3.1 The minipig is now considered as a useful alternative non-rodent species for safety testing of pharmaceuticals. Human parallels diseases in pigs and xenotransplantations, the development of the immune system in juvenile Göttingen minipigs is still unknown or was evaluated with very limited immune developmental endpoints. Characterization. Although the immune system of the adult pig has been studied, particularly in relation to different infectious toxicology studies, the development of main organs or systems, including the immune system, of the minipig still requires further protection properties, the immune protection being one of its most important functions throughout life acting as a first line of immunological defense and involving both innate and adaptive responses. The objective of this preliminary study was to identify the main epidermis and dermis immune cells (including Langerhans cells, dermal dendritic cells, CD4+ and/or CD8+ T cells and B cells) in the juvenile Göttingen minipigs using flow cytometry and immunohistofluorescence (IHF) methods. The proportions of T, B and NK cells analyzed in the dermis and epidermis were also compared with the same populations in selected lymphoid organs and in blood from the same animals. This work was performed at different ages from 3 days to 6 months after birth. In addition, a comparative analysis between healthy skin and chemically-induced inflamed skin was further investigated in 2-month and 6-month-old minipigs.

3.2 Comparative analysis of αβ- and γδ-TCR cell subsets (Figure 3): - The proportion of αβ T cells is higher in blood than in the other matrices. - The proportion of αβ T cells in the secondary lymphoid organs is higher than in the skin and lymph nodes. - Separation between macrophages and dendritic cells was difficult since these two populations share similar cell surface markers (CD14, CD163). - Inflammatory dendritic cells will also be further defined after separation from macrophages. Both cytometry analysis and IHF methods confirmed the presence of the main immune cell populations in the epidermis and dermis of healthy minipigs. There were no major differences in the proportion of immune cells across different ages. However, one of the limits of this preliminary investigation is the small number of animals analyzed on each occasion. The next study will include at least 3 males and 3 females per occasion for a more comprehensive analysis. The conventional dendritic cells (CD11c and CD83) will be further characterized using additional cell surface markers (such as MHCII and CD3). Inflammatory dendritic cells will also be further defined after separation from macrophages. In addition, cell phenotype, the immune response of the Göttingen minipig will be evaluated using an immunomodulator and a new set of animal facilities.