Changes in the gene expression profile of equine mesenchymal stem cells (MSCs) after their allogeneic administration in horses matched or mismatched for the major histocompatibility complex (MHC)

Alina Cequier*†, Elvira Bernad*, Mirta García*, Belén Serrano*, Francisco José Vázquez*†, Antonio Romero*†, Arantza Vitoria*†, Laura Barrachina*†, Clementina Rodellar*

(*) Laboratorio de Genética Bioquímica LAGENBIO – Instituto Agroalimentario de Aragón – IA2 (Universidad de Zaragoza – CITA) – Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain

(†) Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain **Contact: alinacs@unizar.es**

INTRODUCTION

- **Musculoskeletal injuries** have a **great impact** in **equine industry** but there are not effective treatments.
- Allogeneic mesenchymal stem cells (**MSCs**) are a promising therapy but they are not truly immune-privileged, they are **immune-evasive**.
- Further knowledge on MSC **interactions** with the horse immune system *in vivo* is required. These interactions are influenced by:

Donor-receptor MHC-matching/mismatching | MHC expression level | MSC immunomodulatory ability

Donor-specific

Donor-specific + Influenced by external factors

Inflammation | Differentiation

- Horse is highly relevant both as patient as translational model.

OBJECTIVE

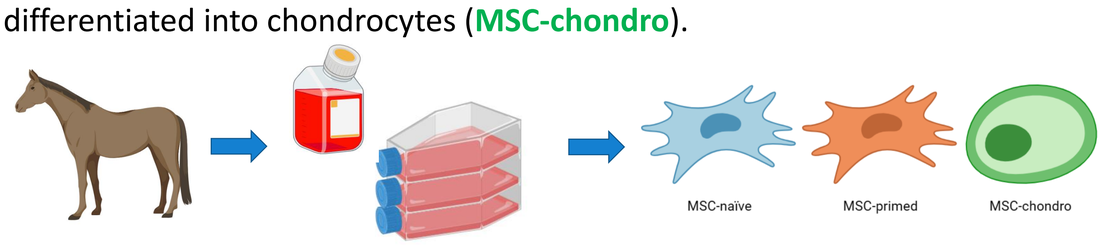
To evaluate the expression of genes related to the immunomodulatory and immunogenic profiles of equine MSCs after their exposure to the immune system of the horse *in vivo*.

METHODS

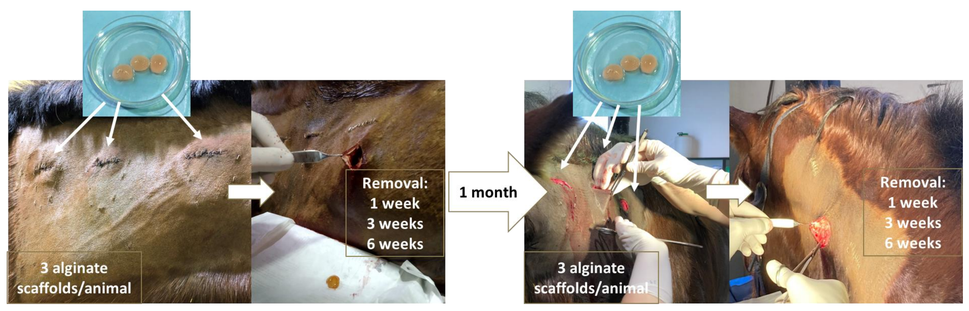
1. **Horse selection** according to their **MHC haplotype** (microsatellite markers).

MSCs donors	MHC-matched allogeneic receptors			MHC-mismatched allogeneic receptors		
D1 MSC-chondro	R1	R2	R3	X1	X2	X3
D2 MSC-primed	A1	A2		Y1	Y2	Y3
D3 MSC-naïve	C1	C2	C3	Z1	Z2	Z3

2. MSCs obtention and encapsulation in alginate scaffolds in three conditions: basal conditions (**MSC-naïve**), pro-inflammatory primed (**MSC-primed**) or differentiated into chondrocytes (**MSC-chondro**).



3. **Placement** of the **alginate scaffolds** and **removal** after 1, 3 and 6 weeks. **Re-exposure** to the same type of stem cells.



4. Gene expression analysis of MSCs retrieved from the scaffolds by **RT-qPCR**

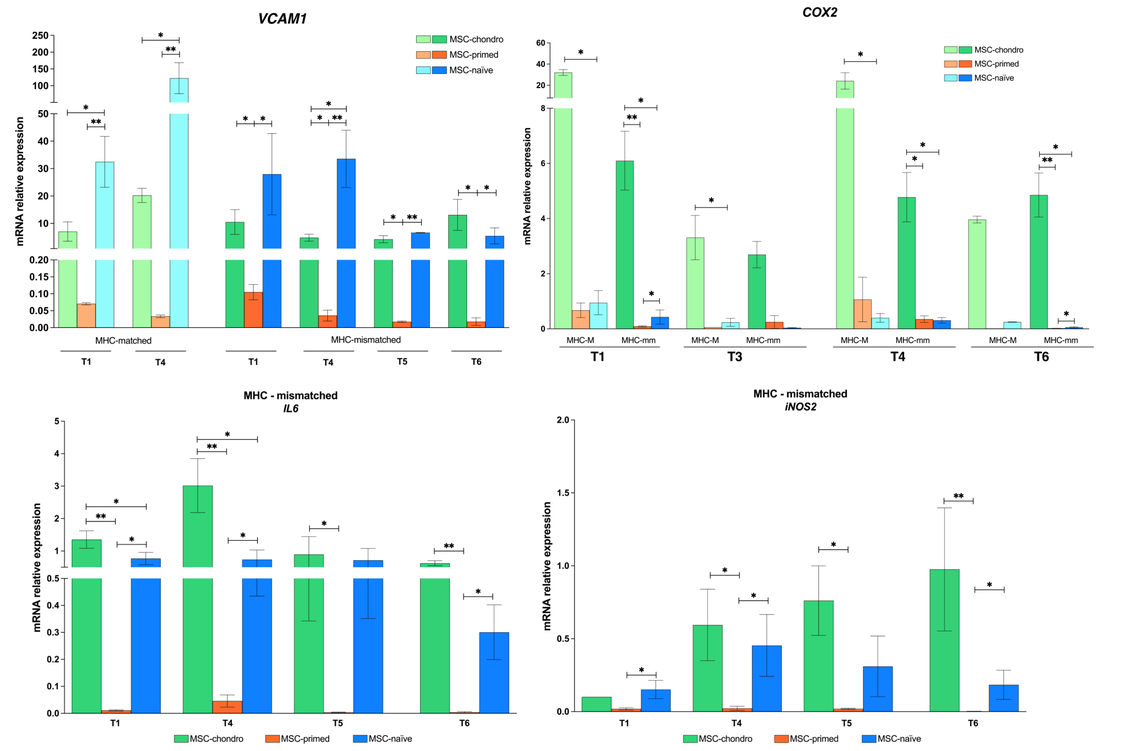
QuantStudio3 Real Time PCR System

Immunomodulation: *VCAM1*, *COX2*, *IDO1*, *iNOS2*, *IL6*

Immunogenicity: *MHC-I*, *MHC-II*, *CD40, CD80*

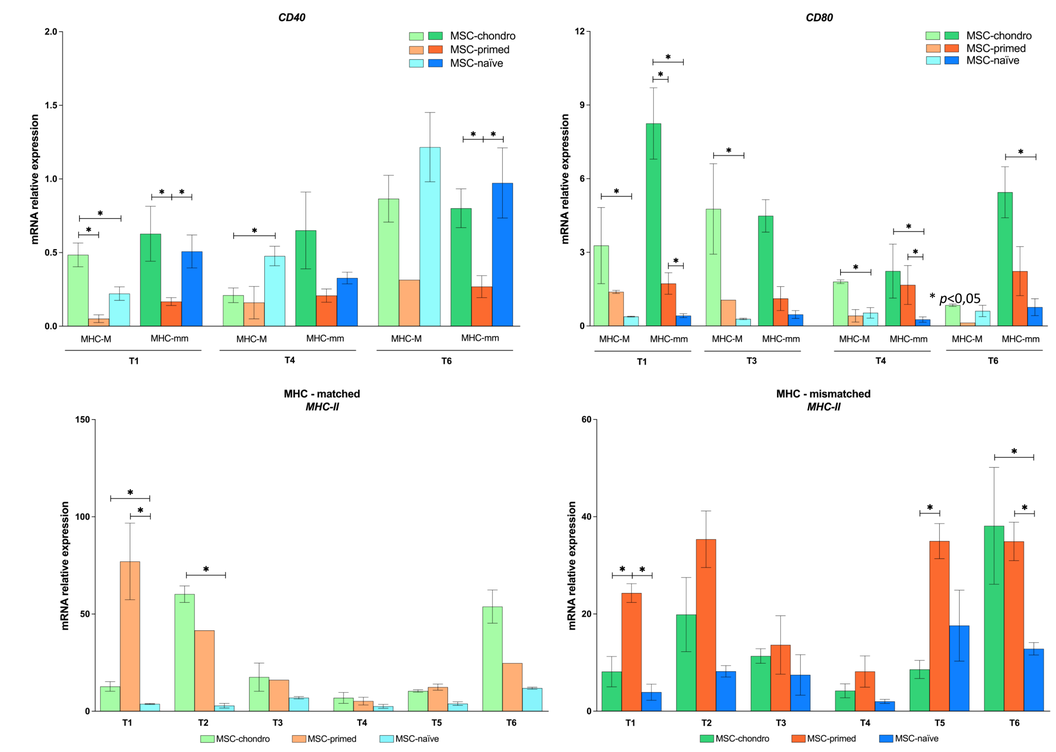
RESULTS AND DISCUSSION

1. Genes coding for immunomodulatory molecules



MSC-Chondro showed the **highest** immunomodulatory profile.
MSC-Primed expressed the **lowest** levels of immunomodulatory genes.

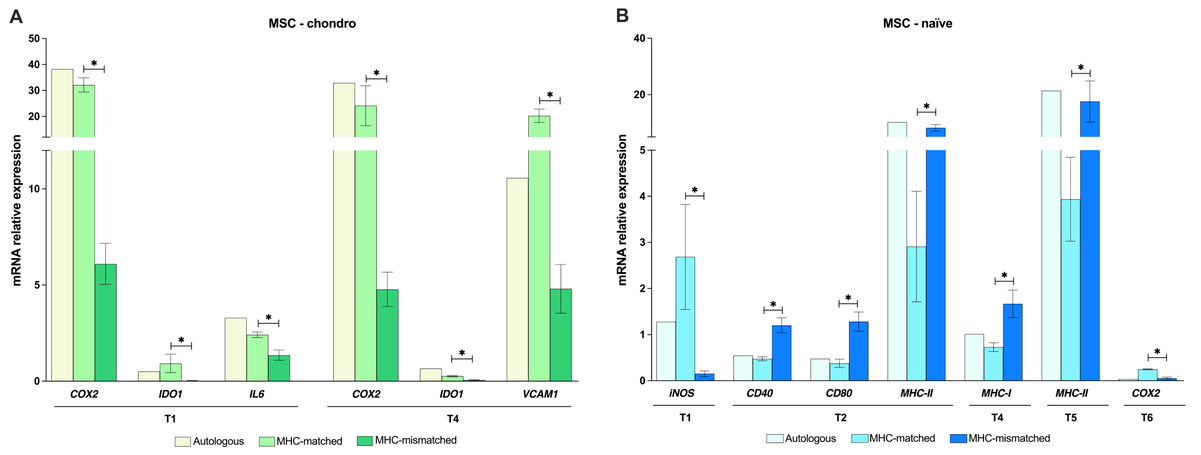
2. Genes coding for immunogenic markers



MSC-Chondro showed the **highest** expression levels of the costimulatory molecules *CD40* and *CD80.*

MSC-Primed expressed the **highest** levels of *MHC-II*.

3. Effect of MHC compatibility



MSC-chondro: MHC-compatibility increased their **immunomodulatory** profile.

MSC-naïve: MHC-incompatibility increased their **immunogenicity**.

T1 = 1 week after 1st administration; T2 = 3 weeks after 1st administration; T3 = 6 weeks after 1st administration; T4 = 1 week after 2nd administration; T5 = 3 weeks after 2nd administration; T6 = 6 weeks after 2nd administration; * = $p < 0.05$; ** = $p < 0.01$

CONCLUSIONS

- The MHC haplotype, the inflammatory exposure and the chondrogenic differentiation of equine MSCs affect their immune profile *in vivo*.
- MSC-chondro may offer advantages for allogeneic cell therapy, contrary to previous *in vitro* findings suggesting higher immunogenicity and lower immunomodulatory capacity
- Understanding the immune response *in vivo* to allogeneic equine MSCs is key for managing this response and design more effective and safer therapies.

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