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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 Gq-dependent inside-out signaling. To limit and terminate Gq-mediated signaling, cells can use several mechanisms, including the action of regulator of G protein signaling (RGS) proteins, which accelerate the GTPase activity of Gq subunits. Using human T cells silenced for or overexpressing RGS10, we show in this article that RGS10 functions as an inhibitor of Gq-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 Gq-dependent signaling during chemokine-activated α4β1- and αLβ2-dependent T cell adhesion.

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Chemokines 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—Gαi, Gαo, 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 Ser106 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 for such regulatory roles.
candidates to regulate the strength and duration of GPCR-depen-
dent 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 medi-
ated 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 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 micro-
melts (Miltenyi Biotec, Auburn, CA). Control P3X63 anti-αb, anti-β1 TS2/16, and anti-CD45 RP2/21 mAb were gifts from Dr. Francisco Sánchez-Madrid (Hospital de la Princesa, Madrid, Spain), and polyclonal anti-β1A Abs were from Dr. Guido Tarone (Turin University, Turin, Italy). The MEM-148 anti-β2 mAb and Abs to RGS10, Gαi, Vav1, phosphotyro-
sine, 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-GFPA was from Molecular Probes (Eugene, OR). Abs to β-actin, hemagglutinin (HA), paxillin, and talin clone 8D4 were obtained from Sigma-Aldrich (St. Louis, MO), and anti-p Elk1/2 and Elk1/2 Abs were from Cell Signaling Technology (Danvers, MA). Anti-CXCR4 mAb and CXCL12 were purchased from R&D Systems (Minneapolis, MN). C2L1 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 cDNA Resource Center (Rolla, MO), and vectors coding for wild-
type and active (V12) Rac1 fused with GFP were from Dr. F. Sánchez-
Madrid. Two different interfering RNA (siRNA) duplexes against human
RGS10 (RGS10.1, sense: 5′-CAGAAAGGACAGUCAUCAUGCt-3′; and
RGS10.2, sense: 5′-GAAACCCGCCCUCUUGUt-3′) were purchased
from Dharmacco (Chicago, IL). Synthetic oligonucleotides (64-mer) that
included the 19-mer sequence from the RGS10.1 siRNA sequence were
synthesized, annealed, and ligated into 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 cells were transfected with siRNA and secondary Abs. Protein
expression of RGS10 was determined by Western blotting using an
anti-GFP mAb (Molecular Probes, Eugene, OR).

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 fluores-
cent microscope (×40; Leica, Bensheim, Germany). For flow
chamber adhesion assays, we followed the reported protocol (33). In brief,
cells were incubated at a flow rate of 1 dyne/cm² into flow chambers con-
taining coimmobilized VCAM-1 and CXCL12. Rolling cells that sub-
sequently 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 to be subjected to exponential 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
procedure (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 described (33). For cell spreading on VCAM-1 (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 ⁴²Ca²⁺
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 Laborato-
ries, West Grove, PA).

Flow cytometry and calcium mobilization assays

For detection of high-affinity LFA-1 cells, resuspended in HEPES buffer
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 Immu-
noResearch 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 Fluo-3-AM and Fura-Red-AM
(Molecular Probes) to a final concentration of 4 µM and 10 µM, re-
spectively. After cell stimulation with CXCL12, we measured the ratio of
Fluo-3-AM/FuraRed-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 sec-
ondary Abs. Proteins were visualized using SuperSignal Chemiluminescent Substitute (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 in-
cubation either with GST-PAK-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/iblurectin
(FN-H89) and to VCAM-1. RGS10 siRNA reduced the ex-
pression of RGS10 by 70% and 90% in Molt-4 and PBL-T cells,
respectively, whereas transfection with RGS10 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 signi-
icantly stronger attachment to FN-H89 and VCAM-1 immobi-
лизованный с CXCL12 чем к контрольной siRNA или shRNA трансфекциям (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

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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 when exposed to CXCL12; conversely, transfectants overexpressing RGS10 displayed a significant inhibition of CXCL12-triggered adhesion, compared with control transfectants (Fig. 3A, 3B). In addition, RGS10 silencing led to stronger adhesion to ICAM-1 triggered by CCL21 than to cells transfected with control siRNA (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 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-β1 association in response to chemokines constitute early events that lead to integrin activation (13). Neither Vav1–talin dissociation nor talin-β1 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-β2 mAb that recognizes high-affinity states of αLβ2 (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 α4β1 and αLβ2 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 α4β1-mediated T cell adhesion following stimulation by chemokines are targeted by RGS10 activity.

Control by RGS10 of α4β1-dependent T cell adhesion involves regulation of Vav1–Rac1 activation

Activation by chemokines of the Vav1–Rac1 pathway is required for α4β1-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 activation 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 α4β1-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\(^{2+}\) mobilization triggered by CXCL12 (Fig. 6E). Thus, RGS10 is capable of regulating not only \(\alpha_4\beta_1\)- and \(\alpha_L\beta_2\)-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 \(\alpha_4\beta_1\) and \(\alpha_L\beta_2\) 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 \(\beta\) 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 \(\beta\) cytoplasmic domains, finally stimulating the transition to high-affinity \(\alpha_4\beta_1\) and \(\alpha_L\beta_2\) conformations (10–12).

The transient nature of integrin activation by chemokines indicates that an active molecular machinery limits and ultimately inhibits the signaling stimulated following G\(_{\alpha}\)-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\(_{\alpha}\) subunits (19, 21). In this work, we show that RGS10 opposes the chemokine-stimulated signaling that is needed for T cell adhesion mediated by \(\alpha_4\beta_1\) and \(\alpha_L\beta_2\). Thus, upregulation of adhesion to \(\alpha_4\beta_1\) and \(\alpha_L\beta_2\) 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\(_{\alpha_i}\) in T cells following exposure to CXCL12, and pertussis toxin blocked chemokine-upregulated adhesion to VCAM-1 of both control and RGS10-silenced cells.
suggesting that RGS10 is inhibiting the adhesion mediated by α4β1 by repressing Goi-dependent signaling.

Flow chamber adhesion experiments that measure rapid firm cell attachment under shear stress revealed that RGS10 silencing caused only moderate and statistically nonsignificant increases in T cell firm arrest over 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 knockdown 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-silenced 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 transfecants. The regulation of Rac1 activation by RGS10 was most likely a consequence of RGS10-dependent control of Vav1 tyrosine phosphorylation, as it was increased or decreased in cells silenced or overexpressing RGS10, respectively. At present, we have not experimentally addressed the mechanisms underlying the control by RGS10 of Vav1 phosphorylation by CXCL12, but on the basis of published work, it is possible that RGS10 might control the duration and/or intensity of Goi-dependent activation 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 phosphorylation 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 indicated 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 knockdown cells.

In addition to repressing chemokine-upregulated T cell adhesion dependent on α4β1 and αLβ2, RGS10 also inhibited adhesion-independent 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 activation 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-
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, 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 αβ2-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 chemotactant 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 GTase activity, but they are also capable of acting as effector antagonists by competing with effectors for GTP-bound Go subunits, and they can also directly interact with effectors (21, 45, 46). For instance, RGS2 binds adenyl cyclase (47), and RGS4 interacts with phospholipase Cβ and phosphatidic acid (48, 49). In addition, RGS14 binds activated H-Ras forms, as do Raf kinases (50, 51), and regulates their subcellular localization and interaction. RGS10 belongs to the same D/R12 subfamily of RGS14, kinases (50, 51), and regulates their subcellular localization and interaction. RGS10 belongs to the same D/R12 subfamily of RGS14, kinases (50, 51), and regulates their subcellular localization and interaction.


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