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CRC protocol write-up
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The role of XLF in murine hematopoietic stem cells in age-dependent progressive primary immunodeficiency

A. Study purpose and rationale.

The purpose of the study is to elucidate the role of XLF protein in murine hematopoietic stem cell function and how this may contribute to the progressive primary immunodeficiency (ID) seen in XLF-deficiency. XLF deficiency is one of the primary immunodeficiencies involving the non-homologous end joining (NHEJ) pathway of DNA repair (1-3). The type of immunodeficiency in patients with XLF deficiency is unique in that it is progressive and age-dependent, diagnosed only after 5 years of age, while most others are symptomatic in the first year of life (4). The mechanism of this unique immunodeficiency is yet unknown.

Studies in XLF deficient mice have shown similar age-dependent progressive lymphocytopenia, but interestingly peripheral lymphocytes in these mice have normal V(D)J recombination, which is the hallmark of other primary IDs caused by mutations in the 6 other proteins involved in NHEJ repair pathway (5). Another interesting aspect of XLF deficiency is the age-dependent myeloid expansion and occasional myeloproliferative disease (4, 5). Aging hematopoietic stem cells (HSCs) show these two characteristics as well- impairment of lymphopoiesis and increased myeolopoiesis in an age-dependent fashion (6,7). Thus, a process that prematurely ages the HSCs could result in such a phenotype. As XLF is involved in a DNA double stranded break repair mechanism, it is possible that deficiency of this protein in HSCs leads to premature aging of these cells in a cell-autonomous manner due to defective DNA repair and accumulation of double strand breaks.

B. Study design and statistical analysis:

Bone marrow hematopoietic stem cells (HSCs) from XLF-deficient mice and their wild-type littermates will be transplanted into irradiated recipients along with supporting

competitor total bone marrow cells from a wild-type mouse expressing a different CD45 allele. Donor HSCs express the CD45.2 allele, recipients express the CD45.1 allele, while competitor cells express both CD45.1 and CD45.2 alleles, which can be easily differentiated in FACS analysis using allele specific antibodies. HSCs will be isolated from the bone marrow, first by lineage depleting the total bone marrow cells using antibodies against mature T-, B-, myeloid cells and erythrocytes, and then by cell sorting of an enriched population defined by the immunophenotype of lineage$^-$ Sca1$^+$cKit$^+$CD150$^+$CD48$^-$, which has been showed to have 47% stem cell activity *in vivo* (8). Recipient mice will be lethally irradiated with total of 12Gy (2 x 6 Gy) prior to the transplantation. HSC from each donor mouse with the competitor total bone marrow cells will be transplanted into 3 recipients. At least 6 donors per group will be used for transplantation, with total of 18 recipients per group.

Short-term and long-term reconstitution of different lineages will be analyzed at 2, 6 and 12 months after transplantation. Peripheral blood from the tail vein will be collected from the recipient mice, and analyzed for lymphoid and myeloid cells within the mononuclear cell fraction using cell specific antibodies, including anti-B220 for B-cells, anti-Thy1 for T-cells, anti-NK1 for NK cells and anti-Mac1/anti-Gr1 for myeloid cells. In addition to these markers the mononuclear cells will be stained for CD45.1 and CD45.2 alleles to differentiate the origin of each cell coming from the donor, the competitor or the recipient mouse. Although lethal irradiation does eradicate almost all hematopoietic cells from the recipient mice, it is well known that some memory T cells and NK cells are radioresistent and should be identified in the analysis. Within each cell type, the ratio of percent contribution form donor to competitor cells will be calculated from the FACS analysis. The log of that ratio will be used in statistical calculations, to equally distribute both increasing and decreasing differences between groups.

Using power calculation for unpaired t-test, 18 mice per group should be sufficient to detect an effect between the two groups equal to the standard deviation observed in the wild-type mice:

$$N = 1 + 16\,(\text{st dev} / \text{effect})^2 = 1 + 16\,(0.5/0.5) = 17$$

Unpaired t-test will be used to compare log (donor/competitor) values in lymphoid and myeloid cells between wild-type and XLF-deficient HSC transplantations, with p-value < 0.025 considered as significant, given the two variables tested: decreased lymphoid and increased myeloid reconstitution.

C. Study procedure:

$Lineage^{-}Sca1^{+}cKit^{+}CD150^{+}CD48^{-}$ cells (HSPCs= hematopoietic stem and progenitor cells) from total bone marrow of XLF-deficient mice and their wild-type littermates will be collected using cell sorting. For each transplantation 1000 HSPCs (wild-type or XLF-deficient, $CD45.2^{+}$) will be mixed with $2x10^{6}$ total bone marrow cells from competitor wild-type mouse ($CD45.1^{+}CD45.2^{+}$) containing about equal number of functional HSCs (500-600 HSCs), and injected via tail-vain into 1 lethally irradiated mouse. Each donor will be used to transplant 3 recipient mice. One competitor mouse will be used per littermate pair of wild-type and XLF-deficient mouse. Experiments will be done using young 2-3-month-old donor mice, 6-8-week-old recipients which will be purchased from Jackson Laboratories.

Sections D- Q:

Not applicable.

R. Radiation:

Small animal irradiator, Atomic Energy of Canada Gammacell 40 Cesium Unit with a dose rate of 1 Gy/min, will be used to irradiate recipient mice at total of 12Gy, with two rounds of 6 Gy each, 2 hours apart.

References:

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