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HTLV tax tails

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lymphoid progenitor (CLP) phenotype or a pre-pro-B phenotype, whereas rIL-7 primarily induced expansion of later stage pro-B and pre-B subsets. Furthermore, transfer of marrow cultured with the hybrid cytokine into irradiated hosts generated significantly increased spleen colony-forming units (CFU-S₁₂) compared with results using control marrow or marrow cultured with rIL-7 alone. The numeric advantage of the hybrid's effects persists throughout B-cell development since larger numbers of mature B cells were also present 3 weeks following transfer in mice that received marrows cultured with the hybrid cytokine compared with controls or those receiving marrow cultured with rIL-7 alone. Mechanistically, the data suggest that the hybrid cytokine signals through both the high-affinity IL-7R (IL-7R α and γ c) and c-Met and that the pairing of the chains leads to IL-7R and c-Met aggregation and capping on the surface of stimulated cells.

This work provides a novel insight into yet another mechanism by which cytokines can mediate specificity in vivo. In this case, postsecretion processing leads to selective pairing of genetically unrelated molecules, rendering the resultant signal distinct and greater than the sum of its parts. Whether HGF β or IL-7 is generally promiscuous and can associate with multiple partners in other settings is yet to be determined. Certainly the previous studies demonstrating synergy between HGF and other cytokines raise the question of whether HGF might also pair with other hematopoietic

cytokines to mediate its diverse effects on marrow progenitors. It seems plausible that other cytokines might also use the trick of entering into a low-energy association in specific microenvironments with different partners to mediate their own unique biology. ■

REFERENCES

- Zarnegar R, Michalopoulos GK. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. *J Cell Biol.* 1995;129:1177-1180.
- Matsuda-Hashii Y, Takai K, Ohta H, et al. Hepatocyte growth factor plays roles in the induction and autocrine maintenance of bone marrow stromal cell IL-11, SDF-1 alpha, and stem cell factor. *Exp Hematol.* 2004;32:955-961.
- Kmiciek TE, Keller JR, Rosen E, Vande Woude GF. Hepatocyte growth factor is a synergistic factor for the growth of hematopoietic progenitor cells. *Blood.* 1992;80:2454-2457.
- Weimar IS, Miranda N, Muller EJ, et al. Hepatocyte growth factor/scatter factor (HGF/SF) is produced by human bone marrow stromal cells and promotes proliferation, adhesion and survival of human hematopoietic progenitor cells (CD34+). *Exp Hematol.* 1998;26:885-894.
- Nishino T, Hisha H, Nishino N, Adachi M, Ikehara S. Hepatocyte growth factor as a hematopoietic regulator. *Blood.* 1995;85:3093-3100.
- McKenna SD, Chen F, Lai L, Goldschneider I. Identification of an IL-7-associated pre-pro-B cell growth-stimulating factor (PPBSF), I: production of the non-IL-7 component by bone marrow stromal cells from IL-7 gene-deleted mice. *J Immunol.* 1998;160:2272-2279.
- Lai L, Chen F, McKenna S, Goldschneider I. Identification of an IL-7-associated pre-pro-B cell growth-stimulating factor (PPBSF), II: PPBSF is a covalently linked heterodimer of IL-7 and a Mr 30,000 cofactor. *J Immunol.* 1998;160:2280-2286.
- Lai L, Goldschneider I. Cutting edge: identification of a hybrid cytokine consisting of IL-7 and the beta-chain of the hepatocyte growth factor/scatter factor. *J Immunol.* 2001;167:3550-3554.

HTLV-2 are close relatives, with about 70% sequence similarity, but only HTLV-1 is firmly associated with adult T-cell leukemia or inflammatory neurologic diseases. The Tax1 protein—but not Tax2—contains a PBM at its C-terminus that binds to proteins that contain PDZ domains.²⁻⁴ Of interest, the E6 oncoproteins from high-risk human papilloma virus (HPV) strains contain a PBM, whereas those from low-risk isolates do not. The adenovirus 9 (Ad9) ORF1 oncoprotein also contains a PBM, and HTLV-1 Tax, HPV18 E6, and Ad9 ORF1 proteins all associate with a PDZ domain in the human Dlg protein. Yeast 2-hybrid screens with Tax1 as bait have also recovered other PDZ-containing proteins. The hDlg protein is of interest here because it forms a complex with adenomatous polyposis coli protein (APC), which negatively regulates cell cycle progression. The APC gene is mutated in colon cancers and the mutated gene products generally lack a PBM. Moreover, the PBMs of Tax1, HPV E6, and Ad9 ORF1 interact with the same PDZ domain on Dlg as APC, suggesting that they may disrupt the APC-Dlg complex and deregulate cell cycle progression.

The paper by Xie and colleagues is the first to examine how the Tax1 PBM affects the course of virus infection in primary human T cells, the natural target for HTLV-1 replication. Previous studies on viral PBMs and Dlg have been done in nonlymphoid cells, including work from Fujii's laboratory (Hirata et al⁵), which showed that Tax1 PBM was necessary for efficient transformation of Rat 1 fibroblasts in vitro. Xie et al have now done experiments with wild-type and mutant HTLV-1 and HTLV-2 viruses in primary T cells and in a rabbit model system. Deletion of the Tax PBM in HTLV-1 rendered the mutant virus unable to establish persistent infections in rabbits. Although important, this result does not provide mechanistic insights because we do not know whether the mutation affects cell proliferation, cell survival, immune response, or virus spread. The really interesting studies here addressed Tax1 PBM function by infecting primary lymphocytes and monitoring cell growth and survival. These experiments indicated that the Tax1 PBM contributes to an early boost in cell proliferation, reminiscent of the increased cell proliferation early after HPV31 infection of human keratinocytes. The HTLV-1 experiments are challenging on many levels, because of limitations in the cell

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Comment on Xie et al, page 1980

HTLV tax tails

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Xie and colleagues examine the contribution to viral pathogenesis of an important protein-protein interaction motif in the transforming protein of HTLV-1.

Human T-cell leukemia viruses encode a protein called Tax that is the consummate adaptor protein. Interactions between Tax and a variety of protein partners activate transcription of virus and cellular genes, redirect signal transduction pathways, and disable cell cycle regulators. A transforming protein in vitro and in vivo, Tax sets the stage for multistep carcinogenesis by increasing cell proliferation and diminishing

DNA repair processes.¹ Several years ago, a new protein interaction motif was identified in HTLV-1 Tax (Tax1) that is shared with oncoproteins from DNA tumor viruses.²⁻⁴ In this issue of *Blood*, Xie and colleagues begin to unravel the actions of this PDZ-binding motif (PBM) in HTLV-1-infected T cells.

There are compelling reasons to believe that the PBM is important. HTLV-1 and

culture and animal models and because Tax is a multifunctional protein and the effects of the PBM are subtle.

The convergence of HTLV-1 with DNA tumor viruses at the level of oncoproteins that target the same PDZ-containing cellular factors is intriguing. There are many compelling reasons to believe that the Tax1 PBM contributes to HTLV-1 pathogenesis and the preliminary data are consistent with this notion, but there is much work to do. The studies described here illustrate how future work should proceed—that is, by examining primary T cells infected with recombinant HTLV. There is a need to characterize the functions of Dlg and other PDZ domain-containing proteins in T lymphocytes so that we can better understand the consequences of Tax1 association. In these ways, we may soon discover which of the many PDZ-containing proteins is the real target of Tax1 and why it is necessary for the virus to interact with it. Furthermore, we would

like to know how HTLV-2 establishes a life-long persistent infection without this function; after all, why does HTLV-2 not cause leukemia? ■

REFERENCES

1. Yoshida M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol*. 2001;19:475-496.
2. Lee SS, Weiss RS, Javier RT. Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of *Drosophila* discs large tumor suppressor protein. *Proc Natl Acad Sci U S A*. 1997;94:6670-6675.
3. Rousset R, Fabre S, Desbois C, Bantignies F, Jalinet, P. The C-terminus of the HTLV-1 Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. *Oncogene*. 1998;16:643-654.
4. Suzuki T, Ohsugi Y, Uchida-Toita M, Akiyama T, Yoshida M. Tax oncoprotein of HTLV-1 binds to the human homologue of *Drosophila* discs large tumor suppressor protein, hDLG, and perturbs its function in cell growth control. *Oncogene*. 1999;18:5967-5972.
5. Hirata A, Higuchi M, Niinuma A, et al. PDZ domain-binding motif of human T-cell leukemia virus type 1 Tax oncoprotein augments the transforming activity in a rat fibroblast cell line. *Virology*. 2004;318:327-336.

formed *ex vivo*, there is a high likelihood that components of the host immune system will be activated by the viral vector, the transgene product, or both.

Of the currently available viral vectors, adeno-associated virus (AAV) has proved the least immunogenic option. Studies of the cellular response to AAV transduction have indicated that this process is far less disruptive than adenoviral transduction,¹ and indeed, there appears to be little, if any, activation of innate immunity following *in vivo* AAV delivery. Nevertheless, there is an adaptive immune response to AAV vectors that is likely to have an adverse influence on both the transduction efficiency and the long-term persistence of transduced cells following AAV gene transfer. This consequence has been demonstrated recently in a clinical trial of AAV2-mediated gene transfer to the liver in hemophilia B. In this study, one patient achieved factor IX levels of 12% within 2 weeks of gene transfer only to experience a transient episode of hepatotoxicity and loss of transgene expression that has been presumed to be related to preexisting cellular immunity to AAV capsid proteins.² Unfortunately, this outcome was not predicted by preclinical studies in large-animal models of hemophilia³ in which long-term expression of the therapeutic transgene has now been documented in the absence of any preexisting AAV immunity.⁴

Scallan and colleagues have now explored the influence of AAV immunity on gene transfer efficacy in a novel mouse model described in this issue of *Blood* (see figure). In this model of passive immunity, using pooled human immunoglobulins infused into SCID or NOD-SCID mice, the authors have been able to demonstrate a greater than expected inhibition of AAV-mediated gene transfer than previously suggested from *in vitro* neutralization assays. This inhibitory effect was most apparent with AAV2 and less evident with pseudotypes AAV6 and AAV8. The use of NOD-SCID mice in this study has also shown that there is an adverse influence of innate immunity on the efficacy of gene transfer with AAV vectors.

In addition to its description of the mouse model of AAV immunity, this article also confirms that a significant number of adults have preexisting neutralizing antibodies (and presumably cellular immunity) to AAV2 (62%).

● ● ● GENE THERAPY

Comment on Scallan et al, page 1810

AAV immunity: more than meets the eye

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Gene transfer with viral vectors is compromised by preexisting host immunity. A novel passive immunity mouse model now provides evidence that anti-AAV humoral responses may be more limiting than previously recognized.

In the pursuit of successful strategies for gene transfer approaches to treat inherited diseases, challenges from the host immune response continue to represent a major obstacle. Until there has been a significant im-

provement in the ability of nonviral protocols to deliver transgenes, most gene transfer studies will continue to use various forms of viral vectors to effect gene delivery. With this in mind, unless the gene transfer protocol is per-



Passive AAV immunity model.