THE ROLE OF PEYER’S PATCHES AND MESENTERIC LYMPH NODES IN ACUTE GVHD: A STUDY GUIDED BY BIOLUMINESCENCE IMAGING IN VIVO

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Acute graft versus host disease (aGVHD) represents one of the major obstacles in allogeneic bone marrow transplantation. Our aim was to precisely determine the spatiotemporal evolution of aGVHD in vivo, with special regard to the role of mucosa associated lymphatic tissues, particularly Peyer’s patches (PP), and mesenteric lymph nodes (mLN).

To visualize immune responses in living animals we developed and characterized a luciferase expressing (Luc+) transgenic mouse line (FVB/N, H-2Kq). Bioluminescence imaging (BLI) and luciferase assays on purified transgenic donor cell populations revealed luciferase expression in T, B, NK cells, granulocytes and macrophages. Recipient mice (Balb/c, H-2Kd) were total body irradiated (800rads) and subsequently transplanted with 4 x 10⁶ Luc+ spleen cells (Splec).

We detected the first bioluminescent signals in PP and mLN within 12 hours after transfer of Luc+ allogeneic Splec. Using triple-color immunofluorescence microscopy (IFM), we could show that the majority of infiltrating donor cells in PP consisted of CD4+ T-cells (>80%). During these early time points (12h-day 2), the CD4+ donor T cells were clearly restricted to parafollicular T cell areas where they proliferated, while completely sparing B cell follicles and subepithelial dome regions. On day 4 we observed a rapid emigration of donor T cells from PP, which coincided with enhanced mucosal infiltration of the small bowel, as shown by BLI.

These findings correlate well with the picture in mLN, where until day 3 after infusion donor T cells were clearly restricted to parafollicular T cell areas where they proliferated. By day 4 where up to 40% of the donor T cells were CD8+, while the cellularity remained fairly high.

Experiments with syngeneic Luc+ Splec showed preferential homing to the liver, but only transient migration to PP and mLN without signs of proliferation at these sites. After day 4 syngeneic Luc+ Splec recipients displayed signs of hematopoietic engraftment. In summary, we showed that homing and proliferation of alloreactive CD4+ donor lymphocytes in specific T cell areas of PP and mLN are crucial events in aGVHD development. FACS analysis revealed distinct changes of the activation markers and homing receptors of the alloreactive T cells. Furthermore BLI provided valuable temporal and spatial guidance for more detailed analysis by IFM and FACS of local environments and enabled us to pinpoint critical events in the induction and extension of aGVHD.

EXPANSION OF HUMAN UMBILICAL CORD BLOOD-DERIVED CD34+ STEM/PROGENITOR CELLS TO TREAT MYOCARDIAL INFARCTION

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We aimed to test the hypothesis that ex-vivo expanded human umbilical cord blood (CB)-derived Stem/Progenitor cells (S/P) can...