

Cytokine Immune Response in Mexican Creole and Commercial Pigs Under Uncontrolled Conditions

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Abstract: The aim of the study was to measure the cytokine immune response in 2 Mexican creole biotypes and compare it to commercial pigs (COM) as disease resistance indicators. Twenty six commercial (COM), 25 Cuino (CP) and 25 Mexican hairless (MHP) pigs were vaccinated with a commercial bacterin containing *Salmonella*, *E. coli* and *Pasteurella* at 45 days of age; a sample was taken a week later to test serum levels of interleukin 1 β (IL-1 β), interleukin 4 (IL-4), interferon-gamma (INF- γ) and alfa tumor necrosis factor (TNF- α). Cytokine quantification serum tests were carried out with commercial kits using the ELISA sandwich method. The Kruskal-Wallis test was used to compare cytokine concentrations and the Wilcoxon test was run to look for differences among breeds. IL-1 β and IL-4 production was higher in HMS as compared to the other breeds. No significant differences between INF- γ and TNF- α production were seen. Results suggest that MHP have a higher response capacity before an infection, compared to the other breeds.

Key words: Cytokines, humoral immune response, creole pigs

INTRODUCTION

A high percentage of domestic breeds for human consumption are in danger of extinction. Native breeds commonly have valuable traits such as easy adaptation to difficult conditions, including tolerance to parasites and infections, drought and poor food quality (CVID, 2003; Sierra *et al.*, 2003). Therefore, there is a need to rescue and take advantage of natural genetics found in Mexican hairless pig (MHP) which could be a specimen worthy of investigation for years to come. Recently in Mexico the study of animal genetic resources have become very important, in parallel, extensive genetic variance has become equally as relevant, but so poorly characterized, even more so when regarding creole or native populations (Mariscal, 1998; Sierra *et al.*, 2005).

The Cuino pig (CP) was reported by FAO (2000) as an endangered swine species; it is not highly valued, it is believed to have been introduced into Mexico in the Conquest days and has survived through various difficult ecological conditions, including infectious and limited nutritional factors. It is a source of great biological diversity and some suppose that it has a high resistance

to disease (Flores, 1992; Lemus *et al.*, 2003). The importance of investigation on the immunological system of pigs has increased notably for this animal. However, reduction of the enormous economical loss cannot be achieved without a better understanding of the immunological system of swine (Saalmüller, 1998).

Development of an effective immune reaction includes lymphoid, inflammatory and hematopoietic cells. A group of proteins are designated together with cytokine that indicates intracellular communication, through complex interaction between cells (Margni, 1996). Cytokines are a group of chemically synthesized polypeptides that respond to different immunological stimulus. They can affect different cellular functions and are involved in the immunity and inflammatory response. These proteins regulate immune initiation, maintenance; determine the type of immune response and resistance mechanism against pathogens (Fresno *et al.*, 1997).

With the current pressure in selection for highly exploited commercial swine breeds, notable diminished genetic variety has been observed, causing undesirable genetic mutations, as well as diminishing resistance to disease (Fujii *et al.*, 1991; O'Brien *et al.*, 1993). Molecular

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studies (Martínez *et al.*, 2000, 2005; Lemus *et al.*, 2001; Canul *et al.*, 2005) show that creole pigs have greater heterozygosity, which could be deduced as a possible resistance to disease. In Mexico, as in other Latin American countries, creole swine have survived more than 500 years without systematic production or health control that leads us to believe that they have a high resistance to disease (Benitez and Sanchez, 2001; Lemus and Alonso, 2005).

MATERIALS AND METHODS

The study was ethically and methodologically approved by the Postgraduate Committee of Production Science and Animal Health, from the Animal Husbandry, Veterinary Medical Academy at the Autonomous University of Nayarit.

Seventy six pigs were monitored in the study and divided in 3 groups: 26 commercial breed (F1 Yorkshire x Landrace) (COM); 25 Cuino and 25 Mexican hairless pigs. Animals were born and raised at the university farm facilities and vaccinated with a commercial bacterin (Intervet/SAGARPA-B-0273-133) containing *Salmonella*, *Escherichia coli* and *Pasteurella*, when they reached 45 days of age in order to establish an immune reaction.

Eight days after the vaccination, 5 mL of blood were extracted from the cava vein without anti-coagulant to a vacutainer. Blood was centrifuged at 1,500 rpm for 15 min (Hermle Z160M) and serum was stored at -20°C until its analysis.

Serum cytokine quantification was carried out with commercial kits that use the ELISA sandwich technique according to the manufacturer instructions. For the IL-1β and TNF-α cytokine quantification, R and D systems' kits on undiluted serum were used; the wave length readings were 450 nm with a correction of 540 nm. For RF or rearing IL-4 quantification, a Biosource kit specifically made for swine was used on undiluted serum, wave length readings of 450 nm were observed. Lastly, INF-γ cytokine serum quantification was carried out with a kit (Pierce endogen) specifically for swine; undiluted serum was also used and meter readings were carried out with a 450 nm filter. Seven days post vaccination, another sampling was carried out in order to determine cytokine levels.

The Kruskal-Wallis test was run to compare cytokine values in the various swine breeds and the Wilcoxon test, to look for differences between distinct pairs of racial combinations. The SAS software was used in all cases.

RESULTS

Commercial (16.9730±9.160) and Cuino (17.097±14.833), showed no significant differences in IL-1β levels, whereas the MHP breed (21.848±10.275) had the highest levels (p<0.05) compared to the other 2 breeds (Fig. 1).

The MHP breed (11.804±3.491) had greater response in the production of IL-4 cytokine, whereas in the CP (8.423±4.437) and COM breeds (9±3.780) IL-4 levels were similar (Fig. 2).

No significant differences among breeds analyzed in the statistical analysis carried out for the values obtained in pg/mL of INF-γ post-vaccination for COM (13.362±10.226), CP(10.520±7.107)andMHP(7.469±4.494) breeds (Fig. 3).

Seven days post-vaccination we proceeded to determine TNF-α concentration values for the 3 swine breeds. We observed the following in the statistical analysis carried out for the values obtained in pg/mL of TNFα after the vaccination: the COM breed (59.953±24.184), MHP (66.747±23.559) and CP (66.783±20.943) (Fig. 4) showed no significant statistical differences.

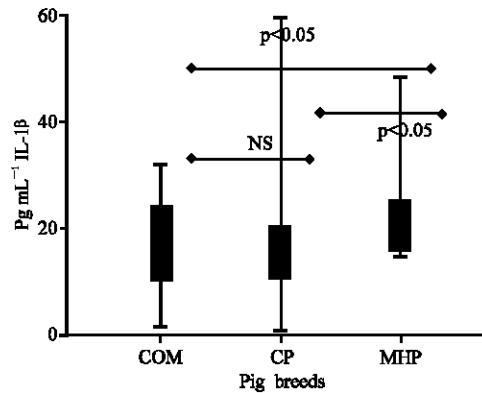


Fig. 1: IL-1β values in pg/mL at seven days post vaccination in COM (commercial), CP (Cuino pig) and MHP (Mexican hairless pig), with an absorbance of 450 nm and correction to 540 nm

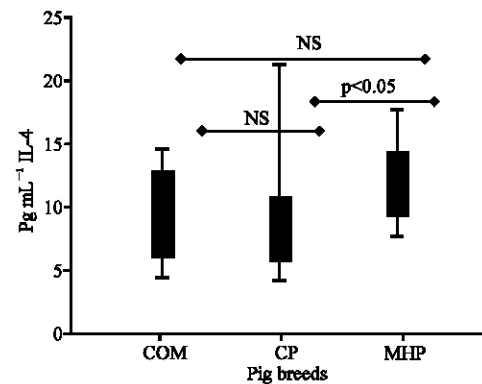


Fig. 2: IL-4 values in pg/mL at seven days post vaccination in COM (commercial), CP (Cuino pig) and MHP (Mexican hairless pig), with an absorbance of 450 nm

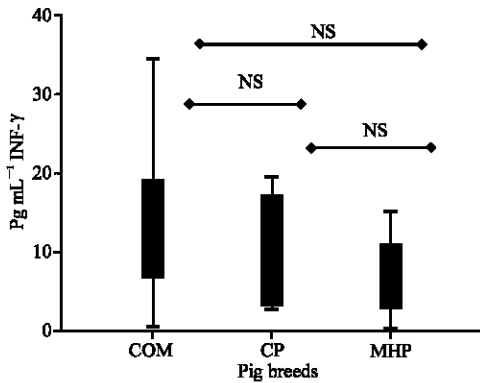


Fig. 3: INF- γ values in pg/mL at seven days post vaccination in COM (commercial pig), CP (Cuino pig) and MHP (Mexican hairless pig), with an absorbance of 450 nm

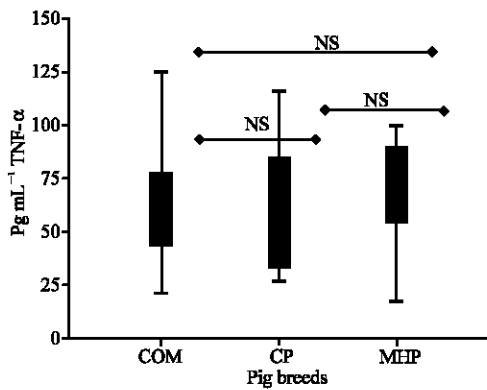


Fig. 4: TNF- α values in pg/mL at seven days post vaccination in COM (commercial), CP (Cuino pig) and MHP (Mexican hairless pig), with an absorbance of 450 nm and correction to 540 nm

DISCUSSION

Even though there are very few comparative studies between commercial and creole pigs, there is a study published by Guerrero-Quiroz *et al.* (2006) carried out in order to compare the humoral immune response through quantification of the IL-1 β levels using 12 MHP and 12 CP, using a triple vaccination with the following protocol: before weaning, after weaning, after the vaccination and during immunological maturing (28, 32, 45 and 60 days of age); authors found higher IL-1 β levels in the MHP breed, too; however, the levels reported are much higher than the ones observed in the present study.

IL-1 β plays an important role in the defense against gram-negative bacteria (Abbas and Lichtman, 2004) and the bacteria contained in the commercial vaccine that was

used; however, we cannot be sure that the cytokine levels reported are due to the vaccination, since pigs were tested in an uncontrolled environment and any stimulation (infection or contact) could raise the levels. However, the report is the same as the before mentioned study, that IL-1 β levels do exist and that the MHP breed has the highest levels. This emphasizes the need of another study with special technical handling; infecting the swine and taking samples before and after the infection would be opportune.

Huang *et al.* (1999) carried out an *in vitro* cytokine proinflammatory production in swine cells, infecting them with *Actinobacillus pleuropneumoniae* under controlled conditions, showing increases in IL-1 levels, coinciding with acute clinical illness.

Although, there are no similar reports determining IL-4 levels, Brown *et al.* (2006) carried out work evaluating the intestinal morphological characteristics and development of the swine immune system at various ages, measuring IL-2 and IL-4 levels in commercial pigs (at 7, 14 and 18 days after birth), no differences were found in said levels. It is worth mentioning that this study only measured cytokine levels in very young pigs, which could be why these results do not coincide with ours.

Just as in the IL-1 β , we cannot be sure that the IL-4 levels in the pigs of our study are due to the vaccination, since the animals were not in a controlled environment, with the possibility of non-controlled stimulation triggering cytokine, but it is worth mentioning that the MHP responded better to this type of stimulation, since higher levels were observed for this creole breed.

Since, INF- γ is a toxic cytokine in high concentrations, we did not expect its production (Fresno *et al.*, 1997). INF- γ is produced as genetic response to infection. When animals are not found sick, we would expect the cytokine levels to be normal; however, we show that in this study there was INF- γ production in all swine breeds under study and there was no difference among breeds.

Xiao *et al.* (2004) carried out a study on 4 month old commercial swine, infecting them with porcine reproductive and respiratory syndrome virus; authors observed differences between levels before and after infection, differences that could not be compared to in our study, since the pigs used were in a controlled environment free of external stimulus. Even though they did not report studies similar to this one, Huang *et al.* (1999) carried out sampling of swine cells *in vitro* before and after stimulation and observed an increase in TNF- α cytokine, concluding that the rise occurred coincidentally when the acute clinical infection manifested itself. Although no differences were observed between TNF- α

and INF levels the 3 breeds used in the study produced them. Further studies measuring cytokine values before and after vaccination with creole pigs under controlled conditions, are needed.

Swine creole population genetic variation in Mexico, Cuba as in Spain has been found to be higher than in commercial breeds, a predisposing factor that could lead to improved immunological response. Mexican creole swine are phylogenetically separated genetically from modern pigs, suggesting that this is how they survived even without systematically improved genetics (Martinez *et al.*, 2000; Lemus *et al.*, 2001). Mexico has not applied reproductive selection programs for this type of creole swine; however they have survived for more than 500 years of zoonosis problems, which leads us to believe that they have a high resistance to illness, in addition to representing a genetic reservoir to pull from in order to obtain improved national varieties (Benitez and Sanchez, 2001; Lemus *et al.*, 2001; Canul *et al.*, 2005; Sierra *et al.*, 2005).

CONCLUSION

The commercial and creole pig breeds used in this study showed cytokine levels. The MHP breed had the highest IL-1 β and IL-4 levels in comparison to the CP and COM breeds. No significant differences were found between the INF- γ and TNF- α cytokine levels in any breed under study. The MHP breed had the best humoral response in comparison to the CP and COM breeds.

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