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TARGETED DISRUPTION OF THE FLT3 LIGAND GENE IN MICE AFFECTS MULTIPLE HEMATOPOIETIC LINEAGES, INCLUDING NATURAL KILLER CELLS, B LYMPHOCYTES, AND DENDRITIC CELLS. H.J. McKenna*, R.E. Miller*, K. Brasel*, E. Maraskovsky,* C. Maliszewski*, B. Pulendran*, D. Lynch*, M. Teepe*, E.R. Roux*, J. Smith*, D.E. Williams, S.D. Lyman*, J.J. Peschon*, K. Stocking*. Immunex Corporation, Seattle, WA.

Flt3 ligand (flt3L) is a hematopoietic growth factor that has effects on early events in the development of a number of blood cell lineages, including B lymphocytes and myeloid cells. Flt3L is primarily expressed as a cell membrane protein that can give rise to a soluble protein *in vivo*. We have generated mice lacking flt3L using homologous recombination. The resulting flt3L -/- animals are viable but have multiple hematopoietic lineages affected. Total cellularity in hematopoietic tissues is reduced compared to strain, age and sex matched control mice (flt3L +/+); bone marrow cellularity is 70% of normal, splenocyte numbers are 54% of normal, and circulating WBC numbers are 32% of normal. The hematocrit levels are unaffected, and blood smears show that the ratio of lymphocytes, monocytes and granulocytes is comparable to that of the controls. There is a lack of NK1.1^{high} cells in the spleen, suggesting an absence of natural killer cells in the flt3L -/- mice. Overnight treatment of flt3L -/- mice with poly-IC, or rhuIL-15, or 3 days of treatment with rhuIL-15, did not induce activation of NK cells able to lyse YAC targets *in vitro*, whereas good levels of lytic activity were noted in the flt3L +/+ mice following each of these treatments. Within the bone marrow, there is a 5-fold reduction in the number of immature B cells ($B220^+$, IgM^-), and the number of clonogenic B cell precursors responsive to IL-7 or combinations of IL-7 plus flt3L or Steel Factor (SLF) are reduced 15-50-fold. However flt3L -/- and flt3L +/+ animals immunized with TNP-KLH in alum gave equivalent titers of TNP-specific IgM, IgA and IgG antibodies after both primary and secondary challenges, and equivalent numbers of germinal centers were noted in the spleen after secondary challenge. Within the spleen of flt3L -/- animals the number of $CD11c^+$ dendritic cells is reduced to 37% of normal. However, on a per cell basis, the dendritic cells are not affected in their ability to stimulate the proliferation of allo-reactive T cells. In summary, mice deficient in flt3L, though viable, are deficient in splenic NK cells, and have reduced numbers of immature B lymphocytes and mature dendritic cells, suggesting an important role for flt3L in the development of these three hematopoietic lineages.

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IDENTIFICATION OF A NEW SIGNAL TRANSDUCTION COMPLEX ASSOCIATED WITH C-KIT AND ACTIVATED BY STEEL FACTOR. M. Sattler*, G. Shrikhande*, S. Verma*, E. Pisick*, K. Prasad*, R. Salgia, and J.D. Griffin. Dana-Farber Cancer Institute, Boston, MA.

Steel factor (SF) is a growth and survival factor for hematopoietic cells. The receptor for SF, c-kit, contains intrinsic tyrosine kinase activity, and binding of SF induces rapid tyrosine phosphorylation of several cellular proteins, including c-kit itself. We found that activation of c-kit induces tyrosine phosphorylation of an unusual adapter protein, CRKL, which has one SH2 domain and two SH3 domains. Further, CRKL coprecipitated with c-kit through an interaction which required the CRKL SH3 domains and not the SH2 domain. A third major protein in this complex was identified as the p85 regulatory subunit of phosphatidylinositol-3' kinase (p85PI3K). Previous studies have shown that the SH2 domain of p85PI3K binds to c-kit at Y719 (murine sequence) after receptor activation, and that mutation of this tyrosine causes defects in adhesion and induction of early response genes. Since p85PI3K contains two proline rich motifs, we asked whether the interaction between c-kit and CRKL-SH3 was mediated through binding to p85PI3K. Far-Western blotting with a CRKL-SH3 GST fusion protein showed that CRKL can bind directly to p85PI3K *in vitro*. However, although CRKL was pre-associated with p85PI3K, the interaction was increased after SF stimulation, suggesting that the interactions of these 3 proteins are complex. Since the binding of CRKL to this complex is mediated by SH3 interactions, the CRKL-SH2 domain is available to recruit other signaling proteins, such as p120CBL, into the complex. We conclude that the signaling pathway of c-kit involving PI3K involves CRKL and predict that CRKL will recruit additional signaling molecules into this complex. Signaling molecules recruited by CRKL may well be involved in propagating signals involving PI3K such as viability and adhesion.

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A MUTATION IN THE JAK KINASE JH2 DOMAIN HYPERACTIVATES *DROSOPHILA* AND MAMMALIAN JAK-STAT PATHWAYS. H. Luo,* P. Rose,* D.L. Barber,* W.P. Hanratty,* S. Lee,* T.M. Roberts,* A.D. D'Andrea, C.R Dearolf.* Developmental Genetics Group, Cancer Biology Section, Joint Cancer for Radiation Therapy, Division of Cellular and Molecular Biology, Department of Pathology, Division of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

The Jak (Janus) family of nonreceptor tyrosine kinases plays a critical role in cytokine signal transduction pathways. Members of this family include the mammalian Jak kinases and the *Drosophila* Jak kinase, Hop. The dominant Hop^{Tum-l} mutation in the Hop Jak kinase causes leukemia-like and other developmental defects in *Drosophila*. We and others have predicted that the Hop^{Tum-l} protein is a hyperactive kinase, although this had not been proven. In the work described here, we report on the new dominant mutation Hop^{T42}, which causes abnormalities that are similar to but more extreme than Hop^{Tum-l}. We determined that Hop^{T42} contains a glutamic acid to lysine substitution at amino acid residue 695 (E695K). This residue occurs in the JH2 (kinase-like) domain, and is conserved among all Jak family members. We determined that Hop^{Tum-l} and Hop^{T42} both hyperphosphorylated and hyperactivated the Drosophila Stat protein, D-Stat, when overexpressed in *Drosophila* cells. Moreover, we found that the Hop^{T42} phenotype was partially rescued by a reduction of wild type D-Stat activity. Finally, generation of the corresponding E695K mutation in murine Jak2 resulted in increased autophosphorylation and increased activation of Stat5 in COS cells. These results demonstrate that the mutant Hop proteins do indeed have increased tyrosine kinase activity, that the mutations hyperactivate the Hop - D-Stat pathway, and that *Drosophila* is a relevant system for the functional dissection of mammalian Jak-Stat pathways. Finally, we propose a model for the role of Hop and Hop - D-Stat pathway in *Drosophila* hematopoiesis.

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ANTI-LFA-1 BLOCKING ANTIBODIES PREVENT MOBILIZATION OF HEMATOPOIETIC PROGENITOR CELLS INDUCED BY INTERLEUKIN-8. I.F.M. Pruijt,* C.G. Figdor,* Y. van Kooyk,* R. Willemze and W.E. Fibbe. Lab of Exp Hematology, Dept of Hematology, Univ Hospital Leiden, Dept of Tumor Immunology, Univ Hospital Nijmegen, The Netherlands.

Previously, we have demonstrated that Interleukin-8 (IL-8) induces rapid (15-30 minutes) mobilization of hematopoietic progenitor cells (HPC) with colony forming, radioprotective and lympho-myeloid repopulating ability (Blood 85: 2269, 1995). Since β_1- and β_2-integrins are essential for adhesion and transendothelial migration of HPC, we studied the involvement of LFA-1 in IL-8-induced mobilization. Balb/c mice (age 8-12 weeks, weight 20-25 gram) were treated with an intraperitoneal injection (i.p.) of 100 μg anti-LFA-1 (H154.163). Following a single injection of anti-LFA-1, no mobilization of colony forming cells (CFU-GM) was observed within 24 hours. These mice showed a consistent thrombocytopenia (anti-LFA-1 296 $\pm$ 65, saline 749 $\pm$ 115x10^9/ml, mean $\pm$ SD, $p < 0.01$); no effect on the white blood cell count was observed. Mice were then pretreated with 100μg of anti-LFA-1 or saline and subsequently injected with 30 μg of IL-8 i.p., administered 20 min. prior to harvesting peripheral blood. Pretreatment with anti-LFA-1 completely prevented IL-8-induced mobilization of HPC (anti-LFA-1 + IL-8 42 $\pm$ 28, n=18 v. saline + IL-8 590 $\pm$ 771 CFU-GM/ml blood, n=17; mean $\pm$ SD, $p<0.001$). In contrast, anti-LFA-1 pretreatment did not influence IL-1 (1 μg i.p. at -360 min.) induced mobilization of HPC (anti-LFA-1 + IL-1 679, saline + IL-1 600 CFU-GM/ml blood, mean, n=2). Addition of anti-LFA-1 antibodies to colony cultures in semisolid medium had no inhibitory activity. Since LFA-1 is reported to be expressed on more differentiated HPC, it was considered that the IL-8-induced mobilization of more primitive HPC was not blocked by anti-LFA-1. Transplantation of 5x10^5 blood-derived mononuclear cells (MNC) from IL-8 mobilized animals pretreated with anti-LFA-1 or saline controls protected 25 and 19% of lethally irradiated recipient mice respectively. In contrast, the survival of recipients transplanted with (5x10^5) MNC derived from IL-8 mobilized animals was 86% (n=20 per group in two experiments, $p<0.01$). We conclude that anti-LFA-1 antibodies completely prevent the rapid mobilization of colony forming cells and cells with radioprotective capacity induced by IL-8. These results indicate a major role for the β_2-integrin LFA-1 in the IL-8 induced-mobilization of HPC.