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Laboratory of Comparative Genomics

Host-Microbe Interactions: Addressing Genetic and Environmental Interactions Utilizing Cloned Pigs

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Abstract

Addressing host microbe interactions through immune responses in out bred species is limited by the need to use replicates to overcome genetic variance and issues associated with conducting studies in multiple environments. In order to overcome these issues, we initiated a study to determine the intrinsic immune response variance in cloned pigs during development and under various challenge conditions. Nuclear transfer cloned Duroc gilts were used in a comparison to age matched Yorkshires littlemates. To establish intrinsic immune variations, blood samples were drawn to evaluate the animals for certain immunological parameters and individual peripheral blood lymphocyte (PBL) immune responses in vitro to transmissible gastroenteritis virus (coronavirus) (TGE) or a synthetic oligonucleotide containing a CpG motif. Additionally the animals were challenged in vivo with an ascaris antigen, and the allergic response of the animals was determined by skin testing. Our results suggest that clonal quantitative variance is a function of antigenic complexity with environmental factors contributing to the qualitative differences observed.

Key words: Cloned pigs, variability, genetics, environment, TGE, CpG motif

Introduction

As shown, environmental factors not only influence gene expression, they also regulate the development of cell lineages, including those of the immune system. The development of the immune system is largely based on exposure to certain stimuli. For instance mast cells of the immune system will release histamine after previous exposure to a particular antigen. When they are exposed a second time to that same antigen, pollen for instance, the IgE antibodies bind to the Fcε receptor causing a release of histamine leading to an allergic reaction (Janeway, Travers, and Walport 2005). Previous sensitization may cause one individual to mount a response to a certain antigen, while another does not. This causes much variation between individuals when trying to asses interactions with certain stimuli or microbes.

To compare the interactions between individuals and their responses to certain microbes, has been extremely difficult in the past. Many parameters had to be controlled for in order to make direct comparisons between individuals. One would have to study the subjects in different environments, and have multiple replicates to be able to overcome genetic variation. One hypothesis to overcome or lessen this obstacle of variation would be to use cloned animals as experimental models. This would then reduce any host-microbe variability due to genetic variations, while at the same time other variables could then be controlled for (Prather R.S., Hawley R.J., et al. 2003). In order to study the relationship between host-microbe in out-bred animals remained limited to the fact that many variables had to be controlled for. With the use of cloned animals, genetic variation is reduced, while intrinsic immune response variance can be assessed. To test the variation between host-microbe interactions, different immunological parameters were assessed. To be able to establish a baseline level of intrinsic variation, cloned animals were evaluated for various blood parameters and peripheral blood lymphocyte (PBL) immune responses in vitro to TGE and CpG. In addition, pigs were challenged in vivo with ascaris antigen, and their individual allergic responses were measured. Age-matched non-genetically similar control swine were assessed using the same parameters to determine the variation of environmental influences or antigenic differences.

The objective of this study was to show that if cloned animals are used for experimental purposes, variations in immune response due to genetics will be reduced, and the minimal differences found can be attributed to the influences of extraneous variables such as the environment.

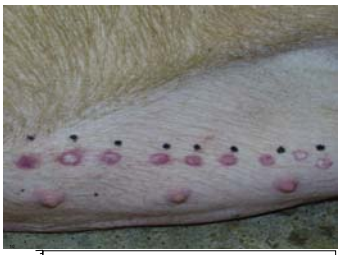
Objectives

- **To be able to reduce biological replicates in experimental research.**
- **To reduce genetic variation among experimental subjects.**
- **Measure different environments using the same sampling population.**
- **Genetic manipulation**

Materials and Methods

A. Ascaris antigen sensitization and skin testing

- An allergic response to Ascaris antigens in these pigs was induced by three bi-weekly subcutaneous injections of an extract of ascaris worms.
- The skin test consisted of 8 adjacent intradermal (ID) injections into the abdominal wall of 100 µl of four-fold serially diluted ascaris antigen extract.
- Allergic response intensity was monitored and measured using calipers at 30 min and 24 hrs.

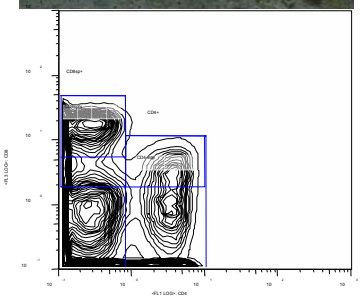


B. Cell isolation and lymphocyte proliferation assay

- **Swine peripheral blood mononuclear cells (PBMC) were isolated from fresh venous blood.**
- Mitogen stimulation assays were conducted using staphylococcal enterotoxin B (SEB 5 ug/ml; Amersham Pharmacia Biotech), and phytohaemagglutinin (PHA 10 ug/ml; Wellcome, Dartford, UK).
- PBMC Proliferation was determined at 72 hr of blank at 37 °C by a 24 hr pulse with 0.5 µCi 3[H]-thymidine.

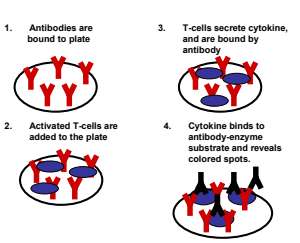
C. Flow cytometry

- **Peripheral blood CD4+CD8+, CD4+CD8 and CD4 CD8+ cells were isolated by two color FACS.**
- Cells were reacted with 10 µg/tube of anti-CD8 mAb in a 100 µl volume and later reacted with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse immuno-globulin F(ab')2 antibody.
- Cells were suspended at 10^7/ml in Flow PBS and sorted by gating on small lymphocytes based on their forward angle and 90° angle light-scatter.



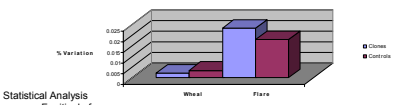
D. Detection of porcine IFN-γ and α secreting lymphocyte

- **Cellular immune responses for individual pigs were quantified using an IFN γor α ELISPOT assay.**
- PBMC from cloned or control pigs were plated at 5 x 10^5 viable cells per well (>98% viability)..
- Hydrolysis of Blue Membrane Substrate (BMS) results in the development of blue spots whose size and intensity are directly proportional to the amount of bound IFN-γ or α.



Results

A. Ascaris antigen sensitization and skin testing (clones vs. controls)



Statistical Analysis
- F-critical of 15.44
- No sig. differences found

Fig. A. Analysis of allergic variation comparing the groups clones and the controls

B. Blastogenesis (clones vs. controls)

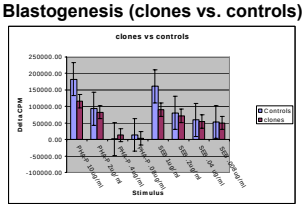


Fig B. PBL Blastogenesis analysis stimulated by PHA and SEB

C1. Phenotypic Analysis (clones vs. controls)

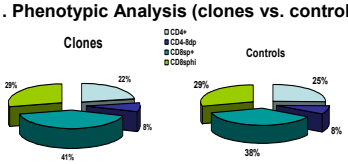


Fig. C1. Comparison of cell types present in PBL

C2. Phenotypic Analysis (indoor vs. outdoor clones)

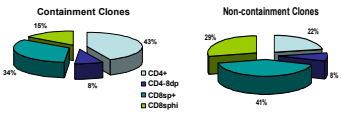


Fig. C2. Comparison of cell types present in PBL

C3. Flow Cytometry (clones vs. controls)

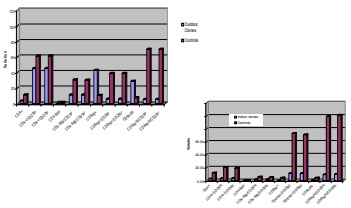
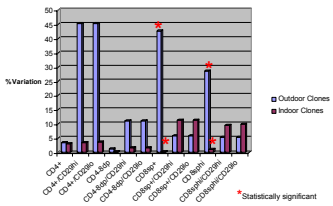


Fig. C3. Full comparison of cell types present in PBL, including CD 29.

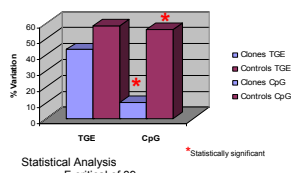
C4. Flow Cytometry (indoor vs. outdoor clones)



*Statistically significant

Fig. C4. Full comparison of cell types present in PBL, including CD 29.

D1. IFN-α stimulation (clones vs. controls)

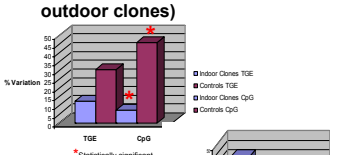


*Statistically significant

Statistical Analysis
- F-critical of 39

Fig. D1. Variations in IFN producing cells that have been stimulated with TGE or CpG

D2. IFN-α stimulation (indoor vs. outdoor clones)



*Statistically significant

Fig. D2. Variations in IFN producing cells that have been stimulated with TGE or CpG

Conclusions

A. From this analysis there was no significant difference (F-critical $_{\alpha 0.05,\ 4,4}$ is 15.44) in allergic response variation within the clones, or within the control groups.
   - demonstrating that cloned pigs respond similarly to antigenic challenges as out-bred pigs, environment has an effect on the development of allergic immune responses.

B. Statistically, there appeared to be no significant differences overall between the immune variations of the controls versus the clones as measured in the blastogenesis.

C. Environment appears to play a part in T-cell development and maturation.
   - Seen with the differences in T-cell populations between clones.

D. IFN-α producing cells appear to be highly influenced by genetics independant of environment.
   - No significant difference in variability between the cloned swine in both environmental situations.

Acknowledgements

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References

1. Janeway, Travers, Walport, and Shlomchik. Immunobiology: the immune system in health and disease. New York: Garland Science Publishing, 2005
2. Nowacki W., Cederblad B. et al. (1993) *Age-related increase of porcine natural interferon α producing cell frequency and of interferon yield per cell.* Veterinary Immunology and Immunopathology, 37:113-122
3. Zuckermann F.A., Husmann R.J. (1996) *Functional and phenotypic analysis of porcine blood CD4/CD8 double-positive T cells.* Immunology 87:500-512