findings suggest a relationship among dye efflux capacity, repopulating capacity, and stem cell homing. Whether these stem cell functions are related to bcrp-1 (Zhou et al., 2001), a transporter responsible for the SP phenotype in HSCs and possibly other adult stem cells, poses a challenging question for stem cell biology.

Matsuzaki et al. have not only shown us how to isolate pure HSCs, which should aid studies of cell fate decision and stem cell regulation. They have provided important clues to understanding stem cell homing and the HSC niche.

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IL-6 *trans*-Signaling: The Heat Is On

The molecular consequence of the fever response has been illuminated by a recent study showing that a temperature shift to 40°C resulted in increased leukocyte adhesion to tissue sections, which was mediated by L-selectin activation in lymphocytes. This L-selectin activation during heat responses was dependent on IL-6 *trans*-signaling via the soluble IL-6R.

Febrile viral infections such as influenza cause significant morbidity, mortality, and economic disruption worldwide, particularly in the elderly and in patients with underlying chronic medical conditions, including pulmonary diseases and diabetes mellitus. In spite of vaccination, a very recent report showed that 7% of U.S. deaths are still attributable to pneumonia and influenza infections (CDC, 2003). Anyone who has experienced a seasonal flu might have wondered about the reason behind the febrile response of our body. Fever responses occur in the course of viral and bacterial infections and as a consequence of tumor growth. Although the fever response is evolutionarily conserved, it is the least understood component of the acute inflammatory response. This may have changed with the work of Sharon Evans and colleagues, which is published in this issue of *Immunity* (Chen et al., 2004).

The authors show that the adhesion of leukocytes to tissue sections via the L-selectin homing receptor is increased when the temperature is raised from 37°C to 40°C. The activation of L-selectin is dependent on the cytoplasmic portion of the L-selectin protein, pointing to an intracellular signaling mechanism. Surprisingly, the authors demonstrate that L-selectin activation is mediated through stimulation of the cytokine receptor gp130 by interleukin-6 (IL-6) in combination with its soluble (sIL-6R) rather than its membrane-bound receptor. Thus IL-6-driven immune responses mediated by the soluble IL-6R emerge as a molecular consequence of the febrile reaction in the human body.

gp130 is the common receptor subunit for the cytokines IL-6, IL-11, IL-27, CNTF, CT-1, CLC, LIF, and OSM. On target cells, IL-6 first binds to the membrane-bound IL-6R. The complex of IL-6 and membrane-bound IL-6R associates with gp130, inducing gp130 dimerization and the initiation of intracellular signaling. gp130 is expressed by all cells in the body, whereas the membrane-bound IL-6R is mainly expressed by hepatocytes, monocytes/macrophages, and some lymphocytes (Taga and Kishimoto, 1997). Interestingly, IL-6 has been found to use an alternative pathway to activate target cells lacking the membrane-bound IL-6 receptor via a naturally occurring soluble form of the IL-6R (sIL-6R). The sIL-6R has been found in various body fluids and is generated by two independent mechanisms: limited proteolysis of the membrane protein and translation from an alternatively spliced mRNA. The sIL-6R together with IL-6 stimulates cells that only express gp130, a process that has been named IL-6 *trans*-signaling (Figure 1A) (Jones and Rose-John, 2002). Importantly, it has been shown that the soluble form of gp130, which is also found in various body fluids including plasma, exclusively inhibits IL-6/sIL-6R *trans*-signaling responses, whereas the stimulation via the membrane-bound IL-6R (classic IL-6 signaling) is unaffected (Figure 1B). This selectivity is due to the fact that IL-6 alone does not bind to membrane-

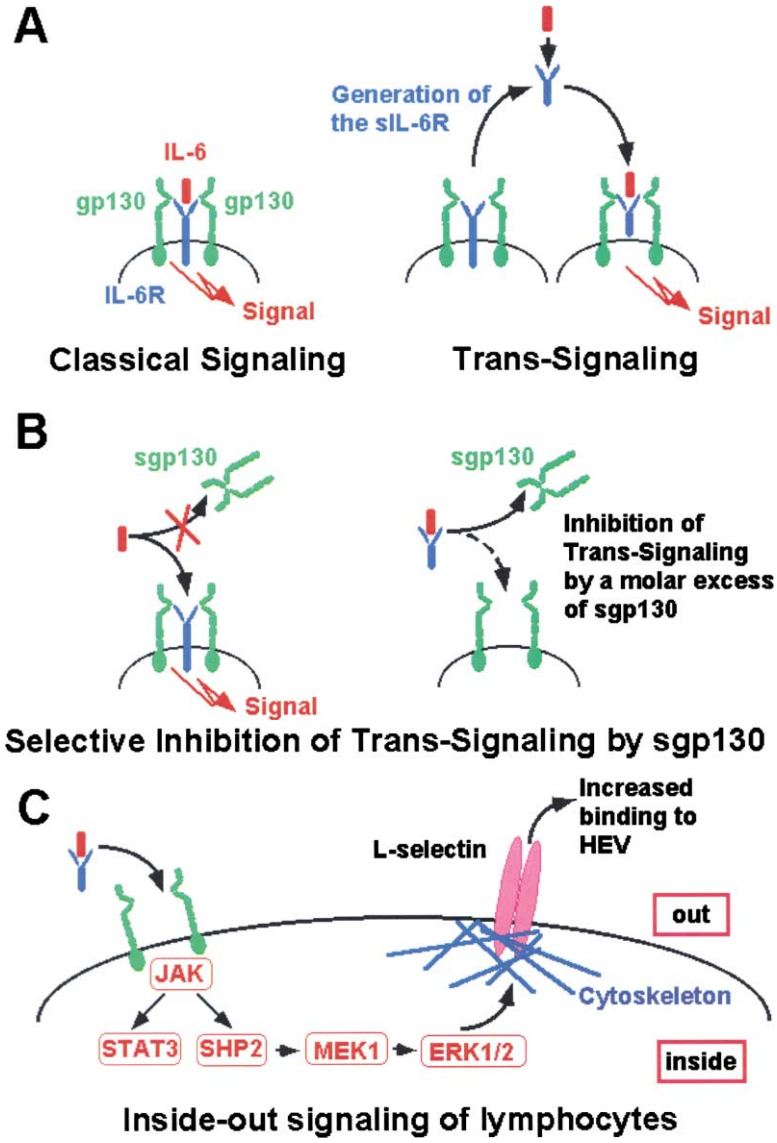

Figure 1. IL-6R *trans*-Signaling and Its Inhibition by sgp130 in Lymphocytes

(A) Classical signaling: IL-6 (red) binds to the membrane-bound IL-6R (blue). Consequently, the IL-6/IL-6R complex binds to gp130 (green), inducing dimerization and initiation of signaling. *trans*-signaling: limited proteolysis and/or alternative splicing lead to the generation of sIL-6R, which binds IL-6. The IL-6/sIL-6R complex can stimulate cells, which only express gp130 but no IL-6R. In the absence of sIL-6R, such cells would not be able to respond to the cytokine IL-6.

(B) IL-6 binds to the membrane-bound IL-6R but not to sgp130. Therefore, sgp130 does not block classical signaling. Because the IL-6/sIL-6R complex binds equally well to membrane-bound and soluble gp130, a molar excess of sgp130 completely blocks *trans*-signaling responses.

(C) Activation of gp130 in lymphocytes leads to activation of JAK kinases resulting in gp130 phosphorylation on tyrosine residues. This leads to STAT3 recruitment and activation, which is involved in antiapoptotic responses via bcl-2 and bcl-xL induction. Activation of the MEK-1 and ERK1/2 pathway leads to enhanced interaction of the cytoplasmic portion of L-selectin and components of the cytoskeleton. As a consequence, enhanced L-selectin-mediated adhesion to high endothelial venules (HEV) can be detected.

bound or soluble gp130, whereas the IL-6/sIL-6R complex binds with equal affinity to both forms of gp130 (Figure 1B). Therefore, sgp130 is the natural inhibitor of IL-6/sIL-6R *trans*-signaling responses and can be used as a molecular tool to discriminate between classic signaling and *trans*-signaling (Jostock et al., 2001).

This remarkable feature of the sgp130 protein was exploited by Chen et al. in their study on IL-6 signaling in heat responses. They establish that multiple lymphocyte subsets within human peripheral blood are responsive to thermal stress, resulting in cell adhesion. This process is mediated by L-selectin, because it can be completely blocked by a neutralizing L-selectin antibody. In contrast to lymphocytes, $CD14^{+}$ monocytes do not adhere after heat stress, which is in line with the failure of these cells to extravasate across lymph node high endothelial venules. Because conditioned media from thermally activated human peripheral blood leukocytes (PBL) also induced L-selectin-mediated adhesion, a soluble factor(s) such as cytokines secreted by these cells was considered likely to be responsible for the effect. Indeed, the authors identified IL-6 as the cytokine involved in L-selectin activation, demonstrating that neutralizing antibodies to IL-6 and IL-6 receptor subunits inhibit thermal activation of L-selectin adhesion. Moreover, the authors demonstrated that activation of MEK1 and ERK1-2 kinases in the gp130-signaling pathway lies upstream of activation of L-selectin/cytoskeleton interactions and enhancement of L-selectin avidity/affinity.

Intriguingly, recombinant sgp130 blocked temperature-induced L-selectin adhesion of lymphocytes in vitro as well as in vivo, indicating that gp130 activation occurred via *trans*-signaling. This was to be expected, because most lymphocytes do not express the membrane-bound IL-6R. Therefore, the authors could conclude that thermal activation of L-selectin adhesion was mediated by IL-6/sIL-6R *trans*-signaling (Figure 1C). The proposed scenario of *trans*-signaling during febrile responses is described in Figure 5 of the report by Chen et al. (2004).

What is so important about *trans*-signaling? First, there are certainly therapeutic implications for specific

blockade of IL-6 signaling in vivo. Recent phase I/II clinical trials have shown that a neutralizing humanized anti-IL-6R monoclonal antibody (MRA) is beneficial for the treatment of rheumatoid arthritis, Castleman's disease, and potentially Crohn's disease (Ding and Jones, 2003; Ito, 2003). Moreover, using recombinant sgp130 protein, animal studies have revealed that the progression of chronic inflammatory diseases like Crohn's disease, peritonitis, and rheumatoid arthritis depends on IL-6/sIL-6R *trans*-signaling (Atreya et al., 2000; Hurst et al., 2001; Nowell et al., 2003), at least partially due to gp130's capacity to block IL-6-dependent STAT3 and bcl-xL activation in T cells and thus to induce T cell apoptosis. Therefore, it can be anticipated that the sgp130 protein can be used as a more specific therapeutic agent to treat these chronic diseases as compared to a neutralizing IL-6R mAb, because it does not interfere with classic IL-6 signaling via the membrane-bound IL-6R, which is important, for example, for the hepatic acute phase response and for B cell stimulation. On the other hand, IL-6R *trans*-signaling responses seem to occur during stress responses including chronic inflammatory diseases, liver regeneration, cardiac stress situations, and as now shown by Chen et al., during febrile responses. Therefore, it will be interesting to analyze whether additional as yet unidentified stress situations or other inflammatory processes also depend on the *trans*-signaling mechanism.

Several open questions remain. The authors provide some evidence that lymphocytes are the most likely cellular source of IL-6. It is, however, unclear where the sIL-6R, which is found at nanomolar levels in the blood, comes from. It is known that inflammatory situations lead to the presence of large amounts of C-reactive protein (CRP) in the blood (Jones and Rose-John, 2002). This protein has been shown to induce the generation of sIL-6R via a limited proteolysis mechanism that can involve neutrophils and macrophages, making these cells likely candidates for a source of sIL-6R. Furthermore, the authors show that supernatants from heat-treated lymphocytes show no measurable (by ELISA) increase in concentrations of IL-6, sIL-6R, and sgp130. There is, however, a detectable increase in the IL-6 bioactivity as measured by the sensitive cellular B9 bioassay. Possible explanations would be that IL-6 binding protein(s) exist that block the binding of mAbs used in commercial ELISA systems. Alternatively, serum factors might enhance the IL-6 bioactivity in a cooperative fashion. Such apparent masking of IL-6 detectability has been reported, but the molecular mechanism has not yet been elucidated (see Sehgal, 1996, reference in Chen et al., 2004).

Chen et al. show that in mice, temperature induced L-selectin activation can be completely blocked by a neutralizing anti-IL-6 mAb as well as by recombinant sgp130, indicating that IL-6 is the only cytokine involved in this reaction. In IL-$6^{-/-}$ mice, however, L-selectin activation is controlled by OSM, LIF, and IL-11, as demonstrated by neutralizing mAbs, pointing to a compensatory mechanism by these gp130 cytokines. It is noteworthy that in other *trans*-signaling models like the peritonitis and rheumatoid arthritis models (Hurst et al., 2001; Nowell et al., 2003), no such compensatory mechanisms have been found. This might indicate that the febrile response during infections is of particular importance. This is underlined by the fact that fever has been conserved for millions of years in very diverse groups of animals.

Chen et al. demonstrate that gp130-mediated Erk1/2 stimulation leads to L-selectin activation and that the COOH terminal 11 amino acids of L-selectin are necessary for this effect. It is, however, unclear whether phosphorylation of L-selectin and/or components of the cytoskeleton occurs during this process (Figure 1C). Therefore, the molecular mechanism of the inside-out signaling from gp130 stimulation to L-selectin activation will have to be elucidated in more detail. Furthermore, it is so far unclear, whether—as a consequence of Erk1/2 signaling—the affinity, the subcellular localization, or the oligmerization of L-selectin is affected.

The described unexpected connection between the heat danger signal and IL-6R *trans*-signaling makes it likely that more examples of this unusual mechanism will be reported in the future and that the story of IL-6 *trans*-signaling is not completed yet: The heat is on.

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