Lost GRP on cytotoxicity?

Jeroen P Roose

Deficiency in the RASGRP1 guanine-nucleotide-exchange factor leads to a novel primary immunodeficiency with impaired activation and proliferation of T cells and B cells and defective killing by cytotoxic T cells and natural killer cells.

RASGRP1 ('RAS guanine-nucleotide-releasing protein 1') is a guanine-nucleotide-exchange factor for the small GTPase RAS and is best known for its function in coupling incoming T cell antigen receptor (TCR) signals to the canonical RASGTP-RAF-MEK-ERK kinase pathway[1] (**Fig. 1**). In resting cells, RASGRP1 sits in an autoinhibited dimer that is opened up following TCR stimulation[2]. The thymocytes of mice deficient in *Rasgrp1* demonstrate impaired TCR-induced ERK activation and a striking block in T cell development, which results in reduced numbers of peripheral T cells[3,4]. *Rasgrp1* deficiency leads to more moderate developmental defects in mouse B cells that also express RASGRP3 (refs. 5,6). How *RASGRP1* deficiency might affect humans has remained unknown. In this issue of *Nature Immunology*, Saltzer *et al.* describe a novel primary immunodeficiency in a patient with loss of RASGRP1 (ref. 7). Their studies confirm the anticipated role of RASGRP1 in the RAS-ERK pathway in T cells and B cells. Quite surprisingly, they also reveal a novel role for RASGRP1 in regulating the microtubule and actin cytoskeleton in $CD8^+$ T cells and natural killer (NK) cells.

Saltzer *et al.* study a 12-year old patient with recurrent infections[7]. Multiple episodes of pneumonia, severe failure to thrive and a progressive decrease in $CD4^+$ T cells observed in serial immunological analyses suggested an immunodeficiency. They identify a homozygous nonsense variant in *RASGRP1* that introduces a premature stop codon that affects position 246 in the protein encoded (**Fig. 1**) and results in very low expression of a truncated RASGRP1 protein. Both parents and all three healthy siblings are heterozygous for this recessive variant, whereas three older siblings of the patient died in their first 2 years of life without genetic testing. Immuno-phenotyping of the patient reveals $CD4^+$ T cell lymphopenia with a relative increase in $CD8^+$ T cells, signs of exhaustion for both $CD4^+$ T cells and $CD8^+$ T cells, an increase in $\gamma\delta^+$ $CD8^+$ T cells, normal numbers of NK cells but very low numbers of invariant NK T cells, normal proportions of regulatory T cells, fewer memory B cells, and a greater abundance of transitional B cells as well as $CD21^{lo}$ B cells that arise with chronic stimulation of the immune system[7].

Mechanistically, the deficiency in *RASGRP1* results in defective proliferation of the patient's T cells and B cells as well as impaired upregulation of the expression of activation markers and diminished phosphorylation of ERK kinases in these cells when antigen receptors are stimulated. These defects confirm the known role of RASGRP1 in activating the ERK kinase pathway that helps drive cell proliferation and also match the results of studies of T cells and B cells from *Rasgrp1*-deficient mice[3–6] (**Fig. 1**). When the authors investigate $CD8^+$ T cells and NK cells, more surprising novel defects emerge[7]. The patient's $CD8^+$ cytotoxic T cells are defective in killing of target cells despite showing increased expression of the lytic proteins perforin and granzyme B. Similarly, the patient's NK cells also have large amounts of intracellular granzyme B and perforin but impaired cytolytic function. Further analyses of the patient's NK cells reveal decreased accumulation of F-actin and impaired convergence of cytotoxic granules toward presented target cells but normal expression of activating receptors on NK cells. Saltzer *et al.* therefore postulate that the deficiency in RASGRP1 perhaps causes these defects in a manner other than through loss of canonical RASGRP1-RASGTP-RAF-MEK-ERK signaling.

The authors turn to an unbiased mass spectrometry approach to analyze HEK293 human embryonic kidney cells and Jurkat T cell leukemia cells and identify the dynein light chain DYNLL1 as an interaction partner of RASGRP1 (**Fig. 1**). A similar RASGRP3-DYNLL1 interaction has been identified previously through a yeast two-hybrid screen showing a QATQT sequence in the carboxyl terminus of RASGRP3 that matches a consensus DYNLL1-binding motif[8]. Deletion of the carboxyl terminus of RASGRP3 encompassing QATQT severely alters the subcellular localization of RASGRP3 but has only modest effects on RAS activation[8]. Saltzer *et al.* reveal that alteration of a KATQT motif in RASGRP1 (**Fig. 1**) leads to loss of its interaction with DYNLL1 (ref. 7). DYNLL1 belongs to the LC8 family of light chains that have been described as 'cargo adaptors' because of their binding to dyneins, myosin 5a or other proteins known to be transported on microtubules or on actin filaments[9]. The authors imply that NK cells from the patient show defective cytolytic killing because DYNLL1's link to the transport of cargo along the cytoskeleton is now lost (**Fig. 1**). How exactly loss of the RASGRP1-DYNLL interaction affects these processes and whether this is through the connection to dynein and/or myosin 5a remains to be investigated. The DYNLL1 or LC8-binding motif is often observed close to a coiled-coil or other dimerization domain of the interacting protein, and LC8 has been described as 'molecular Velcro' because it promotes dimerization[9]. It is intriguing that the KATQT motif in RASGRP1 lies in close proximity to its carboxy-terminal coiled-coil domain identified in its crystal structure[2] (**Fig. 1**). Thus, the binding of DYNLL1 to RASGRP1 might couple RASGRP1 signals to cytoskeletal processes and cytolytic killing, and/or DYNLL1 might stabilize the dimerization of RASGRP1.

Finally, Saltzer *et al.* go back to their studies of $CD8^+$ T cells and focus on actin turnover, activation of the small GTPase RhoA and cell motility[7], probably inspired by a published study of mast cells from mice deficient in *Rasgrp1* (ref. 10). That study established that *Rasgrp1*-deficient mast cells are defective in granule translocation, microtubule formation and RhoA activation[10]. Saltzer *et al.* find that following stimulation with the chemokine CXCL12, $CD8^+$ T cells from the patient demonstrate diminished retrograde actin flow and slower migration on plates coated with the adhesion molecule ICAM-1, concomitant with less CXCL12-induced activation of RhoA[7]. Treatment of these impaired $CD8^+$ T cells with the RhoA activator nocodazole or the immunomodulator lenalidomide restores the migration and RhoA activation, which demonstrates that these impairments are, in principle, reversible.

From a signaling point of view, it has become clear that RASGRP1 can connect

Jeroen P. Roose is in the Department of Anatomy, University of California San Francisco, San Francisco, California, USA.
e-mail: jeroen.roose@ucsf.edu

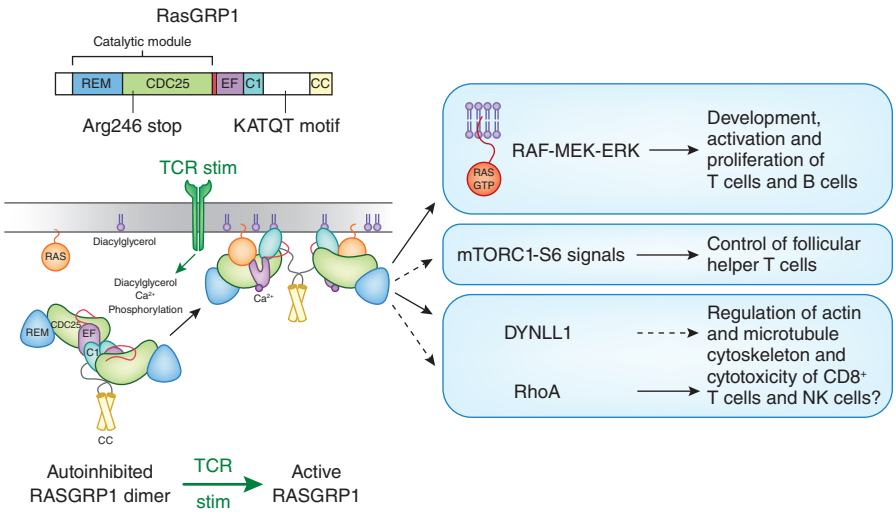

Figure 1 RasGRP1's domains, regulation, signaling and function in immune cells. RASGRP1 contains a catalytic module of the REM and CDC25 domains (top left) responsible for the exchange of GDP for GTP on RAS, followed by an inhibitor linker (red), calcium-binding EF hands, a diacylglycerol-binding C1 domain, and a C-terminal coiled-coil (CC) dimerization motif[1]. Saltzer *et al.* map a stop codon that affects position 246 in the protein encoded in a patient with primary immunodeficiency who is homozygous for this variant and reveal that a KATQT motif mediates binding to DYNLL1 (ref. 7). In an autoinhibited RASGRP1 dimer (middle and bottom left), the inhibitory linker (red) blocks the RAS-binding site and the EF hands obscure the C1 domain[2]. TCR stimulation (stim) results in increases in intracellular calcium (Ca^{2+}) and allosteric changes in RASGRP1 (ref. 2). The generation of diacylglycerol promotes the recruitment of active RASGRP1 to the membrane and its phosphorylation[1]. Active RASGRP1 is known for its role in the canonical RASGTP-RAF-MEK-ERK pathway that influences T cells and B cells[3–6]. The existence of less-well-established pathways through mTORC1-S6 (ref. 11) and DYNLL1 and RhoA[7] indicates that RASGRP1 can fulfill additional functions in other immune cells. Part of the middle and bottom left is adapted from ref. 2.

to more pathways than just the canonical RASGTP-RAF-MEK-ERK kinase pathway. That last pathways involves the recruitment of RASGRP1 to the membrane by the second messenger diacylglycerol[1]. RASGRP1 is controlled through autoinhibition; RASGRP1's EF hands serve a crucial role in this by covering up the C1 domain so that binding to diacylglycerol cannot occur[2]. A mouse model with a point mutation in these EF hands (*Rasgrp1*^{Anaef}) develops spontaneous signaling in resting T cells. Intriguingly, this signaling occurs mostly through a pathway of the metabolic checkpoint kinase complex mTORC1 and ribosomal protein S6 and leads to the spontaneous development of follicular helper T cells and the emergence of autoantibodies in *Rasgrp1*^{Anaef} mice[11]. The study by Saltzer *et al.* adds DYNLL1 and RhoA to RASGRP1's list of possible effector molecules[7]. The molecular connections of mTORC1 and RhoA to RASGRP1 are not clear at all (dotted lines in **Fig. 1**), but the cellular consequences of the RASGRP1-dependent signals are evident. The molecular interaction between RASGRP1 or RASGRP3 and DYNLL1 is firmly established, but future work will need to sort out the specific signaling pathways and cellular functions that rely on this interaction.

From an immunological point of view, Saltzer *et al.* reveal a novel immunodeficiency through their analysis of a single patient with loss of RASGRP1 (ref. 7). Their patient has a partial T cell defect that differs from the severe impairment in T cell development in the mouse model of *Rasgrp1* deficiency[3,4]. How T cells develop relatively efficiently in this patient without RASGRP1 is a puzzle. In addition, the authors comment that the patient has no signs of autoimmune disease. Partial T cell immunodeficiencies in patients are commonly associated with somewhat counterintuitive manifestations of autoimmunity[12]. Features of autoimmunity arise in aged *Rasgrp1*-deficient mice[3,4] as well as in *Rasgrp1*^{Anaef} mice[11], and an altered TCR repertoire with selection of T cells that recognize self antigens probably has a major role here. Whether loss of RASGRP1 in patients can also result in autoimmune disorders remains to be determined, but *RASGRP1* should be considered a gene that is potentially affected in patients with unexplained immunodeficiencies or autoimmune diseases.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Ksionda, O., Limnander, A. & Roose, J.P. *Front. Biol. (Beijing)* **8**, 508–532 (2013).
2. Iwig, J.S. *et al. eLife* **2**, e00813 (2013).
3. Priatel, J.J., Teh, S.J., Dower, N.A., Stone, J.C. & Teh, H.S. *Immunity* **17**, 617–627 (2002).
4. Dower, N.A. *et al. Nat. Immunol.* **1**, 317–321 (2000).
5. Coughlin, J.J., Stang, S.L., Dower, N.A. & Stone, J.C. *J. Immunol.* **175**, 7179–7184 (2005).
6. Bartlett, A., Buhlmann, J.E., Stone, J., Lim, B. & Barrington, R.A. *J. Immunol.* **191**, 3605–3613 (2013).
7. Saltzer, E. *et al. Nat. Immunol.* **17**, 1352–1360 (2016).
8. Okamura, S.M., Oki-Idouchi, C.E. & Lorenzo, P.S. *J. Biol. Chem.* **281**, 36132–36139 (2006).
9. Rapali, P. *et al. FEBS J.* **278**, 2980–2996 (2011).
10. Liu, Y., Zhu, M., Nishida, K., Hirano, T. & Zhang, W. *J. Exp. Med.* **204**, 93–103 (2007).
11. Daley, S.R. *et al. eLife* **2**, e01020 (2013).
12. Liston, A., Enders, A. & Siggs, O.M. *Nat. Rev. Immunol.* **8**, 545–558 (2008).