A Novel Hematopoietic Adaptor Protein, Chat-H, Positively Regulates T Cell Receptor-mediated Interleukin-2 Production by Jurkat Cells*

Akira Sakakibara‡§, Seisuke Hattori‡**, Shun Nakamura‡, and Takuya Katagiri¶**

From the §Division of Biochemistry and Cellular Biology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo 187-8502, Japan, the ¥Department of Cell Biology, University of Virginia, Charlottesville, Virginia 22908, and the $Division of Cellular Proteomics, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Chat (Cas/HEF1-associated signal transducer) is a novel adaptor protein with an N-terminal Src homology-2 domain and C-terminal Cas/HEF1 association domain. We report here the molecular cloning of Chat-H, the hematopoietic isoform of Chat. Chat-H has an extended N-terminal domain besides the known Chat domain structures, suggesting a unique function of Chat-H in hematopoietic cells. Jurkat transfectants overexpressing Chat-H show a marked increase in interleukin-2 production after costimulation of T cell receptor and CD28. The degree of JNK activation is enhanced substantially in the Chat-H transfectants upon costimulation. The Src homology-2 domain mutant of Chat-H loses this signal modulating activity. Expression of the Cas/HEF1 association domain mutant exhibits a dominant negative effect on both JNK activation and interleukin-2 production. We further found that Chat-H forms a complex with Pyk2H and enhances its tyrosine 402 phosphorylation, an up-regulator of the JNK pathway. These results suggest that Chat-H positively controls T cell function via integrating the costimulatory signals.

The antigen-induced activation of T cell receptor (TCR)1 triggers a variety of T cell responses such as cytokine production and cell proliferation, differentiation, adhesion, and migration (1–3). It has been well characterized that nonreceptor type tyrosine kinases including Lck, Fyn, and Zap70 play pivotal roles in initiating the TCR signals (4, 5). Several adaptor proteins that comprise various signaling modules mediate the TCR-mediated induction of cell migration and activation (18, 19). Cas-L/HEF1 functions in TCR and 1-integrin costimulation-mediated induction of cell migration and activation (18, 19). HEF1 belongs to the Cas family of docking proteins consisting of an SH3 domain, substrate domain, and Src family kinase-binding domain (20, 21). The substrate domain includes numerous tyrosine phosphorylation motifs that serve as binding sites for CrkL when phosphorylated by Src family kinase(s) in TCR-mediated signaling (22). Binding of CrkL to HEF1 induces the recruitment of downstream effectors to the HEF1 sites. Two potent CrkL effectors in T cells, C3G and DOCK2, activate the small GTPases Rap1 and Rac, respectively (23–25). This activation further controls cell adhesion and gene expression (26, 27).

In our previous study, we identified Chat as a Cas/HEF1-associated adaptor protein (28). Chat consists of an N-terminal SH2 domain, a C-terminal Cas/HEF1 association domain, and a central serine/proline-rich region containing four potential ERK phosphorylation sites. Indeed, Chat is phosphorylated by
ERK when stimulated with epidermal growth factor or nerve growth factor. Overexpression of Chat induces up-regulation of JNK in COS7 cells. Another group has reported the cDNA cloning of NSP3, the human ortholog of Chat (29). Association of the NSP3 relative, NSP1, with tyrosine kinase receptors of the epidermal growth factor or insulin has been demonstrated (29). Chat also shares the most C-terminal sequence with SHEP1, which binds through its SH2 domain to a tyrosine-phosphorylated motif of Eph receptors (30). These data indicate that Chat family proteins are implicated in the signaling pathways of tyrosine kinases and MAPKs.

We have also described a 115-kDa Chat isoform, which is expressed primarily in hematopoietic cells and forms a complex with HEF1 (28). In this study, we report the molecular cloning of the hematopoietic Chat isoform, Chat-H. Jurkat transfectant cells overexpressing Chat-H show a marked up-regulation of IL-2 production when stimulated with a concurrent ligation of TCR and CD28. IL-2 synthesis is suppressed significantly in cells overexpressing the C-terminal deletion mutant of Chat-H. The activation of JNK in these transfectants is well synchronized with the IL-2 production. Chat-H also promotes the phosphorylation of its tyrosine 402, an intermediate of the JNK activation. Taken together, we propose a role for Chat-H as a positive regulator of TCR signaling in Jurkat cells.

EXPERIMENTAL PROCEDURES

Antibodies—Anti-Chat SH and anti-Chat CT polyclonal antibodies that recognize both Chat and Chat-H were raised as described in our previous work (28). Anti-human CD3 monoclonal antibody (OKT3) was obtained from the American Type Culture Collection, and anti-human CD28 (CD82.2) was obtained from Immunotech. Anti-Pyk2 (N-19) and anti-Pyk2 (C-19) were purchased from Santa Cruz. Anti-phosphotyrosine 402 Pyk2 was from BIOSOURCE. Anti-FLAG (M2) was purchased from Sigma. Anti-phosphotyrosine (4G10) was from Upstate Biotechnology Inc. Antibodies detecting the active form of ERK, JNK, or p38 MAPK were obtained from Promega Corp., and anti-ERK, anti-JNK, and anti-p38 MAPK were from Santa Cruz.

cDNA Cloning and Establishment of Stable Transfectants—Mouse Chat-H cDNA was isolated from a mouse spleen cDNA library (Stratagene) by hybridization screening using the mouse Chat cDNA (GenBank AB030442) probe, followed by nucleotide sequencing. N-terminally FLAG-tagged expression plasmids of Chat-H and its variants, Chat-H-ER238K (the SH2 domain arginine 238 was mutated to lysine), Chat-H-ΔMAPK (amino acid residues 324–456 including four MAPK phosphorylation sites were deleted), or Chat-H-ΔCT (C-terminal 81 amino acid residues essential for Cas/HEF1 association for Chat-H was deleted) were constructed as described (28); a schematic representation is depicted in Fig. 2A. The human acute T-cell leukemia Jurkat clone E6-1 was obtained from the American Type Culture Collection. Jurkat cells were transfected with the expression plasmid together with pcDNA3.1-Hygro by electroporation (17). The transfected were selected by 250 μg/ml hygromycin B (Wako Chemicals). The expressed protein level of each hygromycin-resistant clone was examined by immunoblot analysis against the FLAG tag. The expression levels of CD3 and CD28 were determined by flow cytometry as described previously (17). The clones expressing FLAG-tagged Chat-H mutants with amounts of CD3 and CD28 equivalent to those of parental Jurkat cells transfected with pcDNA3.1-Hygro vector alone were selected for the following analyses. Established clones were maintained in 250 μg/ml hygromycin B-containing medium.

Cell Stimulation and Preparation of Cell Lysates—Jurkat cells (5 × 10^6–10^7 cells) were starved for 4–6 h to become “resting” cells and then were treated on ice for 15 min with a saturating concentration (10 μg/ml) and/or CD28-2 mAb antibodies. Goat anti-mouse IgG (10 μg/ml, Cappel) was added to the cell suspensions to cross-link CD3 and/or CD28 on the cells. The cells were then immediately placed at 37 °C for the indicated time periods. After the desired time point, the cells were lysed in lysis buffer (1% Triton X-100, 25 mM Heps, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM Na$_3$VO$_4$, 10 mM β-glycerophosphate, 10 mM sodium pyrophosphate, 100 units/ml aprotinin, 10 μM leupeptin, 25 μM p-nitrophenyl p’-guanidinobenzoate, 1 mM phenylmethylsulfonyl fluoride, 10 mM iodoacetamide) for 30 min at 4 °C. For treatment with a MAPK inhibitor, Jurkat cells were incubated with 50 μM PD98059 or a control solvent (dimethyl sulfoxide) for 30 min at 37 °C, washed, and then stimulated with anti-CD3.

Immunoprecipitation and Immunoblotting—Immunoprecipitation was carried out by using protein G-Sepharose 4B (Amersham Biosciences) as described above (17). Alkaline phosphatase treatment of immunoprecipitates was described previously (31). For in vitro phosphorylation by active ERK, the immunoprecipitates were washed three times with ERK buffer (50 mM Heps, pH 7.5, 1 mM MgCl$_2$, 1 mM dithiothreitol) followed by resuspension in ERK buffer containing 100 units of recombinant active ERK (Calbiochem) and 50 μM ATP. After a 1-h incubation at 30 °C, samples were treated with or without alkaline phosphatase and eluted by SDS sample buffer. Immunoblotting was performed by the Renaissance enhanced chemiluminescence detection system (PerkinElmer Life Sciences) with a polyvinylidene difluoride membrane (Millipore) as described (17). Determination of ERK, JNK, and p38 MAP Kinase Activities—The activated state of MAPKs (ERK, JNK, and p38 MAPK) was determined by immunoblotting using polyclonal antibodies that recognize the dually phosphorylated, active form of ERK, JNK, or p38 MAPK. To normalize the amount of loaded samples, the blots were reprobed with anti-ERK, anti-JNK, or anti-p38 MAPK antibodies. JNK activity was also determined by a solid phase kinase assay using glutathione S-transferase (GST)-c-Jun$_{1–69}$ (Santa Cruz) and glutathione-Sepharose 4B (Amersham Biosciences) as described (17). IL-2 Assay—Jurkat transfectants (2 × 10^6 cells) were stimulated with either monoclonal antibodies OKT3 and CD28.2 cross-linked by goat anti-mouse IgG or with 10 nM phorbol 12-myristate 13-acetate (Sigma) and 1 μM calcium ionophore (A23187; Calbiochem), as described above (17). After a 24-h incubation, the concentration of IL-2 secreted in the culture supernatant was measured by using human IL-2 enzyme-linked immunosorbent assay kit (Endogen Inc.) according to the manufacturer's instructions.

RESULTS

Isolation of Hematopoietic Cell-specific Chat-H cDNA—Recently, we identified a novel adaptor protein, Cas/HEF1- associated signal transducer, Chat (28). Chat is expressed in a wide range of tissues with an apparent molecular mass of 78 kDa. Interestingly, a 115-kDa Chat isoform is detected in hematopoietic tissues by using anti-Chat antibodies. Because it is expressed exclusively in hematopoietic cells, we tentatively named this protein Chat-H, a hematopoietic cell-specific Chat. In this study, we isolated a cDNA encoding Chat-H from a mouse spleen cDNA library. Chat-H is encoded by an mRNA isoform that shares most of the 3′-sequence with Chat. The unique 5′-sequence implies differential initiation and splicing of the Chat-H transcript in hematopoietic cells. The Chat-H cDNA sequence is identical to that of SHEP1, reported as an activated Eph receptor-binding protein (30). Chat-H consists of 854 amino acid residues; the molecular mass is ~94 kDa (Fig. 1). However, when the Chat-H mRNA was expressed ectopically in 293T cells, the translated product showed an apparent molecular mass of around 115 kDa on SDS-PAGE as observed in hematopoietic tissues (data not shown). The N-terminal amino acid sequence specific for Chat-H (amino acid residues 1–166) does not show any significant similarity to other known protein sequences.

Establishment of Jurkat Clones Expressing Chat-H Variants—To investigate the role of Chat-H in T cell activation, we established Jurkat transfectants overexpressing Chat-H or its mutants (Fig. 2A). An SH2 domain mutant of Chat-H, Chat-H-Δct83, was engineered by replacing a conserved arginine with lysine. The loss of function was verified by its abrogated binding to tyrosine-phosphorylated EphA4 in 293T cell expression system (data not shown). In Chat-H-ΔMAPK, the central region of Chat-H consisting of four ERK phosphorylation consensus sites was deleted. Based on our previous observation, the deletion of the Chat C-terminal 83 amino acid residues is enough to abolish its Cas binding activity (28). Therefore, we constructed Chat-H-ΔCT, the analogous region was deleted. Impaired interaction between Chat-H-ΔCT and HEF1 was confirmed by a communoprecipitation assay (data not shown). The Jurkat clones expressing these Chat-H mutants were
The amino acid sequence of Chat-H deduced from the nucleotide sequence of mouse Chat-H cDNA is shown. The SH2 domain sequence is underlined. MAPK phosphorylation consensus sites are shown by double underlines. The region that corresponds to the Cas/HEF1 association domain is indicated on the left side of the sequence. The junction site between the N-terminal Chat-H-specific sequence and Chat/Chat-H common region is marked with an arrowhead.

FIG. 1. Primary structure of Chat-H. The amino acid sequence of Chat-H deduced from the nucleotide sequence of mouse Chat-H cDNA is shown. The SH2 domain sequence is underlined. MAPK phosphorylation consensus sites are shown by double underlines. The region that corresponds to the Cas/HEF1 association domain is indicated on the left side of the sequence. The junction site between the N-terminal Chat-H-specific sequence and Chat/Chat-H common region is marked with an arrowhead.

FIG. 2. Establishment of Jurkat transfectants overexpressing Chat-H variants. A, schematic representation of Chat-H and its mutants used for establishment of Jurkat transfectants. Full-length Chat-H (ChatH), the SH2 domain point mutant (R238K), deletion mutant of four ERK phosphorylation sites (ΔMAPK), and the C-terminal Cas/HEF1 association domain mutant (ΔCT) are shown. B, expression level of Chat-H variant in each transfectant analyzed by anti-Chat-H immunoblotting.

expression levels of both CD3 and CD28 to the control cells and established three to four independent clones expressing each Chat-H-related protein (data not shown).

TCR Engagement Induces ERK-dependent Phosphorylation of Chat-H—First, we examined whether Chat-H becomes tyrosine phosphorylated in response to TCR stimulation because Chat-H has some potential target motifs for tyrosine kinases. However, we did not detect phosphorylation of these tyrosine residues upon TCR engagement (data not shown). Instead, we found that Chat-H in TCR-stimulated Jurkat cells and mouse splenic T cells exhibits reduced mobility on SDS-PAGE (data not shown). To evaluate the effect of a MEK inhibitor on Chat-H phosphorylation, the cells were incubated with 50 μM PD98059 (PD) for 30 min before stimulation. B, Jurkat-Chat-H cells were incubated with (αCD3) or without (None) anti-CD3 for 3 min. For in vitro phosphorylation and dephosphorylation experiments, anti-Chat immunoprecipitates were treated with alkaline phosphatase (+AP), alkaline phosphatase with phosphatase inhibitors (+AP+PI), active ERK (+ERK), or active ERK and alkaline phosphatase (+ERK+AP). The mobility of each Chat-H band was detected by anti-Chat immunoblotting.

FIG. 3. ERK-mediated phosphorylation of Chat-H induced by TCR activation. A, Jurkat transfectants expressing Chat-H (JT-ChatH) or Chat-H-ΔMAPK (JT-ΔMAPK) were incubated with (+Stim.) or without (−Stim.) anti-CD3 for 3 min. Anti-Chat immunoprecipitates of each stimulated sample were immunoblotted with anti-Chat antibody. To evaluate the effect of a MEK inhibitor on Chat-H phosphorylation, the cells were incubated with 50 μM PD98059 (+PD) for 30 min before stimulation. B, JT-Chat-H cells were incubated with (αCD3) or without (None) anti-CD3 for 3 min. For in vitro phosphorylation and dephosphorylation experiments, anti-Chat immunoprecipitates were treated with alkaline phosphatase (+AP), alkaline phosphatase with phosphatase inhibitors (+AP+PI), active ERK (+ERK), or active ERK and alkaline phosphatase (+ERK+AP). The mobility of each Chat-H band was detected by anti-Chat immunoblotting.
FIG. 4. Overexpression of Chat-H promotes IL-2 synthesis after costimulation of TCR and CD28. Jurkat transfectants (2 × 10⁶ cells/well) expressing vector alone (JT-Vector), Chat-H (JT-ChatH), Chat-H-R238K (JT-R238K), Chat-H-D MAPK (JT-D MAPK), and Chat-H-ΔCT (JT-ΔCT) were incubated with (gray columns) or without (black columns) anti-CD3 and anti-CD28, or phorbol 12-myristate 13-acetate and A23187 (open columns) for 24 h. The concentration of IL-2 in the supernatants was measured by enzyme-linked immunosorbent assay. The values shown are mean values ± S.D. (n = 3).

FIG. 5. Chat-H is not involved in the activation of ERK and p38 MAPK downstream of TCR signaling. A, Jurkat transfectants expressing vector alone (JT-Vector) or Chat-H (JT-ChatH) were incubated with anti-CD3 and anti-CD28 for the indicated times. The whole cell lysates were immunoblotted with anti-active ERKs (phospho-ERK) or anti-active p38 MAPK (phospho-p38). B, Jurkat transfectants expressing vector alone (JT-Vector), Chat-H (JT-ChatH), Chat-H-R238K (JT-R238K), Chat-H-Δ MAPK (JT-Δ MAPK), and Chat-H-ΔCT (JT-ΔCT) were incubated with (+ Stim.) or without (− Stim.) anti-CD3 for 5 min followed by immunoblotting the cell lysates with anti-active ERKs (upper panel; Phospho-ERK). The expression level of ERKs in these cells is shown (lower panel; ERK1, ERK2), C, the same transfectants used in B were costimulated with (+ Stim.) or without (− Stim.) anti-CD3 and anti-CD28 for 5 min, then immunoblotted with anti-active p38 MAPK (upper panel; Phospho-p38). These transfectants express similar amounts of p38 MAPK (lower panel; p38 MAPK).

The JNK activity in JT-Chat-H cells was 5–8-fold higher at 10–40 min after stimulation than those in the Jurkat control (Fig. 6A). As shown in Fig. 6B, the Jurkat transfectants over-expressing Chat-H and Chat-H-Δ MAPK exhibited 3–7-fold higher levels of JNK activity over that of the control cells. In contrast, the JNK activation level of JT-ΔCT was reduced to 30–40% of the controls. The JT-R238K showed almost the control level of JNK activity. The degrees of JNK activation in different Jurkat transfectants agree well with their IL-2 production levels (Figs. 4 and 6B). This strongly suggests the possible augmentation of IL-2 production by Chat-H via up-regulation of the JNK activity.

Chat-H Forms a Complex with Pyk2H and Enhances Phosphorylation of Its Tyrosine 402—To reveal the mechanism underlying the JNK activation by Chat-H, we examined the effect of Chat-H expression on the tyrosine phosphorylation state of several TCR signal-related molecules. Vav, SLP-76, LAT, and protein kinase Cθ are tyrosine phosphorylated upon TCR stimulation and implicated in the activation of MAPKs (4–8, 11). However, we did not detect significant alterations in their phosphorylation levels between the control and Chat-H-over-expressing cells (data not shown). Tyrosine phosphorylation levels of Lck, ZAP-70, Fyn, phospholipase C-γ, and phosphatidylinositol 3-kinase also did not show any significant changes (data not shown).

Nonreceptor tyrosine kinase Pyk2, highly expressed in cells of the hematopoietic lineage and in the central nervous system, is proposed as a key regulator of the JNK signaling pathway in various cellular responses (32–34). We demonstrated recently that Pyk2H, an alternatively spliced isoform of Pyk2, is involved in JNK activation in T cells (17). We further found that the phosphorylation of Pyk2H tyrosine 402 is crucial for the

Chat-H Is Not Involved in the Activation of ERK and p38 MAPK—The MAPK family comprises three distinct kinases, ERK, JNK, and p38 MAPK. The activation of all three kinases is required for the full activation of T cells (11–15). Therefore, we studied the activation of MAPKs in the Jurkat transfectants upon costimulation of TCR and CD28 and examined the role of Chat-H in IL-2 production by Jurkat cells. We first compared the activation kinetics of ERK and p38 MAPK between Jurkat and Chat-H transfectants upon costimulation in Fig. 5A; the detection was carried out by the antibodies specific for their activated forms. The activation of ERK and p38 MAPK reached its maximum at 5 min and decreased to less than 50% at 20 min. Several independent experiments (data not shown) gave a consensus that there was virtually no difference in the activation kinetics between these transfectants. Next, we examined whether ERK and p38 activation is influenced by the presence of various Chat-H mutants. All of the transfectants were found to be similar in the TCR-induced ERK activation and costimulation-elicited p38 MAPK activation at 5 min (Fig. 5, B and C) and at 20 min (data not shown). Here we show ERK activation data from TCR-stimulated transfectants because TCR-stimulated cells (Fig. 5B) and TCR/CD28-costimulated cells (data not shown) gave essentially the same results. Thus, the activation of ERK and p38 MAPK downstream of the TCR is not affected by the function of Chat-H.

Chat-H Is Essential for the Activation of JNK upon Costimulation of TCR and CD28—We next addressed the activation of another MAPK, JNK, in these transfectants. After concurrent ligation of TCR and CD28, JNK activation estimated by activated JNK-specific antibody was promoted significantly in Chat-H-overexpressing cells compared with the control Jurkat cells (data not shown). To quantify the JNK activation level in various transfectants, we employed a solid phase JNK assay.

The phosphorylation of Chat-H tyrosine 402 is crucial for the
FIG. 6. Chat-H overexpression enhances JNK activation upon costimulation of TCR and CD28. A, Jurkat transfectants expressing vector alone (JT-Vector) and Chat-H (JT-ChatH) were stimulated with anti-CD3 and anti-CD28 for the indicated times, and the lysates were subjected to a solid phase JNK assay. The activity of endogenous JNK pulled down with GST-c-Jun was evaluated by an autoradiogram of 32P-labeled GST-c-Jun (Phospho-GST-c-Jun). B, Jurkat transfectants expressing vector alone (JT-Vector), Chat-H (JT-ChatH), Chat-H-R238K (JT-ChatH-R238K), Chat-H-3MAKP (JT-3MAKP), and Chat-H-ΔCT (JT-ΔCT) were incubated with (+Stim.) or without (−Stim.) anti-CD3 and anti-CD28 for 5 min followed by the JNK assay described above. Relative JNK activities are indicated below.

![Diagram](image-url)

DISCUSSION

Recently, we identified a novel adaptor protein, Chat, which forms a complex with Cas family docking proteins. We have also described a Chat isoform, Chat-H, abundantly expressed in hematopoietic cells. In the present study, we report the molecular cloning of the Chat-H cDNA. Chat-H is encoded by a mRNA isoform that shares most of the 3'-sequence with Chat. In other words, Chat-H has an extra N-terminal domain absent in Chat. Chat-H is associated with tyrosine-phosphorylated HEF1 in both splenic T cells and thymocytes (Ref. 28 and data not shown), suggesting a possible interplay between Chat-H and HEF1 in T cell development. By using various Jurkat transfectants overexpressing Chat-H or its mutants, we provide evidence that Chat-H is involved in the positive regulation of T cell signaling, which activates IL-2 gene expression: 1) Chat-H overexpression induces a marked enhancement of IL-2 production under costimulation with TCR and CD28; 2) the function of the Chat-H SH2 domain is essential for this up-regulation; and 3) Chat-H-ΔCT, the Cas/HEF1 association domain mutant, shows a dominant negative effect on the IL-2 production. Thus, Chat-H is likely to play a key role in coupling costimulatory signals to IL-2 gene expression collaborating with HEF1 and unknown target(s) of its SH2 domain.

The activation of ERK, JNK, and p38 MAPK is required for adequate induction of IL-2 synthesis in T cells (11–15). Indeed, the enzymatic activities of ERK and JNK are reduced after costimulation of TCR and CD28 in murine anergic T cells (16).

These data suggest the close correlation between the activities of the MAPK superfamily and T cell activation. The identification of the signaling components, which integrate costimulatory signals for the differential activation of MAPKs, is a key for clarifying T cell fate determination. Intriguingly, we found that the activation level of JNK, but not of ERK or p38, is greatly augmented in response to the costimulation in Jurkat transfectants overexpressing Chat-H. These cells also exhibit a dramatic increase in their IL-2 production level. In addition, we demonstrated the importance of Chat-H-HEF1-interaction in both the up-regulation of JNK pathway and IL-2 production. Because the members of CrkL-DOCK2-Rac pathway, downstream of HEF1 in T cells, are implicated in JNK activation and IL-2 gene expression (24, 25, 27), it is plausible that Chat-H-HEF1 complexes participate in the positive regulation of IL-2 synthesis via activation of the JNK pathway.

Pyk2H has been shown to up-regulate JNK activation and subsequent IL-2 production after costimulation of TCR and CD28 (17). This process is mediated by phosphorylation of Pyk2H tyrosine 402. Interestingly, Chat-H was found to interact with Pyk2H in a phosphorylation-independent manner,
overexpression of Chat-H further enhances the tyrosine 402 phosphorylation of Pyk2H upon costimulation. Both activities of Chat-H require the functional Cas/HEF1 association do-

phosphorylation of Pyk2H upon costimulation. Both activities overexpression of Chat-H further enhances the tyrosine 402

1. Paul, W. E., and Seder, R. A. (1994) Cell 76, 241–251
2. Woods, M. L., and Shimizu, Y. (2001) J. Leukocyte Biol. 69, 874–880
3. Serrador, J. M., Nieto, M., and Sánchez-Madrid, F. (1999) Trends Cell Biol. 9, 228–232
4. Qian, D., and Weiss, A. (1997) Curr. Opin. Cell Biol. 9, 205–212
5. Werlen, G., and Palmer, E. (2002) Curr. Opin. Immunol. 14, 299–305
6. Rudd, C. E. (1999) Cell 96, 5–8
7. Leo, A., Wieranders, J., Baier, G., Horejsi, V., and Schraven, B. (2002) J. Clin. Invest. 109, 301–309
8. Samelson, L. E. (2002) Annu. Rev. Immunol. 20, 371–394
9. Prauwirth, K. A., and Thompson, C. B. (2002) J. Clin. Invest. 109, 295–299
10. Powell, J., Kagey, J. A., Kitagawa-Sakakida, S., and Schwartz, R. H. (1998) Immunol. Rev. 165, 297–300
11. Rincón, M., Flavell, R. A., and Davis, R. J. (2001) Oncogene 20, 2490–2497
12. Franklin, R. A., Tordai, A., Patel, H., Gardner, A. M., Johnson, G. L., and Gelfand, E. W. (1994) J. Clin. Invest. 93, 2134–2140
13. Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M., and Ben-Neria, Y. (1994) Cell 77, 727–736
14. Nishina, H., Bachmann, M., Oliveira-dos-Santos, A. J., Kozieradzki, I., Fischer, K. D., Odermatt, B., Wakeham, A., Shahinian, A., Takimoto, H., Bernstein, A., Mak, T. W., Woodgett, J. R., Ohashi, P. S., and Penninger, J. M. (1997) J. Exp. Med. 186, 941–953
15. Matsuda, S., Moriguchi, T., Koyanagi, S., and Nishida, E. (1998) J. Biol. Chem. 273, 12378–12382
16. Li, W., Whaley, C. D., Mendeine, A., and Mueller, D. L. (1996) Science 271, 1272–1276
17. Katagiri, T., Takahashi, T., Sasaki, T., Nakamura, S., and Hattori, S. (2000) J. Biol. Chem. 275, 19645–19652
18. Ohashi, Y., Iwata, S., Kamiguchi, K., and Morimoto, C. (1999) J. Immunol. 163, 3727–3734
19. Kamiguchi, K., Tachibana, K., Iwata, S., Ohashi, Y., and Morimoto, C. (1999) J. Immunol. 163, 563–568
20. Minegishi, M., Tachibana, K., Sato, T., Iwata, S., Noyima, Y., and Morimoto, C. (1998) J. Exp. Med. 184, 1365–1375
21. Bouton, A. H., Riggins, R. B., and Bruce-Staskal, P. J. (2001) Oncogene 20, 6448–6458
22. Kanda, H., Misuma, T., Harasaki, K., Yamamoto, K., Yazaki, Y., Hirai, H., and Noyima, Y. (1999) Immunology 97, 56–61
23. Ohashi, Y., Tachibana, K., Kamiguchi, K., Fujita, H., and Morimoto, C. (1998) J. Biol. Chem. 273, 6446–6451
24. Nishihara, H., Maeda, M., Oda, A., Tsuchida, M., Sawa, H., Nagashima, K., and Tanaka, S. (2002) Blood 100, 3968–3974
25. Nishihara, H., Maeda, M., Oda, A., Tsuchida, M., Sawa, H., Nagashima, K., and Tanaka, S. (2002) Biochem. Biophys. Res. Commun. 296, 716–720
26. Bos, J. L., de Rooij, J., and Reeuwijk, K. A. (2001) Nat. Rev. Mol. Cell Biol. 2, 369–377
27. Takai, Y., Sasaki, T., and Matsuzaki, T. (2001) Physiol. Rev. 81, 153–208
28. Sakakibara, A., and Hattori, S. (2000) J. Biol. Chem. 275, 6404–6410
29. Lu, Y., Brush, J., and Stewart, T. A. (1999) J. Biol. Chem. 274, 10047–10052
30. Dedelet, V. C., Pazzagli, C., Zisch, A. H., Hauser, C. A., and Pasquale, E. B. (1999) J. Biol. Chem. 274, 31941–31946
31. Sakakibara, A., Furuse, M., Saito, M., Ando-Akatsuka, Y., and Tsukita, S. (1996) J. Cell Biol. 137, 1393–1401
32. Tokawa, G., Dick, I., Lee, S., and Schlesinger, J. (1986) Science 232, 792–794
33. Yu, H., Li, X., Marchetto, G. S., Dey, R., Hunter, D., Calf, B., Dawson, T. L., Wilm, M., Anderseg, R. J., Graves, L. M., and Earp, H. S. (1996) J. Biol. Chem. 271, 29993–29998
34. Avraham, H., Park, S.-Y., Schinkmann, K., and Avraham, S. (2000) Cell. Signal. 12, 123–133
35. Frank, G. D., Eguchi, S., Motley, E. D., Sasaki, T., and Inagami, T. (2001) Biochem. Biophys. Res. Commun. 286, 692–696
36. Helder, N., and Klein, R. (1999) Development 203, 2033–2044
37. Schmocker, D., and Zipsurgy, S. L. (2001) Cell 105, 701–704

Tyrosine kinases and adaptor proteins play crucial roles in diverse immune cell signaling pathways (4–8). Our data clearly indicate the functional requirement of the Chat-H SH2 domain in the Chat-H-mediated up-regulation of JNK activation and IL-2 synthesis after costimulation. However, Chat-H SH2 domain target(s) responsible for the augmentation of these T cell responses have not yet been identified. Fascinatingly, Chat-H is identical to SHEP1, which binds to activated Eph receptors via its SH2 domain (30). Eph receptor tyrosine kinases function as receptors for ephrins, and the ephrin-Eph system is implicated in a wide range of intercellular communications (36, 37). However, little is known about the role of the ephrin-Eph system in hematopoietic cells. It is very intriguing to assess whether the ephrin-Eph system is involved in T cell activation and differentiation.

Chat-H is phosphorylated by ERK upon TCR stimulation. This raises the possibility that ERK regulates Chat-H function. Chat-H promotes both JNK activation and IL-2 production in Chat-H. Identification of effector protein(s) whose function is controlled by ERK-mediated Chat-H phosphorylation will provide some insights into its significance in the TCR signaling.

In this report, we illuminated the positive regulatory role of a novel hematopoietic adaptor protein Chat-H in the T cell activation. We also revealed that interactions via its domain structures are crucial for the up-regulation of costimulatory signals. Further investigations on the molecular mechanism of signal integration by Chat-H and in vivo studies using transgenic mice will be the next approaches to understanding the physiological function of Chat-H in the T cell fate determination.

Acknowledgements—We thank D. J. Webb, K. Momotani, R. B. Riggins, and A. H. Bouton for critical reading of the manuscript. We also thank the members of Division of Biochemistry and Cellular Biology, National Institute of Neuroscience for helpful discussions and technical advice.

REFERENCES
A Novel Hematopoietic Adaptor Protein, Chat-H, Positively Regulates T Cell Receptor-mediated Interleukin-2 Production by Jurkat Cells
Akira Sakakibara, Seisuke Hattori, Shun Nakamura and Takuya Katagiri

J. Biol. Chem. 2003, 278:6012-6017.
doi: 10.1074/jbc.M207942200 originally published online December 14, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207942200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 16 of which can be accessed free at http://www.jbc.org/content/278/8/6012.full.html#ref-list-1