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RGS10 Restricts Upregulation by Chemokines of T Cell Adhesion Mediated by α4β1 and αLβ2 Integrins

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Chemokines rapidly and transiently upregulate α4β1 and αLβ2 integrin-mediated adhesion during T lymphocyte extravasation by activating Gα-dependent inside-out signaling. To limit and terminate Gα-mediated signaling, cells can use several mechanisms, including the action of regulator of G protein signaling (RGS) proteins, which accelerate the GTPase activity of Gα subunits. Using human T cells silenced for or overexpressing RGS10, we show in this article that RGS10 functions as an inhibitor of Gα-dependent, chemokine-upregulated T cell adhesion mediated by α4β1 and αLβ2. Shear stress-dependent detachment and cell spreading analyses revealed that RGS10 action mainly targets the adhesion strengthening and spreading phases of α4β1-mediated cell attachment. Associated with these observations, chemokine-stimulated Vav1–Rac1 activation was longer sustained and of higher intensity in RGS10-silenced T cells, or inhibited in cells overexpressing RGS10. Of importance, expression of constitutively activated Rac1 forms in cells overexpressing RGS10 led to the rescue of CXCL12-stimulated adhesion to VCAM-1 to levels similar to those in control transfectants. Instead, adhesion under flow conditions, soluble binding experiment, flow cytometry, and biochemical analyses revealed that the earlier chemokine-triggered integrin activation step was mostly independent of RGS10 actions. The data strongly suggest that RGS10 opposes activation by chemokines of the Vav1–Rac1 pathway in T cells, leading to repression of adhesion strengthening mediated by α4β1. In addition to control chemokine-upregulated T cell attachment, RGS10 also limited adhesion-independent cell chemotaxis and activation of cdc42. These results identify RGS10 as a key molecule that contributes to the termination of Gα-dependent signaling during chemokine-activated α4β1- and αLβ2-dependent T cell adhesion. The Journal of Immunology, 2011, 187: 1264–1272.

C hemokines stimulate cell migration and activation, and exert their functions upon binding to heterotrimeric guanine nucleotide-binding (G) protein-coupled receptors (GPCR) (1–3). In the immune system, chemokines promote the migration of immune cells from lymph and blood circulation into lymphoid tissues and sites of inflammation during immune surveillance (4–7). For T lymphocytes, this process is achieved after rapid and transient stimulation of α4β1 and αLβ2 integrin activity by chemokines presented on the endothelium. Thus, chemokine binding to GPCR induces activation of intracellular effector molecules that lead to integrin-mediated upregulation of lymphocyte adhesion, a process called inside-out signaling (8, 9). Key inside-out molecules that regulate the activation of α4β1 and αLβ2 include talin and kindlin-3, as well as the Vav1–Rac1 and RAPL–Rap1 pathways (10–15).

Heterotrimeric G proteins consist of an α subunit and a complex formed by β and γ subunits (16–18). Basally, Gβγ and GDP-bound Gα are associated, and following interaction with an activated receptor, GTP replaces GDP and GTP-Gα dissociates from the Gβγ dimer. These two elements interact with effector proteins, leading to the activation of distinct signaling pathways. The GTPase activity inherent to Gα limits G protein activation, as GTP hydrolysis causes reassociation of GDP-Gα and Gβγ. G proteins are classified into four subfamilies—Gαs, Gαi/o, Gαq/11, and Gα12/13—according to the Gα protein present in the complex (17, 18).

Heterotrimeric G proteins can be regulated by members of the regulators of G protein signaling (RGS) family (19–22). RGS proteins contain a 120-aa-long region called RGS domain, which is responsible for binding to Gα subunits and for GTPase accelerating activity (21). This activity promotes the return of Gα to its inactive form, which leads to faster termination of G protein-dependent signaling. Therefore, RGS proteins control the timing and duration of specific responses involving GPCR signaling. In T lymphocytes, constitutive or regulated expression of RGS2, RGS3, RGS4, RGS14, and RGS16 has been previously reported (23–27). Both migration and G protein-mediated signaling activation has been shown to be a target of regulation by these RGS proteins.

RGS10 belongs to the R12 subfamily of the RGS family, which also includes RGS12 and RGS14 (21), and it has been found to be expressed on brain, thymus, and lymph nodes (28, 29). RGS10 mainly accelerates the GTPase activity of Gαi (28). RGS10 is phosphorylated at Ser168 by cAMP-dependent protein kinase A, and this phosphorylation has been proposed to regulate RGS10 cell localization and function (30).

Although considerable effort has been made to identify molecules that are required for integrin activation in T lymphocytes, little is known about the mechanisms controlling the termination of integrin-mediated adhesion, a process that contributes to the homeostasis of the immune response. RGS proteins represent good
candidates to regulate the strength and duration of GPCR-dependent signaling leading to integrin activation in lymphocytes. In the present work, we have investigated whether RGS10 could represent such a regulatory molecule in the control of integrin-dependent T cell adhesion. The results indicate that RGS10 represses the strength of chemokine-dependent T lymphocyte adhesion mediated by α4β1, and therefore suggest that RGS10 may control the duration of GPCR-dependent signaling required for upregulation of this adhesion.

Materials and Methods

Cells, Abs, and reagents

Human Molt-4 and Jurkat T cell lines, as well as peripheral blood T lymphocytes (PBL-Ts), were cultured and prepared as described (31). The Consejo Superior de Investigaciones Científicas Ethics Committee (Madrid, Spain) approved the protocols used to obtain and process the human blood samples. Human CD4+ T cells were purified using anti-CD4-coated microbeads (Miltenyi Biotec, Auburn, CA). Control P3X63 anti-α4 HPI/2, anti-β1 TS1/26, and anti-CD45 RP/21 mAb were gifts from Dr. Francisco Sánchez-Madrid (Hospital de la Princesa, Madrid, Spain), and polyclonal anti-β1A Abs were from Dr. Guido Taron (Unirivers University, Turin, Italy). The MEM-148 anti-β2 mAb and Abs to RGS10, Goα, Vav1, phosphotyrosine, Rap1, and RhoA were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-Rac1 and anti-Cdc42 were from BD Biosciences Pharmingen (San Diego, CA); and anti-GFP was from Molecular Probes (Eugene, OR). Anti-Rac1 and anti-Cdc42 were from BD Biosciences Pharmingen (San Diego, CA); anti-Rap1, or anti-RhoA Abs. were from Cell Signaling Technology (Danvers, MA). Anti-CXCR4 mAb and CXCL12 were purchased from R&D Systems (Minneapolis, MN). CCL21 was obtained from Peprotech (Rocky Hill, NJ), and pertussis toxin was from Sigma-Aldrich.

Transfections and RNA interference

HA-fused RGS10 in the pcdNA3.1 vector was obtained from Missouri S&T DNA Resource Center (Rolla, MO), and vectors coding for wild-type and active (V12) Rac1 fused with GFP were from Dr. F. Sánchez-Madrid. Two small interfering RNA (siRNA) duplexes against human RGS10 (RGS10.1, sense: 5'-CAAAGGAGACUCAUGCAT-3'; and RGS10.2, sense: 5'-GAACCGCAUCCACGUAUtt-3') were purchased from Dharmaco (Chicago, IL). Synthetic oligonucleotides (64-mer) that included the 19-mer sequence from the RGS10.1 siRNA sequence were synthesized, annealed, and ligated into the pSuper vector, as described (32), to generate the pSuper-RGS10 short hairpin RNA (shRNA). The pSuper vector was used as the control in transfections involving pSuper-RGS10. Human Rac1 and Rap1, or control siRNA (31), were purchased from Ambion (Austin, TX). Vectors and siRNA were nucleofected (Amaxa, Cologne, Germany) following the described procedure (31). Molt-4 or PBL-T siRNA transfectants were assayed 22–24 h posttransfection, and transfection did not affect cell viability, as assessed in flow cytometry cell-cycle analyses.

Cell adhesion, chemotaxis, and spreading assays

For static adhesions, cells were plated on wells coated with CXCL12 or CCL21 (650 ng/ml), together with the fibronectin fragment FN-H89 or VCAM-1, and plates were incubated for 2 min at 37°C, according to the described method (31). Extent of adhesion was quantified with a fluorescence analyzer (BMG Labtechnologies, Offenburg, Germany). For flow cytometry cell-cycle analyses, we followed the reported protocol (33). In brief, cells were infused at a flow rate of 1 dye/μm² into flow chambers containing coimmobilized VCAM-1 and CXCL12. Rolling cells that subsequently firmly attached for at least 20 s were expressed as stable arrest, whereas tethering cells that did not arrest at any moment were expressed as rolling cells. To evaluate shear resistance, cells were allowed to attach and then were exposed to sequential increases of the flow rate. The number of cells remaining bound was determined as the percentage of total adhered cells after the adhesion step. For chemotaxis assays, we used the reported protocol (34). Migrated cells were counted in a flow cytometer (Coulter Epics XL; Beckman Coulter, Miami, FL) by passing each sample in the same predetermined time and flow conditions. For cellular spreading, CXCL12-stimulated cells attached on VCAM-1 were fixed and analyzed as previously described (33). For cell binding to VCAM-1–Fc (R&D Systems), cells were stimulated for 45 s with CXCL12 before adding VCAM-1–Fc for 75 s. For cell binding to ICAM-1–Fc (R&D Systems), cells (2 × 10⁵) were resuspended in HEPES buffer (HEPES 20 mM, pH 7.4, NaCl 140 mM; glucose 2 g/l; BSA 0.1%) with or without 5 mM Mg²⁺ and 1 mM EGTA. ICAM-1–Fc (200 μg/ml) was added to samples, and after a 30-min incubation at 37°C, cells were stimulated for 60 s with CXCL12. Detection of VCAM-1–Fc and ICAM-1–Fc was performed by flow cytometry using PE-conjugated AffiniPure F(ab')₂ fragment goat anti-human IgG, Fcy fragment specific (Jackson ImmunoResearch Laboratories, West Grove, PA).

Flow cytometry and calcium mobilization assays

For detection of high-affinity LFA-1, cells resuspended in HEPES buffer containing 1% dimethyl sulfoxide (DMSO) were incubated for 10 min at 37°C with the MEM-148 mAb (10 μg/ml), followed by exposure for 5 min to CXCL12. After washing, cells were incubated with FITC-conjugated rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories) for 30 min at 4°C. For calcium mobilization, cells were washed with labeling medium (HBSS containing 1% FBS, CaCl₂ 1 mM, MgCl₂ 1 mM), followed by an incubation step of 45 min at 37°C with the Ca²⁺-sensitive fluorescent dyes Fluo3-AM and Fura-AM (Molecular Probes) to a final concentration of 0.4 μM and 10 μM, respectively. After cell stimulation with CXCL12, we measured the ratio of Fluo3-AM/Fura-AM fluorescence by flow cytometry. Ionomycin (Sigma-Aldrich) was used as a positive control for Ca²⁺ mobilization.

Immunoprecipitation, immunoblotting, and GTPase assays

For immunoprecipitation, cells were solubilized in lysis buffer containing 1% digitonin, and after preclearing with protein G-Sepharose beads (Amersham Pharmacia Biotech, Uppsala, Sweden), supernatants were incubated with Abs followed by coupling to protein G-Sepharose. Proteins were resolved by SDS-PAGE, then transferred to membranes that were sequentially incubated with primary Abs and with HRP-conjugated secondary Abs. Proteins were visualized using SuperSignal Chemiluminescent Substrate (Pierce, Rockford, IL). For GTPase assays, we followed the method described (31). Briefly, cells exposed to CXCL12 were lysed, and aliquots from extracts were separated for total lysate controls and for incubation either with GST-PK-CD (for active Rac1 and cdc42), GST-Ral-GDS (for active Rap1), or GST-C21 (for active RhoA) fusion proteins, followed by incubation with glutathione-agarose beads. Bound proteins were eluted and subjected to immunoblotting using anti-Rac1, anti-cdc42, anti-Rap1, or anti-RhoA Abs.

Statistical analyses

Data were analyzed by one-way ANOVA, followed by Tukey–Kramer multiple comparisons. In both analyses, the minimum acceptable level of significance was p < 0.05.

Results

RGS10 controls chemokine-stimulated T cell adhesion mediated by α4β1 and αLβ2 integrins

RGS10 was found to be expressed in PBL-Ts and in the CD4⁺ subpopulation, as well as in the human T cell lines Molt-4 and Jurkat (Fig. 1A). CXCL12 rapidly and transiently stimulated the association of RGS10 with Goα (Fig. 1B), suggesting that RGS10 might regulate Goα-dependent signaling pathways in T cells. To investigate whether adhesion of T cells to α4β1 ligands that is upregulated by CXCL12 can be controlled by RGS10, we silenced its expression in Molt-4 and PBL-T cells by siRNA or shRNA, and tested transfectants in static adhesion assays to CS-1/fibronectin (FN-H89) and to VCAM-1. RGS10 shRNA reduced the expression of RGS10 by 70% and 90% in Molt-4 and PBL-T cells, respectively, whereas transfection with RGS10.2 siRNA led to a 50% decrease in the expression of this protein (Fig. 1C). When RGS10 shRNA was transfected into Molt-4 cells, a 50% reduction in RGS10 expression was observed (Fig. 1C, bottom panel). Molt-4 and PBL-T cells silenced for RGS10 expression showed significantly stronger attachment to FN-H89 and VCAM-1 immobilized with CXCL12 than to control siRNA or shRNA transfectants (Fig. 1D, 1E). Under basal conditions (no CXCL12 stimulation), the attachment of RGS10 knockdown T cells to VCAM-1 was slightly increased compared with control siRNA transfectants, although the extent of this increase was always smaller than the upregulation of adhesion seen in RGS10-depleted cells incubated...
with CXCL12. Pertussis toxin, an inhibitor of Goi-triggered signaling, blocked both the CXCL12-stimulated T cell adhesion to VCAM-1 in control shRNA transfectants and the further upregulation of adhesion seen in RGS10 shRNA counterparts (Fig. 1F), indicating that RGS10 was exerting its regulatory actions on adhesion through Goi. Flow cytometry control experiments indicated that RGS10 depletion did not alter the expression of CXCR4, α4 or β1 (not shown).

To determine whether the regulation by RGS10 of α4β1-dependent T cell adhesion could also be detected with chemokines other than CXCL12, we used CCL21, a chemokine that binds to the CCR7 receptor on the surface of T lymphocytes and that has been demonstrated to activate integrin-mediated lymphocyte adhesion (35, 36). The results indicated that RGS10-silenced T cells attached to VCAM-1 more strongly than did control siRNA transfectants when exposed to CCL21 (Fig. 1G), indicating that RGS10 controls α4β1-dependent T cell adhesion in response to multiple Goi-coupled chemokine receptors.

Further support for the involvement of RGS10 in the control of T cell adhesion mediated by α4β1 in response to chemokines came from experiments using T cells that overexpressed RGS10. Thus, Molt-4 cells overexpressing RGS10-HA displayed a significant reduction in CXCL12-upregulated attachment to FN-89 and VCAM-1, compared with mock transfectants (Fig. 2A, 2B). To analyze whether RGS10 could regulate other adhesions in T cells, in addition to α4β1-dependent attachment, we studied the effect of silencing or overexpressing RGS10 on αLβ2-mediated adhesion. RGS10-depleted Molt-4 and PBL-T cells displayed higher attachment to ICAM-1 than did control siRNA transfectants (Fig. 3A, 3B). In addition, RGS10 silencing led to stronger adhesion to ICAM-1 triggered by CCL21 than in control siRNA-transfected cells (Fig. 3C).

Together, these results indicate that RGS10 functions as a controller of chemokine-stimulated T cell adhesion involving integrins α4β1 and αLβ2.

**RGS10 controls the chemokine-upregulated T cell adhesion strengthening and spreading steps mediated by α4β1**

The adhesion process dependent on lymphocyte integrins can be divided into an initial step involving generation of high-affinity activated integrins that support firm interaction with their ligands, a subsequent phase of increase in avidity that includes...
enhanced valency of the adhesions, followed by a phase of adhesion strengthening that supports final cell spreading (9). To study which of these steps can be regulated by RGS10, we first performed adhesion assays under flow conditions to mimic the initial phases of the adhesion cascade. Adhesion under shear stress revealed a moderate and statistically nonsignificant \( p > 0.05 \) increase in rapid \((<20\text{s})\) stable cell arrest on VCAM-1 coimmobilized with CXCL12 of both PBL-T and Molt-4 RGS10 siRNA transfectants, when compared with control counterparts (Fig. 4A). Control experiments indicated that adhesion was abolished by pretreatment of cells with pertussis toxin (not shown). Dissociation of Vav1 from talin and increase in talin-\( \beta \)-1 association in response to chemokines constitute early events that lead to integrin activation (13). Neither Vav1–talin dissociation nor talin-\( \beta \)-1 assembly was significantly affected by RGS10 silencing (Fig. 4B); furthermore, RGS10-depleted T cells displayed binding capacity to soluble VCAM-1–Fc comparable to that in control counterparts (Fig. 4C). In addition, soluble binding to Molt-4 cells of ICAM-1–Fc or MEM-148, an anti-\( \beta \)-2 mAb that recognizes high-affinity states of \( \alpha \)L\( \beta \)2 (37), was not altered by RGS10 knocking down (Fig. 4D). These results strongly suggest that the initial steps of chemokine-triggered T cell adhesion that generate active \( \alpha \)4\( \beta \)1 and \( \alpha \)L\( \beta \)2 are not significantly controlled by RGS10 function.

Notably, RGS10-silenced PBL-T and Molt-4 cells developed higher resistance to detachment at increased shear stress than did control siRNA transfectants (Fig. 4E). Moreover, RGS10 knockdown cells displayed higher spreading on VCAM-1 than did control siRNA transfectants, and interference reflection microscopy analyses showed that the ligand-bound focal plane of RGS10-depleted cells had a larger area than did control transfectants (Fig. 4F). Therefore, these data indicate that the adhesion strengthening and spreading steps of \( \alpha \)4\( \beta \)1-mediated T cell adhesion following stimulation by chemokines are targeted by RGS10 activity.

**Control by RGS10 of \( \alpha \)4\( \beta \)1-dependent T cell adhesion involves regulation of Vav1–Rac1 activation**

Activation by chemokines of the Vav1–Rac1 pathway is required for \( \alpha \)4\( \beta \)1-mediated T cell adhesion strengthening (31). RGS10-depleted Molt-4 cells displayed a more sustained Vav1 tyrosine phosphorylation and Rac1 activation in response to CXCL12 than did control siRNA transfectants (Fig. 5A). On the contrary, RGS10 overexpression led to reduced Vav1 phosphorylation levels and to a decrease in Rac1 activity compared with findings in control transfectants (Fig. 5B). As also shown before (31), Rac1 silencing completely abolished CXCL12-stimulated Molt-4 cell adhesion to VCAM-1 (Supplemental Fig. 1). Remarkably, reduction in CXCL12-stimulated adhesion to VCAM-1 in cells overexpressing RGS10 was rescued by coexpression of GFP-fused active Rac1 (V12), under both static and flow conditions (Fig. 5C, 5D). These data indicate that control of Vav1–Rac1 activation by RGS10 in chemokine-dependent signaling underlies the regulation of \( \alpha \)4\( \beta \)1-mediated T cell adhesion by RGS10.

Rap1 activation in response to CXCL12 was only minimally increased at 2.5 min in RGS10 knockdown cells compared with control transfectants (Fig. 5E, left panel), whereas longer incubations with the chemokine caused no changes in Rap1 activation between these transfectants (not shown). In addition, Rap1 depletion led to partial (40%) inhibition of CXCL12-stimulated Molt-4 cell adhesion to VCAM-1 (Fig. 5E, right panel).
Therefore, these results indicate that a major consequence of RGS10 actions is the limitation of Vav1–Rac1 activation by chemokines, which then results in reduction in α4β1-dependent T cell attachment.

**RGS10 regulates T cell chemotaxis and cdc42 activation**

Finally, we investigated whether RGS10 is capable of controlling T cell responses to chemokines other than cell adhesion. Chemotaxis to CXCL12 of RGS10 knockdown Molt-4 transfectants was significantly higher than that of control siRNA counterparts, whereas cells overexpressing RGS10 migrated clearly less than mock transfectants (Fig. 6A). In addition, RGS10 silencing led to sustained cdc42 activation in response to CXCL12 (Fig. 6B), whereas no substantial alterations were detected for RhoA activation (Fig. 6C). Phosphorylation of the Erk1/2 MAPK was only modestly increased in RGS10 knockdown cells (Fig. 6D), and no...
changes in the activation of the PI3K downstream effector Akt were observed (not shown). Furthermore, RGS10 depletion did not significantly affect Ca\textsuperscript{2+} mobilization triggered by CXCL12 (Fig. 6E). Thus, RGS10 is capable of regulating not only α\textsubscript{4}β\textsubscript{1}- and αLβ\textsubscript{2}-dependent T cell adhesion but also migration and activation of specific signaling pathways.

**Discussion**

Lymphocyte extravasation at sites of tissue injury or at lymphoid organs depends on chemokine-activated adhesion mediated by α\textsubscript{4}β\textsubscript{1} and αLβ\textsubscript{2} integrins (8, 9). This activation must be rapid to deliver tight cell attachment to resist the blood shear stress, but also transient to allow lymphocyte locomotion on and diapedesis across endothelial layers. The chemokine-dependent integrin activation step in lymphocytes requires an inside-out signaling that impinges on the integrin β subunit cytoplasmic domains, a key event for transmitting conformational changes to the extracellular regions that leads to increase in integrin affinity (11). Talin and kindlins are recipients of this inside-out signaling, which promotes their binding to the β cytoplasmic domains, finally stimulating the transition to high-affinity α\textsubscript{4}β\textsubscript{1} and αLβ\textsubscript{2} conformations (10–12).

The transient nature of integrin activation by chemokines indicates that an active molecular machinery limits and ultimately inhibit the signaling stimulated following G\textsubscript{a}-coupled chemokine receptor interaction with their ligands. To limit and terminate this signaling, cells use several mechanisms, including the action of RGS proteins, which accelerate the GTPase activity of G\textsubscript{a} subunits (19, 21). In this work, we show that RGS10 opposes the chemokine-stimulated signaling that is needed for T cell adhesion mediated by α\textsubscript{4}β\textsubscript{1} and αLβ\textsubscript{2}. Thus, upregulation of adhesion to α\textsubscript{4}β\textsubscript{1} and αLβ\textsubscript{2} ligands in response to CXCL12 and CCL21 was significantly stronger in RGS10-depleted cells than in control transfectants. On the contrary, when RGS10 was overexpressed, stimulation of adhesion by these chemokines was limited. RGS10 transiently associated to G\textsubscript{a} in T cells following exposure to CXCL12, and pertussis toxin blocked chemokine-upregulated adhesion to VCAM-1 of both control and RGS10-silenced cells,
sustained and of higher intensity in RGS10-depleted than control
mutants in response to CXCL12 (200 ng/ml).

suggesting that RGS10 is inhibiting the adhesion mediated by

Flow chamber adhesion experiments that measure rapid firm

attachment under shear stress revealed that RGS10 silencing

caused only moderate and statistically nonsignificant increases in

T cell firm arrest to VCAM-1 following the rolling step. Moreover,

chemokine-stimulated increase in talin-β1 association, an early

event leading to high-affinity α4β1 that is competent for VCAM-1

binding and that is linked with Vav1–talin dissociation (13), was

not altered by RGS10 depletion. Correlating with this observation,

binding of soluble VCAM-1–Fc, as well as interaction of ICAM-

1–Fc with αLβ2, was comparable in control and RGS10 knock-
down T cells. In addition, generation of αLβ2 high-affinity states

was not affected by RGS10 depletion. Instead, chemokine-
stimulated Rac1 activation, a process taking place during the

strengthening of adhesion after the integrin activation step (31),

was longer sustained and of higher intensity in RGS10-depleted

T cells, without further increase in talin–talin association, an early

event leading to high-affinity states of the ZAP-70 kinase in response to CXCL12 (38, 39). Therefore, these results strongly suggest that RGS10 actions mostly oppose

the Vav1–Rac1-dependent adhesion strengthening and spreading

steps of α4β1-mediated T cell adhesion triggered by chemokines,

with no or minor involvement of RGS10 in the initial integrin

activation phases.

VCAM-1 binding triggered by chemokines is inhibited in T cells

silenced for Vav1 or talin, owing to the requirement of a preformed

Vav1–talin complex for α4β1 activation (13). This activation step

is optimally achieved following increase in talin binding to α4β1

upon chemokine-promoted Vav1–talin disassembly involving Vav1

tyrosine phosphorylation by ZAP-70. The fact that phosphoryla-
tion of Vav1 is of higher intensity in RGS10-depleted than control

cells, without further increase in talin-β1 binding, suggests a more

sustained Goi-dependent activation in cells silenced for RGS10,

with no alterations in the rapid and earlier α4β1 activation.

Rap1 activation has been reported to mediate chemokine-
promoted, integrin-dependent T cell adhesion (14, 40, 41). We

found that Rap1 silencing partially (40%) affects CXCL12-
stimulated T cell adhesion to VCAM-1. However, our results in-
dicated that Rap1 activation by CXCL12 was not significantly

altered in RGS10 knockdown T cells, suggesting minor roles for

Rap1 in mediating the increased adhesion seen in RGS10 knock-
down cells.

In addition to repressing chemokine-upregulated T cell adhesion

dependent on α4β1 and αLβ2, RGS10 also inhibited adhesion-
dependent cell chemotaxis and cdc42 activation in response to

CXCL12. The mechanisms underlying the control by RGS10 of

these two processes has not been addressed in the current study,

but they also likely involve termination of Goi-dependent acti-

vation of downstream signaling.

The following model can be proposed from present and earlier

data. Chemokine binding to their receptors triggers Goi-dependent

signaling that leads to changes in molecular associations between

Vav1, talin, and β1, resulting in early α4β1 activation (13), but

also promotes Goi association with RGS10. Although the initial

steps of adhesion are not targeted following RGS10–Goi, associ-

FIGURE 6. RGS10 controls chemokine-triggered T cell chemotaxis and cdc42 activation. A. Molt-4 cells were transfected with control or RGS10 siRNA (left) or with mock or RGS10-HA vectors (right), and subsequently subjected to chemotaxis toward CXCL12. Chemotaxis was significantly upregulated (*) or inhibited (+), p < 0.05. B–D, Control or RGS10 siRNA transfectants were subjected to cdc42 (B) or RhoA GTPase (C) assays, or tested by immuno-
blotting for Erk1/2 and phospho-Erk1/2 expression levels (D). E, Control or RGS10 siRNA transfectants were subjected to Ca2+ mobilization experi-
ments in response to CXCL12 (200 ng/ml).
ation, the consequence of the assembly of this complex may well be the gradual termination of Vav1–Rac1 activation that triggers chemokine-upregulated strengthening of the adhesion mediated by α4β1.

Other RGS proteins, such as RGS2, RGS3, RGS4, RGS14, and RGS16, have been reported to be expressed in T cells, either resting or activated (23–27). Therefore, it could be speculated that some of them might contribute together with RGS10 to the inhibition of chemokine-stimulated, α4β1- and αEβ2-dependent T cell adhesion. Thus, an early work showed that overexpression of RGS1, RGS3, and RGS4 in a pre-B cell line led to reduced adhesion to VCAM-1 following chemoattractant activation (42). In addition, overexpression of RGS16 results in inhibition of CXCL12-induced migration and MAPK and Akt activation in the progenitor Mo7e model cell line (43). Furthermore, RGS1 and RGS3 inhibit CXCL12-triggered B cell chemotaxis (44). Therefore, RGS protein function in lymphoid cells appears to be highly relevant for physiological termination of chemokine signals that control important processes such as cell adhesion and migration, as well as cell activation.

Not only do RGS proteins associate to Go subunits and accelerate their GTPase activity, but they are also capable of acting as effector antagonists by competing with effector molecules for GTP-bound Go subunits, and they can also directly interact with effectors (21, 45, 46). For instance, RGS2 binds adenylyl cyclase (47), and RGS4 interacts with phospholipase Cβ and phospholipase acid (48, 49).

In addition, RGS14 binds activated H-Ras forms, as well as Raf kinases (50, 51), and regulates their subcellular localization and activities. RGS10 belongs to the same D/R12 subfamily of RGS14, kinases (50, 51), and regulates their subcellular localization and fine-tuning by RGS proteins.

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Disclosures

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