Poster presentation abstract

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An alternative in vitro method to evaluate IL-1ß for toxicity test of autogenous vaccines

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In Italy the legislation provides that Experimental Zooprophilactic Institutes (II.ZZ.SS.) are the only agencies authorized by the Ministry of Health for the production of veterinary autogenous and autologous vaccines. Autogenous vaccines are prepared for immunization against pathogenic microorganisms isolated from sick animals of one herd and only usable in the same farm. The II.ZZ.SS. must perform the in vivo toxicity test for each lot of vaccine produced as laid down in the Decree of 17 March 1994 [1]. According to the 3R principle (Replacement, Refinement, Reduction), we have proposed an alternative in vitro method for testing toxicity of autogenous vaccines. The method described in this work measures IL-1ß production by macrophages in response to vaccine antigens. In this study, macrophages were obtained after differentiation from pig monocytes in peripheral blood mononuclear cells (PBMC) frozen in liquid nitrogen, using 10 ng/ ml of Macrophage-Colony Stimulating Factor (M-CSF). Differentiated macrophages were reacted with the antigens at different dilutions for 24 hours, followed by quantification of released IL-1ß. Samples were analyzed for IL-1ß by "Duo set ELISA for Porcine IL-1ß/IL-1F2" (R&D System) [2]. Preliminary results indicate different levels of activation of each macrophage population, which bears on sensitivity to vaccine antigens. It is therefore crucial for standardization to start from a single batch of low-activation cells, to standardize the period of cell differentiation and to choose a batch of suitable FCS, in order to have representative and replicable data. The study aims to present the potential of this methodology for replacement of the current in vivo test. It can be used in association with other in vitro tests to get an overview of the potential toxicity of the vaccines.

[1] Ministerial Decree 17 March 1994. [2] Fabio Martinon, Kimberly Burns, and Jürg Tschopp. The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of pro- IL-1ß. Molecular Cell, Vol. 10, 417–426, August, 2002

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