

tissues (CCL19, CCL20, CCL21, and CCL25) and plasma (CCL3, CCL4, and CXCL12). The physiologic significance of these observations was demonstrated by T-lymphocyte infiltration in lymph nodes, gut and skin lymphoid tissue. T-cell cycling, a known outcome of IL-7 administration, was noted in these same tissues prior to detection of cycling T cells in the peripheral blood.

This work is important for many reasons: (1) it provides us with data supporting tissue redistribution as the explanation of lymphopenia observed in recombinant human (rh) IL-7 clinical trials; (2) it shows potential mechanisms that could account for some of the side effects observed in preclinical and clinical studies of IL-7, specifically skin rashes, diarrhea, and possibly the elevation of liver enzymes (however, no liver biopsies were shown); (3) it gives insight into the homing of T cells in response to homeostatic cytokine signals that are relevant in both normal and lymphopenic conditions; (4) it shows that IL-7 induces T-cell cycling in lymph nodes, skin and gut, suggesting that the T-cell expansions seen in rhIL-7-treated subjects occur at the tissue level and are not due to redistribution; and (5) it suggests mechanisms that could explain lack of response to endogenous or exogenous IL-7 such as destruction of tissue or lymph node architecture or disruption of chemokine receptor-chemokine interactions. Finally, the study also highlights our shortcomings in assessing total body lymphocytes by demonstrating how peripheral blood T lymphocyte observations may not be representative and may even be misleading in disease states characterized by altered levels of cytokines, chemokines, and chemokine receptor expression.

Some questions remain: Why didn't CCR7 (an important molecule for homing to lymph nodes) increase on T cells? Why were there no increases of CD3⁺ cells in lymph node biopsies at 24 hours despite demonstrable increases in Ki67 expression? Why were there significant differences in chemokine receptor expression between CD4 and CD8 T cells despite identical disappearance and recovery rates from the circulation? Nevertheless, this type of detailed work with frequent peripheral blood and tissue sampling would not be possible in a clinical study. Although the authors contrast their observations to the IL-2 effects, suggesting that apoptosis explained the lymphopenia induced by IL-2, one could argue

that similar trafficking phenomena may also have occurred in IL-2-treated subjects, in addition to the enhanced apoptosis that followed the observed lymphopenia.⁴

Better understanding of the mechanisms of action of cytokines can help interpret clinical observations, improve future clinical study designs, ameliorate concerns about lymphopenia or other transient side effects, and further elucidate the role of cytokines in normal T-cell homeostasis and lymphopenia. Phase I clinical studies of rhIL-7 have shown significant expansion of both CD4 and CD8 T-cell subsets, suggesting a potential role for rhIL-7 in treatment of lymphopenic diseases such as HIV infection.^{3,5} After the recent failure of IL-2 to show any clinical benefit in large phase III clinical trials in HIV infection^{6,7} despite significant CD4 T-cell increases, it will be essential to demonstrate that cytokine-induced T-cell expansions in peripheral blood reflect a normal T-cell tissue distribution and function with a diverse T-cell repertoire.

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● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Park et al, page 860

Efferocytosis: another function of uPAR

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uPAR, the receptor for urokinase plasminogen activator, is a regulator of the uptake by macrophages of apoptotic neutrophils (efferocytosis). Its role and mechanism appear to be complex and possibly controversial.

The urokinase plasminogen activator was originally thought to function primarily by concentrating urokinase-dependent proteolytic activity on the surface of cells, hence, increasing the potential of cells to move and migrate through barriers. Over the years, however, it has been firmly established that uPAR is also a signaling receptor, albeit missing an intracellular domain, therefore needing to interact with other extracellular/transmembrane proteins to activate signaling pathways. The development of uPAR Ko mice did not move the field forward initially because the mice appeared normal. However, a subsequent series of phenotypes have been reported on closer study, showing that uPAR is required in vivo for the homeostasis of a wide variety of

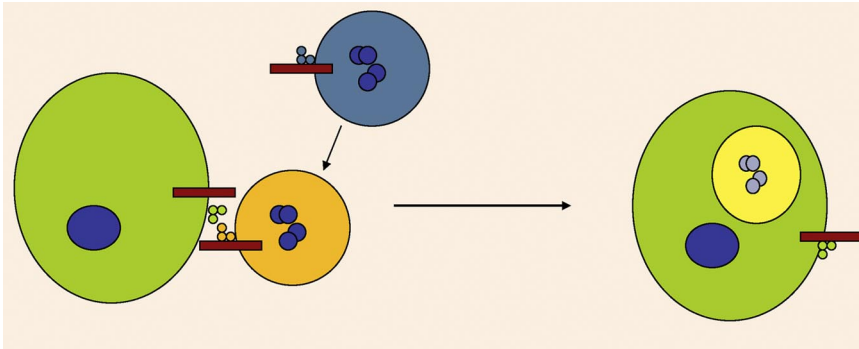
Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Beq S, Rozlan S, Gautier D, et al. Injection of glycosylated recombinant simian IL-7 provokes rapid and massive T-cell homing in rhesus macaques. *Blood*. 2009;114:816-825.
2. Sportes C, Hakim FT, Memon SA, et al. Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. *J Exp Med*. 2008;205:1701-1714.
3. Sereti I, Dunham RM, Spritzler J, et al. IL-7 administration drives T cell cycle entry and expansion in HIV-1 infection. *Blood*. 2009;113:6304-6314.
4. Sereti I, Herpin B, Metcalf JA, et al. CD4 T cell expansions are associated with increased apoptosis rates of T lymphocytes during IL-2 cycles in HIV infected patients. *AIDS*. 2001;15:1765-1775.
5. Levy Y, Lacarabatz C, Weiss L, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J Clin Invest*. 2009;119:997-1007.
6. Lusso M, Abrams D, INSIGHT ESPRIT Study Group. Effect of IL-2 on clinical outcomes in patients with a CD4+ cell count of 300/mm³. Primary results of the ESPRIT study. Paper presented at: Conference on Retroviruses and Opportunistic Infections; February 10, 2009; Montreal, Canada.
7. Levy Y, SILCAAT Sci Committee. Effect of IL-2 on clinical outcomes in patients with a CD4+ cell count 50-299 cells/mm³. Primary results of the SILCAAT study. Paper presented at: Conference on Retroviruses and Opportunistic Infections; February 10, 2009; Montreal, Canada.

cells including hematopoietic stem cells, osteoblasts, osteoclasts, macrophages, and others. uPAR Ko mice are deficient in a series of important functions (inflammation, bone homeostasis, kidney and hematopoietic stem cells mobilization and homing).¹⁻⁴ Some of these have been linked to human pathology.

uPAR is an adhesion receptor. It directly binds with high affinity to the extracellular matrix component, vitronectin, and this appears to be essential for uPAR dimerization and signaling.⁵⁻⁷ A direct interaction between uPAR and different integrins has been suggested by many publications; however, in our opinion, while there is no doubt of a functional interaction, there is no real evidence that the link is direct.



Scheme showing a neutrophil (on top) undergoing apoptosis (the color change indicates the apoptosis), and encountering a macrophage (left). Both express uPAR (3 little spheres of the same color of the cytoplasm) and integrins (red bars). Through the interaction of these 2 proteins the neutrophil is efferocytosed into the macrophage (on the right). The scheme does not necessarily imply a direct uPAR-integrin interaction.

Clearance of apoptotic neutrophils by macrophages (efferocytosis) is an important mechanism regulating inflammation, host responses, and cancer. Clearance of dead cells is important to avoid unwanted inflammatory responses. In this issue, Park and colleagues show that uPAR modulates neutrophils efferocytosis exploiting macrophages and neutrophils isolated from uPAR^{Ko} mice.⁸ Indeed, uPAR^{-/-} macrophages show increased engulfing activity of viable (uPAR^{+/+}) neutrophils both in vivo and in vitro. A similar increase in neutrophil uptake is observed when using uPAR^{-/-} neutrophils and uPAR^{+/+} macrophages, but not when both cell types are uPAR-negative. The data suggest a mechanism different from the “eat me” or “don’t eat me.”⁹ Interestingly, administration of exogenous suPAR (a soluble version of uPAR) reverses both phenotypes. Indeed, suPAR inhibits the increased uptake of uPAR^{+/+} neutrophils by uPAR^{-/-} macrophages as well as the increased uptake of uPAR^{-/-} neutrophils by uPAR^{+/+} macrophages. The authors’ interpretation is that suPAR modulates the adhesion of neutrophils/macrophages through direct interactions with integrins both in *cis* and *trans*. Remarkably, the positive effect of unilateral uPAR deficiency on neutrophil uptake by macrophages seems to require the nonredundant functions of a large number of integrins including α M, α V, β 1, β 2, and β 3 on both the neutrophil and the macrophage, as well as the LDL receptor-related protein, LRP. A direct effect of exogenous suPAR on the activity of Mac1 was previously described.¹⁰

The complexity of the uPAR/efferocytosis relationship is further underscored by another paper published almost at the same time.¹¹ D’Mello et al show that uPAR overexpression

in nonprofessional phagocytes stimulates efferocytosis of apoptotic cells by cancer cells, specifically. While in this work the cells used are not professional phagocytes, the result is nevertheless opposite that of Park et al. Moreover, in this effect, a direct role of integrins seems to be excluded. It is important to realize that uPAR overexpression is a frequent event in cancer cells and that its presence may induce an environment more favorable for cancer invasion by regulating the infiltration and clearance of inflammatory cells.

Although the precise mechanism underlying uPAR function in efferocytosis still remains to be elucidated, the data from these papers clearly point to an unrecognized role in heterotypic cell-to-cell adhesion.

The role of the uPAR ligand, uPA, was not addressed in either of the 2 papers. However, contact between apoptotic cells and macrophages induces IL10, which is required for efferocytosis. In turn, IL10 induction requires the activity (and the tyrosine phosphorylation) of a transcription factor, Prep1.¹² Prep1 (at that time called UEF3) was discovered years ago as a transcription factor binding to the enhancer region of the uPA gene in a region

that serves to modulate the response to various proliferation (and other) stimuli.¹³ Is it possible that there is a connection between Prep1-dependent uPA expression, uPAR, and efferocytosis?

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REFERENCES

- Wei C, Möller CC, Altintas MM, et al. Modification of kidney barrier function by the urokinase receptor. *Nat Med*. 2008;14:55-63.
- Furlan F, Galbati C, Jorgensen NR, et al. Role of urokinase plasminogen activator receptor in bone homeostasis. *J Bone Min Res*. 2007;22:1387-1396.
- Tjwa M, Sidenius N, Moura R, et al. Membrane-anchored uPAR regulates the proliferation, marrow pool size, engraftment, and mobilization of hematopoietic stem/progenitor cells. *J Clin Invest*. 2009;119:1008-1018.
- May AE, Kanse SM, Lund LR, et al. Urokinase receptor (CD87) regulates leukocyte recruitment via beta 2 integrins in vivo. *J Exp Med*. 1998;188:1029-1037.
- Wei Y, Waltz DA, Rao N, et al. Identification of the urokinase receptor as an adhesion receptor for vitronectin. *J Biol Chem*. 1994;269:32380-32388.
- Madsen CD, Ferraris GM, Andolfo A, et al. uPAR-induced cell adhesion and migration: vitronectin provides the key. *J Cell Biol*. 2007;177:927-939.
- Caiolfa V, Zamai M, Malengo G, et al. Monomer-dimer dynamics and distribution of GPI-anchored uPAR are determined by cell surface protein assemblies. *J Cell Biol*. 2007;179:1067-1082.
- Park Y-J, Liu G, Tsuruta Y, Lorne E, Abraham E. Participation of the urokinase receptor in neutrophil efferocytosis. *Blood*. 2009;114:860-870.
- Henson PM, Tuder RM. Apoptosis in the lung: induction, clearance and detection. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:L601-611.
- Simon DI, Rao NK, Xu H, et al. Mac-1 (CD11b/CD18) and the urokinase receptor (CD87) form a functional unit on monocytic cells. *Blood*. 1996;88:3185-3194.
- D’Mello V, Singh S, Wu Y, Birge RB. The urokinase plasminogen activator receptor (uPAR) promotes efferocytosis of apoptotic cells. *J Biol Chem*. 2009;284:17030-17038.
- Chung EY, et al. Interleukin-10 expression in macrophages during phagocytosis of apoptotic cells is mediated by homeodomain proteins Pbx1 and Prep-1. *Immunity*. 2007;27:952-964.
- Berthelsen J, Vandekerckhove J, Blasi F. Purification and characterization of UEF3, a novel factor involved in the regulation of the urokinase and other controlled promoters. *J Biol Chem*. 1996;271:3822-3830.

● ● ● THROMBOSIS & HEMOSTASIS

Comment on Qadura et al, page 871

Plasma-derived and recombinant FVIII

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While plasma-derived and recombinant coagulation FVIII may largely share the same amino acid sequence and restore coagulation equally well, Qadura and colleagues demonstrate in this issue of *Blood* that these molecules appear quite different to the immune system.