To help clarify this rather complex situation, Tan and colleagues now bring the novel strategy of binding and destroying the B7 molecules in DCs before they have a chance to reach the cell surface. The authors used the B7-binding portion of CTLA4 but modified it to include a retention signal that targets it to the endoplasmic reticulum. When this construct was transfected into human monocyte-derived DCs, it markedly reduced the cell-surface expression of B7-1 and B7-2, and—just as theory would predict—rendered the DCs tolerogenic in vitro. This was accomplished without inducing IDO in the DCs (unlike CTLA4-Ig) and apparently also without inducing other changes to the basic DC biology (at least not obvious ones).

Thus, this study offers novel support for the hypothesis that it is simply the absence of B7 expression that renders the DCs tolerogenic. This interpretation of the authors' data remains still somewhat speculative and will need to be more directly tested in the future (eg, by artificially ligating the appropriate co-stimulatory counter-receptor on the T cells to test whether this bypasses the tolerogenic effects of the transfected DCs). It will also be important to definitively rule out that the formation and degradation of intracellular CTLA4/B7 complexes do not somehow alter the basic biology of the DCs. But these questions notwithstanding, from a therapeutic standpoint the results of Tan and colleagues suggest a novel and intriguing strategy that might render human monocyte-derived DCs stably tolerogenic in vivo. ■

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PHAGOCYTES

Comment on Cao et al, page 3234

Mac-1 mediates migration to lymph nodes

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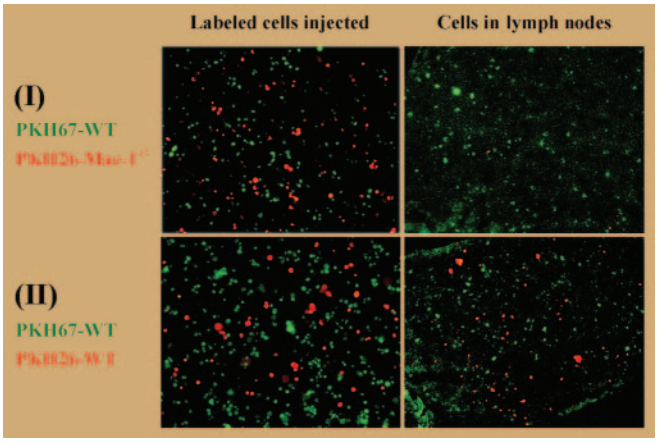
Bacterial lipopolysaccharide induces macrophages to migrate from inflamed tissues to lymph nodes using the adhesion molecule Mac-1.

Cell migration requires attachments to cells and matrix by adhesion molecules. Mac-1, also known as CD11b/CD18, $\alpha_M\beta_2$ integrin, or complement receptor 3, is an adhesion molecule expressed by a variety of phagocytes including macrophages in inflammatory sites. In the present issue, Cao and colleagues demonstrate that Mac-1 mediates a bacterial lipopolysaccharide (LPS)–induced migration of macrophages out of inflamed tissues and into the lymph nodes.



Macrophage migration from the peritoneum to the lymph nodes. See the complete figure in the article beginning on page 3234.

Proinflammatory stimuli including LPS can render macrophages difficult to extract from body cavities using lavage. In the present studies, Cao and colleagues observed that LPS decreased the numbers of macrophages that could be lavaged from thioglycollate-inflamed wild-type (WT) mice, but LPS did not affect the numbers of thioglycollate-elicited macrophages lavaged from Mac-1–deficient mice. Therefore, Mac-1 is necessary to whatever makes thioglycollate-elicited macrophages refractory to lavage after LPS stimulation.

To determine how LPS affected such macrophages, Cao and colleagues transferred fluorescent thioglycollate-elicited macrophages to thioglycollate-inflamed peritoneal spaces and tracked them after injecting saline or LPS. LPS but not saline induced the fluorescent macrophages to adhere to peritoneal surfaces within 5 minutes. However, by lavage time (4 hours after LPS injection), these cells were no longer present in either the peritoneal lavage fluid or on the peritoneal surfaces; instead, they were in the draining lymph nodes and the circulating blood. When Mac-1 was inhibited in these WT mice by a soluble antagonist of Mac-1 (neutrophil inhibitory factor), more macrophages could be recovered by lavage and fewer macrophages appeared in the lymph nodes. When fluorescent Mac-1–deficient macrophages were injected into Mac-1–deficient hosts, LPS failed to induce the adhesion of fluorescent cells to the peritoneal surfaces and fluorescent cells did not appear in the lymph nodes or blood. Labeling thioglycollate-elicited WT macrophages green and thioglycollate-elicited Mac-1–deficient macrophages red allowed these different cells to be mixed together and then studied within the same WT mice with thioglycollate-induced peritonitis. After LPS injection, red Mac-1–deficient macrophages but not green WT macrophages were recovered by lavage, whereas green WT but not red Mac-1–deficient macrophages appeared in the lymph nodes (see figure). Altogether, these data make a compelling argument for Mac-1–mediated migration of macrophages to the lymph nodes in this inflammatory setting.

Mechanisms by which macrophages migrate to the lymph nodes are beginning to be elucidated.[1] LPS must induce changes in chemokines, adhesion molecules, and other factors to mediate this transit of inflammatory macrophages. The initial tight adhesion to peritoneal surfaces mediated by Mac-1 may be critical to the ensuing migration. This initial adhesion occurs within 5 minutes after LPS injection, suggesting that it does not require new gene expression. The Mac-1 ligands essential for attachment to peritoneal surfaces and migration to the lymph

nodes, and the mechanisms by which LPS rapidly alters their availability or function, remain to be determined.

Agents targeting Mac-1 (via CD11b or CD18) and Mac-1 ligands are planned and ongoing in clinical trials.[2] The findings of Cao and colleagues suggest that therapies inhibiting Mac-1 interaction with ligands could compromise macrophage migration from inflammatory sites to lymph nodes for some patients, which could influence the resolution of inflammation and/or antigen presentation and adaptive immunity. Thus, the discovery that inflammatory macrophages can be stimulated to migrate to lymph nodes using Mac-1–dependent pathways has implications for experimental medicine as well as fundamental immunology. ■

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CHEMOKINES, CYTOKINES, AND INTERLEUKINS

Comment on Berthebaud et al, page 2962

RGS16 "tightens the reins" on CXCR4

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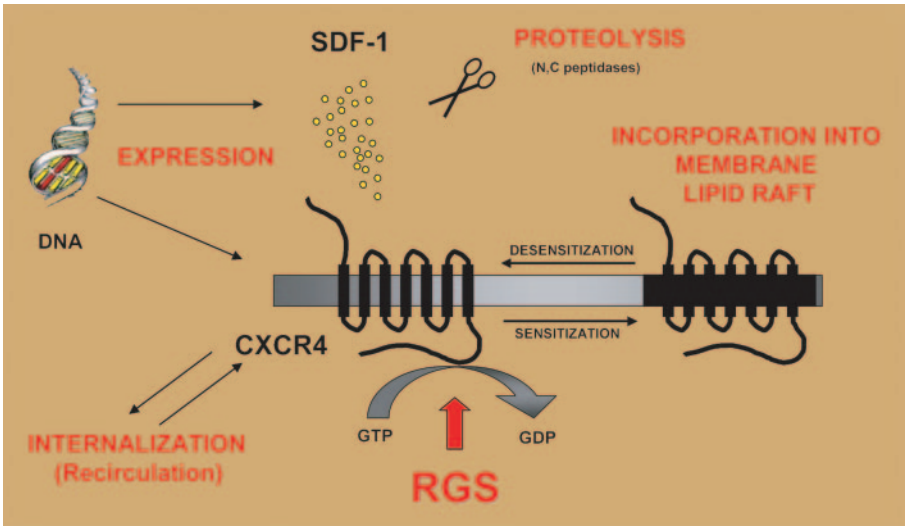
While expression of CXCR4, the receptor for the α-chemokine stromal-derived factor 1 (SDF-1), is relatively high on mature megakaryocytes, these cells lose their responsiveness to stimulation by SDF-1 compared with young megakaryoblasts. RGS16, a member of the regulators of G-protein signaling (RGS) family, is found to be responsible for this effect.

The CXC chemokine receptor 4 (CXCR4)–stromal-derived factor 1 (SDF-1) axis plays an important role in the maturation of megakaryocytes (MKs) by promoting their developmental translocation from the osteoblastic niche to the endothelial niche and is thus involved in MK maturation and proplatelet formation.[1] CXCR4 is highly expressed on MKs; however, the chemotactic responsiveness of these cells to an SDF-1 gradient, which is robust with early megakaryoblasts, decreases with maturation of MKs.[2] A similar phenomenon was found for maturing B-lymphocytic cells in bone marrow (BM).[3] Involvement of regulators of G-protein signaling (RGS) proteins was suspected in these phenomena and a hunt to identify these proteins began. The RGS proteins function as guanosine triphosphate (GTP)–activating proteins (GAPs) for Gα subunits, accelerating the inactivation of Gα-GTP. RGS may also block signaling by acting as effector antagonists. Expression of RGS proteins seems to be hematopoietic-lineage specific and cell-maturation dependent.



Different levels of regulation of CXCR4 function on hematopoietic cells. First, expression of CXCR4 is regulated at the transcriptional level by several factors (eg, hypoxia). Second, CXCR4 as well as its ligand SDF-1 are subject to proteolytic degradation by several proteases that are expressed in the hematopoietic microenvironment and serum. Third, CXCR4 after interaction with SDF-1 is internalized from the surface and is recirculated from the endosomal compartment at different rates. Fourth, functionality of the CXCR4 receptor depends on its incorporation into membrane lipid rafts, and several signals from other membrane receptors or integrins may increase the incorporation of CXCR4 into membrane lipid rafts, increasing its signaling.[4] Finally, as demonstrated by Berthebaud et al, CXCR4 is the subject of negative regulation by RGS16. It is possible that this mechanism plays a role in heterologous desensitization or negative cross-talk of CXCR4 after stimulation of MKs by other chemokines (eg, macrophage inflammatory protein 1β [MIP-1β] or interleukin-8 [IL-8]).

An RGS protein that modulates the chemotactic responsiveness of MKs to SDF-1 has been identified in an elegant study by Berthebaud and colleagues. Using several complementary strategies, they identified RGS16 as responsible for this effect. They found RGS16 to be up-regulated during MK maturation and differentiation. Overexpressing RGS16 mRNA in the megakaryocytic MO7e cell line inhibited SDF-1–induced migration. On the other hand, knocking-down RGS16 mRNA via lentiviral-mediated RNA interference increased CXCR4 signaling both in MO7e cells and primary MKs. Based on this, Berthebaud et al postulate that RGS16 regulation is a mechanism that controls MK chemotaxis to an SDF-1 gradient and MK developmental migration within the BM microenvironment. RGS16 inhibits SDF-1–mediated phosphorylation of mitogen-activated protein kinase p42/44 (MAPKp42/44) and protein kinase B (AKT) in MO7e cells. Surprisingly, however, RGS16 does not seem to influence colony-forming unit (CFU)–MK growth, MK adhesion to either fibronectin or collagen I, and proplatelet formation.

Thus, a multilevel model of regulation of the CXCR4–SDF-1 axis on MKs and other hematopoietic cells emerges from this and other studies, and, as is shown in the figure, RGS16 emerges as a pivotal negative regulator of CXCR4 signaling in MKs.

Such regulation of CXCR4 signaling in MKs by RGS16 raises further questions. First, it would be important to identify factors/cytokines that directly modulate the expression of RGS16 in MKs. Second, since CXCR4 is also highly expressed on platelets,