Host Langerhans Cells (LCs) Can Be Therapeutically Manipulated in Vivo with Imiquimod (TLR7 Agonist) to Augment DLI-Mediated GVHD and GVVL Reactivity

Poster Session I

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We recently found in murine models that after MHC-matched allografting, the residual host LCs, the major dendritic cells (DCs) of the skin, survive in epidermis despite the presence of large numbers of peripheral donor T cells in the graft and conversion to the full donor DC chimerism in blood. This observation led us to hypothesize that in vivo manipulation of residual host LCs, which persist in complete donor chimeras after MHC-matched allografting, may have a central role in augmenting DLI-mediated alloimmune responses. We tested our hypothesis in two murine models of MHC-matched allografting. To manipulate T cell-LC interaction in vivo we used the Toll-like receptor 7 (TLR7) ligand imiquimod. Topical application of imiquimod is known to augment in situ maturation of the LCs and enhance their emigration from the skin to the skin-draining lymph nodes (LNs). We first tracked the in vivo fate of DLI-derived T cells after their administration to 8-week-old B6.SJL→C3.H.SW complete donor chimeras that were pretreated with vehicle or imiquimod. As DLI, we used purified donor T cells from the B6.PL-Thy1.1 mice that differ in the expression of Thy 1.1 allele. In the imiquimod-treated group, the expansion of DLI-derived Thy1.1+ T cells in LNs and spleen was significantly better than that of the vehicle-treated group. This augmented DLI-mediated GVHD response was also reflected by a higher number of DLI-derived CD8+ INF-γ secreting T cells and by an increase in donor-derived LC chimerism in the imiquimod-treated group. Next, we tested the effect of imiquimod on the GVL reactivity. Four-week-old C3.H.SW→C57BL/6 mice chimeras constructed after lethal conditioning were pretreated with imiquimod prior to DLI administration and lethal challenge with C1498 leukemia cells. Chimeras that received imiquimod and DLI had superior leukemia-free survival in comparison to animals that received DLI plus vehicle (P < .01) or imiquimod alone (P < .02). The superior leukemia-free survival in the group that received DLI plus imiquimod correlated also with faster conversion to full donor CD8+ T cell chimerism in comparison to the DLI plus vehicle group (P < .01). In both models, we have not observed any significant clinical signs of GVHD. These results indicate that imiquimod, through its action on LCs, can be used to enhance the DLI-mediated alloimmune responses including their GVL reactivity without exacerbating GVHD.

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It has been recognized that dysfunction of CB cellular immunity is in part due to the immaturity of the CB cellular immune system (Cairo, 1997). However, biological pathways and molecular mechanisms associated with the immaturity of CB cellular immunity are still poorly understood. Recently we have utilized oligonucleotide microarray to examine gene expression profile of CB vs APB Mo and have demonstrated significant differential gene expression patterns (Jiang/Cairo, 2004). In the current study, differential expressed genes and proteins were examined in Mo-derived CB vs APB DC by means of oligonucleotide microarray and proteomics. Briefly, Mo were purified and cultured for 8 days with GM-CSF, IL-4 and LPS. Oligonucleotide microarray was carried out (Affymetrix). The proteomic study was conducted by LC-MS/MS chromatography and tandem mass spectrometry. We identified gene expression patterns that were significantly lower in CB
vs APB DC including surface molecules HLA-DQA1 (4F), HLA-DR (4D), CD70, and chemokine genes IL6 (2F), IL8 (3F), CXCL1 (10F), CXCL2 (8F), immunoregulatory gene ISG20 (11F), TNFSF10 (4.5F). The proteomic results indicated several zinc finger proteins (29, 221), (2-3F) and interleukin-4 precursor (7.7F) were expressed higher in APB vs CB. In contrast, cell cycle regulators cyclin I (3F), Rh-like protein 2 (4.3F) were significantly lower in APB vs CB. We then compared CB vs APB DC antigen presentation activity to APB CD8 T cells by ELISPOT assay for interferon-γ (IFNγ) production (BD Pharmingen). Briefly, CD8 T cells (MHC HLA A2) were incubated with CB or APB DC that were loaded with GCV or untreated DC. GCV treated DC significantly reduced the ability to induce CD8 T cells to produce IFNγ compared with APB mDC (3.5F). We postulate that decreased expression of specific surface molecules and other genes and proteins resulting in lower surface protein expression in CB DC may in part be responsible for the lack of initiation of cell surface signaling events to trigger CB-DC to induce activation of CD8 T cells. Furthermore, these significantly decreased expressed genes and proteins in LPS-CB vs APB DC may also partially be responsible for differential innate and adaptive immune function and properties of CB vs. APB.

**148 EX VIVO ACTIVATED AND TRANSDUCED HUMAN T CELLS GENERATE LETHAL GVHD IN A MOUSE MODEL, AND ARE EFFICIENTLY ELIMINATED IN VIVO WITH SUICIDE GENE THERAPY**


No in vivo models exist to consistently examine the efficacy of ex vivo manipulation of human T cells (huT) on T cell function. NOD SCID 

**149 PERSISTENT MIXED CHIMERISM IN PLASMA CELLS FOLLOWING ALLOGENEIC STEM-CELL TRANSPLANTATION (SCT) IN PATIENTS WITH ACUTE LEUKEMIA IS A SURROGATE MARKER FOR RECURRENT PROLIFERATION**

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Chimerism within cellular subsets following allologenic SCT has been studied extensively, yet there is only limited data on chimerism kinetics within plasma cells (PC) and its prognostic significance. In this study we prospectively analyzed the relative ratios of recipient and donor-derived PC in pts with acute leukemia at serial time-points following SCT from a sex-matched donor in relation to SCT outcomes. Bone-marrow (BM) preparations were evaluated by a Duet combined cytogenetic/morphologic analysis system (Bioview Ltd, Israel). The system scans BM preparations and saves all cell coordinates. PC are detected and marked by their morphology. The stain is then removed and FISH for X and Y markers is applied to the same slide allowing differentiation of recipient and donor PC by their gender. The study included 50 pts following myeloablative (n = 22) or reduced-intensity conditioning (n = 28). Thirty-six pts (72%) had recipient PC detected early after SCT, constituting 0.01-1.6% of BM cells. This was often associated with low level recipient chimerism (<1%) in lymphocytes. Early detection of recipient PC was not related to donor type, conditioning regimen or acute GVHD, and had no prognostic significance. The median time to disappearance of recipient PC was 12 months. In 16 of the 50 pts with recipient PC they persisted beyond 6 months (and up to >18 months), in 10 they disappeared by this time period, 6 died before 6 months with recipient PC and 4 have insufficient follow-up. Persistence of recipient PC beyond 6 months was not associated with mixed-chimerism in other subsets at this stage. BM tests beyond 6 months are available in 30 pts of all 50 pts. The outcome of 16 pts with recipient PC persisting beyond 6 months was significantly inferior to 14 pts with no recipient PC at this stage; 8 patients in the first group relapsed compared to only 1 pt in the second. The 2-year DFS was 35% (7-62), and 91% (74-100), respectively (P = .02). Donor derived PC were detected during the course in 27 pts. The estimated median time to first detection of donor PC was 6 months (1-15). Engraftment kinetics of donor PC had no relation to SCT outcomes. In conclusion, recipient PC may persist for long durations after allologenic SCT and are relatively resistant to conditioning and to allogeneic responses. Persistence of recipient PC beyond 6 months is a surrogate marker for ineffective GVL, even in pts with GVHD, and is therefore associated with an increased risk for leukemia relapse.

**150 INTENTIONAL INDUCTION OF IMMUNE-HEMATOPOIETIC MIXED CHIMERISM AS A PLATFORM FOR EARLY CELLULAR THERAPY IN PEDiatric LEUKEMIA PATENTS AFTER ALLOGENEIC TRANSPLANTATION: ENHANCING GVL EFFECT WHILE AVOIDING GVHD**


To maximize graft-versus-leukemia (GvL) effect while minimizing transplant-related morbidity and mortality, we designed a study of allologenic PBSC CD34+ selected transplantation followed by DLI. PBSC CD34+ selection was performed by CliniMACS device. Between June 2004 and July 2005, sixteen consecutive patients (4 females and 12 males) aged between 1-12 years (median 6 years) diagnosed with AML 6, ALL 10 were conditioned with fludarabine 30 mg/m2/day × 4 days and melphalan 140 mg/m2/day × 1 day. Status at transplantation was 1st CR 10, 2nd CR 5 and 3rd CR 1. GvHD prophylaxis consisted of human GvHD, and causing the death of mice. Interestingly, Td T cells could be efficiently eliminated in vivo by treatment with GCV, meaning we could potentially control human GVHD with the suicide system.