ERK1, ERK2, JNK and p38 MAPK was reached between 10 and 30 min after CD99 engagement. ERK activation appeared to be much stronger than those of JNK and p38 MAPK.

3.2. CD99-induced activation of ERK and JNK, but not of p38 MAPK, requires PKC activity

Next we addressed if all three MAPKs were activated in a similar manner or differentially regulated. We utilized several

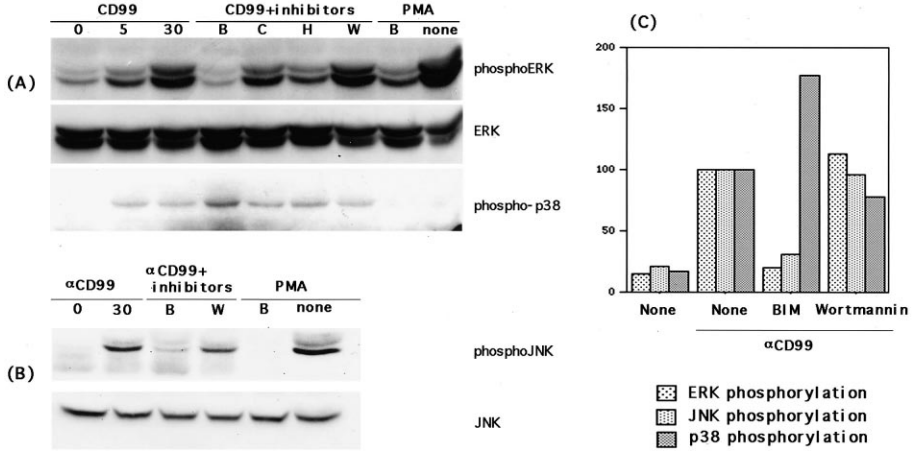

Fig. 2. Effect of chemical inhibitors on CD99-induced MAPK phosphorylation. A: Jurkat T cells were stimulated with anti-CD99 mAb 1 h after pretreatment of chemical inhibitors as shown (B, bisindolylmaleimide I 10 μM; C, chelerythrine chloride 4 μM; H, herbimycin A 2 μM; W, wortmannin 100 nM). Duration of stimulation with anti-CD99 mAb was given in minutes. All experiments with chemical inhibitors and PMA were done with stimulation for 30 min. As a positive control, Jurkat cells were stimulated with 50 nM PMA for 30 min with or without pretreatment of bisindolylmaleimide I (right two lanes). Cell lysates were immunoblotted by anti-phospho-ERK (upper panel), anti-ERK antibody (middle panel) and anti-phospho-p38 MAPK (lower panel). B: Lysates from Jurkat cells stimulated with anti-CD99 mAb in the absence or presence of given chemical inhibitors were immunoblotted by anti-phospho-JNK (upper panel), anti-JNK antibody (lower panel). C: The relative intensity of MAPK (ERK, JNK, and p38 MAPK) phosphorylation. The extent of MAPK phosphorylation obtained from anti-CD99 stimulation in the absence of any inhibitors was arbitrarily shown as 100. The relative intensity of the bands was determined by NIH image. For each analysis, the extent of MAPK phosphorylation was corrected by the intensity of total MAPK level.

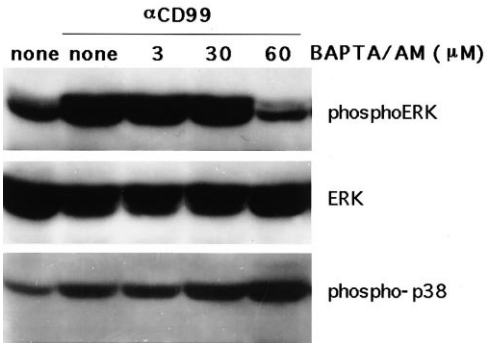

Fig. 3. Effect of BAPTA-AM on CD99-induced phosphorylation of ERK and p38 MAPK. Jurkat T cells were stimulated for 30 min with anti-CD99 mAb 1 h after pretreatment of BAPTA-AM at given final concentrations as indicated. Cell lysates were immunoblotted by anti-phospho-ERK (upper panel), anti-ERK antibody (middle panel) and anti-phospho-p38 MAPK (lower panel).

inhibitors to test whether they block CD99-mediated MAPK activation. The requirement of PKC and PI3-K for CD99-mediated activation of MAPKs was investigated by bisindolylmaleimide I, a specific inhibitor of PKC, and wortmannin, a PI3-K inhibitor, respectively. As shown in Fig. 2A, the pretreatment of Jurkat cells with bisindolylmaleimide I resulted in a substantial blockade of CD99-mediated activation of ERK. However, ERK phosphorylation was slightly inhibited by chelerythrine chloride or herbimycin and was not blocked by wortmannin. These results suggest that ERK activation is dependent on the PKC activity. JNK phosphorylation was also a similar case to ERK phosphorylation (Fig. 2B). JNK activity was greatly inhibited by bisindolylmaleimide I and only very slightly by wortmannin. Therefore, ERK and JNK appeared to be activated downstream of some forms of bisindolylmaleimide I-inhibitable PKC.

In contrast to ERK and JNK, p38 MAPK phosphorylation was not inhibited, but enhanced by the pretreatment of bisindolylmaleimide I (Fig. 2A), suggesting that p38 MAPK is regulated independently of ERK or JNK. The relative phosphorylation of MAPKs was summarized in Fig. 2C, by adjusting so that the level of MAPK phosphorylation is 100 when cells were stimulated with anti-CD99 mAb in the absence of any inhibitor. The differential regulation of of ERK and p38 MAPK was further revealed by the dependence on intracytoplasmic Ca^{2+}. Based on the report that CD99 engagement elevates intracytoplasmic Ca^{2+} [4], we tested whether activation of ERK and p38 MAPK was inhibited by a intracytoplasmic Ca^{2+} inhibitor, BAPTA-AM. As shown in Fig. 3, ERK phosphorylation was downregulated with the pretreatment of a high concentration (60 μM) of BAPTA-AM, but p38 phosphorylation was enhanced by the same treatment.

Therefore, we showed that ERK, JNK, and p38 MAPK were differentially regulated based on the difference in PKC and intracytoplasmic Ca^{2+} dependency. It appeared that phosphorylation of ERK and JNK was inversely correlated with that of p38 MAPK in some situations such as PKC or Ca^{2+} inhibition, although all MAPKs are activated by CD99 engagement.

3.3. CD3 engagement activates all three MAPK members, but in a different manner

Since CD99 was recently reported to be a costimulatory molecule which lowers the requirement of the extent of

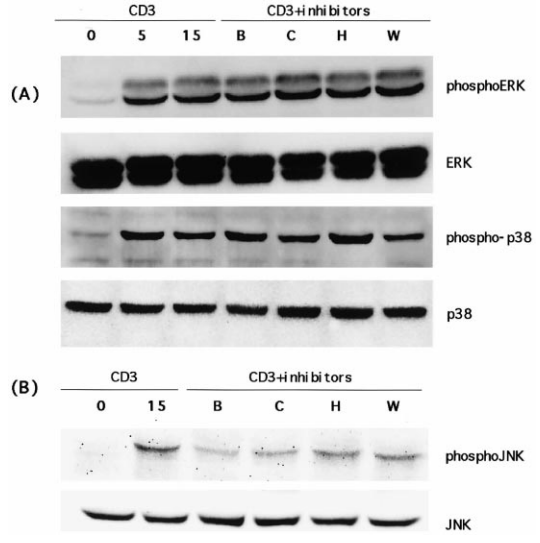

Fig. 4. Effect of chemical inhibitors on CD3-induced MAPK phosphorylation. Jurkat T cells were stimulated with OKT3 anti-CD3 mAb 1 h after pretreatment of chemical inhibitors as shown (B, bisindolylmaleimide I 10 μM; C, chelerythrine chloride 4 μM; H, herbimycin A 2 μM; W, wortmannin 100 nM). Duration of stimulation with anti-CD3 was given in minutes. All experiments with chemical inhibitors were done with stimulation for 15 min. Cell lysates were immunoblotted by antibodies as indicated.

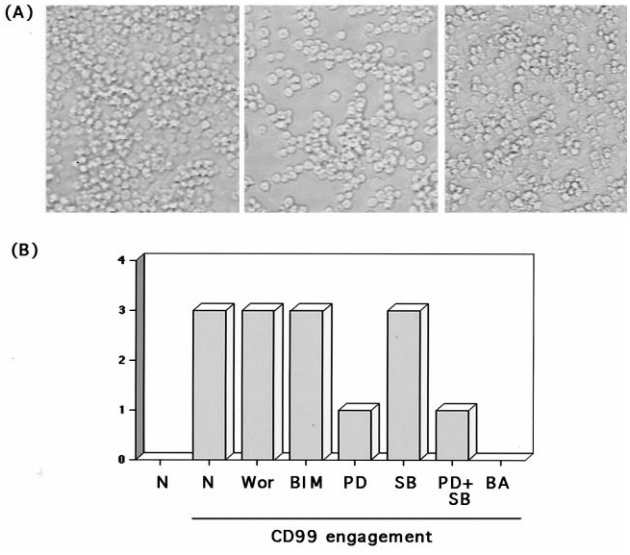

Fig. 5. Effect of chemical inhibitors on CD99-induced homotypic aggregation. Jurkat T cells were stimulated in the absence of serum with DN16 anti-CD99 mAb 1 h after pretreatment of various chemical inhibitors. Photography taken at 2 h after CD99 engagement or control treatment. A: left panel; no stimulation, middle panel; CD99 engagement, right panel; CD99 engagement after the pretreatment of PD98059. Aggregation score was measured according to the previously described criteria in B. CD99 engagement was performed 1 h after the pretreatment of given chemical inhibitors. (N, no treatment; Wor, wortmannin 100 nM; BIM, bisindolylmaleimide I 10 μM; PD, PD98059 50 μM; SB, SB203580 10 μM; PD+SB, combined treatment of PD98059 50 μM and SB203580 10 μM; BA, BAPTA-AM 30 μM).

TCR stimulation which is well known to activate MAPKs [7,17–20], we compared the signaling requirements for MAPK activation by CD99 ligation to those of CD3 engagement. As shown in Fig. 4, ligation of CD3 led to activation of all three MAPKs; ERK, JNK, and p38 MAPK. However, the signaling requirements for MAPKs were different from those of CD99 engagement. ERK phosphorylation was not inhibited by bisindolylmaleimide I or wortmannin, but JNK phosphorylation was inhibited by either bisindolylmaleimide I or wortmannin. These results indicate that the signaling pathway leading to the activation of ERK and JNK by CD3 is composed of at least two components; PKC-dependent and PI3-K-dependent ones. In the case of p38 MAPK, the activation of p38 MAPK by CD3 was not blocked by bisindolylmaleimide I, but slightly inhibited by wortmannin. With these results, we could interpret that CD3 and CD99 utilize different signaling pathways to activate ERK and JNK.

3.4. CD99-induced homotypic aggregation is dependent on MAPK kinase (MEK) and calcium, but not on PKC

To investigate the signaling pathway for CD99-induced homotypic aggregation, we tested if pharmacological signaling inhibitors blocked CD99-induced homotypic aggregation in Jurkat and Molt-4 T cells. Wortmannin for PI3-K, PD98059 for MEK-1, SB203580 for p38 MAPK, BAPTA-AM for intracellular Ca^{2+}, and bisindolylmaleimide I for PKC were used in the indicated concentrations. In both cell lines, the treatment with effective concentration of wortmannin or bisindolylmaleimide I did not inhibit homotypic aggregation at all and SB203580 inhibited homotypic aggregation only very slightly. In contrast, BAPTA-AM and PD98059 blocked homotypic aggregation significantly (Fig. 5). These data indicated that the signaling pathway leading to homotypic aggregation is largely dependent on MEK-1/ERK pathway and intracytoplasmic Ca^{2+}.

4. Discussion

The function of CD99 began to be elucidated recently and its role in pathogenesis in Hodgkin's disease was suggested [21]. It is likely that CD99 plays a role in the maintenance of cell shape and cell division. So far, the natural ligand for CD99 is unknown, but it appeared that CD99 has a certain function operating perpetually in in vitro cultured cells since its ablation by anti-sense CD99 construct produced a distinct phenotype as reported [21]. It could be speculated that the ligand for CD99 may be present in the usual culture condition; contained in serum, secreted by cells, or expressed on the cell surface. Fortunately, antibodies against CD99 act as a strong agonist for CD99 and stimulate intracellular signaling events. In this study, DN16 and YG32 anti-CD99 antibodies were used for elucidation of the signaling pathways activated by CD99 ligation. The effect was not clearly Fc receptor-mediated since isotype-matched control antibody did not activate the signaling pathways induced by anti-CD99 antibody.

In this study, we paid attention to homotypic aggregation along with activation of MAPKs. In lymphocytes, LFA-1, a heterodimer of integrins αL and β2, has a crucial role in intercellular adhesion and was shown to be also essential in CD99-induced homotypic aggregation [6]. Signalling pathway leading to LFA-1 activation or signaling from LFA-1 are well described [22,23]. Many signaling and cytoskeletal molecules are shown to interact with the cytoplasmic domains of LFA-1, which include ILK, FAK, α actinin, talin, RACK-1, and so on. It is thought that most of them are players of post-receptor events while some of them are involved in the induction of high avidity state of LFA-1. Cytohesin was implicated in the pathway for conversion of LFA-1 from low avidity to high avidity status and was shown to be downstream of phosphatidylinositol-3 kinase [24,25]. PI 3-kinase was implicated in integrin-mediated adhesion triggered by various kinds of stimuli [26]. On the other hand, PKC plays a crucial role in homotypic aggregation in some occasions. Phorbol esters were reported to cause formation of LFA-1/ICAM-1-dependent cell aggregation in some types of cells although it did not in Jurkat T cells in our laboratory [27]. It appeared that PI 3-kinase and PKC are responsible for affinity change of LFA-1 and post-receptor events, respectively. In this study, we showed that homotypic aggregation induced by CD99 was independent of both PI 3-kinase and PKC. Therefore, it appeared that CD99 induced homotypic aggregation in a signaling pathway which is different from the ways described above.

The homotypic aggregation triggered by CD99 ligation was shown to be dependent on MEK-1 and Ca^{2+}. We performed homotypic aggregation assays repeatedly and found that some sorts of serum factors supplemented the requirement for MEK since the homotypic aggregation was not inhibited by PD098059 in the presence of serum, but it was still blocked by the addition of BAPTA-AM, implicating the absolute requirement of Ca^{2+} (data not shown). Our data presented here were obtained in the absence of serum to exclude these complicating factors in serum. With respect to PKC, we also obtained some conflicting data in that the inhibition of PKC by bisindolylmaleimide I blocked ERK activation, but not homotypic aggregation. We think that the elucidation of the exact PKC isoforms which is activated by CD99 ligation is mandatory to resolve this dilemma. It is one of several possible explanations that the residual activity of ERK in the presence of bisindolylmaleimide I could be due to some fractions or subtype(s) of PKC which could not be inhibited by bisindolylmaleimide I and this residual ERK activity was sufficient to induce homotypic aggregation. It is also possible that some isoforms of PKC promote homotypic aggregation, but other isoforms inhibit aggregation and bisindolylmaleimide I operated in an overall direction so that it blocked PKCs which inhibited homotypic aggregation. The study exploiting the PKC isoform will be needed to understand the exact signaling pathway downstream of CD99.

In this report, we showed that three different MAPKs were activated in response to CD99 ligation. We do not know whether CD99 elicits all these MAPKs in physiologic situations or activates only some of them depending on environmental and cellular conditions. Although anti-CD99 antibody may give a very strong stimulus which is not achieved in the physiological conditions, it is thought to be significant to note that CD99 was capable of eliciting activation of these MAPKs. Among these, ERK appeared to be the principal MAPK and MEK-1 which is upstream of ERK was essential for homotypic aggregation along with Ca^{2+}.

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References

[1] Bernard, G., Breittmayer, J.P., de Matteis, M., Trampont, P., Hofman, P., Senik, A. and Bernard, A. (1997) J. Immunol. 158, 2543–2550.
[2] Sohn, H.W., Choi, E.Y., Kim, S.H., Lee, I.S., Chung, D.H., Sung, U.A., Hwang, D.H., Cho, S.S., Jun, B.H., Jang, J.J., Chi, J.G. and Park, S.H. (1998) Am. J. Pathol. 153, 1937–1945.
[3] Choi, E.Y., Park, W.S., Jung, K.C., Kim, S.H., Kim, Y.Y., Lee, W.J. and Park, S.H. (1998) J. Immunol. 161, 749–754.
[4] Waclavicek, M., Majdic, O., Stulnig, T., Berger, M., Sunder-Plassmann, R., Zlabinge, G.J., Baumruker, T., Stökl, J., Ebner, C., Knapp, W. and Pickl, W.F. (1998) J. Immunol. 161, 4671–4678.
[5] Bernard, G., Zoccola, D., Deckert, M., Breittmayer, J.-P., Aussel, C. and Bernard, A. (1995) J. Immunol. 154, 26–32.
[6] Hahn, J.H., Kim, M.K., Choi, E.Y., Kim, S.H., Sohn, H.W., Ham, D.I., Chung, D.H., Kim, T.J., Lee, W.J., Park, C.K., Ree, H.J. and Park, S.H. (1997) J. Immunol. 159, 2250–2258.
[7] Wingett, D., Forcier, K. and Nielson, C.P. (1999) Cell. Immunol. 193, 17–23.
[8] Ferrell, J.E. (1996) Curr. Top. Dev. Biol. 33, 1–30.
[9] Garrington, T.P. and Johnson, G.L. (1999) Curr. Opin. Cell. Biol. 11, 211.
[10] Tordai, A., Franklin, R.A., Patel, H., Gardner, A.M., Johnson, G.L. and Gelfand, E.W. (1994) J. Biol. Chem. 269, 7538–7543.
[11] Campbell, K.S. (1999) Curr. Opin. Immunol. 11, 256–264.
[12] Egea, J., Espinet, C. and Comella, J.X. (1999) J. Biol. Chem. 274, 75–85.
[13] Hawes, B.E., van Biesen, T., Koch, W.J., Luttrell, L.M. and Lef, R.J. (1995) J. Biol. Chem. 270, 17148–17153.
[14] Sun, H., Tonks, N.K. and Bar-Sagi, D. (1994) Science 266, 285–288.
[15] Alberola-Ila, J., Forbush, K.A., Seger, R., Krebs, E.G. and Perlmutter, R.M. (1995) Nature 373, 620–623.
[16] Reszka, A.A., Seger, R., Diltz, C.D., Krebs, E.G. and Fischer, E.H. (1995) Proc. Natl. Acad. Sci. USA 92, 8881–8885.
[17] Gupta, S., Weiss, A., Kumar, G., Wang, S. and Nel, A. (1994) J. Biol. Chem. 269, 17349–17357.
[18] Von Willebrand, M., Jascur, T., Bonnefoy-Berard, N., Yano, H., Altman, A., Matsuda, Y. and Mustelin, T. (1996) Eur. J. Biochem. 235, 828–835.
[19] Li, W., Whaley, C.D., Mondino, A. and Mueller, D.L. (1996) Science 271, 1272–1276.
[20] Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M. and Ben-Neriah, Y. (1994) Cell 77, 727–736.
[21] Kim, S.H., Choi, E.Y., Shin, Y.K., Kim, T.J., Chung, D.H., Chang, S.I., Kim, N.K. and Park, S.H. (1998) Blood 92, 4287–4295.
[22] Dedhar, S. and Hannigan, G.E. (1996) Curr. Opin. Cell Biol. 8, 657–669.
[23] Parsons, J.T. (1996) Curr. Opin. Cell Biol. 8, 146–152.
[24] Kolanus, W., Nagel, W., Schiller, B., Zeitlmann, L., Godar, S., Stockinger, H. and Seed, B. (1996) Cell 86, 233–242.
[25] Nagel, W., Zeitlmann, L., Schilcher, P., Geiger, C., Kolanus, J. and Kolanus, W. (1998) J. Biol. Chem. 273, 14853–14861.
[26] Shimizu, Y. and Hunt III, S.W. (1996) Immunol. Today 17, 565–573.
[27] Mobley, J.L., Ennis, E. and Shimizu, Y. (1996) J. Immunol. 156, 948–956.