POSTER

LEUKOCYTE ANTIGENS AS CANDIDATE GENES TO IMPROVE THE IMMUNORESPONSE IN SWINE

ANTÍGENOS LEUCOCITARIOS COMO GENES CANDIDATOS PARA MEJORAR LA RESPUESTA INMUNE EN CERDOS

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PALABRAS CLAVE ADICONALES

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SUMMARY

Leukocyte antigens (CD) have functions related to immune response and are of interest as *classical* candidate genes for health. Polymorphisms (e.g. SNPs) in these genes may be associated with variation in the immune response and consequently in disease response. This approach is being taken in search of susceptibility genes for swine disease. In addition, these genes may vary between populations, especially where specific adaptation to pathogens has occurred, and are of potential interest in characterising pig biodiversity.

RESUMEN

Los antígenos leucocitarios (CD) tienen funciones relacionadas con la respuesta inmune y son de interés con genes candidatos *clásicos* para la salud. Los Polimorfismos (ej. SNPs) en estos genes pueden estar asociados con variaciones en la respuesta inmune y consecuentemente con la respuesta a la enfermedad. Este ensayo se está desarrollando en la búsqueda de susceptibilidades genéticas a enfermedades porcinas.

Adicionalmente, estos genes pueden variar entre poblaciones, especialmente en la que han ocurrido adaptaciones a patógenos, y suponen un interés potencial para la caracterización de la biodiversidad porcina.

In the last twenty years the number of leukocyte receptors discovered in humans and domestic animals have been increasing enormously. The first International Workshop and conference on Human Leukocyte differentiation antigens (HLDA), which was held in Paris in 1982, proposed the *Cluster Differentiation* (CD) nomenclature for the leukocyte surface antigens recognized by at least two independent antibodies. The number of defined CD has been growing since then with each

workshop; 151 CD clusters in the 5th Boston workshop in 1993 and 240 in the 7th Harrogate workshop in 2000. The same nomenclature was adopted for leukocyte antigens in different animal species. In swine 38 CD clusters were described at the last workshop held in Amsterdam in 1999 (Haverson *et al*., 2001) .

Many different biological functions have been described for these receptors that include adhesion structures, B cells receptors, cytokine receptors, endothelial cells receptors, myeloid cells receptors, NK antigens, non-lineage antigens, platelet receptors and T cell receptors. All of them are of fundamental importance in the control of the immune response. In certain cases changes in these receptors have severe consequences for an individual, e.g. CD18 (®2 integrin) deficiency in humans causes a severe granulocyte disorder with susceptibility to bacterial infections and high morbidity and mortality. This deficiency causes leukocyte adhesion deficiency disease (BLAD) in the bovine specie and CLAD in the canine specie (Jeyaseelan *et al.,* 2000). In swine the complex CD11/CD18 mediates the cellular adhesion of PRV (pseudorabies virus) infected monocytes to endothelial cells and subsequently the virus transmission to endothelial cells (Van de Walle *et al*., 2003).

Recent advances in DNA technology have shown that a large repertoire of functional variants exists in CD receptor genes as is mentioned by Iida and Nakamura (2003), Iida *et al*. (2003), Mackelprang *et al*. (2002), Meller *et al*. (2001) and Tomer *et al*. (2002).

Most of the CD receptor studied are polymorphic at the cDNA level, showing single nucleotide polymorphisms (SNPs) and some of them also present variability at the protein level (coding SNPs). This polymorphism may influence CD gene expression resulting in a change in the immune response and consequently in the disease response

Studies using single nucleotide polymorphisms (SNPs) or microsatellite polymorphisms have now become technologically realistic and our group proposes to use CD gene variants in the *classical* candidate gene approach in search of susceptibility genes to disease and other related characters such as fertility in swine populations (Tsuchiya *et al*., 2002).

Our group is specialized in the study of differentiation antigens of the immune system of domestic animals, especially in swine. The first step in our goal has been to characterize functionally and phenotypically the cells by means of monoclonal antibodies from the immune system of these animals. So during the past ten years we have characterized many swine leucocytes antigens, among them CD5; CD11; CD18; CD29; MHC Class I; CD41/61; CD46; CD230 and Fibrinogen.

In a second step we have characterized the genes that controlled swine CD receptors using a general protocol presented in **figure 1**.

Following this protocol swine CD9, (accession number Genbank, AY072785) CD29, (accession number Genbank, AF192528) CD51 (accession number Genbank, pending insertion), CD61 (accession number Genbank,

AF282890) and CD97 (accession number Genbank, AF413207) have been characterized in our laboratory.

At this moment we are obtaining our first results about CD gene polymorphism. We have described (Morera *et al*., 2002) a microsatellite in the 3'-UTR region of the swine CD61. Two SNP have been detected after a partial screening of the coding and regulatory regions of the swine CD61 gene. Our group is carrying out more studies to detect variability in the coding and regulatory regions of the previously characterized CD9, CD29 and CD51 genes.

These markers may be a valuable tool to be used in case control association analyses in various swine populations, e.g. in the case of CD9

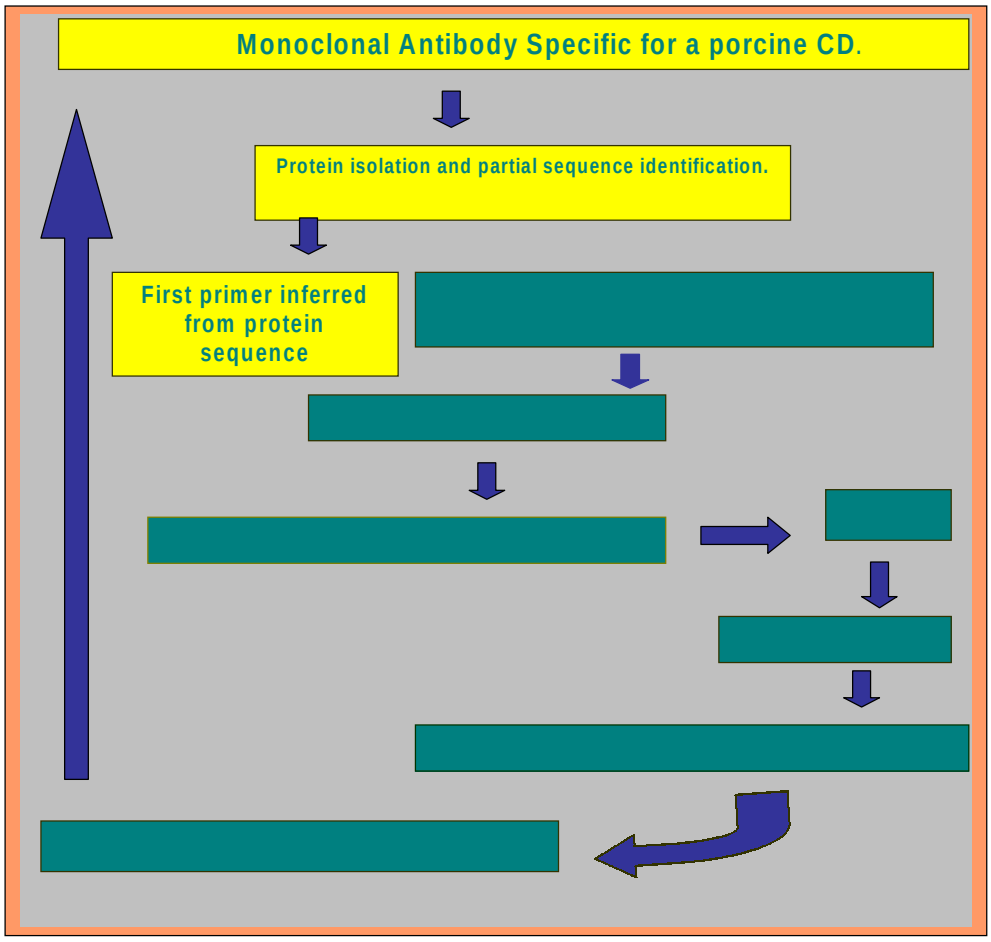

Figure 1*.Protocol to clone candidate genes for swine leukocyte antigens.* (Protocolo seguido para la clonación de un gen candidato presente en leucocitos porcinos).

gene for fertility and with different resistance to various infectious diseases in the case of CD29, CD51 and CD61 genes.

In addition, such polymorphism may be useful in characterising diversity within pig populations (see Ciobanu *et al*., 2001; Blott *et al*., 2003; Rothschild, 2003). Of particular interest in such studies is adaptation to local conditions, including disease agents that are geographically restricted, therefore the analysis of variation in CD genes may be an important component of this research.

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