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Skin Immune System In The Juvenile Göttingen Minipig

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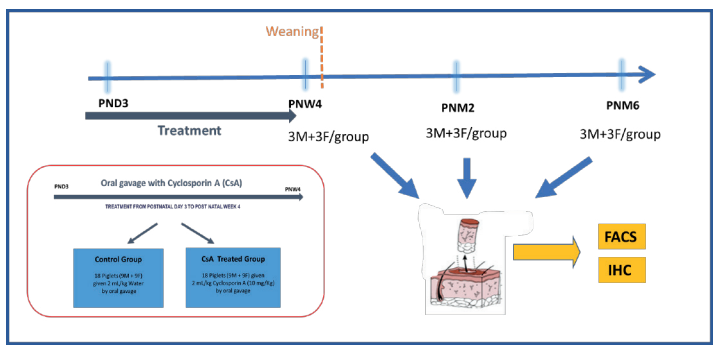
1 INTRODUCTION

Some skin diseases need to be treated early in childhood by dermal application or, for severe cases, by the systemic route using immunosuppressants, such as cyclosporin A. Safety evaluation of new pediatric medicines is performed by the conduct of toxicology studies using juvenile animals. The minipig is now considered as a useful alternative non-rodent species for safety testing of pharmaceuticals. Human parallels in many features of its anatomy, physiology and biochemistry make the minipig a good model for man. There is a real need to better understand the immune system organization and response in the Göttingen minipig to better evaluate the toxicological effect of new pharmaceuticals in development. This project specifically focused on the **skin immune system** in the Göttingen minipig from birth to the adult age.

2 MATERIALS AND METHODS



Thirty six piglets, 18 males and 18 females, were born in our animal facilities from six Gottingen sows (Ellegaard, Denmark) and were raised with their respective mothers under optimal housing conditions until weaning, i.e. 4 weeks after birth. After weaning, the animals were group-housed per gender. The study design was approved by the Animal Ethical Committee and conducted in compliance with the European Animal Welfare Guidelines in an AAALAC accredited Test Facility. Half of the piglets were given **cyclosporin A (CsA)** at the dose level of 10 mg/kg/day by gavage **from 3 days to 4 weeks of age**. The other half was given water as negative control.
On each occasion, i.e. **PND3, PND7, PNW2, PNW4, PNM2 and PNM6**, the selected animals were euthanized and necropsied. Skin biopsies and other selected tissues/matrices (not part of this poster) were collected and prepared, as follows:

- frozen for **IHC staining**,
- or immediately processed in cell suspensions for **FACS analysis** (Fortessa, BD Biosciences) and FACSDiva software (BD Biosciences). The epidermis was separated from the dermis after overnight digestion with Dispase II.

The following skin immune cell subsets were identified and counted: **T cells** ($CD45^+CD3^+$) and **γδ-T cells** ($CD45^+CD3^+$γδ-TCR^+) in both dermis and epidermis, **conventional dendritic cells** (**cDC1** population identified as $MHCII^+CD172a^{neg/low}CADM1^+$ subset; **cDC2** population identified as $MHCII^+CD172a^{high}CADM1^{neg/low}$ subset) and '**inflammatory" cells** ($MHCII^{high}CD163^+$ and $CD209^+$ subsets) in the dermis, and **Langherans cells** ($MHCII^+CD172a^+CADM1^+CD207^+$) in the epidermis.

3 RESULTS

T cells in Dermis and Epidermis

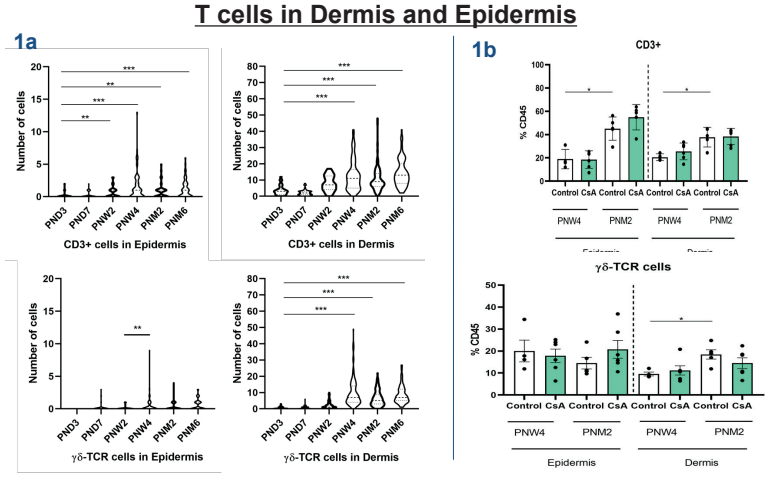


Figure 1a (IHC method):

→ *higher numbers of T cells (x3 to x9) and γδ-T cells (x5) were noted in the dermis compared to the epidermis.*

→ *T cells and γδ-T cells were greater in both dermis and epidermis of 2- or 4-week old and older minipigs than neonatal and 7-day old piglets ($p \leq 0.01$ or $p \leq 0.001$).*

Figure 1b (FACS method):

→ *higher proportions of T cells and γδ-T cells were noted in 2-month old minipigs, when compared with 4-week old minipigs, for both dermis and epidermis (T cells) or dermis only (γδ-T cells) ($p \leq 0.01$).*

→ *dermal and epidermal T cell and γδ-T cell proportions were not affected by the treatment of CsA.*

Conventional Dermal Dendritic cells and Langherans cells in Epidermis

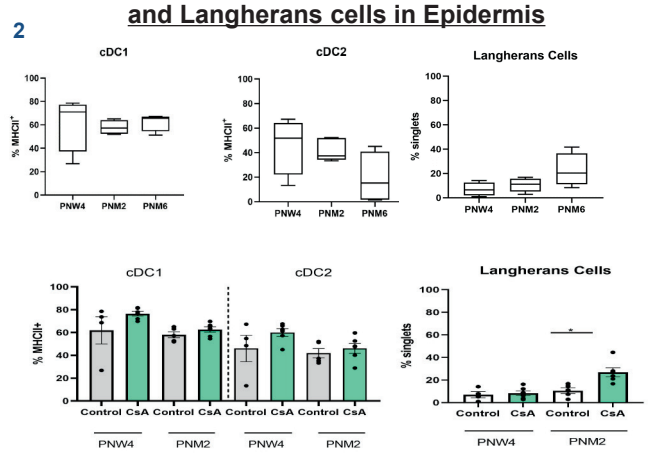


Figure 2 (FACS method):

→ *no differences in proportions of conventional dermal dendritic cells or Langherans cells were noted across age.*

→ *dermal conventional dendritic cells were not affected by the treatment of CsA.*

→ *higher ($p \leq 0.05$) percentage in Langherans cells was noted in the epidermis of 2-month old minipigs previously treated with CsA, whereas no relevant changes were seen in 4-week old minipigs treated with CsA, when compared with their respective control groups.*

Inflammatory cells in Dermis

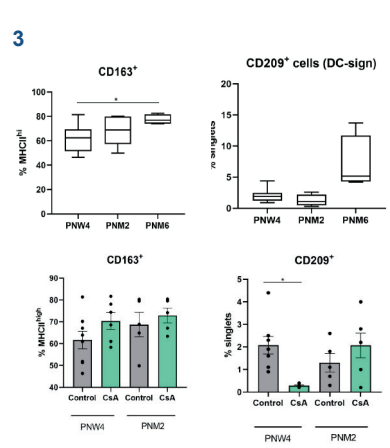


Figure 3 (FACS method):

→ *higher percentages in dermal $MHCII^{high}CD163^+$ and $CD209^+$ populations were noted in 6-month old minipigs when compared with younger animals ($p \leq 0.05$ for $MHCII^{high}CD163^+$; not statistically significant for $CD209^+$).*

→ *lower percentage in dermal CD209+ subset is noted in 4-week old minipigs treated with CsA, when compared with the control group.*

4 CONCLUSION

The above results demonstrate that the proportions of main immune cells in the dermis and epidermis of 2- or 4-week old Göttingen piglets are close to those in the adult skin, on the basis of immunohistochemistry (IHC) and cytometry (FACS) analyses. Oral gavage with cyclosporin A at the dose level of 10 mg/kg/day in juvenile minipigs was associated with a reduced percentage in dermal CD209+ cells (DC-sign) at the end of the 4-week treatment period and with a slightly increased percentage in epidermal Langherans cells four weeks after the end of the treatment period.