Evidence for a novel function of the CD40 ligand as a signalling molecule in T-lymphocytes

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Abstract The interaction of the CD40 receptor with its ligand has been shown to be crucial for the activation of B-lymphocytes. Here, we provide evidence that the pg39 molecule/CD40 ligand (gp39/CD40L) also functions as a stimulatory molecule for T-lymphocytes. Activation of T-lymphocytes via gp39/CD40L induced a strong activation of Jun-N-terminal kinase (JNK) and p38-K. Activation of these kinases correlates with a stimulation of Rac1 and inhibition of Rac1 prevents gp39/CD40L triggered JNK/p38-K activation. Further, cellular stimulation via the CD40 ligand results in tyrosine phosphorylation of cellular proteins and the activation of $p56^{lck}$. Inhibition of src-like kinases inhibits Rac1 as well as JNK/p38-K stimulation suggesting a signalling cascade from the gp39/CD40L via $p56^{lck}$ and Rac1 to JNK/p38-K.

Key words: CD40 ligand; gp39; T-lymphocytes; Tyrosine kinase; Jun-N-terminal kinase

1. Introduction

The interaction of B- and T-lymphocytes is essential for proliferation, differentiation, isotype switching and survival of B-lymphocytes [1,2]. In particular, the binding of the CD40 receptor on B-cells to the CD40 ligand (CD40L, also named gp39 or TRAP) expressed on T-lymphocytes seems to be very important for B-lymphocyte activation, since mutations of gp39/CD40L result in the X-linked hyper-IgM syndrome. This immunodeficiency syndrome is characterized by normal or elevated IgM levels and inefficient isotype switching due to impaired stimulation of B-lymphocytes [3–6]. Patients suffer from severe, recurrent bacterial infections and a higher incidence of autoimmune diseases and malignancies.

However, some data indicate that binding of the gp39/CD40L to the CD40 receptor may activate not only B- but also T-lymphocytes [7–10]. The best evidence for this hypothesis is provided by CD40 receptor knock-out mice. These mice lack germinal centers and the formation of germinal centers during an immune response can be initiated by injection of soluble CD40-Fc molecules. This implies that cellular stimulation of T-lymphocytes via the gp39/CD40L has a crucial function in the initiation of germinal center formation.

The gp39/CD40L is a 30 kDa type II membrane glycoprotein expressed on T-lymphocytes, but also on mast cells, basophils, NK cells, monocytes and even on B-lymphocytes [11–15]. Human or mouse gp39/CD40L consists of 261 or 260 amino acids, respectively, with a 22 amino acid cytoplasmic amino-terminal tail lacking any known enzymatic activity [2]. The cytoplasmic domains of mouse and human gp39/CD40L are highly homologous with 82% identical residues [2] implying an important role of the cytoplasmic domain for the function of gp39/CD40L. Furthermore, gp39/CD40L exhibits significant homology to the tumor necrosis factor, Fas and CD30 ligands [2].

In the present study we tested the hypothesis that gp39/CD40L functions as a stimulatory molecule for T-lymphocytes and we aimed to identify signalling events initiated by gp39/CD40L. The results show a gp39/CD40L triggered activation of JNK/p38 kinases, which seems to be mediated by the src-like tyrosine kinase $p56^{lck}$ and the small G-protein Rac1.

2. Materials and methods

2.1. Cells, immunoprecipitation and immunoblotting

All reagents were purchased from Sigma, if not otherwise cited. Human leukemic Jurkat or murine gp39/CD40L positive or negative EL4 T-lymphocytes [16] were grown in RPMI 1640 medium, 10% fetal calf serum, 10 mM HEPES (pH 7.4), 2 mM L-glutamine, 100 μM non-essential amino acids, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin (all from Life Technologies, Eggenstein, Germany) and 50 μM β-mercaptoethanol. Murine splenic T-lymphocytes were purified by Ficoll gradient centrifugation and three times sorting by 1 h incubation with immobilized anti-CD3 (2C11). Finally, cells were incubated for 8 h with 10 μg/ml phytohemagglutinin. FACS analysis showed that more than 90% of the cells were T-cell receptor (TCR)/CD3 and gp39/CD40L positive. For activation, cells were washed twice and stimulated at 37°C with 2 μg/ml monoclonal anti-human or anti-mouse gp39/CD40L antibody (Pharmingen, San Diego, CA, USA) or isotype matched hamster or mouse immunoglobulins (Dianova, Hamburg, Germany). In addition, EL4 cells were also activated by 1 μg/ml purified CD40 receptor. Stimulation was terminated by lysis in 25 mM HEPES (pH 7.4), 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100, 125 mM NaCl, 10 mM each sodium fluoride, Na_3VO_4 and sodium pyrophosphate and 10 μg/ml each of aprotinin and leupeptin (RIPA buffer). Post-centrifugation supernatants were added to 5×SDS sample buffer and 5% β-mercaptoethanol (total cell lysates) or subjected to immunoprecipitation overnight at 4°C using 3 μg of each antibody/sample. For control immunoprecipitates, anti-gp39/CD40L stimulated samples were incubated with irrelevant control immunoglobulins as indicated. Immunocomplexes were immobilized by addition of protein A/G coupled agarose (Santa Cruz Inc.), washed, resuspended in reducing SDS sample buffer, separated by SDS/PAGE and transferred to polyvinyldifluoride membranes (BioRad). Blots were incubated overnight at 4°C with the appropriate antibody (0.5 μg/ml) and developed by incubation with horseradish peroxidase conjugated protein G (BioRad) and use of a chemoluminescence kit (Amersham).

CD40 receptor was purified from mouse splenic B-lymphocytes by immunoprecipitation with a monoclonal anti-CD40 receptor antibody (Pharmingen) as above. Immunoprecipitates were immobilized by addition of protein A/G coupled agarose followed by six times washing in RIPA buffer and three times in HEPES buffered saline solution (H/S). CD40 receptor protein was eluted in 100 mM glycine (pH 3.0), immediately neutralized and dialysed against H/S. The purity of the

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CD40 receptor preparation was determined by biotinylation, separation on 10% SDS/PAGE and visualization with horseradish peroxidase coupled streptavidin (Amersham). The blots showed a single bland (plus the heavy and light chains of the anti-CD40 receptor antibody used for purification) indicating the purity of the CD40 receptor preparation. The concentration of CD40 was calculated using a BioRad protein assay. The immunoglobulin concentration was determined by comparison with the values obtained using an irrelevant, isotype matched immunoglobulin in the purification process. The immunoglobulin content in the preparation was also estimated on the blots by comparison of the signal after HRP-streptavidin visualization with the signal of different amounts of normal, isotype matched immunoglobulin loaded on the same gel. The values were subtracted to get the CD40 receptor concentration. Since we used B-lymphocytes for the purification, this procedure should result in the purification of membrane CD40, which was confirmed by the size of the protein in blots. Cells were stimulated with 0.5 μg/ml of the purified receptor. CD40 receptor purification from splenocytes was performed to obtain stimulation of T-lymphocytes with a molecule displaying all natural modifications.

2.2. Kinase and Rac1 assays

For determination of JNK, p38-K or src-like tyrosine kinase activity, 8×10^6 cells were stimulated as above, lysed in RIPA buffer and subjected to immunoprecipitation using agarose coupled anti-$p56^{lck}$ or -$p59^{fyn}$, -JNK or -p38-K antibodies as described [17,18]. Immunoprecipitates were washed three times in lysis buffer and twice in kinase buffer (25 mM HEPES (pH 7.0), 150 mM NaCl, 10 mM $MnCl_2$, 1 mM Na_3VO_4, 5 mM DTT, 0.5% NP40) for src-like kinases or twice in H/S, 1% NP-40, 2 mM Na_3VO_4, once in 100 mM Tris (pH 7.5), 0.1 M LiCl and twice in kinase buffer 12.5 mM MOPS (pH 7.5), 7.5 mM $MgCl_2$, 12.5 mM β-glycerophosphate and 0.5 mM each of NaF, EGTA and Na_3VO_4 for JNK and p38-K. Kinase reaction was started by addition of 30 μl kinase buffer supplemented with 10 μM ATP, [^{32}P]γATP

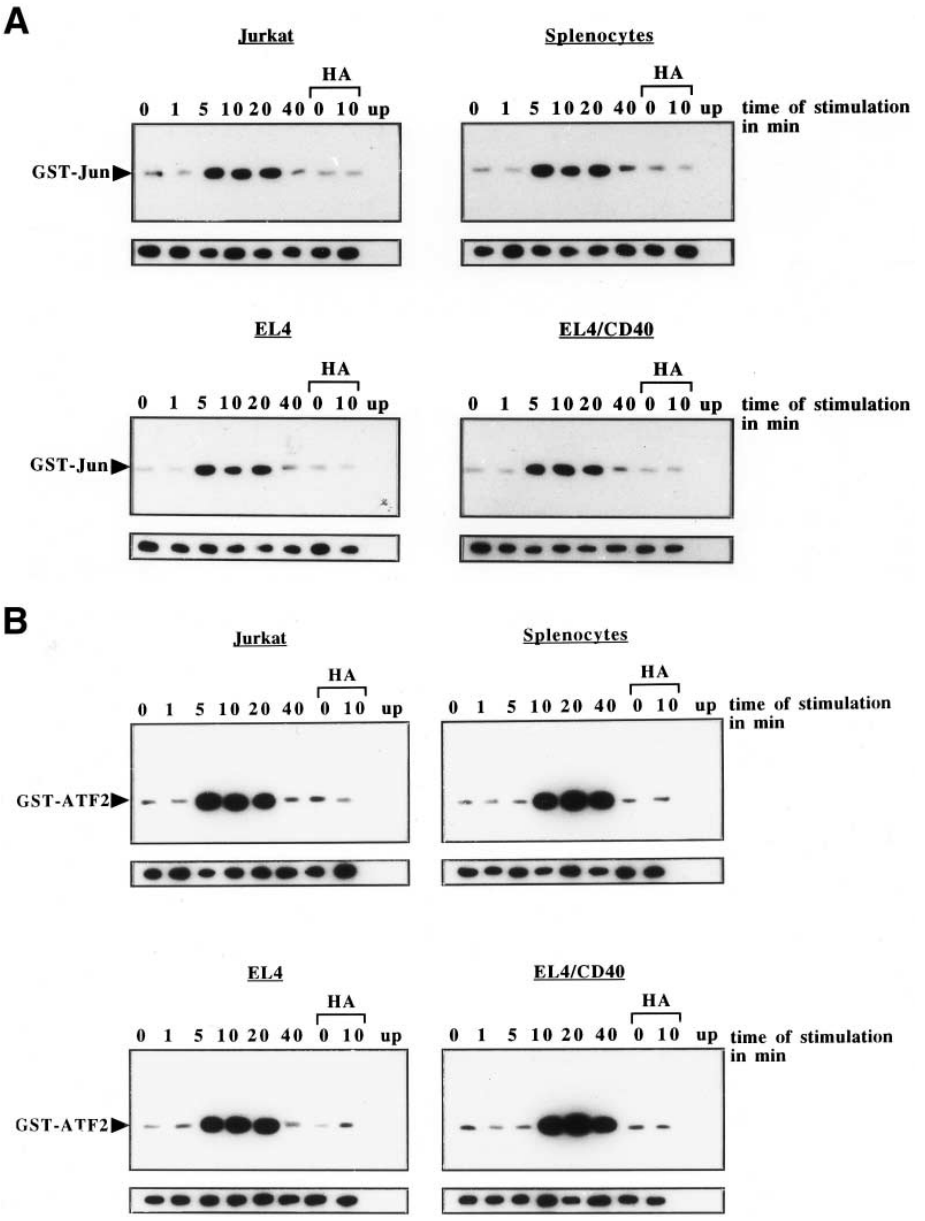

Fig. 1. Activation of T-lymphocytes via gp39/CD40L with anti-gp39/CD40L antibodies or purified CD40 receptor proteins (EL4/CD40) stimulates JNK (A) and p38-K (B). The activation of both kinases is inhibited by preincubation of the cells with the src-like tyrosine kinase inhibitor Herbimycin A (HA). Cells were lysed after stimulation via gp39/CD40L, JNK and p38-K were immunoprecipitated and subjected to kinase assays with [^{32}P]γATP and GST-c-Jun or GST-ATF-2, respectively. Samples were separated by SDS-PAGE, blotted and analyzed by autoradiography. Aliquots of the immunoprecipitates were blotted with anti-JNK or anti-p38-K to test for similar amounts of protein in all lanes (small blots below).

(10 μCi/sample; 3000 Ci/mmol; NEN/DuPont) and 1 μg/ml GST-c-Jun (amino acids 1–79) (for JNK) or GST-ATF-2 (amino acids 1–96) (for p38-K). Src-like kinase activity was determined by autophosphorylation. After 15 min incubation reducing SDS sample buffer was added, samples were separated on SDS/PAGE, blotted and autoradiography was performed. The substrates GST-c-Jun and GST-ATF-2 were expressed as described [18].

Rac1 activity was determined in untransfected or N17Rac1 or pCEV and CD20 co-transfected Jurkat or EL4 cells after 4 h labelling with 1 mCi/ml $^{32}P_i$. Cells were stimulated as indicated and lysed in 25 mM Tris-HCl (pH 7.5), 1% Triton X-100, 0.2% SDS, 0.5% sodium deoxycholate, 20 mM $MgCl_2$, 450 mM NaCl and 100 μg each of aprotinin and leupeptin. Rac1 was immunoprecipitated using a polyclonal, affinity purified anti-Rac1 antibody (Santa Cruz Inc.) and protein A/G conjugated agarose as described [18]. Immunoprecipitates were washed seven times in lysis buffer, once in 25 mM Tris-HCl (pH 7.5), 0.1% Triton X-100, 1 mM $MgCl_2$ and 125 mM NaCl and bound nucleotides were eluted in 1 mM EDTA at 68°C for 20 min. Samples were normalized for radioactivity and nucleotides were separated on polyethyleneimine-cellulose plates (Machery&Nagel) with 0.75 M KH_2PO_4 (pH 3.5). The thin layer chromatography plates were analyzed by autoradiography.

2.3. Transfection assays

Jurkat or EL4 cells were co-transfected with transdominant inhibitory pCEV/N17*Rac1* or vector control pCEV (each 50 μg/20×10^6 cells) with an expression vector for CD20 (pRc/CMV-*cd20*) (10 μg) as previously described [18,19]. Since the ratio of 5:1 pCEV/N17*Rac1*:pRc/CMV-*cd20* drives expression of N17Rac1 in any CD20 positive cell, the selection of CD20 positive cells via magnetic beads permits effective sorting for N17Rac1 expressing cells. In vivo labelling experiments showed that approximately 10% of all cells were CD20 positive, non-specific binding of the cells to the magnetic beads did not exceed 0.5% of all cells. Purified cells were allowed to recover for 30 min at 37°C and were then used for determination of JNK, p38-K or Rac1 activity.

3. Results

In order to identify signalling molecules which might be regulated by gp39/CD40L in T-lymphocytes, gp39/CD40L positive mouse EL4, human Jurkat or activated murine T-lymphocytes were stimulated with monoclonal anti-gp39/CD40L antibodies or purified CD40 receptor. Flow cytometry analysis revealed similar levels of gp39/CD40L expression on EL4, Jurkat and activated murine T-lymphocytes. Cellular stimulation via gp39/CD40L resulted in a rapid and strong activation of Jun-N-terminal kinase (JNK) and p38-K in all cell types (Fig. 1A,B).

The activation of JNK-p38-K is regulated via different mechanisms including src-like tyrosine kinases [20]. We therefore investigated whether Herbimycin A, a src-like tyrosine kinase inhibitor, influences the activation of JNK/p38-K (Fig. 1A,B). Preincubation with Herbimycin A resulted in an almost complete inhibition of gp39/CD40L induced JNK/p38-K activation. To further investigate a possible role of tyrosine kinases by the gp30/CD40L, we analyzed the activation of the src-like tyrosine kinases $p56^{lck}$ and $p59^{fyn}$ by gp39/CD40L. These two kinases have been shown to have a crucial function in several signal transduction pathways in T-lymphocytes [21]. Cellular stimulation via gp39/CD40L using monoclonal anti-gp39/CD40L antibodies or purified CD40 receptor molecules induced an approximately 8-fold activation of $p56^{lck}$ in EL4 and Jurkat cells as well as in ex vivo mouse splenocytes (Fig. 2), whereas $p59^{fyn}$ was not stimulated by cellular triggering via gp39/CD40L. Activation of $p56^{lck}$ correlated with a tyrosine phosphorylation of several cellular proteins determined by blotting of whole cell lysates with the monoclonal anti-phosphotyrosine antibody 4G10 (not shown).

In addition to a regulation by src-like tyrosine kinases, it has been reported that the activation of JNK and p38K depends, at least under certain conditions, on the activity of Rac1 [20]. We therefore tested whether CD40 triggering stimulates Rac1. To this end Rac1 was immunoprecipitated from ^{32}P-labelled cells, bound guanine nucleotides were eluted and

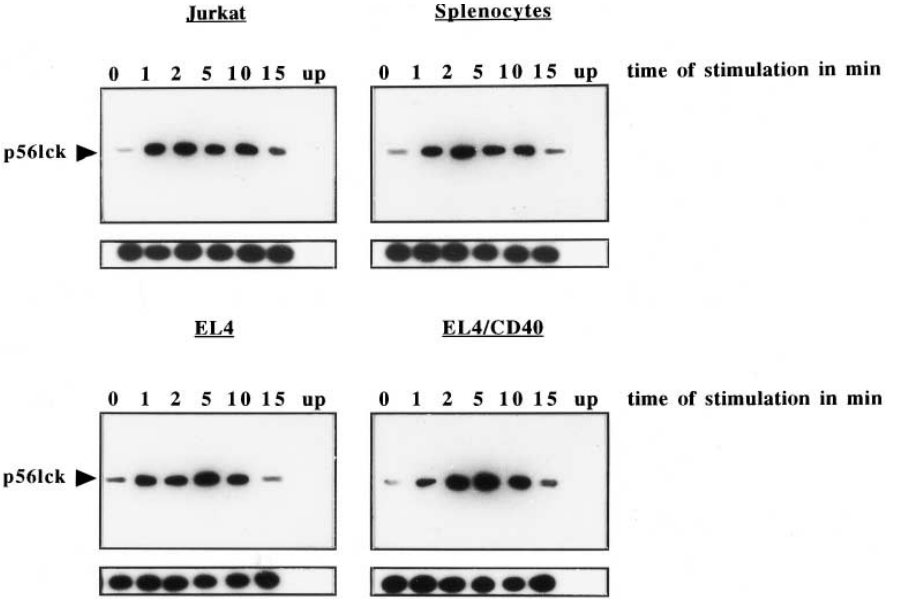

Fig. 2. Stimulation of Jurkat, EL4 or splenocytes via the gp39/CD40L results in activation of the tyrosine kinase $p56^{lck}$. Jurkat, murine EL4 cells or PHA stimulated murine splenocytes were incubated with monoclonal anti-gp39/CD40L antibodies or purified CD40 receptor (EL4/CD40), lysed and subjected to immunoprecipitation with an anti-$p56^{lck}$. The activity of immunoprecipitated $p56^{lck}$ was measured by autophosphorylation of the kinase followed by Western blotting and autoradiography. Non-specific immunoprecipitates (up) were performed with an irrelevant rabbit polyclonal antibody. An aliquot of the immunoprecipitates was blotted with anti-$p56^{lck}$ to test for similar amounts of $p56^{lck}$ in all lanes (small blots below). Shown are representative blots of three independent experiments.

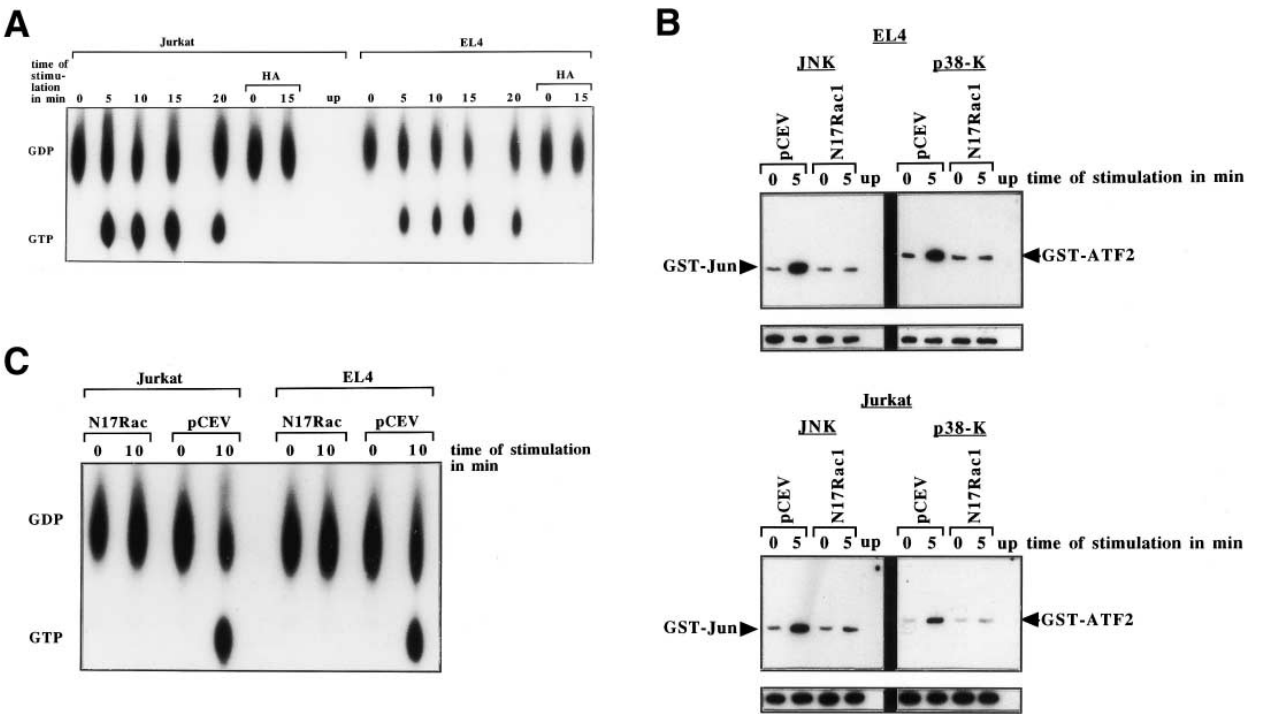

Fig. 3. Gp39/CD40L triggering activates the small G-protein Rac1 which is prevented by preincubation with Herbimycin A (HA) (A). Inhibition of Rac1 by transfection of transdominant inhibitory N17Rac1 blocks activation of JNK and p38-K upon gp39/CD40L mediated cell activation (B). The efficiency of N17Rac1 expression is indicated by an almost complete inhibition of endogenous Rac1 activation after gp39/CD40L triggering (C). For determination of Rac1 activity cells were transfected with N17Rac1 or vector control, or left untransfected, metabolically labelled with $^{32}P_i$, stimulated via gp39/CD40L and Rac1 was immunoprecipitated. Guanine nucleotides bound to immunoprecipitated Rac1 were eluted, separated by thin layer chromatography and analyzed by autoradiography. Experiments were repeated three times. JNK and p38-K activity was determined as above.

separated by TLC. The results show that gp39/CD40L triggering results in a rapid stimulation of Rac1, which was inhibited by Herbimycin A pretreatment (Fig. 3A). This suggests that src-like tyrosine kinases are involved in Rac1 stimulation by gp39/CD40L. To further test the function of Rac1 for JNK/p38-K activation, we transiently transfected a transdominant inhibitory N17Rac1 construct blocking the activation of endogenous Rac1. These inhibitory constructs bind guanine nucleotide exchange factors for small G-proteins with a very high affinity and specificity. This prevents the exchange of guanine nucleotides on a small G-protein without affecting the binding of GDP to e.g. endogenous Rac1 [22]. Expression of this construct almost completely prevented the activation of JNK and p38-K by gp39/CD40L (Fig. 3B). The efficiency of N17Rac1 transfection was determined by measuring Rac1 activity after gp39/CD40L triggering. The results show an inhibition of Rac1 stimulation in cells expressing N17Rac1, whereas transfection of the control vector did not alter anti-gp39/CD40L induced Rac1 activation (Fig. 3C).

The results presented above point to a novel pathway elicited by gp39/CD40L in T-lymphocytes. To further prove the specificity of the observed signalling events, gp39/CD40L negative or positive EL4 and Jurkat cells were stimulated with anti-gp39/CD40L antibodies or isotype matched irrelevant immunoglobulins. Treatment of gp39/CD40L negative EL4 cells did not result in any of the activation events observed after stimulation of gp39/CD40L positive EL4 or Jurkat cells (Fig. 4A,B). Likewise, incubation of gp39/CD40L positive T-cells with isotype matched irrelevant immunoglobulins did not trigger the stimulation of JNK, p38-K, $p56^{lck}$ (Fig. 4A) or Rac1 (Fig. 4B). These results indicate a specific activation of the observed signalling cascade by the gp39/CD40L.

4. Discussion

The present study provides evidence for a novel role of the gp39/CD40L: in addition to its function as a ligand for the CD40 receptor on B-lymphocytes it also seems to function as a signalling molecule on T-lymphocytes mediating T-cell activation. The blockade of JNK/p38-K stimulation by Herbimycin A pretreatment and inhibition of Rac1/2 points to a sequential activation of JNK and p38-K via Rac proteins and Herbimycin A sensitive kinases, e.g. $p56^{lck}$.

Activation of JNK/p38-K after gp39/CD40L triggering was observed in mouse and human T-lymphocytes as well as in ex vivo splenic T-lymphocytes. Since two different monoclonal antibodies specific for human or mouse gp39/CD40L, respectively, were applied in the present study, it seems unlikely that the observed activation signals are mediated by a non-specific cross-reaction with another surface molecule. In addition, isotype matched immunoglobulins did not activate gp39/CD40L positive EL4 or Jurkat T-lymphocytes. Finally, gp39/CD40L negative EL4 cells did not respond to stimulation with anti-gp39/CD40L antibodies. However, to further exclude the possibility of cellular stimulation via cross-reaction of the antibody with another surface molecule we purified CD40 receptor from mouse splenic B-lymphocytes and used the purified CD40 receptor molecules for stimulation of EL4 cells. Purified CD40 receptor induced the same signalling events as observed upon stimulation with the antibodies indicating the specificity

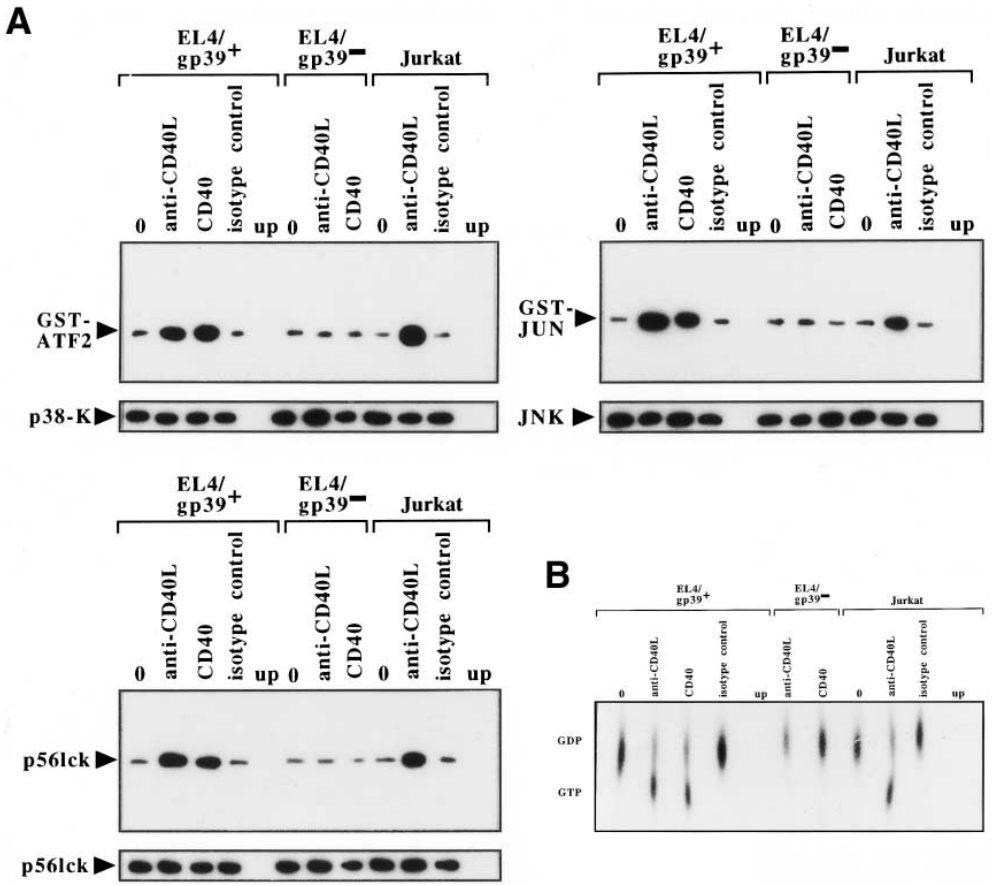

Fig. 4. Activation of human Jurkat or murine gp39/CD40L positive (gp39/CD40L$^+$) EL4 T-lymphocytes via p39/CD40L specifically activates JNK, p38-K, p56^{lck} (A) and Rac1 (B), whereas gp39/CD40L negative (gp39/CD40L$^-$) EL4 cells did not respond to stimulation with anti-gp39/CD40L or purified CD40. Likewise, incubation of gp39/CD40L positive EL4 or Jurkat cells with isotype matched immunoglobulins does not result in cellular stimulation. Western blotting of aliquots of the indicated kinases shows that similar amounts of protein were loaded in all lanes (small blots). These results show the specificity of the observed signalling cascade upon activation of T-lymphocytes via gp39/CD40L.

of the observed activation events for the gp39/CD40L. The anti-CD40 receptor antibody used for purification, which is also present in the CD40 preparation does not bind to EL4 or Jurkat cells as determined by FACS analysis.

The stimulation of JNK and p38-K upon gp39/CD40L triggering might be involved in the regulation of gene expression, in particular in the formation of a functional AP-1 complex. Activation of AP-1 after gp39/CD40L triggering may result in IL-2 synthesis, a crucial event for T-cell stimulation [23]. The stimulation of p38-K may trigger phosphorylation of ATF-1 and ATF-2, which might also be involved in the formation of functional AP-1 or might interact with NF-κB [24,25]. Thus, p38-K may synergistically interact with JNK in the regulation of transcription. Further, the stimulation of p38-K could have additional functions, in particular phosphorylation of heat shock protein HSP 25/27 [26]. However, the exact role of these kinases for gp39/CD40L signalling has yet to be elucidated.

Several receptors including the B-cell receptor (BCR), TCR/CD3, CD40 or Fas have been shown to activate src-like tyrosine kinases, whereas only some of these receptors, e.g. the CD40 or Fas receptor, stimulate JNK/p38-K without co-stimulation [27–30]. Thus, p56^{lck} might be necessary but not sufficient for JNK/p38-K stimulation and additional gp39/CD40L but not TCR/CD3 or BCR elicited signals may exist and act together with p56^{lck}.

Several studies support our results that gp39/CD40L has a dual function resulting in activation of both T- and B-lymphocytes, upon binding to the CD40 receptor.

First, the best evidence for a stimulatory function of the gp39/CD40L for T-lymphocytes is provided by CD40 receptor knock-out mice, which lack germinal centers [7]. Co-injection of the antigen DNP-KLH with CD40-Fc restores the formation of germinal centers suggesting that stimulation of T-lymphocytes via gp39/CD40L has a crucial function in the initiation of germinal center formation.

Second, stimulation of activated peripheral T-cells via gp39/CD40L increased TCR/CD3 induced IL-4 synthesis and proliferation [8,9].

Third, a signalling function of the gp39/CD40L is also supported by the finding that other ligands of the tumor necrosis factor/nerve growth factor- eceptor family, in particular the CD30 ligand, exhibit similar dual functions [31,32].

However, application of activating CD40 receptor antibodies to gp39/CD40L knock-out mice restores cellular and humoral immune responses to adenoviral vectors via a B7.2-CD28 dependent mechanism [33]. Thus, gp39/CD40L may not be absolutely required for the stimulation of T-cells under these conditions and gp39/CD40L may function predominantly as a co-stimulatory molecule which can be replaced by other strong co-stimuli or by upregulation of other stimulatory receptors replacing the function of gp39/CD40L in these knock-out mice. It might also be possible that the func-

tion of gp39/CD40L as a signalling molecule is restricted to T-lymphocytes in the germinal center [7] or to a certain subpopulation of peripheral T-lymphocytes.

Since interaction of gp39/CD40L with the CD40 receptor results in a downregulation and internalization of the gp39/CD40L [10], the observed signalling events elicited by gp39/CD40L may be involved in a negative regulation of the interaction between gp39/CD40L and the CD40 receptor. In particular, Rac1 has been shown to play an important role in actin cytoskeleton regulation [34] and Rac1 activation might be involved in the internalization of the gp39/CD40L. This may limit the activation of B-lymphocytes. Thus, the observed signalling events could be involved in a negative regulation of B-lymphocyte activation by T-lymphocytes via the CD40 ligand/CD40 receptor system.

The present study suggests that gp39/CD40L activates JNK/p38-K via the src-like tyrosine kinase $p56^{lck}$ and Rac proteins. Since gp39/CD40L is predominantly expressed on activated, $CD4^+$ T-lymphocytes [9,35,36], the stimulation of these cells by gp39/CD40L may result in a selective activation and proliferation of antigen specific T-lymphocytes, at least in germinal centers, after interaction with CD40 receptor positive antigen presenting cells.

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References

[1] Banchereau, J., Bazan, F., Blanchard, D., Brière, F., Galizzi, J.P., van Kooten, C., Liu, Y.J., Rousset, F. and Saeland, S. (1994) Annu. Rev. Immunol. 12, 881–922.
[2] Laman, J.D., Claasen, E. and Noelle, R.J. (1996) Crit. Rev. Immunol. 16, 59–108.
[3] Allen, R.C., Armitage, R.J., Conley, M.J., Rosenblatt, H., Jenkins, M.A., Copeland, N.G., Bedell, M.A., Edelhoff, S., Disteche, C.M., Simoneaux, D.K., Fanslow, W.C., Belmont, J. and Spriggs, M.K. (1993) Science 259, 990–993.
[4] Aruffo, A., Farrington, M., Hollenbaugh, D., Li, X., Milatovitch, A., Nonoyama, S., Bajorath, J., Grossmaire, L.S., Stenkamp, R., Neubauer, M., Roberts, R.L., Noelle, R.J., Ledbetter, J.A., Franke, U. and Ochs, H.D. (1993) Cell 72, 291–300.
[5] Fuleihan, R., Ramesh, N., Loh, R., Jabara, H., Rosen, R.S., Chatila, T., Fu, S.M., Stamenkovic, I. and Geha, R.S. (1993) Proc. Natl. Acad. Sci. USA 90, 2170–2173.
[6] Korthäuer, U., Graf, D., Mages, H.W., Brière, F., Padyachee, M., Malcolm, S., Ugazio, A.G., Notarangelo, L.D., Levinsky, R.J. and Kroczek, R.A. (1993) Nature 361, 539–541.
[7] Van Essen, D., Kikutani, H. and Gray, D. (1996) Nature 378, 620–623.
[8] Blotta, M.H., Marshall, J.D., DeKruyf, R.H. and Umetsu, D.T. (1996) J. Immunol. 156, 3133–3140.
[9] Cayabyab, M., Phillips, J.H. and Lanier, L.L. (1994) J. Immunol. 152, 1523–1531.
[10] Yellin, M.J., Sippel, K., Inghirami, G., Covey, L.R., Lee, J.J., Sinning, J., Clark, E.A., Chess, L. and Lederman, S. (1994) J. Immunol. 152, 598–608.
[11] Armitage, R.J., Fanslow, W.C., Strockbine, L., Sato, T.A., Clifford, K.N., Macduff, B.M., Anderson, D.M., Gimpel, S.D., David-Smith, T., Maliszewski, C.R., Clark, E.A., Smith, C.A., Grabstein, K.H., Cosman, D. and Spriggs, M. (1992) Nature 357, 80–82.
[12] Gauchat, J.F., Mazzei, G., Life, P., Henchoz, S., Peitsch, M.C., Aubry, J.P., Jomotte, T. and Bonnefoy, J.Y. (1994) Res. Immunol. 145, 240–244.
[13] Graf, D., Korthäuer, U., Mages, H.W., Senger, G. and Kroczek, R.A. (1992) Eur. J. Immunol. 22, 3191–3194.
[14] Grammer, A.C., Bergman, M.C., Miura, Y., Fujita, K., Davis, L.S. and Lipsky, P.E. (1995) J. Immunol. 154, 4996–5010.
[15] Spriggs, M.K., Armitage, R.J., Strockbine, L., Clifford, K.N., Macduff, B.M., Sato, T.A., Maliszewski, C.R. and Fanslow, W.C. (1992) J. Exp. Med. 176, 1543–1550.
[16] Werner-Favre, C., Gauchat, J.F., Mazzei, G., Aubry, J.P., Bonnefoy, J.Y. and Zubler, R.H. (1994) Immunology 81, 111–114.
[17] Gulbins, E., Brenner, B., Schlottmann, K., Koppenhoefer, U., Linderkamp, O., Coggeshall, K.M. and Lang, F. (1996) J. Immunol. 157, 2844–2850.
[18] Brenner, B., Koppenhoefer, U., Weinstock, C., Linderkamp, O., Lang, F. and Gulbins, E. (1997) J. Biol. Chem. 272, 22173–22181.
[19] Gulbins, E., Bissonette, R., Mahboubi, A., Martin, S., Nishioka, W., Brunner, T., Baier, G., Baier-Bitterlich, G., Byrd, C., Lang, F., Kolesnick, R., Altman, A. and Green, D. (1995) Immunity 2, 341–351.
[20] Minden, A., Lin, A., Claret, F.X., Abo, A. and Karin, M. (1995) Cell 81, 1147–1157.
[21] Weiss, A. and Littman, D.R. (1994) Cell 76, 263–274.
[22] Feig, L.A. and Cooper, G.M. (1988) Mol. Cell. Biol. 8, 3235–3243.
[23] Crabtree, G.R. (1989) Science 243, 355–361.
[24] Du, W., Thanos, D. and Maniatis, T. (1993) Cell 74, 887–898.
[25] Kaszubska, W., Hooft van Huijsduijnen, R., Ghersa, P., DeRaemy-Schenk, A.M., Chen, B.P.C., Hai, T., CeLamarter, J.F. and Whelan, J. (1993) Mol. Cell. Biol. 13, 7180–7190.
[26] Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, T. and Nebreda, A.R. (1994) Cell 78, 1027–1037.
[27] Ren, C.L., Morio, T., Fu, S.M. and Geha, R.S. (1994) J. Exp. Med. 179, 673–680.
[28] Berberich, I., Shu, G., Siebelt, F., Woodgett, J.R., Kyriakis, J.M. and Clark, E.A. (1996) EMBO J. 15, 92–101.
[29] Latinis, K.M. and Koretzky, G.A. (1996) Blood 87, 871–875.
[30] Sakata, N., Patel, H.R., Terada, N., Aruffo, A., Johnson, G.L. and Gelfand, E.W. (1995) J. Biol. Chem. 270, 30823–30828.
[31] Stueber, E., Neurath, M., Calderhead, D., Fell, H.P. and Strober, W. (1995) Immunity 2, 507–521.
[32] Wiley, S.R., Goodwin, R.G. and Smith, C.A. (1996) J. Immunol. 157, 3635–3639.
[33] Yang, Y. and Wilson, J.M. (1996) Science 273, 1862–1864.
[34] Ridley, A.J., Paterson, H.F., Johnston, C.J., Diekmann, D. and Hall, A. (1992) Cell 70, 401–410.
[35] Lederman, S., Yellin, M.J., Krichesky, A., Belko, J., Lee, J.J. and Chess, L. (1992) J. Exp. Med. 175, 1091–1101.
[36] Lane, P., Traunecker, H.S., Inui, S., Lanzavecchia, A. and Gray, D. (1992) Eur. J. Immunol. 22, 2573–2578.