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The early in vivo effects of a single antiemetic dose of dexamethasone on innate immune cell gene expression and activation in healthy volunteers

C. R. Bain,[1] D. F. Draxler,[2] R. Taylor,[3] S. Wallace,[4] O. Gouldthorpe,[5] T. B. Corcoran,[6] P. S. Myles,[7] R. L Medcalf,[8] K. Bozaoglu[9]

1 Specialist Anaesthetist and Researcher, Department of Anaesthesia and Perioperative Medicine, The Alfred Hospital and Monash University, Melbourne, Australia,
2 Senior Researcher, Molecular Neurotrauma and Haemostasis laboratory, Australian Centre for Blood Diseases and Monash University, Melbourne, Australia,
3 Research Assistant, Genomics and Systems Biology Laboratory, Baker IDI Heart and Diabetes Institute Victoria, Australia,
4 Research Manager, Department of Anaesthesia and Perioperative Medicine, The Alfred Hospital and Monash University, Melbourne, Australia
5 Anaesthetic Registrar, Department of Anaesthesia and Perioperative Medicine, The Alfred Hospital and Monash University, Melbourne, Australia,
6 Professor and Director of Research, Department of Anaesthesia & Pain Medicine Royal Perth Hospital and University of Western Australia, Perth, Australia,
7 Professor and Director, Department of Anaesthesia and Perioperative Medicine, The Alfred Hospital and Monash University, Melbourne, Australia,
8 Professor and Head, Molecular Neurotrauma and Haemostasis laboratory, Australian Centre for Blood Diseases and Monash University, Melbourne, Australia,
9 Senior Research Officer, Bruce Lefroy Centre for Genetic Health Research, Murdoch Children's Research Institute and Department of Paediatrics, University of Melbourne, Victoria, Australia

Correspondence to: **Chris R. Bain**
Department of Anaesthesia and Perioperative Medicine, The Alfred Hospital and Monash University, 55 Commercial Road, Melbourne Victoria 3004, Australia
Email: c.bain@alfred.org.au

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Summary

Dexamethasone is often administered to surgical patients for antiemetic prophylaxis. This study examined the early (up to 24h) *in vivo* effects of dexamethasone (8mg), to demonstrate the magnitude and temporal nature of changes, on circulating peripheral blood mononuclear cell gene expression and activation in ten healthy male volunteers. Blood samples were drawn at baseline, 2h, 4h and 24h. Gene expression was measured using quantitative real-time polymerase chain reaction. Cytokine expression was measured using multiplex immunoassays. Innate immune cell phenotypes were examined with flow cytometry. Dexamethasone resulted in rapid transient changes in Immunophilin (p=0.0247), Plasminogen Activator Inhibitor 1(p=0.0004), Forkhead Box P3 (p=0.0068), and Dual Specific Phosphatase 1 (p=0.0157) gene expression at 4h compared to pre-dexamethasone. Plasma Interleukin 10 levels increased within 2h (p=0.0071) and returned to baseline at 24h. Reductions in classical (p=0.0009) and intermediate monocytes (p=0.0178) and dendritic cells (p=0.0012) were followed by increases in the level of these populations at 24h compared to pre-dexamethasone (classical monocytes p=0.0073, intermediate monocytes p=0.0271, dendritic cells p=0.0142). There was a profound reduction in the mean fluorescence intensity of the maturation marker, human histocompatibility leukocyte antigen, at 24h in all monocyte subsets (p=0.0002 for classical and non-classical monocytes,

p=0.0001 for intermediate monocytes) and dendritic cells (p=0.0001). This study confirms rapid transient effects of 8 mg dexamethasone on innate immune cells with the potential to alter the inflammatory response to surgery and provides support for the hypothesis that intraoperative administration may be both immunosuppressive and immune-activating in the immediate perioperative period.

Dexamethasone is a potent synthetic glucocorticosteroid frequently administered intravenously during surgery (4-8 mg) as a first line antiemetic [1] that has potentially significant immunological effects [2]. However, little has actually been described of the magnitude and timing of the *in vivo* effects of a single perioperative dose of dexamethasone on innate immune cell gene expression and activation or how these actions may influence the response to surgical stress.

Endogenous glucocorticoid (cortisol) at basal levels is critical for normal metabolic and inflammatory function . During an inflammatory stimulus, glucocorticoid levels rise and act to suppress inflammation and limit the damage thereof [3]. “Stress associated” concentrations of cortisol typical of the surgical response, induce transient but significant monocytopenia, lymphopenia and neutrophilia within 6 hours [4]. Surgery induces sterile inflammation with tissue bound and circulating innate immune cells (dendritic cells, monocytes and macrophages) responding to tissue damage. These cells, by means of antigen presentation, stimulate an adaptive immune response. T lymphocytes known as T Helper type 1 (Th-1) cells then release the proinflammatory cytokines interferon-γ (INF-γ), interleukin-2 (IL-2) and tumour necrosis factor-α (TNF-α) promoting cell mediated immunity, whereas T Helper Type 2 cells (Th-2) secrete anti-inflammatory cytokines (interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13)) to stimulate humoral immunity and depress cell mediated immunity [2,5].

Dexamethasone administration may rapidly modify this response and the magnitude of the effect will depend on the timing of administration, the dose and the background cortisol levels. *In vitro*, in murine mammary and anterior pituitary corticotroph cells, John and colleagues [6] demonstrated that glucocorticoid receptor (GR) mediated stimulation of gene expression by dexamethasone (100 nM) resulted in complex regulation profiles. Rather than

simple repression or activation of gene expression, for some genes the changes were transient peaking within 4h then returning to baseline and, in other cases, the changes persisted and continued beyond 24h [6]. *In vivo*, Menke and colleagues [7] demonstrated rapid (within 3h) changes in whole blood gene expression profiles following dexamethasone (1.5 mg) in a cohort of patients with depression. However, to begin to understand the impact of these effects on immunity it is necessary to focus on immune cell subsets. Galon and colleagues [8] examined the effects of dexamethasone (10^{-7}M), *ex vivo*, on peripheral blood mononuclear cells (PBMCs). They demonstrated a bidirectional action on gene expression that was both immune-stimulatory and immunosuppressive with patterns suggestive of increased innate compared to adaptive immune activity.

Given the above data it is evident that dexamethasone has important genomic and cellular actions on PBMCs and these effects are likely to rapidly follow intraoperative administration. However, currently there is no data on the early (2-24h) *in vivo* effects on PBMCs. We, therefore, conducted a preliminary analysis to determine the magnitude and timing of dexamethasone induced changes in PBMC gene expression and activation at 2h, 4h and 24h in otherwise healthy volunteers.

METHODS

Following approval by The Alfred Hospital Ethics Committee (HREC Number: 493/15), ten healthy male volunteers aged between 20-35 years old provided written informed consent for inclusion in this study. No clinical (phenotype) data was collected, however each participant was specifically questioned regarding concurrent illness. One participant was recovering from a recent viral illness and was excluded from the study. Each participant was examined by a medical practitioner with blood pressure, heart rate, temperature and oxygen saturation being measured. Baseline blood was collected after a 22g intravenous cannula was inserted. Dexamethasone (8 mg) was mixed with saline (100 ml) and administered intravenously over 15 minutes. Participants were required to stay in the clinical area for 30 minutes after the infusion was completed and the cannula was then removed. Participants returned to the research area at 2 hours (h), 4h and 24h for blood collection. The baseline and 24h bloods were collected between 0645 and 1100h. All participants recruited to the study completed the protocol. There were no adverse drug reactions or effects reported.

Ten mls of blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes (BD Bioscience, New Jersey, USA), and placed on ice immediately. One ml of blood was taken from this EDTA tube and stored at -80^{o}C in an eppendorf tube. Then 4 mls of whole blood was transferred into a 15ml falcon tube and subjected to PBMC fractionation using the Ficoll-Paque density centrifugation protocol as per manufacturer's instructions (GE Healthcare, Chicago, USA). The pellets were resuspended in Trizol and stored at -80°C.

The remaining 4ml of blood was used to isolate plasma, buffy coat and red blood cells (RBCs). The blood was centrifuged at 3000 rpm for 20 mins at 22°C, deceleration=1. Then plasma, buffy coat, and RBCs were collected and stored in Eppendorf tubes at -80°C. The buffy coat was stored in 10% dimethyl sulphoxide (DMSO)/90% foetal bovine serum (FBS) in a cryovial and allowed to cool in a commercially available freezing container (Nalgene® Mr. Frosty® Cryo 1°C Freezing Containers, Thermo Fisher Scientific, Massachusetts, USA) to achieve a cooling rate of -1°C.min^{-1}.

RNA was extracted from cells using Trizol (Life Technologies, California, USA) followed by column purification using Direct-zol™ RNA MiniPrep kit (Zymo Research) as per manufacturer's instructions. Complementary DNA (cDNA) was prepared from the same amount of ribonucleic acid (RNA) template (2 µg) with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, California, USA). Primer sequences used for quantitative real-time polymerase chain reaction (qRT-PCR) are described in supplementary Table 1. QuantiNova SYBR Green PCR Kit (QIAGEN, Hilden, Germany) was used to report relative gene expression levels in duplicate using the ABI 7500 Real-Time PCR machine (Applied Biosystems, California, USA). Gene expression was calculated using the comparative Ct method with normalization to the housekeeping gene Nuclear Receptor Binding Protein 1 (NRBP). We selected genes that were either known to be dexamethasone sensitive and/or where changes represented a potentially significant immunological action (Table 1).

Plasma cytokine levels were measured in duplicate using custom human ProcartaPlex® high sensitivity multiplex immunoassay panels (Affymetrix eBioscience, San Diego, USA) by elisakit.com, as per manufacturer's instructions. The cytokines included the multiplex panel IFN-γ, Interleukin-1 beta (IL-1β), IL-10, IL-4, interleukin-6 (IL-6), TNF-α, soluble tumour necrosis factor receptor-2 (TNF-R2) and transforming growth factor-beta (TGF-β) were chosen to reveal significant anti or proinflammatory effect, potentially mediated by rapid

dexamethasone induced changes in gene expression, or where significant changes have previously been associated with important perioperative outcomes (Table 1).

After thawing, PBMCs were washed twice and transferred to a 96-well round-bottom plate (Corning, New York, USA) to be stained with a fluorochrome-label. We have recently established two antibody cocktails for surface staining of PBMCs to assess the cellular innate immune response [9]. The assessed myeloid and lymphoid cell populations as well as functional markers were chosen as they represent key cells/markers involved in inflammation and immune stimulation as well as immunosuppression (Supplementary Table 2). In brief, one cocktail was used to identify myeloid cell populations and it consisted of Cluster of differentiation (CD) 33 Fluorecein isothiocyanate (FITC), Human histocompatibility leukocyte antigen (HLA-DR) allophycocyanin (APC/Cy7), CD11c brilliant violet (BV) 421, CD141 APC, CD1c phycoerythrin (PE)Cy7, CD14 alexa fluor (AF)700, CD16 BV510, CD83 PE/Dazzle 594, CD86 peridinin chlorophyll protein (PerCP)/Cy5.5 and Zombie Yellow (all BioLegend, San Diego, USA) for exclusion of dead cells. After incubation for 20 mins on ice, cells were washed and resuspended in 100µl of phosphate buffer solution (PBS)+2%FBS+1% paraformaldehyde (PFA).

The second cocktail was used for identification of lymphoid cell populations and consisted of CD3 BV510, CD4 BV650, CD8 AF700, CD56 APC/Cy7, CD25 PerCP/Cy5.5 and Zombie Yellow (all BioLegend, San Diego, U.S.). Cells were incubated for 20 mins on ice, followed by a washing step before they were permeabilised in 200µl of Fix/Perm buffer (eBioscience, San Diego, USA). After incubation for 30-60 mins at room temperature, cells were washed using Perm buffer (eBioscience, San Diego, USA) before 50µl of FoxP3 PE/Dazzle594 antibody diluted in Perm buffer was added for intracellular staining of cells. Samples were incubated for 30 mins at room temperature, and then washed again with Perm buffer, and finally resuspended in 100µl of PBS. Stained samples were acquired on a 4-laser LSR Fortessa (BD Biosciences, New Jersey, USA) with BD FACSDiva software (BD Biosciences, New Jersey, USA). Single stain controls for compensation were generated utilising UltraComp eBeads (eBioscience, San Diego, USA) and the analysis of acquired samples was performed in FlowJo data analysis software (FlowJo, LLC, Ashland, USA).

All statistical analyses were performed within Prism 7, Graphpad software (La Jolla, USA). The data is compared to pre-dexamethasone levels using repeated measure one-way ANOVA with Dunnett's multiple comparisons test and Student’s unpaired t-tests. Differences were

statistically significant if $p<0.05$.

RESULTS

In our healthy volunteers, significant changes in gene expression were observed following dexamethasone administration (Table 2A and Figure 1). Within 2h increased expression of known glucocorticoid responsive genes, Immunophilin (FKBP5) and Plasminogen Activator Inhibitor 1 (PAI-1), confirmed a rapid onset of action at the mRNA level. Expression remained elevated at 4h and returned to baseline pre-administration levels by 24h. A rapid reduction in Forkhead Box P3 (FoxP3) and Dual specific Phosphatase 1 (DUSP1) gene expression was observed at 2h and 4h however, the FoxP3 mRNA levels remained repressed at 24h relative to pre-dexamethasone levels. Gene expression of Antigen Presenting Glycoprotein (CD-1D) increased transiently at 4h and the proinflammatory cytokine gene, interleukin 1β (IL-1β), was reduced within 2h. Tumour Necrosis Factor Receptor 2 (TNFR2) gene expression was also decreased at 4h. This data confirms the rapid and transient *in vivo* genomic action of intravenous dexamethasone (8mg) with significant changes being present within 2h, that return back to baseline by 24h in healthy volunteers.

The effects on plasma cytokine levels was measured using high sensitivity multiplex immunoassays at baseline, 2h, 4h and 24h post treatment (Table 2B and Figure 2). Significant time-dependent increases in the level of the anti-inflammatory cytokine, IL-10 was evident at 2h and 4h and returned to baseline by 24h, whilst soluble TNF-R2 levels decreased over 24h compared to pre-dexamethsone. The proinflammatory cytokine, TNF-α, did not demonstrate any significant changes and, therefore, the IL-10/TNF-α ratio was significantly reduced at 2h and 4h. Other proinflammatory cytokines, (IL-1β and IL-6), as well as the immunosuppressive mediator TGF-β, and the Th-1 linked IFN-γ and Th-2 linked IL-4 showed no significant changes. This data demonstrates a rapid transient *in vivo* anti-inflammatory effect of dexamethasone.

The effects on innate immune cells were examined by flow cytometric phenotyping of PBMCs. First, we assessed the number of PBMCs recovered from 2ml of whole blood (Table 2C and Figure 3) and found a gradual decrease of cells within the first 4h, however by 24 hours, the number of PBMCs were significantly increased compared to baseline. A

significant reduction was observed in classical monocytes and conventional dendritic cells (cDCs) at 2h and 4h post administration. There was also a reduction of intermediate monocytes at 2h. Cell numbers were significantly increased at 24h after administration in classical monocytes, intermediate monocytes, cDCs, but not in non-classical monocytes. Changes in cDC levels affected both the CD1c+ and the CD141+ subset at 4h with a subsequent increase of CD141+ cDC at 24h (supplementary Figure 1). A continuous increase was observed for NK cells within 24h post administration.

HLA-DR mean fluorescence intensity (MFI), representing expression strength, decreased significantly in intermediate monocytes and increased in cDCs within the first 4h. However, by 24h HLA-DR was down regulated in all monocyte subsets as well as cDCs (Table 2D and Figure 3). Interestingly, CD83 expression increased significantly within 4h in intermediate monocytes and cDCs followed by a reduction close to baseline at the 24h time point. In classical monocytes CD83 expression increased gradually up to 24h post administration of dexamethasone . Non-classical monocytes upregulated CD83 expression only at 24h (Table 2D and Figure 3). CD86 was unaffected in all assessed cell populations except for a reduction in non-classical monocytes at 4h post dexamethasone administration (Table 2D). Both, CD4+ T cells and CD8+ T cells were decreased at 4h compared to pre-dex (supplementary Figure 1). Also, Treg cells were reduced at 2h post-dexamethasone administration, which is consistent with the FoxP3 gene expression data. In contrast, the number of immunosuppressive CD14+ CD16+ monocytic myeloid derived suppressor cells (MO-MDSC) showed a gradual increase (per ml blood) over the assessed time with a peak at 24h post administration (supplementary Figure 1).

DISCUSSION

The prophylactic antiemetic effect of 4-8 mg of dexamethasone is well established [1], and has the potential to significantly modify the inflammatory response to surgery [2]. However, little is currently described of early *in vivo* effects on the innate immune system in otherwise well surgical patients.

Changes in PBMC gene expression occur rapidly (within 2h), persist at 4h and mostly resolve within 24h. The pattern of change suggests intracellular signalling shifts towards an immunosuppressive phenotype (e.g. decreased Fox-P3) for some genes, but for other genes

the observed change is potentially immune-stimulatory (e.g. decreased DUSP1). The reduced IL-1β gene expression levels at 2h and 4h is consistent with a rapid anti-inflammatory effect and we observed little effect on other classic pro or anti-inflammatory cytokine genes (TNF-α and IL-10) during the 24h period of this investigation. This is an important finding as changes in the ratio of mRNA for these genes has been associated with a perioperative immunomodulatory state that may increase the risk of infection [10].

At the functional level, whilst we demonstrated striking effects of dexamethasone on plasma IL-10, similar effects were absent in other cytokines including TNF-α. This is consistent with a previously described effect following a much larger dexamethasone dose (100mg) in adult patients following cardiopulmonary bypass that was associated with augmented increases in plasma IL-10 and decreased elevation of TNF-α [11]. Thus, the single antiemetic dose may have an anti-inflammatory effect within 2hrs that can potentially change the perioperative inflammatory response. However, this effect was not reflected by changed PBMC cytokine gene expression, again suggesting that the net effect of an antiemetic dose of dexamethasone is quite variable. This is consistent with the study by Galon *et al*., [8] that demonstrated that dexamethasone can have simultaneous stimulatory and inhibitory effects on various inflammatory pathways in immune cells [8]. Notably, the reduction in soluble TNF-R2 we demonstrate here suggests reduced inhibition of free TNF-α that may result in enhanced TNF-α signalling in target cells [12].

Accompanying these rapid genomic actions were significant *in vivo* changes in levels of innate immune cells. We observed a significant decrease in classical monocytes within 2h of dexamethasone administration. These cells are a major source of proinflammatory cytokines and, due to their capacity to present antigens to lymphocytes, they also function to link the innate and adaptive immune response [13]. A similar effect was observed for cDCs, also potent antigen-presenting cells, consistent with an initial suppressive effect on innate immunity. However, these changes were followed by significant increases above baseline at the 24h time point, suggesting a compensatory response to the early dexamethasone induced effects. These observations may relate to the previously described biphasic response to changing levels of endogenous glucocorticoids [14]. In this model, transient elevation of *in vivo* glucocorticoid concentrations to levels observed during major systemic stress enhances a subsequent delayed *in-vivo* inflammatory response to an innate immune trigger [15-16]. Recent data suggest that, at physiological stress levels, endogenous glucocorticoid may be

able to regulate leukocyte trafficking through changes in the expression of adhesion and chemotaxis molecules such as CCR2 and CX3CR1 [4].

While we did not examine these surface markers directly, the maturation marker HLA-DR, facilitating presentation of extracellular antigens by myeloid cells [13], was upregulated in some (cDCs), and down regulated in other (intermediate monocytes) myeloid subsets in the early phase after dexamethasone administration despite the profound changes in the numbers of corresponding cells. By 24h HLA-DR expression was reduced in all monocyte subsets as well as cDCs. This observation is consistent with previous *ex-vivo* studies where hydrocortisone has been shown to down regulate HLA-DR on all monocyte subsets [17]. The gradual increase in MO-MDSC over 24h is consistent with an immunosuppressive effect of dexamethasone, although significance was not reached in our volunteers. However, the early opposing regulation of HLA-DR expression in different antigen-presenting cells suggests that dexamethasone may initially act to modulate the innate immune response rather than to simply suppress it. Notably, natural killer cells, which are known for their involvement in the innate immune response [18], showed a gradual increase up to 24h after dexamethasone administration. In turn, CD4+ T cells and CD8+ T cells and the immunosuppressive Treg cells were rapidly reduced, but returned to baseline at 24h.

Expression of the activation marker CD83 increased significantly within 4h after dexamethasone injection and returned to baseline at 24h in both intermediate monocytes as well as conventional dendritic cells. In turn, CD83 expression on classical monocytes increased continuously and reached significance 24h post-administration, suggesting time-dependent differences between myeloid cell subsets to respond to dexamethasone-induced modulation of their activation state. However, dexamethasone, surprisingly, seems to act as a myeloid immune cell activator in all of them. Dexamethasone maximally suppresses (<5%) the hypothalamic-pituitary-adrenal axis at 24h [19], an action that is potentially proinflammatory due to decreased endogenous cortisol secretion. It remains to be determined whether late increases in CD83 expression represents a delayed effect of dexamethasone or rather the compensatory response due to dropping endogenous glucocorticoid levels. Further functional testing is required to ascertain the net effect of the changes in HLA-DR and CD83 expression on the responsiveness of monocytes and conventional dendritic cells to immune triggers.

In a recent placebo controlled, clinical study of patients undergoing gynaecological surgery, Corcoran and co-workers demonstrated that dexamethasone 4 mg significantly attenuated surgically induced lymphopenia and C-reactive protein increase post operatively [20]. The changes were detected at 24h, but not at the study's later time points (48h, 72h or 6 weeks), suggesting that the effects of dexamethasone are also transient in the surgical setting. Interestingly they also detected increased monocyte numbers at 24h following dexamethasone [20]. In this study examining the effects of dexamethasone on PBMCs at 2h and 4h as well as 24h, we focused on early innate immune effects. Our data suggests that the monocyte and conventional dendritic cell changes at 24h may be due to compensation from rapid, potent anti-proliferative effects of dexamethasone on myeloid cell subsets, and highlights important potential early effects on innate immunity for surgical patients.

There are a number of limitations to this study. This is a small cohort of healthy male volunteers without a placebo comparison. We, therefore, cannot assume the results are generalizable or reflect what will be observed in surgical patients. We also do not know if this will have any bearing on important outcomes such as surgical site infection and sepsis. A larger study in surgical patients is warranted.

In summary, a single antiemetic dose of dexamethasone in healthy volunteers creates rapid transient effects on PBMC gene expression and plasma cytokines, with dramatic changes in innate immune cell numbers and activation *in vivo*. It is possible that these changes may be relevant in altering the inflammatory response to surgery. Moreover, these findings support the notion that intraoperative dexamethasone may be both immune-activating and immunosuppressive and establishes the timing for future analyses into determining the impact of early effects of dexamethasone on the inflammatory response perioperatively.

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Additional contributor: Mr H Trahair

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Figure 1. **Effects of intravenous dexamethasone (8mg) administration on PBMC gene expression in healthy volunteers.** Gene expression was measured using quantitative real time PCR. Gene expression of A)TNFα, n=9; B) IL-6, n=9; C) DUSP1, n=9; D) IL-10, n=9; E) IL-1β, n=8; F) FoxP3, n=6; G) HLA-DR, n=8; H) TNFR, n=7; I) CD-1D, n= 9; J) PAI-1, n=9; K) FKBP5, n=8. Data is presented as mean (SD). Significance was calculated using a one-way ANOVA with Dunnett's correction; * unpaired t test; pre-dex, pre-dexamethasone administration; h, hour; AU, arbitrary units.

Figure 2. Effects of intravenous dexamethasone (8mg) on plasma cytokine levels in healthy volunteers. A) TNF-α; B) IL-1β; C) IL-6; D) IL-10; E) TNFα/IL-10; F) TGF-β G) INF-γ; H) IL-4; I) TNF-R2. Plasma cytokine levels were measured using a standard (TNF-R2 and TGF-β) and high sensitivity multiplex immunoassay. Data is presented as mean (SD); n=9. One-way ANOVA with Dunnett's correction; * unpaired t test; Pre-dex, pre-dexamethasone administration; h, hour.

Figure 3. Flow-cytometic analysis of PBMCs following intravenous dexamethasone (8mg) in healthy volunteers. Flow-cytometric analysis of cryopreserved PBMCs to assess numbers and activation status of innate immune cells before a single injection of 8mg dexamethasone and 2h, 4h, and 24h after administration. A) PBMCs; B) NK cells C) Treg; D) class monocytes; E) HLA-DR on class monocytes; F) CD83 on class monocytes; G) intermed monocytes; H) HLA-DR on intermed monocytes; I) CD83 on intermed monocytes; J) non-class monocytes; K) HLA-DR on non-class monocytes; L) CD83 on non-class monocytes; M) cDCs; N) HLA-DR on cDCs; O) CD83 on cDCs. Cell numbers represent cells recovered from cryopreserved samples. Data are expressed as mean (SD); n=9; one-way ANOVA with Dunnett's correction; * unpaired t test. pre-dex, pre dexamethasone

administration; h, hour; PBMCs, peripheral blood mononuclear cells; NK cells, natural killer cells; class monocytes, classical monocytes; Treg; regulatory T cells; intermed monocytes, intermediate monocytes; cDCs, conventional dendritic cells; MFI; mean fluorescence intensity.

TABLE 1: GENES AND CYTOKINES ANALYSED BY RT-PCR AND PLASMA MULTIPLEX

IMMUNOASSAY.

	Name	Immune effects	Rationale
IL-10	Interleukin-10	• Anti-inflammatory cytokine produced by Th2 cells. • Stimulates Th2 response and suppresses Treg cells [5] • May be susceptible to TA [21]	• Gene expression may represent an immunosuppressive response to trauma [22] • IL-10 expression in T cells contributes to a Th2 shift [5]
IL-4	Interleukin-4	• Anti-inflammatory cytokine produced by Th2 cells [5]	• GCs mediate upregulation associated with a Th2 shift [5].
TGF-β	Transforming growth factor	• Anti-inflammatory cytokine [5]	• Upregulated by GCs in PBMCs *ex*-vivo [8]
TNF-α	Tumour necrosis factor alpha	• Proinflammatory cytokine upregulated during adaptive immune response. Stimulates cell mediated immunity • Susceptible to TR [21]	• Perioperative *in vivo* changes in the ratios of TNF-α/IL-10 mRNA have been associated with increased perioperative complications and infections [10,23]
IFN-γ	Interferon gamma	• Proinflammatory cytokine [5]	• Susceptible to TR [21]
IL-6	Interleukin-6	• Proinflammatory innate immune cytokine. Stimulates hepatic CRP production • Susceptible to TR [21]	• Classically suppressed by GC during cardiac surgery. Elevated in post-surgical SIRS [24] • Increases in IL-6 may reflect a pro inflammatory effect of GC [14]
IL-1β	Interleukin-1β	• Proinflammatory cytokine, co-stimulator of T cell function [25] • Susceptible to TR [21]	• Levels rise after surgery. Produced by intermediate monocytes in sepsis [24]
TNF-R2	TNF receptor 2	• Receptor principally expressed in T_{reg} Cells [12]	• Soluble form is a biomarker of chronic inflammation [26]

DUSP1	Dual specific phosphatase 1	• Anti-inflammatory. • Negative feedback on MAPK signalling [14]	• Susceptible to TA [21]
PAI-1	Plasminogen activator inhibitor	• Anti-inflammatory effects and inhibition of fibrinolysis [21]	• GC sensitive [27]. Potentially susceptible to TA [21]
FoxP3	Forkhead Box P3	• Anti-inflammatory. A marker of CD4+ CD25+ regulatory T (T_{reg}) cell development and function [28]	• GC sensitive gene [21,29] • Lower *in vivo* mRNA levels with immunosuppression and increased risk of perioperative infection [10]
FKBP5	Immunophilin or FK506 Binding protein 5.	• GR co-chaperone modulates GR/GC signalling [30]	• Classic immediate GR/GC target • Reliable marker of GR activation [7]
CD-1D	Antigen presenting glycoprotein	• Anti-inflammatory • May be susceptible to TA [21]	• Stimulates inhibitory NK and invariant T cells [31]
HLA-DR	Human histocompatibility leukocyte antigen-DR	• Expression on intermediate monocytes during innate immune activation and antigen presentation to T cells	• Down regulated in surgical patients [32] • Predict immunosuppression and systemic inflammation [33]

GR: Glucocorticoid receptor; GC: Glucocorticoid; TR: Transrepression, GR mediated inhibition of gene expression through interaction with transcription factors (e.g. AP-1 and NF-□B); TA: Transactivation, GR mediated activation of anti-inflammatory genes through interaction with glucocorticoid response elements (GLE) within DNA; NK-cells: Natural killer cells; Th1; Helper T cells (Type 1); Th2: Helper T cells (Type 2); mRNA; messenger RNA, MAPK: Mitogen activated protein kinase; SIRS: Systemic inflammatory response syndrome.

TABLE 2: CHANGES IN PBMC INFLAMMATORY A) GENE EXPRESSION, B) CYTOKINE LEVELS, C) CELL NUMBERS AND D) ACTIVATION FOLLOWING DEXAMETHASONE 8MG IN HEALTHY VOLUNTEERS

A

Gene	**pre-dex**	**t=2h**		**t=4h**		**t=24h**	
(AU)	**mean (SD)**	**mean (SD)**	**p value**	**mean (SD)**	**p value**	**mean (SD)**	**p value**
TNF-α	104.5 (35)	79.64 (21)	0.1760	99.56 (34)	0.9496	113.3 (34)	0.4829
IL-6	114 (52)	13 (64)	0.6513	208.8 (125)	0.0827	116.4 (48)	0.9948
DUSP1	141.5 (90)	90.21 (69)	**0.0235**	56.93 (66)	**0.0157**	242.4 (269)	0.4446
IL-10	102.8 (15)	95.43 (19)	0.5005	93.25 (24)	0.4072	101.2 (16)	0.9843
IL-1β	176.7 (133)	60.24 (39)	**0.0328***	72.78 (39)	0.1250	186.5 (130)	0.9789
FoxP3	109 (43)	46.37 (33)	**0.0194***	23.2 (21)	**0.0068**	65.11 (19)	**0.0481***
HLA-DR	117.9 (86)	89.29 (58)	0.1478	71.23 (45)	0.0621	108.4 (80)	0.6094
TNF-R2	149.5 (71)	208.1 (212)	0.8493	102.4 (53)	**0.0388**	225.7 (223)	0.7863

CD-1D	102.3 (22)	134.7 (100)	0.7097	177.7 (81)	**0.0164***	103.4 (60)	0.9999
PAI-1	126 (109)	237.2 (110)	**0.0478***	417.8 (217)	**0.0004**	142 (95)	0.9532
FKBP5	403.8 (406)	1797 (1446)	**0.0248**	1517 (1133)	**0.0247**	214.7 (167)	0.2326

B

Cytokines pg.ml-1	**Pre-dex**	**t=2h**		**t=4h**		**t=24h**	
	mean (SD)	**mean (SD)**	**p value**	**mean (SD)**	**p value**	**mean (SD)**	**p value**
TNF-α	1.209 (0.018)	1.221 (0.227)	0.9900	1.28 (0.206)	0.4952	1.234 (0.093)	0.9410
IL-1β	0.85 (0.199)	0.835 (0.201)	0.9253	0.916 (0.207)	0.5273	0.909 (0.139)	0.3888
IL-6	1.721 (0.229)	1.617 (0.278)	0.4290	1.533 (0.162)	0.2173	1.781 (0.433)	0.9688
IL-10	0.266 (0.107)	0.572 (0.275)	**0.0071**	0.105 (0.969)	**0.0273***	0.223 (0.059)	0.5709
TNFα/IL10	4.996 (1.366)	2.46 (1.00)	**0.011**	1.904 (1.096)	**0.011**	5.78 (1.176)	0.2233
TGF-β	647.4 (289.2)	627.8 (276.7)	0.970	706.8 (332.6)	0.8515	631.6 (264.9)	0.9835
IFN-γ	1.613 (1.257)	1.39 (0.818)	0.368	1.442 (1.255)	0.0710	1.39 (0.954)	0.2386
IL-4	3.309 (0.563)	3.289 (0.394)	0.997	3.324 (0.228)	0.9994	3.147 (0.407)	0.7606
sTNF-R2	745 (41.29)	709.3 (83.84)	0.3528	692.3 (74.17)	0.0557	646.9 (54.7)	**0.0069**

*, unpaired t test, all other p-values are calculated using a repeated measure one-way ANOVA with Dunnett's correction

C

Cell counts No.ml-1	**Pre-dex**	**t=2h**		**t=4h**		**t=24h**	
	mean (SD)	**mean (SD)**	**p value**	**mean (SD)**	**p value**	**mean (SD)**	**p value**
PBMCs	596,600 (329,330)	465,050 (335,018)	0.6094	344,450 177,581	**0.0179**	1,073,500 (499,355)	**0.0220**

Classical monocytes	223,361 (103,954)	107,216 (40,230)	**0.0179**	47,216 (28,947)	**0.0009**	539,571 (209,325)	**0.0073**
intermed. monocytes	9024 (2,620)	5339 (2868)	**0.0178***	6469 (2520)	0.0641	35,412 (22,504)	**0.0271**
non-class. monocytes	9 679 (7175)	5 491 (3017)	0.1738	7606 (3534)	0.3443	8849 (2680)	0.9771
cDC	14,083 (6698)	4240 (2922)	**0.0088**	2750 (1279)	**0.0012**	24,641 (9365)	**0.0142***
CD1c+ cDC	12,660 (6018)	3461 (2305)	**0.0064**	2258 (1056)	**0.0012**	22,412 (8489)	**0.0125***
CD141+ cDC	481 (195)	446 (397)	0.9703	283 (178)	**0.0076**	1183 (748)	**0.0341**
MO-MDSC	3298 (6805)	5720 (15,901)	0.8441	9827(20,412)	0.5063	28,436 (26,719)	**0.0147***
NK cells	5896 (2579)	16,696 (16,122)	0.2293	25,885 (19,357)	**0.0484***	38,069 (31,572)	**0.0213***
T cells	15,4398 (62,390)	109573 (60,149)	0.2969	83,538 (49,469)	**0.0249**	231,819 (104,578)	0.1612
CD4+ T cells	48,138 (35,519)	28,507 (24,280)	0.2166	17,150 (12,476)	**0.0350**	70,609 (44,144)	0.3715
CD8+ T cells	75,036 (40,398)	49,647 (16,333)	0.2067	43,365 (24,833)	**0.0471**	112,412 (74,085)	0.2420
Treg	1604 (1366)	538.6 (636.3)	**0.0500***	652.2 (648.7)	0.0982	1972 (1074)	0.8166

Intermed, intermediate; non-class, non-classical; MO-MDSC, monocytic myeloid-derived suppressor cells

*, unpaired t test, all other p-values are calculated using a repeated measure one-way ANOVA with Dunnett's correction

D

Surface markers (MFI)	**Pre-dex mean(SD)**	**t=2h mean (SD)**	**p value**	**t=4h mean (SD)**	**p value**	**t=24h mean (SD)**	**p value**

				classical monocytes			
HLA-DR	27,356 (3274)	27,359 (4469)	0.9999	28,734 (5459)	0.7276	21,478 (1951)	**0.0002**
CD83	2736 (428.6)	2806 (693.9)	0.9758	3536 (1030)	**0.0472***	4059 (1152)	**0.0176**
CD86	4109 (345.9)	4019 (254.9)	0.4994	3872 (603.2)	0.5583	3899 (311)	0.1379
				intermediate monocytes			
HLA-DR	73,202 (12,437)	68,485 (14,736)	0.1314	60,665 (11,029)	**0.0013**	45,827 (5406)	**0.0001**
CD83	5725 (1260)	7209 (3296)	0.1709	10067 (4940)	**0.0334**	8388 (2495)	**0.0213**
CD86	4590 (516.3)	4948 (1008)	0.3891	5527 (1496)	0.1942	4897 (557.9)	0.2367
				non-classical monocytes			
HLA-DR	30,052 (6243)	27,628(6822)	0.0874	24,713 (5887)	**0.0087**	19,027 (3748)	**0.0002**
CD83	9150 (3357)	9170 (4114)	0.9999	9965 (4536)	0.7155	13,089 (4017)	**0.0274**
CD86	3340 (414)	3217 (468)	0.1313	2905 (506.2)	**0.0032**	3084 (294.7)	0.0750
				cDC			
HLA-DR	57,144 (8857)	63,110 (9865)	**0.0071**	65,738 (6777)	**0.0078**	40,603 (9008)	**0.0001**
CD83	1495 (186.3)	2104 (540.6)	**0.0044**	2228 (460.5)	**0.0010**	1648 (248.8)	0.2114
CD86	1159 (90.39)	1207 (136.5)	0.1768	1198 (145)	0.5209	1130 (117)	0.4004

cDC: conventional dendritic cells

*, unpaired t test, all other p-values are calculated using a repeated measure one-way ANOVA with Dunnett's correction

Figure 1

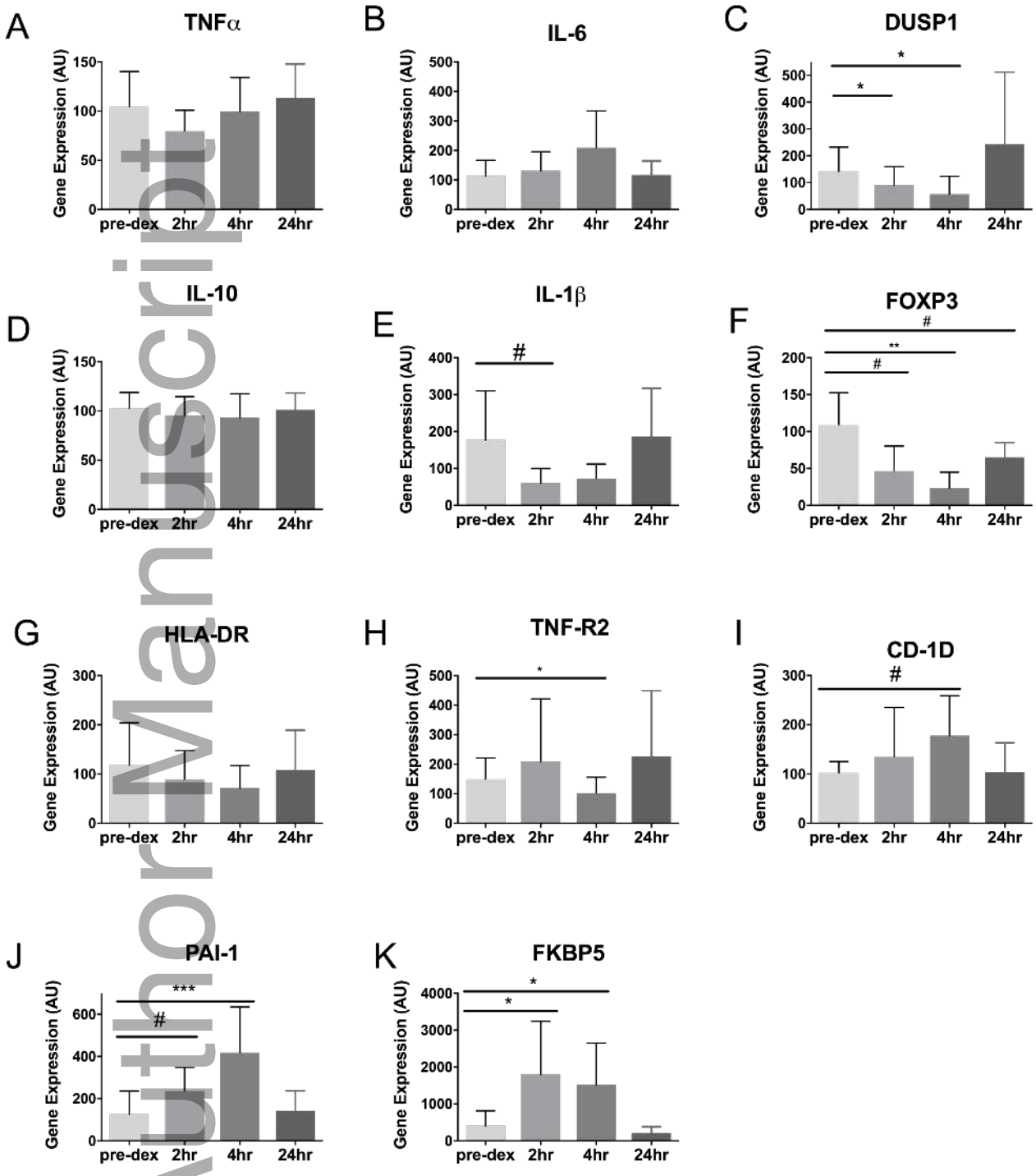

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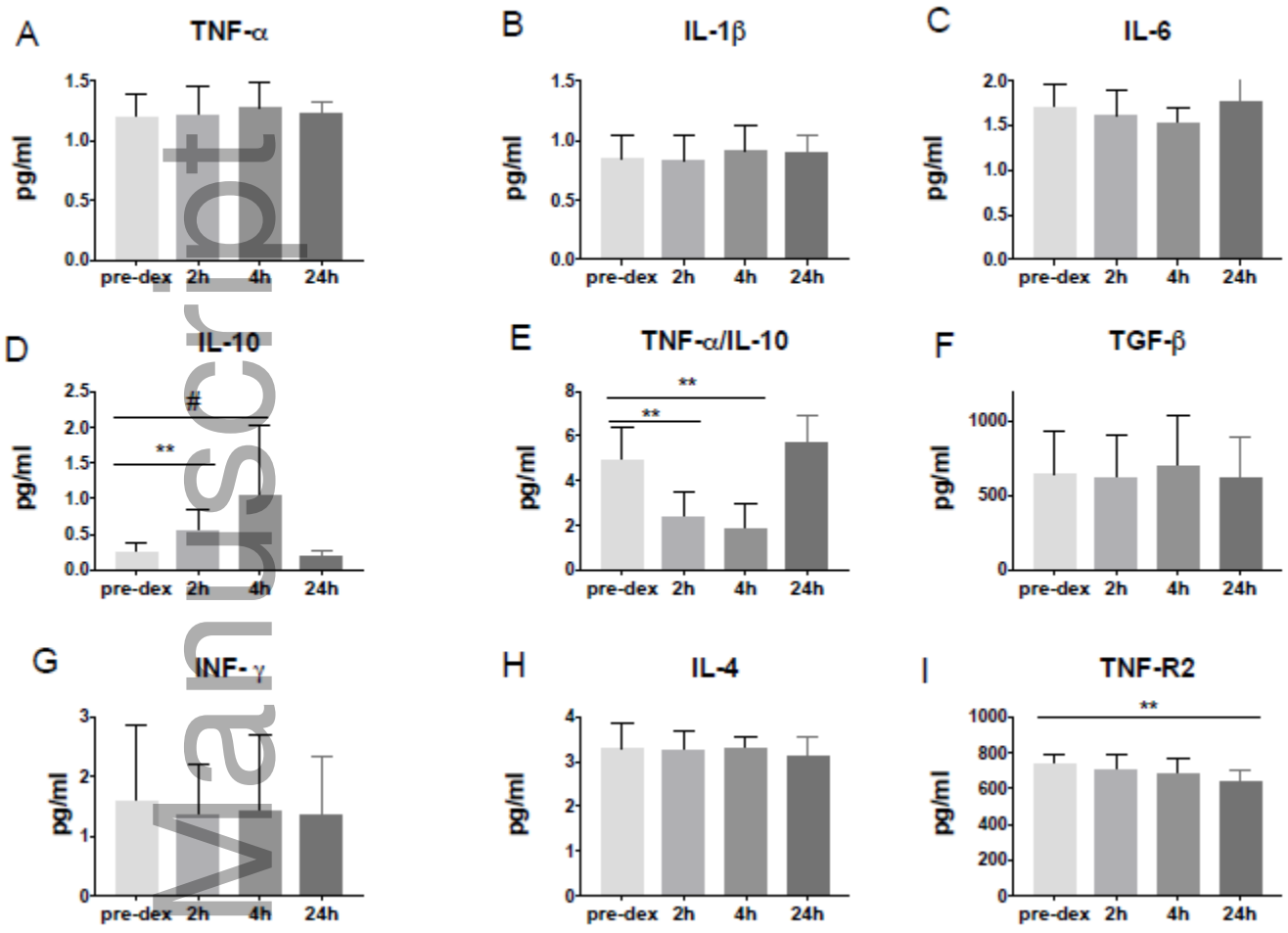
Figure 2



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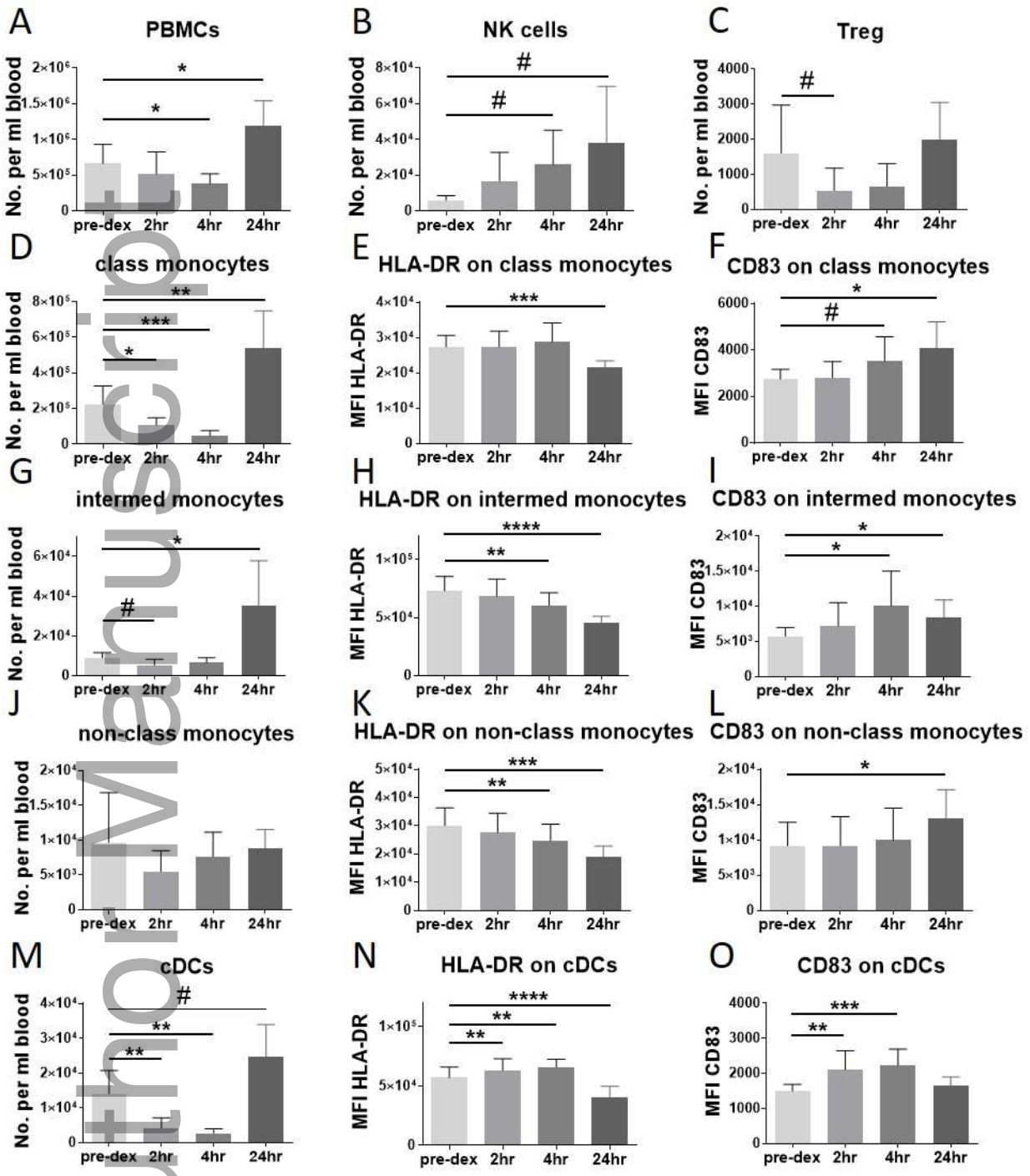

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