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TRANSPLANTABLE MURINE NATURAL KILLER CELL PROGENITORS: IDENTIFICATION AND ENRICHMENT. Moore TA*, Bennett M, Kumar V*. U.T. Southwestern Medical Center, Dallas, Texas, U.S.A.

Little is understood about the earliest stages of natural killer (NK) cell differentiation in bone marrow or the lineage relationship of immature NK cells to other, more well defined lineages of the hematopoietic system.

By sensitivity to antibody + C' depletion, we have previously identified a transplantable NK progenitor population to be heat stable antigen (HSA)+ NK1.1- 3A4-. To measure NK progenitors, recipient mice are treated with anti-NK1.1 mAb to deplete endogenous mature NK cells, lethally irradiated, then injected with syngeneic bone marrow cells. Ten to 14 days later, regeneration of mature NK cell function is assessed by measuring the clearance of injected radiolabelled YAC-1 tumor targets from the lungs.

Using well documented flow cytometric procedures, we have enriched this progenitor from within the blast size population of bone marrow and with the surface phenotype Ly-6 (Sca-1)+ Lin (B220, Gr-1, CD2, CD3, NK1.1)-. This population comprises approximately 0.5-1.0% of total bmc.

Since this population is also greatly enriched for pluripotent stem cells as well as other hematopoietic lineage progenitor cells, we are currently attempting to further subdivide this population in order to identify a cell phenotype capable of generating exclusively NK cells upon in vivo transfer. Antigens to be examined include HSA, Fall-3, and CZ-1.

Finally, since NK progenitors are unresponsive to IL-2 in vitro, we are also examining the cellular and/or cytokine interactions which drive progenitors to differentiate into IL-2 responsive NK precursor cells, which then generate mature NK cells.

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IN VITRO EVIDENCES OF T CELL COMMITMENT IN HUMAN BONE MARROW. Schmitt C*, Dalloul AH, Arock M, Debré P*, Mossalayi MD. Department of Molecular Immunohematology, Pitié-Salpêtrière Hospital, Paris, France.

Human thymus is necessary for normal development of T cell compartment. However, the question remains to whether T cell commitment occurs intrathymically, or that prothymocytes are yet developed in the bone marrow. To approach this question, we have analysed phenotypic and functional capacities of thymic and bone marrow CD2⁻ precursors by taking advantage of CD7 and CD34 expression. Three subsets were analysed: CD7⁺CD34⁻, CD7⁺CD34⁺ and CD7⁻CD34⁻. Our data show that in the bone marrow CD7⁺CD34⁻ cells were not able to form T cell colonies in a prothymocyte-specific assay while CD7⁺CD34⁺ cells contain 1/35-53 prothymocytes. By contrast to both subsets, CD7⁻CD34⁻ cells were not able to generate myeloid colonies (< 1/100) but had high T cell differentiation potential (1/2-6). Intrathymically, CD7⁺ is expressed by early T cell compartment (subcortex) while CD34 expression is absent or very marginal in this tissue. Thymocytes analysis shows that CD7⁺CD2⁻CD34⁻ (pro T) constitute 0.2-0.4% of total thymocytes while CD7⁺CD34⁺ and CD7⁻CD34⁺ cells are very rare in the thymus (>.01%). As marrow derived counterpart, early CD7⁺CD2⁻ thymocytes show proT cell frequencies of 1/3-6 while myeloid clones did not surpass 1/100. Therefore, thymic CD7⁺ cells are generally committed to T cell lineage with rare exception. In the bone marrow, CD7 prothymocytes are present and seems to be derived from more immature CD34⁺CD7⁺ precursors possessing both myeloid and lymphoid potential. As CD7⁺CD2⁻CD34⁻ cells were previously detected in the upper thorax region of human fetus just before thymus genesis. We therefore suggest that commitment to T cell lineage already occurs in human bone marrow.

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RECOVERY OF MURINE LYMPHOPOIESIS FOLLOWING IN VIVO ABLATION WITH ANTI-L3T4 ANTIBODY. KuKuruga M, Scheduling S, Media JE, Nakeff, A. Division of Hematology and Oncology, Wayne State University, School of Medicine, Detroit, MI, USA

We have utilized multivariate flow cytometry to investigate the recovery of spleen lymphocytes following the in vivo depletion of the L3T4 (murine equivalent to human CD4) subset in mice injected with anti-L3T4 ascites. BDF1 mice received a single i.p. injection of 0.2 ml GK1.5 (anti-L3T4) ascites. L3T4 subsets were ablated from about 30% of total spleen cells to undetectable levels within 3 hrs and remained so for 2 weeks. Since L3T4 cells comprise ~70% of all T-cells, we were surprised to observe that the latter did not differ in number from control values at 3 hrs and at no later time did they decrease more than 15%. No changes in splenic weight, cellularity or the LYT2 subset were detected that could account for the discrepancy between total THY1.2 cells and the corresponding T-cell subsets, nor were any changes noted in the number of IgG, ASIALO-GM1, and MAC-1 cells. By the third week following the initial depletion, L3T4 cells had recovered to 10% of total spleen cells and over the next two weeks increased slowly to about 20%. LYT2 cells were elevated at 4 and 5 weeks post ablation such that the LYT2 plus L3T4 subsets added up to the number of THY1.2 cells. Our results indicate that a considerable fraction of splenic T-cells are detected within the first two weeks following in vivo ablation of the L3T4 subset that remains undetected by monoclonal markers for L3T4 and LYT2.

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ACTIVATION MARKER EXPRESSION ON THE PERIPHERAL BLOOD LYMPHOCYTES OF NORMAL VOLUNTEERS, RECIPIENTS OF INTERLEUKIN 2 AND PATIENTS UNDERGOING BONE MARROW TRANSPLANTATION.

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Dual colour immunophenotyping and cytotoxicity assays on the peripheral blood lymphocytes (PBL) of normal volunteers after culture with or without Interleukin 2 (IL2) demonstrated that the *in vitro* induction of cytotoxicity for a natural killer (NK) resistant cell line was accompanied by an increase in the number of cells positive for the antigen bound by the monoclonal antibody NK1-L16 (17 ± 8% unstimulated, 45 ± 18% IL2 stimulated, (mean ± standard deviation), p<0.008) and an increase in CD71 (transferrin receptor) expression (2 ± 1.6% unstimulated, 12 ± 9% IL2 stimulated, p=0.04). PBL from patients who have undergone bone marrow transplantation (BMT), autologous or allogeneic, also showed increased killing of the NK resistant cell line (1.2 ± 0.9% pre BMT, 9 ± 7.2% post BMT, p=0.004) which was accompanied by increased NK1-L16 binding (33.1 ± 13.8% pre, 50.3 ± 21% post BMT, p=0.063). Increased NK1-L16 binding was predominantly on CD2 positive, CD56 negative cells. After BMT there was no change in K562 killing (16.5 ± 15% pre, 16.5 ± 12.4% post) nor in the percentage of cells expressing CD71. PBL from recipients of IL2 *in vivo* showed significantly increased killing of NK sensitive (8 ± 3.9% pre, 38.5 ± 15% post IL2, p=0.03) and NK resistant (1.9 ± 1.1% pre, 11.6 ± 5% post IL2, p=0.001) targets which was associated with increased NK1-L16 (33.5 ± 14.1% pre IL2, 60 ± 20.3% post IL2, p=0.08) and CD71 binding (2.7 ± 0.8% pre IL2, 5.2 ± 2.6% post IL2, p=0.15). NK1-L16 and CD71 are markers of lymphocyte activation *in vitro*, but only NK1-L16 has been found to be a marker of activation *in vivo*.