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Research paper

Young foal and adult horse monocyte-derived dendritic cells differ by their degree of phenotypic maturity

Catherine Mérant, Cormac C. Breathnach, Katharina Kohler, Cetewayo Rashid, Patricia Van Meter, David W. Horohov *

Department of Veterinary Science, Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546, USA

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ABSTRACT

Newborn foals are very susceptible to infections by opportunistic pathogens such as *Rhodococcus equi*. This susceptibility is thought to be due to the immaturity of their immune system, in particular their inability to produce interferon-gamma. This deficiency may result from an insufficiency in accessory signals. We therefore compared monocyte-derived dendritic cells (MoDC) from foals and from adult horses. CD172, MHC-I and MHC-II were generally expressed on more than 90% MoDC from foals and adults. $CD1w2^+CD86^+$ cells tended to be less represented in 2–3-week-old foals than in adults. This difference was significant among $CD14^-$ cells. The percentage of $CD14^-CD1w2^+CD86^+$ cells tended to be increased at 3 months. This suggests that very young foal dendritic cells are quantitatively less mature than their adult counterparts. The expression of IL-1, IL-12, IL-15 and IL-18 mRNA was not different in foal and adult MoDC, but the levels of TNF-α, IL-10, MCP-1 and TGF-β were lower in foal cells. TNF-α and IL-10 expression was increased by LPS; TNF-α even reached the level of adult MoDC. This may mean that the lack of IFN-γ in foals is not due to decreased levels of IL-12, IL-15 or IL-18, but rather to lower constitutive levels of TNF-α.

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1. Introduction

Dendritic cells (DC) and macrophages are the main antigen presenting cells (APC) in mammalian species. Myeloid DC have been shown to be the only APC able to induce activation of naïve T cells (Banchereau and Steinman, 1998). Human DC originate from a $CD34^+$ bone marrow or blood stem cell which differentiates into a $CD1a^+$ or $CD14^+$ precursor (Caux et al., 1996) under the influence of GM-CSF and TNF-α (Santiago-Schwarz et al., 1992; Szabolcs et al., 1995). These cytokines allow the first precursors to become Langerhans cells ($langerin^+$, $E\text{-}cadherin^+$) and the second ones to become interstitial DC (Caux et al., 1992, 1996). Both cell types express CD1a, CD1c, CD80/86 and MHC-II molecules. After capturing antigens, these immature DC differentiate while migrating through the lymph, where they are called veiled cells. Langerhans cells will form interdigitating DC (Hoefsmit et al., 1982). This is manifested in the loss of CD1a, the acquisition of CD83 and the up-regulation of CD1b, CD1c, CD80/86 and MHC-II. GM-CSF and IL-4 can also induce the differentiation of monocytes into DC (Sallusto and Lanzavecchia, 1994; Romani et al., 1994), and this pathway has been widely used as a convenient method for obtaining DC *in vitro* (Hammond et al., 1999; Mauel et al., 2006; Flaminio et al., 2007).

Mature DC cannot take up antigen, but can stimulate naïve T cells to exert their helper or cytotoxic functions, including the secretion of IFN-γ. Newborn foals are IFN-γ

* Corresponding author at: 108 Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099, USA. Tel.: +1 859 257 4757; fax: +1 859 257 8542.
E-mail address: david.horohov@uky.edu (D.W. Horohov).

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deficient (Breathnach et al., 2006), which makes them very susceptible to infections by opportunistic pathogens such as *Rhodococcus equi*. This IFN-γ deficiency may be explained either by an intrinsic incapacity of their T cells or by an insufficiency in the accessory signals of their DC.

What is known about human and murine neonates would favor more the second hypothesis. Indeed, the expression of IFN-γ and TNF-α in the lungs of 3-week-old mice is lower than in adult's, which explains their incapacity to clear *Pneumocystis carinii* pneumonia. However, neonatal lymphocytes acquire the capacity to produce these cytokines and to resolve the infection after transfer to an adult lung environment (Qureshi and Garvy, 2001). Moreover, dendritic cells from mice of less than 1 week of age have decreased ability to present antigens; this is likely due to the lack of expression of CD80, CD86, and CD11c (Muthukkumar et al., 2000). In humans, DC differentiated from cord blood monocytes do not up-regulate HLA-DR, CD83 and CD86 upon LPS stimulation as much as adult blood monocyte-derived DC (Langrish et al., 2002). They are also unable to down-regulate CCR5 and produce IL-12p70, but their kinetics of secretion of TNF-α and IL-10 are similar to adult DC (Langrish et al., 2002). IL-12 seems to be central, since it induces the production of IFN-γ by cord blood CD4 T cells. However, its effect is synergized by IL-2, IL-1 and TNF-α (Wu et al., 1993).

In order to determine if neonatal foal DC had similar deficiencies, we derived DC from blood monocytes of 2-week-old foals and compared their allo-antigen presentation capacities, surface molecule expressions and cytokine productions to those of adult horse monocyte-derived DC (MoDC).

2. Materials and methods

2.1. Preparation of monocyte-derived dendritic cells (MoDC)

All sample collection procedures have been reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee. Heparinized blood (120–150 ml) was collected from 11 foals of various breeds at 2–3 weeks, then at 3 months of age. Each time the foals were bled, an adult horse was used as control (4 mares were used). The same adult was always used as control for the same foal. The peripheral blood mononuclear cells (PBMC) were isolated from the blood by centrifugation on Ficoll-Paque (GE Healthcare) and cultured at $7.5–15 \times 10^6$ cells/ml in RPMI-1640 supplemented with 10% autologous serum, 1% glutamine-penicillin-streptomycin (PSG; Sigma, St-Louis) 0.1% 2-mercaptoethanol (2-ME; Invitrogen, Carlsbad, CA) and 0.1% amphotericin B (Sigma) in tissue culture grade Petri dishes. After 4 h, the non-adherent cells were removed, and supernatant of CHO cells producing recombinant equine IL-4 and GM-CSF (Hammond et al., 1999) was added at 10% for 4 or 7 days. The non-adherent MoDC were then purified by centrifugation on Nycoprep 1.068 (Axis Shield, Greiner BioOne), and their number and viability were determined by Trypan blue exclusion using an automated cell counter (Beckman Coulter). Cells were resuspended in cRPMI (RPMI-1640 containing 2.5% fetal equine serum (Cellect, MP Biomedicals, Irvine, CA), 1% PSG and 0.1% 2-ME) for mixed leukocyte reaction studies and quantification of cytokine mRNAs or in PBS-BSA 1%-NaN3 0.1% (PBA) for immunostaining. Samples containing enough cells were also processed for electron microscopy.

2.2. Transmission electronic microscopy

Cells were resuspended in Sorensen buffer and fixed in glutaraldehyde for 4 h. They were then dehydrated successively in 40, 70, 90, 95 and 100% ethanol solutions by centrifuging between each bath. The sample was embedded in Spurs (Electron Microscope Sciences) and allowed to harden at 60 °C for 72 h. Ultrathin cuts (110 nm) were performed using a RMC 6000-XL ultramicrotome. Sections were stained with uranyl acetate followed by lead citrate, and placed on copper grids. Cuts were observed using a Hitachi 7000 transmission electron microscope at 75 kV.

2.3. Immunostaining and flow cytometry analysis

The MoDC FcRs were saturated by pre-incubation with equine purified immunoglobulins (Jackson Immunoresearch, West Groove, PA). Cells were then labeled with mouse monoclonal antibodies to: CD172a (clone DH59B), CD14 (big 10), EqWC2 (CVS6), CD1w2 (PE-labeled-CC20), CD86 (PE-Cy5-labeled IT2.2), MHC-I (CVS22) and MHC-II (CVS10). Some MoDC were also stained with mAbs against CD3 (UC F6G-3) and the B cells marker CZ2.1. MAbs were mouse IgG1, except CC20, CVS22, CVS10 (IgG2a) and IT2.2 (IgG2b). DH59B, big 10, CC20 and IT2.2 were purchased form VMRD (Pullman, WA), Biometec/Axxora Platform (San Diego, CA), Biolegend (San Diego, CA) and AbD Serotec (Raleigh, NC), respectively. All CVS mAbs were gifts from Dr Paul Lunn (University of Colorado). Unconjugated mouse IgG1 were detected with FITC-anti-mouse IgG1 goat F(ab′)2 fragments (Southern Biotechnology Associates, Birmingham, AL) whereas unlabeled mAbs of other isotypes were revealed with FITC-anti-mouse IgG (H + L) Ab (Caltag/Invitrogen). The stainings were analyzed on a FACSCalibur flow cytometer (BDBiosciences, San Jose, CA). Putative DC were back gated based on their expression of CD172a.

2.4. Mixed leukocyte reaction

MoDC were mixed with 100,000 allogenic PBMCs at the ratios: 0, 0.01, 0.03, 0.1, 0.3 and 1 DC per 1 PBMC. After 5 days of culture, cells were pulsed with 1 μCi of [^{3}H] thymidine per well for 18 h and their radioactivity was measured in a liquid scintillation counter.

2.5. Cytokine real-time polymerase chain reaction

MoDC cultured for 3 h in complete RPMI alone or with 1 μg LPS/ml were resuspended in RNA-STAT 60 (Tel-Test Inc, Fisher Scientific, Pittsburgh, PA) and frozen at −70 °C. Total RNA was extracted according to the manufacturer's protocol. One microgram of RNA was reverse-transcribed using avian myeloblastosis virus reverse-transcriptase, as previously described (Horohov et al., 2008). The cDNA

Table 1
Primer probe sets designed for measurement of gene expression; FAM: dye; NFQ: non-fluorescent quencher.

Target gene	Sequence or reference	
IL-10	Fwd	AGGACCAGCTGGACAACATG
	Rev	GGTAAAACTGGATCATCTCCGACAA
	Probe	FAM CCAGGTAACCCTTAAAGTC NFQ
MCP-1	Fwd	GCGGCCGCCTTCAG
	Rev	CAGCAGGTGACTGGAGAATTAATTG
	Probe	CAGGTGCTGGCTCAGC NFQ
TGFβ	Fwd	FAM CCCTGCCCCTACATTTGGA
	Rev	TGTACAGGGCCAGGACCTT
	Probe	FAM CCTGGACACGCAGTACAG NFQ
iNOS	Fwd	GCGTTACTCCACCAACAATGG
	Rev	CCAGATCCGGAAGTCATGCTTTC
	Probe	FAM ATGGCCGACCTGATGTT NFQ
CCR5	Fwd	GCAGAGCAGCTGAGACATCT
	Rev	GGACTTGTCGTCTGATAATCCATCT
	Probe	CAACCCAGGAGGCCTT (reverse)
CCR7	Fwd	GTGGTGGCTCTCCTTGTCA
	Rev	AATCGTCCGTGACCTCATCTTG
	Probe	CAGGCACACCTGGAAAA (reverse)
IL-1β	Quinlivan et al. (2007)	
IL-6	Quinlivan et al. (2007)	
IL-12	Horohov et al. (2008)	
IL-15	Horohov et al. (2008)	
IL-18	Horohov et al. (2008)	
IFN-γ	Horohov et al. (2008)	
TNF-α	Horohov et al. (2008)	

were amplified by real-time polymerase chain reaction (RT-PCR) using equine-specific, intron-spanning primers for the housekeeping gene β-GUS (internal control) and various immune-related genes (see Table 1) using an Applied Biosystems 7900 sequence detection system. Relative expression of each gene was determined using the method of Livak and Schmittgen (2001).

2.6. Statistical analyses

Determination of the normality of the data was performed with SigmaStat software version 3.5. For normally distributed data which passed the equality of variance test (percentage of DC sub-populations), the differences between foals and adults were investigated by two-way analysis of variance of repeated measures. The foal RT-PCR data, which did not pass the normality test, were compared to those of adults by performing a Wilcoxon test on the mathematical difference between the RQ obtained a foal and the RQ obtained from its corresponding adult control; the null hypothesis was: difference = 0. LPS-stimulated samples were compared to medium cultured samples the same way.

3. Results

3.1. Morphology of cells

Preliminary experiments indicated that the number of viable cells was often 2–5 times as high after 4 days of culture as after 7 days. After 4 days, cultures showed large elongated cells extending their dendrites onto the plastic. The non-adherent cells collected for phenotypical and functional studies were examined by transmission electron microscopy. Their shape was round to triangular and almost always contained vacuoles and prominent pseudopodia (Fig. 1a), and occasionally structures resembling Birbeck granules (Fig. 1b), all of which are characteristic of dendritic cells.

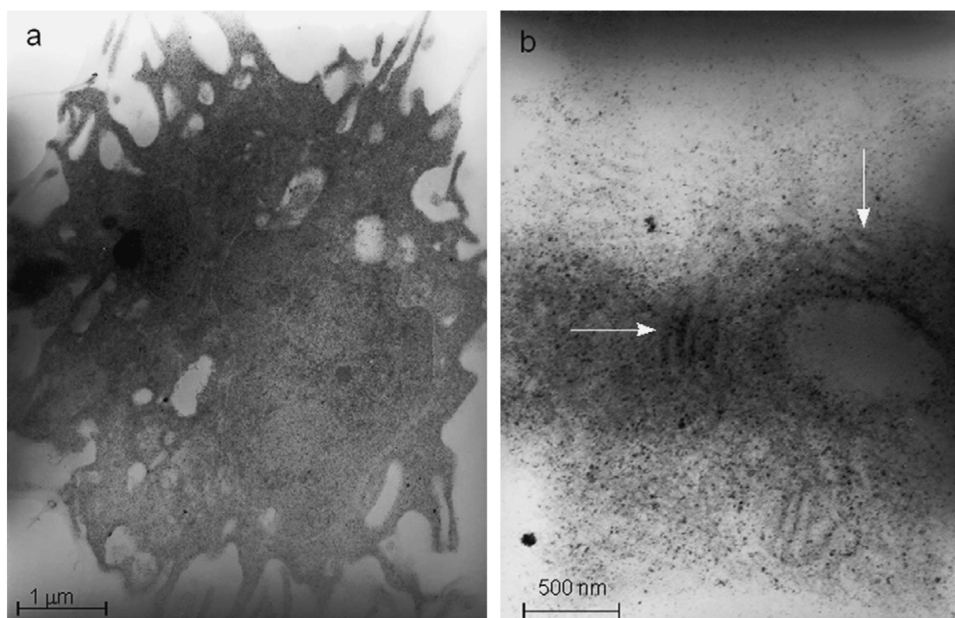

Fig. 1. Transmission electron microscopy observation of equine MoDC. (a) Cells derived from monocytes cultured for 4 days in GM-CSF and IL-4 were surrounded with dendrites. (b) Birbeck granule-like structures (arrows).

Table 2
Sub-populations of healthy foal and adult control MoDC, as defined by their expression of surface antigens.

Cell populations (%)	Adults (2 wk control)		2-week-old foals		Adults (3 month control)		3-month-old foals	
	Average	SD	Average	SD	Average	SD	Average	SD
$CD172^+$ (DH59B)	94.86	2.65	92.01	6.08	92.64	1.05	94.72	2.93
$EqWC2^+$ (CVS6)	ND		ND		23.51	11.40	31.87	15.64
$CDw19^+$ (CZ2.1)	3.43	1.48	10.04	6.75	5.28	2.25	12.23	5.25
$MHC\text{-}I^+$ (CVS22)	96.94	1.91	95.96	2.60	95.89	1.92	96.08	4.65
$MHC\text{-}II^+$ (CVS10)	95.13	2.15	90.25	4.87	85.42	3.59	90.38	9.76
$CD1w2^+$ (CC20)[*]	44.66	13.14	35.84	16.09	47.03	10.54	40.51	14.53
$CD86^+$ (IT2.2)[*]	53.23	16.58	38.81	11.49	50.71	10.21	41.28	13.74
$CD1w2^+$ $CD86^-$	10.86	4.94	14.49	12.27	13.87	6.37	17.27	8.03
$CD1w2^-$ $CD86^+$	18.93	3.93	16.46	6.10	17.17	2.01	17.72	5.78
$CD1w2^+$ $CD86^+$	35.70	13.64	18.94	6.70	32.76	11.43	22.94	12.01
$CD1w2^-$ $CD86^-$	34.51	15.21	50.12	12.81	36.21	8.65	42.07	14.92
Among $CD14^+$ cells	10.03	6.22	16.69	7.93	6.69	2.16	10.49	3.37
$CD1w2^+$ $CD86^-$	6.36	4.55	9.20	6.03	8.18	3.59	11.80	4.35
$CD1w2^-$ $CD86^+$	24.32	6.67	20.27	13.13	24.31	9.58	22.75	11.03
$CD1w2^+$ $CD86^+$	33.62	12.13	22.55	11.16	40.87	12.05	27.54	12.51
$CD1w2^-$ $CD86^-$	32.42	13.97	46.75	15.63	26.65	6.29	37.92	12.25
Among $CD14^-$ cells	89.97	6.22	83.31	7.93	93.31	2.16	89.51	3.37
$CD1w2^+$ $CD86^-$	11.59	5.26	15.79	13.43	14.35	6.86	17.86	8.56
$CD1w2^-$ $CD86^+$	18.08	4.67	14.97	5.47	16.62	1.44	16.96	5.57
$CD1w2^+$ $CD86^+$	35.43	14.67	18.10	7.20	32.26	11.58	22.45	12.16
$CD1w2^-$ CD86-	34.90	16.63	51.14	15.02	36.78	9.32	42.72	15.63
$CD14^-$ $CD1w2^+$ $CD86^+$	32.07	13.99	15.39[a]	6.76	30.21	11.29	20.33	11.57

In parenthesis: clone name and number of animals; SD: standard deviation.
[*] Triple-staining-derived percentages.
[a] $p = 0.006$.

3.2. Surface phenotype of MoDC

The myeloid lineage marker CD172a was expressed on more than 90% cells of the MoDC gate of foals or adults (Table 2). More than 95% of the cells expressed MHC-I and over 85% expressed MHC-II (Fig. 2 and Table 2). EqWC2 showed variable monomodal expression on cells from both foals and adults. Less than 3% T cells were detected, as determined by their expression of CD3. CZ2.1, which recognizes the putative equine CD19, stained 5% mare cells, but 10% foal cells in average (Table 2). $CD1w2^+CD86^+$ cells (Figs. 3 and 4) were less represented in 2–3-week-old foals than in adults. This was significant within the $CD14^-$ cells ($p = 0.006$). The percentage of these cells tended to be increased at 3 months, but the difference with the 2-week-old foals was not significant. However, the difference between 3-month-old foals and mares was not significant.

3.3. Functionality of the MoDC

Cellular proliferation, assessed by [^{3}H] thymidine incorporation, showed that the foal MoDC could induce mixed leukocyte reaction at the same extent as the adult horse MoDC (data not shown).

The expression levels of cytokine, inflammation factor and chemokine receptor genes were determined using PBMC as a calibrator. The chemokine receptors CCR5 and CCR7 were detected in both foal and adult MoDC. The constitutive production of IL-1, IL-6, IL-12, IL-15 (Fig. 5), IL-18 and iNOS was not significantly different between foal and adult MoDC. In contrast, the levels of IFN-γ, TNF-α, IL-10, MCP-1 ($p = 0.016$) and TGF-β ($p = 0.031$) were significantly lower in foal cells (Fig. 5).

No significant difference was observed in the production of IL-18, TGF-β (Fig. 5), MCP-1 and iNOS between

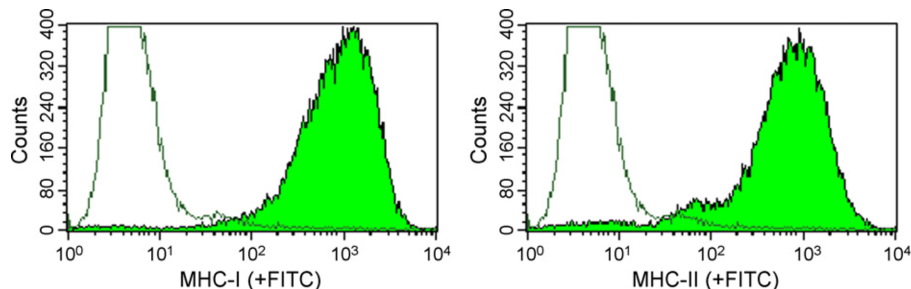

Fig. 2. Expression of MHC class I (left) and class II (right) molecules on the MoDC of a 3-month-old foal, as detected by flow cytometry. The empty histogram represents the staining by an isotype control antibody.

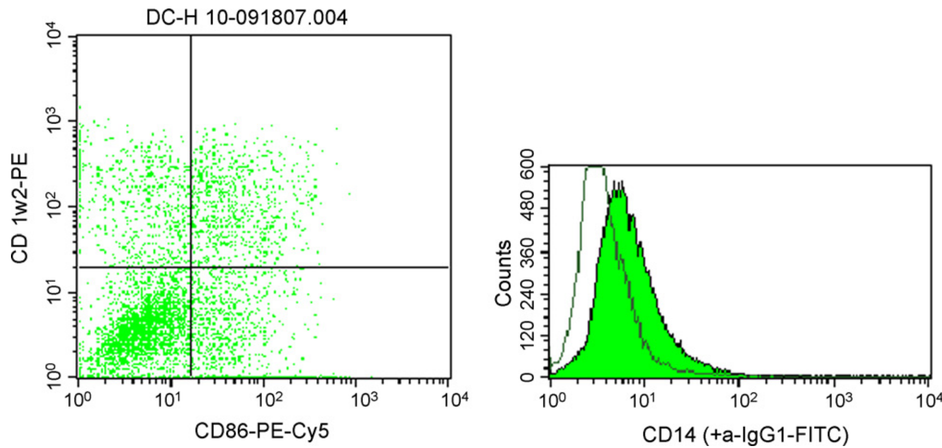

Fig. 3. Flow cytometry analysis of the MoDC of a 3-month-old foal after triple-staining with mAbs directed to CD1w2 (PE-labeled CC20), CD86 (PE-Cy-5-labeled IT2.2) and CD14 (big 10, revealed with an FITC-anti-mouse IgG1 antibody).

medium and LPS-cultured cells from adults or foals. LPS increased TNF-α and IL-6 in foal MoDC (TNF: $p = 0.016$; IL-6: $p = 0.016$). Surprisingly, no significant difference was found between medium and LPS-cultured cells in terms of IL-1β production in foals ($p = 0.938$), nor in adults ($p = 0.063$).

LPS significantly increased the IFN-γ ($p = 0.047$), IL-12, IL-15 and IL-10 ($p = 0.016$), but not MCP-1 mRNA levels in foal cells. IL-10 and IL-15 were also significantly increased in adult cells (Figs. 5 and 6). This was particularly high for IL-10.

However, LPS did not increase foal DC IFN-γ to the levels of adults ($p = 0.016$). The difference of TNF-α production between these groups were not significant after LPS stimulation ($p = 0.297$); the difference in TGF-β production was a little above the significance threshold ($p = 0.078$). The levels of IL-1, IL-6, IL-12, IL-15 and iNOS remained similar between foal and adult cells after culture with LPS, and those of IL-10 and MCP-1 remained significantly different ($p = 0.016$ and 0.031, respectively, Fig. 5).

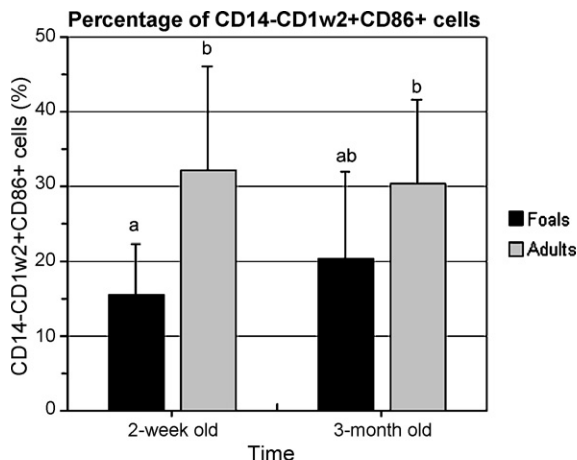

Fig. 4. Percentage of foal and adult horse $CD14^{-}CD1w2^{+}CD86^{+}$ MoDC. Adult mares were used as controls on the days when the foals were bled. Results are shown as average ± standard deviation. Different letters show percentages which are significantly different from each other: "a" is significantly different from "b", but not from "ab".

3.4. Relationship between the percentages of $CD14^{+}$ or $CD14^{-}CD1w2^{+}CD86^{+}$ cells and the cytokine mRNA levels

Low IFN-γ production by MoDC (cultured in medium alone or with LPS) were associated to low percentages (< 25%) of $CD14^{-}CD1w2^{+}CD86^{+}$ cells, whether they were differentiated from foals or from adult horses. MoDC preparations which contained high percentages of $CD14^{-}CD1w2^{+}CD86^{+}$ cells produced 3–50 times more IFN-γ.

The cell percentage/TNF-α mRNA curves of foals and adults were parallel: the TNF production in medium alone was related to the percentage of $CD14^{+}$ cells in foal MoDC, but surprisingly seemed to be inversely correlated to it in adult horse MoDC (Fig. 6). After stimulation with LPS, there was no visible relationship between these parameters.

The relative amount of IL-10 in cells cultured in medium was slightly correlated with the percentage of $CD14^{-}CD1w2^{+}CD86^{+}$ cells. However, except for one adult horse sample, MoDC cultured in LPS produced IL-10 proportionally to this percentage, following one line for both groups (Fig. 6; $p = 0.887$, $R = 0.860$). The same profile was observed for MCP-1 and to some extent for IL-12, both with or without LPS. In contrast, IL-18 (Fig. 6) and IL-6 production did not seem to be linked to the percentage of $CD14^{-}CD1w2^{+}CD86^{+}$ cells.

4. Discussion

In order to determine if the IFN-γ deficiency of newborn foals (Breathnach et al., 2006) was due to an insufficiency in the accessory signals of their DC, we compared foal MoDC to adult MoDC. Hammond et al. (1999) have shown that equine MoDC express high levels of MHC molecules and variable levels of CD86. Our results confirmed these findings, and also showed that foal 4-day MoDC expressed high levels of CD172a, similarly to adults. Moreover, triple-stainings enabled us to confirm the heterogeneity of equine MoDC. Cell surface expression of CD14, CD1w2 and CD86 defined 8 overlapping cell sub-populations in our preparations. Since CD14 is a marker of immature DC and monocytes/macrophages in horses (Mauel et al., 2006), we

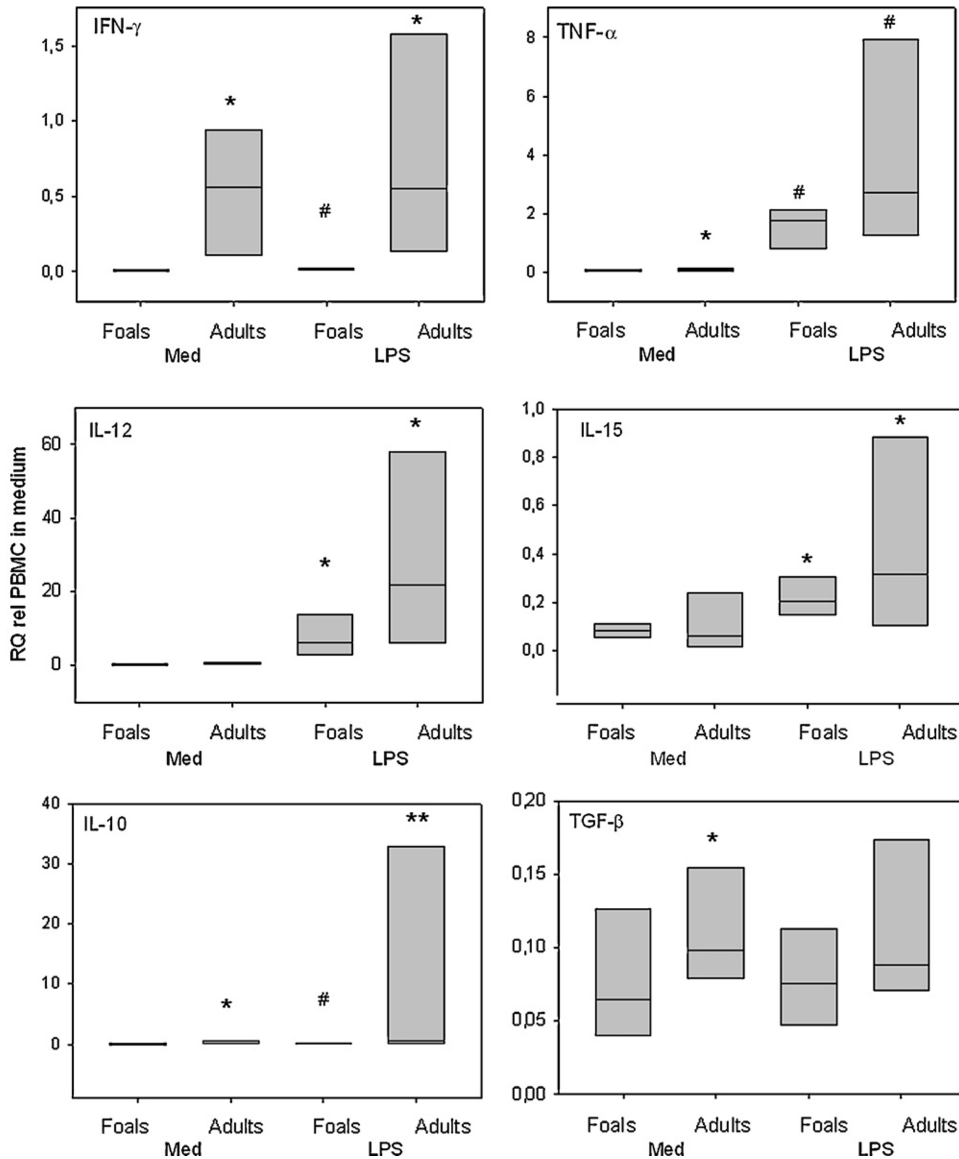

Fig. 5. Cytokine mRNA production by foal and adult horse MoDC cultured in medium alone or with 1 μg LPS/ ml. Results are expressed as median RQ (line within the boxes), 25th percentile (lower boundary of the boxes) and 75th percentile (upper boundary). '*', '#' and '**' show significant differences. Results were obtained from 6 foals and 4 adults.

analyzed the $CD14^-$ cells in detail. CD86 (B7.2) plays an essential co-stimulation role via its ligands CD28 and CTLA-4. It is present from the immature DC stages (Brigl and Brenner, 2004), and its expression increases during the maturation of human DC *in vivo* (Banchereau and Steinman, 1998) and equine MoDC (Mauel et al., 2006). CD1w2, the bovine and equine equivalent of CD1b (Howard et al., 1991), is found on equine lymph node paracortex cells of dendritic aspect (Siedek et al., 1997), which are likely to be interdigitating DC. In humans, CD1b is expressed on DC precursors, and on mature DC. Therefore, the association of CD86 and CD1w2 (CD1b) is likely to be characteristic of mature DC. The $CD1w2^-CD86^+$ cells of our equine MoDC cultures may be the equivalent of interstitial DC or Langerhans cells; the probable presence of Birbeck granules in some cells supports this hypothesis. The $CD1w2^+CD86^-$ cells could be precursors. Our results indicated that the MoDC from young foals contained fewer $CD14^-CD1w2^+CD86^+$ than adult horse MoDC (Fig. 4). This suggests that young foal dendritic cells are quantitatively less mature than their adult counterparts.

The MoDC obtained in our cultures were surprisingly mature, as shown by the strong expression of CD86. Human and mouse MoDC are usually immature before the adding of LPS or other pathogen-associated molecular pathways. Our results on horses were similar to those of Hammond et al. (1999), but Mauel et al. (2006) have obtained immature DC after 6–7 days of culture and sometimes still after 3 more days of culture with LPS. These discrepancies may have been due to the technical

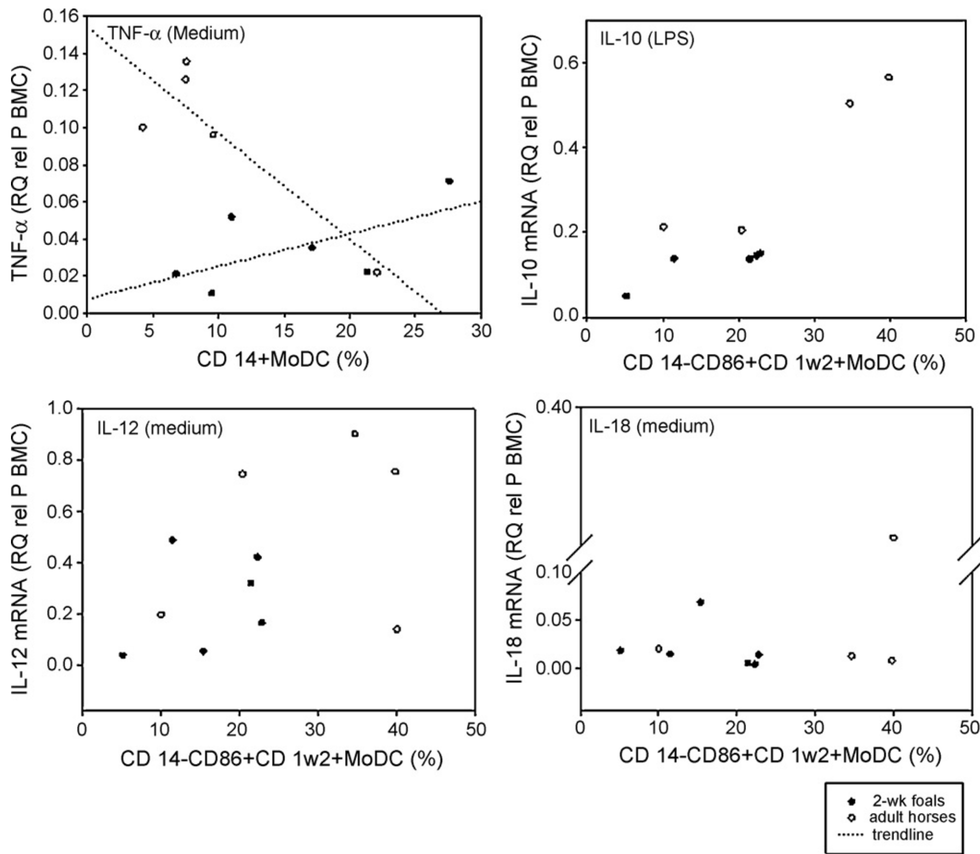

Fig. 6. Relationship between the amounts of TNF-α, IL-10, IL-12 and IL-18 mRNA and the percentage of $CD14^+$ or $CD14^-CD86^+CD1w2^+$ in foal and adult horse MoDC.

differences: Mauel et al. used FBS whereas we used autologous serum. Very low quantities of LPS might have been present in the sera and have accelerated the differentiation process. We however used autologous serum because: (1) it is an equine product; (2) it cannot induce any allogenic stimulation; (3) it contains the molecules monocytes are in contact with *in vivo*. Despite the probable variations of serologic status between the animals we used, the phenotype of their MoDCs showed high qualitative homogeneity within age groups.

The IFN-γ inducing cytokines IL-12, IL-15 and IL-18 were not produced in a significantly different way by foal and adult MoDC. So far, no significant difference between foal and adult horses has been found in terms of IL-12 production by unstimulated, CpG-ODN-stimulated or LPS-sitmulated MoDC (Flaminio et al., 2007). Furthermore, the administration of plasmid encoding equine IL-12 had no Th1 adjuvant effect on a *R. equi* vapA DNA vaccine in neonatal or adult horses (Mealey et al., 2007). The IFN-γ deficiency of foals may therefore not be explained by an insufficient production of IL-12 by DC. The lack of IFN-γ secretion by foals could have been explained by a higher IL-10 or TGF-β production by DC. However, our results show that the amounts of TGF-β were not higher in foal MoDCs and that those of IL-10 were even lower (Fig. 5). These results are consistent with the notion that foal MoDC are not Th2-biased (Flaminio et al., 2007).

The cytokines TNF-α, MCP-1 and IL-10 were three of the four molecules which we found were expressed at lower levels by foal MoDC. Since the amounts of IL-10 and MCP-1 mRNA looked proportional to the percentage of $CD14^-CD1w2^+CD86^+$ cells, especially after LPS stimulation, these are very likely to be the main producers of these cytokines. If this is true, it can be concluded that they respond to LPS by producing more IL-10, but not MCP-1 after 3 h. The cell percentage/RQ dots from most foals and adults were located on the same line on the chart (Fig. 6), which suggests that the relative lack of IL-10 and MCP-1 production by foal MoDC was mainly due to the lower number of $CD14^-CD1w2^+CD86^+$ cells. These cells are likely to respond indirectly to LPS, since they do not express CD14.

The possible $CD14^-CD1w2^+CD86^+$ cells deficiency cannot however explain why unstimulated foal MoDC produced less TNF-α than adult horse MoDC because the amount of TNF mRNAs was inversely correlated to the percentage of $CD14^-CD1w2^+CD86^+$ cells. TNF-α was probably produced by foal $CD14^+$ cells (Fig. 6) and the different aspect of the TNF/CD14+ cell curves between the two groups suggests that these cells have different mechanisms of TNF production depending on age. This may be important because the TNF-α levels were lower in foal cells (Fig. 5) and TNF-α can induce IFN-γ.

Altogether, our results suggest that neonatal foal MoDC do not have a decreased capacity to produce Th1-inducing

cytokines in general, but rather to produce cytokines of the inflammation pathway like IL-10 and TNF-α. LPS might partly trigger this capacity, but bacterial infections may happen too quickly for the foal's immature immune system to raise an adequate immune response.

Conflict of interest

None of the authors has conflict of interests.

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