Journal of Proteomics 135 (2016) 181–190

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Differential proteomics reveals age-dependent liver oxidative costs of innate immune activation in mice

Marine I. Plumel [a,c], Margaux Benhaim-Delarbre [a,c], Magali Rompais [a,c], Danièle Thiersé [a,c], Gabriele Sorci [d], Alain van Dorsselaer [a,c], François Criscuolo [b,c,1], Fabrice Bertile [a,c,*,1]

[a] Institut Pluridisciplinaire Hubert Curien, Département Sciences Analytiques, CNRS UMR7178, 25 rue Becquerel, 67087 Strasbourg Cedex 2, France
[b] Institut Pluridisciplinaire Hubert Curien, Département d'Ecologie, Physiologie et Ethologie, CNRS UMR7178, 23 rue Becquerel, 67087 Strasbourg Cedex 2, France
[c] Université de Strasbourg, 4 rue Blaise Pascal, F-67081 Strasbourg Cedex, France
[d] Biogéosciences, CNRS UMR6282, Université de Bourgogne, 6 boulevard Gabriel, F-21000 Dijon, France

ARTICLE INFO

Article history:
Received 23 July 2015
Received in revised form 1 September 2015
Accepted 7 September 2015
Available online 12 September 2015

Keywords:
Immunosenescence
Liver
Proteomics
Oxidative stress
Ageing
Mice

ABSTRACT

Individual response to an immune challenge results from the optimization of a trade-off between benefits and costs of immune cell activation. Age-related immune disorders may have several mechanistic bases, from immune cell defects to chronic pro-inflammatory status and oxidative imbalance, but we are still lacking experimental data showing the relative importance of each of these mechanisms. Using a proteomic approach and subsequent biochemical validations of proteomics-derived hypotheses, we found age-dependent regulations in the liver of 3-months and 1-year old-mice in response to an acute innate immune activation. Old mice presented a chronic up-regulation of several proteins involved in pathways related to oxidative stress control. Interestingly, these pathways were weakly affected by the innate immune activation in old compared to young individuals. In addition, old mice suffered from lower glutathione-S-transferase activity and from higher oxidative damage at the end of the experiment, thus suggesting that they paid a higher immune-related cost than young individuals. On the whole, our data showed that a substantial fraction of the liver costs elicited by an activation of the innate immune response is effectively related to oxidative stress, and that ageing impairs the capacity of old individuals to control it.

Significance: Our paper tackles the open question of the cost of mounting an innate immune response. Evolutionary biologists are familiar since a long time with the concept of trade-offs among key traits of an organism, trade-offs that shape life history trajectories of species and individuals, ultimately in terms of reproduction and survival. On the other hand, medicine and molecular biologists study the intimate mechanisms of immune senescence and underline that oxidative imbalance is probably playing a key role in the progressive loss of immune function with age. This paper merges the two fields by exploring the nature of the cellular pathways that are mainly affected by age when the innate immunity is triggered. To this purpose, a proteomic approach was used to explore liver protein profiles and provide for the first time convincing data supporting the idea that oxidative stress constitutes a cost of innate immune response in old mice, possibly contributing to senescence. Proteomics-derived hypotheses were furthermore validated using biochemical assays. This paper therefore illustrates the added value of using proteomics to answer evolutionary biology questions, and opens a promising way to study the inter-specific variability in the rates of immune-senescence.

1. Introduction

Senescence is a multi-level phenomenon that starts at the level of the cell, and has ultimate consequences on the entire organism. It has been defined as the accumulation of unrepaired damage on biomolecules and cells causing the progressive decline of both organismal functions and reproductive and survival rates of individuals over time [1]. Among the physiological functions shown to be affected by age, senescence of the immune system has been largely studied [2–4], and old individuals are often characterized by chronic or repeated infections, inflammatory diseases and autoimmune disorders [5]. In addition, the finding that senescent fibroblasts actually express inflammatory genes [6], suggests that even non-immune cells may contribute to mal-adaptive immunity in old-age individuals.

Interestingly, several studies from the molecular biology and medicine fields recently underlined that tackling the question of immune-senescence from an evolutionary point of view may help to better

* Corresponding author at: Institut Pluridisciplinaire Hubert Curien, Département Sciences Analytiques, CNRS UMR7178, 25 rue Becquerel, 67087 Strasbourg Cedex 2, France.
[1] Equal contributors, shared seniorship of the paper.

http://dx.doi.org/10.1016/j.jprot.2015.09.008
1874-3919/

understand which mechanisms are important in the progressive impairment of the immune system [7, 8], and also whether the change in the immune functioning with age is mal-adaptive or not. More particularly, the well-known evolutionary concept of antagonistic pleiotropy [9] has been successfully applied to explain how some cell pathways may contribute to ageing. Those pathways have deleterious impact at old ages, but are still preserved by natural selection because of their beneficial effects on early life traits (e.g. TOR pathways, [10]), and ultimately on overall individual fitness. Antagonistic pleiotropy may also well explain why the control of the immune system is compromised with age, as strong responses are favoured by natural selection because of their role in fighting infectious diseases, but pro-inflammatory processes can also be harmful in terms of increased oxidative stress and immunopathology [11, 12].

This idea of progressive accumulation of damage while growing old has also been theorized in the context of evolutionary biology, leading to the *Disposable Soma* theory [13]. While taking over the global idea of the *Antagonistic Pleiotropy* theory, Kirkwood gave a mechanistic explanation related to metabolism and direct negative impact of one function on another through the production of reactive oxygen species (ROS). ROS are inevitably produced by mitochondria when processing reduced co-enzymes to ATP. Because functions compete for limited resources, this may reduce the investment an organism is currently doing in somatic maintenance, for example in buffering oxidants. This is even more critical when the activated physiological functions entail an increase in energy expenditure or produce reactive species, putatively modulating oxidative balance and ultimately ageing [14]. Such more or less clear relationships have been highlighted in model and non-model species [15–21], leading to the idea that oxidative stress could be one of the mediating mechanisms sustaining life-history trade-offs such as those among reproduction, growth or somatic maintenance and longevity [22].

Within somatic maintenance, the immune system has a preponderant role. It preserves the organism both from external (pathogens) and internal (cancer) threats. Because immune response potentially implies the production of ROS both *via* an increased energy expenditure [23, 24] and/or the activation of immune cells [25, 26], mounting an immune response is likely to be costly and to lead to energy and oxidative based trade-offs. For instance, innate immunity relies on production of nitric oxide and superoxide by macrophages [27, 28] which may have non-specific deleterious impact on host cells [29]. However, individuals are not paying an identical energy cost when responding to an immune challenge, and old individuals may have to face more critical immune-associated trade-offs [24]. Such an observation suggests an ultimate cost of mounting an immune response, an idea further characterized in several taxa where individuals challenged with an immune treatment exhibit a reduced survival rate [30, 31].

We challenged one specific arm of the host immune system of mice using bacterial lipopolysaccharide (LPS), the innate immunity. LPS is recognized by different innate cell receptors (macrophage scavenger receptors, MARCO receptors or toll-like receptor 4) [32], and triggers an innate inflammatory response. Timely innate response to LPS injection guarantees the early clearance of bacterial endotoxin and prevents excessive immune inflammation ultimately leading to a septic shock [33]. Previous studies have shown an increase of oxidative stress in LPS treated individuals using blood markers [34], and successfully characterized proteomic specific patterns induced by LPS exposure [35]. Based on this knowledge, one objective was therefore to decipher here liver regulations that are elicited in response to an immune challenge in mice. Indeed, the liver is a key organ for the maintenance of metabolic homeostasis, but it also has to constantly deal with antigenic loads [36, 37], and oxidative stress plays an important role in the pathogenesis of many liver diseases [38]. More particularly, the aim was to establish whether age-related changes in protein profiles could support the idea that old individuals actually pay a higher cost in terms of deleterious reactions triggered by activation of the immune system (*e.g.* like oxidative imbalance) than young individuals. To this purpose, we took advantage of the benefits a global analytical strategy like proteomics can bring to the evolutionary ecology field [39].

2. Materials and methods

2.1. Experimental procedures

The experiment was conducted using eight 3 month-old (young) and eight one year-old (old) C57BL/6J male mice, reared in our laboratory under constant temperature (24 ± 2 °C) and photoperiod (13:11 L:D cycle), with free access to a standard chow diet consisting (by weight) of 21.4% proteins, 51.7% carbohydrates and 5.1% fat (SAFE A03) and water. Each group was randomly divided in two subgroups (4 mice each kept in individual cages) where animals received a single intraperitoneal injection of either 25 μg/kg body mass of LPS from *Escherichia Coli* (serotype 055-B5, patch 011M400IV, Sigma Aldrich), or phosphate buffered solution (PBS). This LPS dose is largely below the lethal dose for young and 1 year-old mice (see [40]). None of the injected mice died after the injection. Body mass (±0.1 g) of individuals was measured just before the injection and animal death. Mice were sacrificed 24 h after injection by cervical dislocation and their liver was quickly collected, snap-frozen in liquid nitrogen, and several sample aliquots were stored at −80 °C until biochemical and proteomic analyses. The study complied with legislation (L87-848) on animal experimentation in France and was done under the DEPE license obtained from the French Department of Veterinary Service (number G67-482-18). Dr F. Criscuolo is the holder of an animal experimentation license (no. 67–78) delivered by French authorities.

2.2. Liver proteomics

Unless otherwise specified, all chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2.1. Protein extraction

Frozen liver samples were first pulverized using a laboratory ball mill (Mikrodismembrator, Sartorius). ~10 mg of the grinded powders were then dissolved in 400 μL of a buffer composed of 8 M Urea, 2 M Thiourea, 4% Chaps, 1% dithiothreitol, Triton X100 0.5%, TLCK 0.05% and 0.02 to 2 mM protease inhibitors. After sonication on ice (10 s, 135 watts), 9 volumes of cold acetone were added, and samples were kept at −20 °C during 16 h. Proteins were pelleted by centrifugation (14 min, 4 °C, 14000 g), vacuum-dried (Speedvac, Thermoscientific) after discarding supernatants, and then dissolved in a buffer composed of 7 M Urea, 2 M Thiourea, 30 mM Tris (pH 8.5) and 4% Chaps buffer. After adjustment of the pH to 8.5, homogenization was finally completed by sonication on ice (10 s, 135 watts).

After determination of total protein concentrations using the Bio-Rad Protein Assay [41] (BioRad, Hercules, CA, USA), protein integrity and similarity of electrophoretic protein profiles was checked prior to 2D-DIGE analysis [42–44]. To do so, proteins were electrophoresed on a 12% SDS-PAGE acrylamide gel (20 μg loaded; 50 V for 30 min and then 100 V to complete migration) and stained with colloidal Coomassie blue [45] (G 250, Fluka, Buchs, Switzerland).

2.2.2. 2D-DIGE experiment

Protein samples were first labelled using a CyDye DIGE Fluor Minimal Dye Labeling Kit (GE HealthCare, Uppsala, Sweden). More precisely, 400 pmol of Cy3 and Cy5 were used to randomly label 50 μg of protein samples from the different groups, and 3.2 nmol of Cy2 were used to label 400 μg of proteins after having mixed all the samples (25 μg each; internal standard). After incubation in the dark for 30 min on ice, protein labelling was quenched by addition of 10 mM lysine and incubation in the dark for 10 min on ice. Random distribution of samples from the 4 groups (i.e. young PBS, yound LPS, old PBS and old LPS) was