Short communication

Towards a global analysis of porcine alveolar macrophages proteins through two-dimensional electrophoresis and mass spectrometry

Eva Pérez-Reinado[a], María Ramírez-Boo[a], Juan J. Garrido[a], Jesús V. Jorrín[b], Ángela Moreno[a],*

[a]*Unidad Mixta C.S.I.C.-UCO "Marcadores Genéticos Moleculares en Animales Domésticos", Departamento de Genética, Universidad de Córdoba, Córdoba, Spain*
[b]*Grupo de Agricultura y Bioquímica de Plantas, Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, Córdoba, Spain*

Abstract

Alveolar macrophages (AM) are the primary phagocytes of the innate immune systems, constituting a link between innate and adaptive immunity. With the aim of studying the porcine AM biology and the dynamics of pig-pathogen cell interactions, we have obtained a reference 2-DE map of the porcine AM proteins. The proteins were separated by 2-DE using a 5–8 range pH gradient in isoelectric focusing and over 800 spots were detected. A set of proteins, covering the pI 5.2–7.4 and M_W 19 to 106 kDa ranges, was subjected to MS analysis and 106 proteins were assigned identification by PMF, this identification being confirmed by MS/MS. An important number of proteins is involved in immunological functions, signalling process, transport or apoptosis, confirming that macrophages are involved in a wide range of biological functions. This reference map provides a useful tool for identifying protein pattern changes as a result of inflammation, exposure to infectious agents or genetic diseases.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Alveolar macrophages; Innate immunity; Protein profiling; Porcine proteomics; Two-dimensional gel electrophoresis

*Corresponding author. Departamento de Genética, Campus Rabanales, Edificio Gregor Mendel (C-5), 14071 Córdoba, Spain. Tel./fax: +34 957 218730.
E-mail address: ge1moloa@uco.es (Á. Moreno).

1. Introduction

The immune system in vertebrates consists of two related functional arms: the innate and the adaptive immune systems, which function in a coordinated way to protect against infection. Innate immunity acts as the first line of host defense against microbial pathogens and macrophages are the major effector cells of the innate immune system. Many studies have demonstrated the crucial role played by macrophages in infectious, autoimmune and

inflammatory diseases [1], but the cellular mechanisms determining these processes are largely unknown [2]. Alveolar macrophages (AM) are a unique subset of tissue macrophages situated in the alveolar milieu that function primarily in the host defense of the lung against inhaled particulate matter, microorganisms and environmental toxins. The early recognition and response to invading pathogens by the AM is essential, resulting in the generation of signalling cascades and production of cytokines, chemokines, metalloproteinases, grown factors, reactive oxygen and nitrogen species [3]. So, AM constitutes an important link between the innate and adaptive immune response, which is essential for protective immunity [4].

The role of AM in complex disorders is still incompletely known. A global analysis of the AM proteins using two-dimensional electrophoresis (2-DE) is a very good approach to investigate the biological events controlling the defense of the host against pathogens and the protein abundance changes associated with pathological processes. Such studies can be facilitated by comparing the gels obtained with an established 2-DE reference map representing the typical patterns of AM under normal conditions. For this reason, different research projects are now in progress to establish a 2-DE database for different mammalian cells and tissues [5]. However, most of the data are available on samples from humans, where several studies have been reported in order to generate the proteome of AM [1–3]. Animal proteomics could also benefit from any advance in human proteomics, opening up a promising new scenario in veterinary sciences; however, only a limited number of proteomic projects have been reported in domestic animals and bovine species have been selected in most cases for proteomic studies [5].

The importance of the pig in the entire agricultural economy and in the human health (through food consumption) has promoted investigations on fundamental mechanisms controlling animal health and productivity. Also, the interest in porcine immunology has recently increased due to the potential of the pig to serve as a large animal model for biomedical research [6]. Animal genomics and proteomics technologies are promising tools for gaining knowledge of the porcine immune response and a proteomic analysis of porcine AM could be a useful one in this issue. A protein database for radioactive labelled porcine AM, including 995 polypeptides, has been established [7] and the pattern of African swine fever virus (ASFV)-induced polypeptides in porcine AM has been described. These polypeptides could not be assigned identification by mass spectrometry (MS) because the amount of material obtained in this biological system is under detection limit. A proteomic approach has been used to study the dynamics of ASFV and host cell interactions, searching for alterations in cellular protein profile after virus infection [8] in order to determine target proteins for pathogenesis studies.

A research project focused on studying the swine immune response against pathogens is being developed by our group. In this paper, we report a reference 2-DE map of swine AM protein which, together with the map of peripheral blood mononuclear cells (PBMC) previously reported [9], provides a useful tool for studies on AM biology, as well as for identifying the proteins implicated in the immune response or physiological changes that could be correlated with porcine diseases.

2. Material and methods

2.1. Animals and swine AM preparation

Lungs from five healthy pigs of approximately 1 year of age were collected at the slaughterhouse. Porcine AM were isolated according to Carrascosa et al. [10] with modifications. Briefly, freshly isolated lungs were flushed with cold sterile phosphate buffer saline (PBS) containing EDTA 2 mM, 1 mg/ml of glucose, 200 U/ml penicillin and 40 μg/ml gentamycin. Macrophages were recovered following centrifugation and they were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 40 μg/ml gentamycin, 200 U/ml penicillin and 2 mg/ml streptomycin. Cells were incubated at 37 °C in the presence of 5% CO_2 in Teflon flasks (Nunc, UK) for 2 h and washed to remove non-adherent cells. Adherent cells were found to contain >95% macrophages using a monoclonal antibody (BL1H7) directed against SWC3, a monocyte/macrophage lineage marker.

2.2. 2-DE and image analysis

Cells (10^8) were mixed and solubilized in 1 ml of sample buffer (7 M urea, 2 M thiourea, CHAPS 4%, dithiothreitol (DTT) 1%, ampholytes 0.8% and H_2O). Immobilized pH gradient (IPG) strips (17 cm, 5–8 linear pH gradient) (Bio-Rad) were rehydrated