

PBL 16 weeks buffy coat

Flow Cytometry (week 16) [Mice were fed on Mouse Breeder Diet (5021, Labdiet) from weaning]: analysis is performed on the buffy coat obtained from heparinised blood after red blood cell lysis for the following parameters: Percentages of total T cells (CD3⁺), CD4⁺ and CD8⁺ T cells, NKT cells (CD3⁺ CD161⁺), NK cells (CD3⁻ CD161⁺), B cells (CD19⁺), Granulocytes (CD11b⁺ Gr1⁺) and Monocytes (CD11b⁺ Gr1^{dim/neg} CD161⁻) are presented relative to the total viable CD45⁺ population. Percentages of CD44^{hi} CD62L^{lo} CD4⁺ T cells and CD25⁺ regulatory T cells are presented relative to the total CD4⁺ T cell population. Percentages of effector of CD44^{hi} CD62L^{lo} CD8⁺ T cells and mature IgD⁺ B cells are presented relative to the total CD8⁺ T cell and B cell populations respectively. All samples are acquired on a BD LSR II with dead cells excluded with the use of a viability indicator. All analysis is performed using FlowJo software.

PBL 16 weeks whole blood

Peripheral blood leukocyte assay is performed at 16 weeks of age (Mice were fed on Mouse Breeder Diet (5021, Labdiet) from weaning): Non-fasted mice are terminally anaesthetised and blood is collected into EDTA coated tubes via the retro-orbital sinus. Whole blood is stained with two titrated cocktails of antibodies. Panel 1 containing CD45, TCRab, TCRgd, CD161, CD4, CD8, CD25, CD44, CD62L and KLRG1. Panel 2 containing CD45, CD19, IgD, Ly6B, Ly6G, Ly6C, CD11b and I-A/I-E. Samples are fixed and red blood cells lysed prior to acquisition on a BD LSR II flow cytometer after running automated compensation using compbeads and BD FACSDiva software. Data is analysed using FlowJo after singlet doublet discrimination, a time gate is used to exclude HTS issues and leukocytes identified with a SSC and CD45 gate. Total T cells, alpha beta T cells, CD4⁺ alpha beta T cells, CD8⁺ alpha beta T cells, gamma delta T cells, NKT cells, NK cells, B cells, monocytes, granulocytes and eosinophils are reported as percentage of leukocytes. CD4⁺ CD25⁺ regulatory alpha beta T cells, CD4⁺ CD44^{hi} CD62L^{lo} alpha beta T cells, CD4⁺ KLRG1⁺ alpha beta T cells, CD8⁺ CD44^{hi} CD62L^{lo} alpha beta T cells, CD8⁺ KLRG1⁺ alpha beta T cells,

KLRG1⁺ NK cells, IgD⁺ B cells, Ly6C^{hi} I-A/I-E^{lo} monocytes and Ly6C^{lo} I-A/I-E^{lo} monocytes are reported as percentage of parent. Using the white blood cell count obtained from the haematology analysis absolute cell counts are derived for each population and reported as cells/ul.

PBL 6 weeks whole blood

Peripheral blood leukocyte assay is performed at 6 weeks of age (Mice were fed on Mouse Breeder Diet (5021, Labdiet) from weaning): Non-fasted unanaesthetised mice are restrained and blood is collected into EDTA coated tubes via the tail. Whole blood is stained with a titrated cocktail of antibodies including CD45, TCRab, TCRgd, CD161, CD4, CD8, CD19, CD25, CD44 and CD62L. Samples are fixed and red blood cells lysed prior to acquisition on a BD LSR II flow cytometer after running automated compensation using compbeads and BD FACSDiva software. Data is analysed using FlowJo after singlet doublet discrimination, a time gate is used to exclude HTS issues and leukocytes identified with a SSC and CD45 gate. Total T cells, alpha beta T cells, CD4⁺ alpha beta T cells, CD8⁺ alpha beta T cells, gamma delta T cells, NKT cells, NK cells and B cells are reported as percentage of leukocytes. CD4⁺ CD25⁺ regulatory alpha beta T cells, CD4⁺ CD44^{hi} CD62L^{lo} alpha beta T cells and CD8⁺ CD44^{hi} CD62L^{lo} alpha beta T cells are reported as percentage of parent. Using the white blood cell count obtained from the haematology analysis absolute cell counts are derived for each population and reported as cells/ul.