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Role of mitogen-activated protein kinases in Thy-1-induced T-lymphocyte activation

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ABSTRACT

Thy-1 (CD90) crosslinking by monoclonal antibodies (mAb) in the context of costimulation causes the activation of mouse T-lymphocytes; however, the associated signal transduction processes have not been studied in detail. In this study we investigated the role of mitogen-activated protein kinases (MAPKs) in Thy-1-mediated T-lymphocyte activation using mAb-coated polystyrene microspheres to crosslink Thy-1 and costimulatory CD28 on murine T-lymphocytes. Concurrent Thy-1 and CD28 crosslinking induced DNA synthesis by T-lymphocytes, as well as interleukin (IL)-2 and IL-2 receptor (IL-2R) α chain (CD25) expression. Increased phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and c-Jun N-terminal protein kinase (JNK) was also observed. Pharmacologic inhibition of ERK1/2 or JNK activation inhibited Thy-1-induced DNA synthesis and IL-2 production by T-lymphocytes. p38 MAPK inhibition also decreased DNA synthesis in Thy-1-stimulated T-lymphocytes; however, IL-2 production was increased in these cells. Inhibition of JNK, but not ERK1/2 or p38 MAPK, caused a marked reduction in Thy-1-induced CD25 expression. In addition, inhibition of p38 MAPK or JNK, but not ERK1/2, impaired the growth of IL-2-dependent CTLL-2 T-lymphocytes but did not substantially affect CD25 expression. Finally, exogenous IL-2 reversed the inhibitory effect of ERK1/2 or JNK inhibition on Thy-1-stimulated DNA synthesis by T-lymphocytes but did not substantially reverse JNK inhibition of CD25 expression. Collectively, these results suggest that during Thy-1-induced T-lymphocyte activation, ERK1/2 and JNK promoted IL-2 production whereas p38 MAPK negatively regulated IL-2 expression. JNK signalling was also required for CD25 expression. IL-2R signalling involved both p38 MAPK and JNK in CTLL-2 cells, whereas p38 MAPK was most important for IL-2R signalling in primary T-lymphocytes. MAPKs are therefore essential signalling intermediates for the Thy-1-driven proliferation of mouse T-lymphocytes.

1. Introduction

Thy-1 (CD90) is a ~25 kDa glycophosphatidylinositol-anchored protein that is heavily expressed on the surface of thymocytes and peripheral T-lymphocytes in mice, and is involved in the activation of T-lymphocytes [1]. In the context of T-lymphocyte activation, both stimulatory and inhibitory roles have been proposed for Thy-1. For example, thymocytes from Thy-1-deficient mice are hyper-responsive to T cell receptor (TCR) stimulation [2], which suggests that Thy-1 might function as a negative regulator of TCR signalling. The majority of studies on Thy-1, however, support a mitogenic role for Thy-1 in T-lymphocyte activation. Thy-1 signalling in response to Thy-1 crosslinking by monoclonal antibody (mAb) induces cellular proliferation, interleukin (IL)-2 synthesis, and high affinity IL-2 receptor (IL-2R) expression by T-lymphocytes [3,4]. Importantly, anti-Thy-1 mAb-induced T-lymphocyte activation is dependent on costimulation provided by CD28 ligation [5]. Functional studies support the ability of Thy-1 to provide a TCR-like signal to T-lymphocytes. For example, like TCR signalling, Thy-1 stimulation triggers Fas/Fas ligand-mediated cytotoxicity [6]. In contrast, perforin-mediated cytotoxicity is induced by normal TCR stimulation but not by Thy-1 signalling or by low-dose antigen stimulation of the TCR [5,6]. Thy-1 signalling is therefore functionally analogous to a weak TCR-derived activating signal.

Mitogen-activated protein kinases (MAPKs), which include extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal protein kinase (JNK), and p38 MAPK, are serine/threonine kinases that are involved in TCR-driven T-lymphocyte activation [7]. However, there is little consensus in the literature with respect to the role of MAPKs in critical T-lymphocyte activation events, such as IL-2 production, IL-2R expression, and T-lymphocyte proliferation. The ERK1/2 MAPK pathway is generally believed to regulate the proliferative capacity of mammalian cells [8]. In human T-lymphocytes, ERK1/2 mediates enhanced IL-2 production caused by p38 MAPK inhibition [9] whereas pharmacologic inhibition of ERK1/2 results in impaired IL-2 production [10,11]. Decreased ERK1/2 activation in the elderly is also associated

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with impaired IL-2 expression in response to TCR stimulation [12]. Nevertheless, T-lymphocytes from ERK1-deficient mice maintain the ability to produce IL-2 after TCR stimulation [13]. Interestingly, ERK1/2 inhibition has opposing effects on CD25 (α chain of the high affinity IL-2R) expression by anti-CD3 mAb-activated Th1 and Th2 T-lymphocyte subsets [14]. Inhibition of ERK1/2 activity in mouse T-lymphocytes decreases their proliferative response to mitogenic anti-CD3 mAb [15], indicating the involvement of ERK1/2 in cell cycle progression. However, sustained ERK1/2 signalling in T-lymphocytes is also associated with inhibition of cyclin activity, resulting in a lack of T-lymphocyte responsiveness to IL-2 [16].

JNK and p38 MAPK are so-called stress-activated protein kinases that, depending on the circumstances, are involved in regulating both cell proliferation and apoptosis [17]. Pharmacologic blockade of p38 MAPK activity impairs IL-2 production by anti-CD3 and anti-CD28 mAb-stimulated T-lymphocytes [18,19], as well as IL-2 gene transcription in Jurkat T-lymphocytes activated with ionophore plus phorbol ester [11]. Consistent with this finding, T-lymphocyte proliferation in response to TCR/CD3 stimulation is markedly reduced following p38 MAPK inhibition [15,18,20]. However, enhanced IL-2 production by anti-CD3 and anti-CD28 mAb-stimulated human T-lymphocytes is also reported to occur when p38 MAPK is inhibited [9]. In contrast, another study suggests that p38 MAPK is not involved in IL-2 production since there is normal IL-2-dependent proliferation of $CD4^+$ T-lymphocytes from p38 MAPK-deficient mice [21]. IL-2 production is not dramatically altered in JNK1- or JNK2-deficient T-lymphocytes [22,23]; however, under certain circumstances activated T-lymphocytes from JNK1- or JNK2-deficient mice produce less IL-2 than wild-type controls [24,25]. Interestingly, JNK2 acts as a negative regulator of T-lymphocyte activation since JNK2-deficient $CD8^+$ T-lymphocytes are hyper-responsive to stimulation by anti-CD3 and anti-CD38 mAbs, and undergo more proliferation and produce more IL-2 than normal T-lymphocytes [26].

Little is known about the role of MAPKs in Thy-1-driven T-lymphocyte activation. Moreover, although Thy-1 is sometimes described as a TCR analog with respect to its signalling properties and immunological function, the role of MAPKs in TCR/CD3 signal transduction is in fact far from clear. In the present study, pharmacologic inhibition of ERK1/2, p38 MAPK, and JNK was used to delineate the role of these MAPKs in IL-2R expression, IL-2 production, and T-lymphocyte proliferation in response to stimulation with anti-Thy-1 and anti-CD28 mAbs co-immobilized on microspheres. We found that DNA synthesis by T-lymphocytes was dependent on ERK1/2, JNK, and p38 MAPK. ERK1/2 and JNK activity were also required for IL-2 expression, which was negatively regulated by p38 MAPK. In addition, JNK activation promoted CD25 expression whereas p38 MAPK was involved in IL-2R signalling.

2. Materials and methods

2.1. Mice

Female (6–8 week-old) C57BL/6 mice were purchased from Charles River Canada (Lasalle, Quebec, Canada). Mice were housed in the Carleton Animal Care Facility of Dalhousie University. Standard rodent chow and water were supplied ad libitum. Animal protocols were consistent with the Canadian Council on Animal Care guidelines and were approved by the Dalhousie University Committee on Laboratory Animals. Mice used in experiments were 8–12 weeks of age.

2.2. Reagents and antibodies

PD98059, SL327, SB203580, ML3403, L-form JNK Inhibitor I, and SP600125 were purchased from EMD Biosciences, Inc. (San Diego, CA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and brefeldin A were from Sigma-Aldrich (Oakville, Ontario, Canada). Recombinant murine IL-2 was purchased from Peprotech Inc. (Rocky Hill, NJ). Anti-Thy-1 mAb (clone G7), phycoerythrin (PE)-conjugated anti-CD25 mAb (clone 3C7), and fluorescein isothiocyanate (FITC)-conjugated anti-Thy-1.2 mAb (clone 30-H12) were purchased from BD Biosciences (San Jose, CA). Rat IgG_{2c}, anti-CD28 mAb (clone 37.51.1), PE-conjugated anti-TCRβ mAb (clone H57-597), FITC-conjugated anti-CD25 mAb (clone PC61.5.3), FITC-conjugated rat IgG_{2a}, FITC-conjugated rat IgG_{2b}, FITC-conjugated rat IgG_1, PE-conjugated hamster IgG, and Low-Tox rabbit complement were purchased from Cedarlane Laboratories (Burlington, Ontario, Canada). Rat IgG was from Jackson ImmunoResearch (West Grove, PA) while hamster IgG and anti-IL-2 mAb (clone JES6-1A12) were from eBioscience (San Diego, CA). Anti-phospho-JNK (Thr183/Tyr185) mAb (clone G9) was from Cell Signalling Technology (Danvers, MA). Anti-phospho-ERK1/2 mAb (clone E-4), anti-ERK1/2 mAb (clone K-23), anti-JNK mAb (clone F-3), anti-actin mAb (clone I-19), anti-CD25 mAb (clone M-19), anti-p38 MAPK mAb (clone C-20), horseradish peroxidase (HRP)-conjugated bovine anti-goat IgG, HRP-conjugated goat anti-rat IgG, HRP-conjugated donkey anti-rabbit IgG, and HRP-conjugated goat anti-mouse IgG antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-p38 MAPK (Thr180/Tyr182) mAb was from Biosource International (Camarillo, CA). Anti-asialo GM1 antiserum was purchased from Wako Chemicals USA (Richmond, VA).

2.3. T-lymphocyte isolation

Mice were sacrificed by cervical dislocation, spleens were removed under aseptic conditions, homogenized to yield a single-cell suspension, and erythrocytes were eliminated by osmotic shock. Highly purified T-lymphocytes (typically at least 95% $\alpha\beta TCR^+$ and 99% Thy-1.2^+ by flow cytometry) were isolated by passing the resulting spleen cell preparation through $CD3^+$ T-lymphocyte-enrichment immunocolumns (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions, followed by a two-step treatment with anti-asialo GM1 antibodies plus Low-Tox rabbit complement to remove contaminating NK cells. The resulting T-lymphocytes were then washed extensively with phosphate-buffered saline (PBS; pH 7.2) and resuspended in RPMI 1640 medium (Sigma-Aldrich) supplemented with 5% heat-inactivated (at 56 °C for 30 min) fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4) (all from Invitrogen Canada Inc., Burlington, Ontario, Canada) prior to use in experiments. T-lymphocytes prepared in this manner were at least 95% viable by trypan blue dye-exclusion test.

2.4. Cell lines

CTLL-2 cells were obtained from the American Tissue Culture Collection (Manassas, VA) and maintained at 37 °C in a 5% CO_2 humidified atmosphere in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 U/ml recombinant murine IL-2.

2.5. Cell viability assays

T-lymphocyte viability was assessed by trypan blue dye-exclusion test [27] and MTT assay [28].

2.6. Flow cytometry

T-lymphocytes were washed once with PBS and twice with immunofluorescence (IF) buffer (1% bovine serum albumin, 0.2% NaN_3, in PBS), then mixed with fluorochrome-conjugated antibodies or isotype-matched fluorochrome-conjugated control antibodies at a