Malaria-derived hemozoin alters chromatin remodelling and skews dendritic cell responses to subsequent bacterial infections

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Abstract

Hemozoin (HZ), the malaria pigment, is released together with the parasite during the blood stage of the disease, which is connected with the pro-inflammatory response to induce T-cells and B-cells. Co-infections with bacteria lead to a more severe disease progression and the underlying mechanisms are poorly understood. Here, we investigated the impact of HZ on the early response of monocyte-derived dendritic cells (moDC) to a common bacterial component, LPS. A short-term HZ exposure for two hours did not induce an inflammatory response, but it did alter the transcriptional response to LPS. In moDC co-exposed to HZ and LPS, the induction of HLR-DR and PD-L1 gene expression was reduced and associated with decreased binding of RELA compared to LPS-stimulated cells. These gene promoters recruited the silencing chromatin remodelling complex NuRD upon co-exposure, instead of the PBAF complex at the promoter in LPS-stimulated cells. Further, HZ maintained transcription of C-type lectin receptors associated to an immature DC phenotype, DC-SIGN (CD209) and macrophage mannose receptor (MMR/CD206). Here, activated RELA and IRF3 were recruited in a PBAF and NCBAF dependent manner. Upon LPS co-exposure, NuRD was replacing these complexes to allow for a reduced transcriptional level of these immature markers. The association of chromatin remodelling complexes did not alter the chromatin state at the promoters, which was changed during differentiation to DCs or even at an earlier point. In conclusion, HZ exposure primes specific gene promoters at an early time point, which results in a different transcriptional response and may also lead to a changed immune reaction to bacterial co-infections.

Keywords: malaria, hemozoin, dendritic cells, chromatin remodelling, SWI/SNF complex

39 ## Introduction

40 The human immune response to malaria is multifaceted and complex since the malaria parasite,
41 Plasmodium, has many life stages and infect several organs in man. During the blood stage, the burst
42 of infected erythrocyte releases the parasite together with erythrocyte malaria specific content that
43 elicit a strong innate pro-inflammatory response, which leads to activation of T-cells and to the
44 clearance of the parasite (reviewed in Miner, 2018). Co-infections with viruses or bacteria usually
45 accelerate both the malaria and the infection, in particular in children and naïve infected individuals.
46 Several lines of evidence show that co-infection with bacteria increases bacteraemia, suggested to be
47 the result of a Plasmodium suppressed innate response (Harding et al., 2020; Pham et al., 2021).
48
49 The contribution of different Plasmodium and host molecules to the inflammatory response have been
50 thoroughly studied, in particular hemozoin (HZ) (reviewed in Aldridge and Vogel, 2014; Olivier et al.,
51 2014; Pham et al., 2021), a crystalline pigment from Plasmodium digested haem. HZ is quickly
52 phagocytosed by multinucleated cells after release and is responsible for long term suppression of
53 innate cells *in vivo* by reducing the generation of ROS (Parroche et al., 2007; Harding et al., 2020; Pham
54 et al., 2021). As of today, the response of monocytes and macrophages to HZ is contradictory with
55 both pro-inflammatory responses (Guha et al. 2021; Velagapudi et al., 2019) as well as no response
56 (Couper et al., 2010) reported. For dendritic cells (DC), exposure to infected red blood cells (iRBC) or
57 HZ impairs their function at several stages; the differentiation of monocytes to DC (moDC) *in vitro*
58 (Skrokhod et al., 2004; Diou et al., 2010), moDC maturation upon exposure to stimuli (Urban et al.
59 1999; Skorokhod et al., 2004; Elliot et al., 2007; Diou et al., 2010; Giusti et al, 2011; Bujila et al., 2014),
60 and impaired T-cell activation (Urban et al., 1999; Millington et al., 2007; Elliot et al., 2007; Guisti et
61 al., 2011; Bujila et al., 2014). Blood-derived DC stimulated with iRBC do not elicit a proinflammatory
62 response, except for production of the chemokines CXCL9, CXCL10 and CCL2 (Götz et al. 2017), and
63 require extracellular ROS in addition to the parasite erythrocyte lysate to give a pro-inflammatory
64 response (Götz et al. 2017; Ty et al. 2019; Götz et al., 2019). This suggests that the higher level of

65 oxidative stress may be responsible for the early acute inflammatory response and lower the
66 thresholds for other stimuli.
67
68 The cellular response to microbial co-exposure may rely on different pathways. Macrophages and DC
69 exposed to iRBC and HZ increase intracellular ROS production coupled to the activation of the NLPR3
70 inflammasome and secretion of IL-1β (Griffith et al., 2009; Shio et al., 2009; Barrera et al. 2011;
71 Velagapudi et al. 2019), which could influence the response to other stimuli. HZ released from
72 erythrocytes contains parasite and host molecules, including plasmodium DNA, lipids, protein and
73 membranes, not only the crystal (Olivier et al. 2014, Pham et al. 2021). The differential impact of HZ
74 may be caused by the Plasmodium molecules, and both DNA, membrane fractions and host fibrinogen
75 on the crystal trigger a response in innate cells (Barrera et al. 2011). A receptor for HZ is not identified,
76 but when phagocytosed, HZ has been shown to activate NFκB signalling in a MYD88 independent way
77 (Griffith et al. 2009; Velagapudi et al., 2019; Barrera et al., 2011). Pre-incubation of DCs with HZ also
78 affects subsequent responses to LPS, with an impaired maturation (Skorokhad et al., 2004; Gusti et al.,
79 2011). These effects may be a result of priming of innate cells to establish a trained or tolerance states
80 respectively, by altering the histone modification pattern, in particular changing the level of active
81 histone H3K4me3 at promotors (Schrum et al., 2018). The establishment of a trained and tolerance
82 state requires several steps and occurs late in the inflammatory process (Netea et al., 2020). However,
83 iRBC and HZ have been shown to induce early responses in the acute phase, in particular altering the
84 transcription of genes in innate immune cells within a few hours (Götz et al., 2017). Whether these
85 changes lead to an altered response to subsequent infections or co-infections, is not known.
86
87 Here, we show that exposure of crude HZ early in the acute response modulates the LPS-triggered
88 response on a transcriptional level. Pre-exposure to HZ prevents the upregulation of HLR-DR and PD-
89 L1 gene expression, in contrast to inflammatory genes, where HZ cannot influence the subsequent LPS-
90 induced activation. Furthermore, HZ tends to keep moDC in an immature state (Soilleuxet al., 2002)

with maintained transcription of DC-SIGN and MMR genes. Many immune genes are regulated by NFκB and IRFs in an SWI/SNF dependent manner with specific constellations operating on different gene promoters (Loo et al., 2020; Baxter et al., 2023). We show that the HLR-DR and PD-L1 associated with the BAF180-PBAF variant upon LPS stimulation, and this complex was replaced by the silencing NuRD complex in co-exposed cells. NuRD has been reported to antagonise the SWI/SNF on immune genes (Ramirez-Carrozzi et al., 2006). Despite the association of chromatin remodelling factors, the regulation of these genes is not dependent on chromatin alterations. The genes in the DC response either established an open configuration during differentiation from monocytes to moDC, or was already pre-set in a very open state at an even earlier stage.

We propose that HZ primes specific gene promoters to respond differently to subsequent or simultaneous stimulation by bacteria through the recruitment of SWI/SNF complexes and NuRD, with a capacity to transiently alter a following T-cell response. At the same time transcription is actively maintained on immaturity genes important for homeostasis.

106 ## Material and Methods

107 **Human subjects**

108 Peripheral blood cells were retrieved from anonymous, healthy blood donors who cannot be traced
109 back; thus, the project does not require approval from the Swedish ethical review authority.

110

111 **Natural Hz preparation**

112 Natural HZ was prepared as previously described[1–3]. Briefly, 12 h after schizogony of synchronized FCR3
113 parasite cultures, the supernatant was collected. A 10%/40% interphase of a 6% mannitol-containing
114 Percoll gradient was used to collect natural HZ, which was then extensively washed with 10 mM
115 phosphate buffer followed by washing with PBS. HZ was opsonized with equal volume of human AB
116 serum for 30 min at 37°C and then it was quantified according to its haem content. HZ preparation
117 with 4 nmol/μl haem content was used in the study. Prior to use, HZ was resuspended by passage
118 through a 0.4 mm needle fitted to a 1 ml syringe.

119

120 **Cell culture and stimulations**

121 Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats using Ficoll-Hypaque
122 (Cytiva) gradient centrifugation. Monocytes were enriched from PBMC by negative selection using
123 EasySep™ human monocyte kit (STEMCELL Technologies) according to the manufacturer´s
124 instructions. Monocytes were resuspended at $1x10^6$ cells/ml and were seeded in 96-well plates (with
125 200 μl/well) or 6-well plates (with 2 ml/well) using RPMI-1640 culture medium which was
126 supplemented with 20 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (all
127 from Cytiva), 5% human AB serum (Sigma-Aldrich), 50 μM 2-mercaptoethanol, 2% sodium pyruvate
128 (both from Gibco), 35 ng/ml IL-4 and 50 ng/ml GM-CSF (both from PeproTech). On day 3, half of the
129 medium volume was replaced with the fresh medium containing IL-4 and GM-CSF. On day 5, cells were
130 pre-exposed to HZ (5 μl for 96-well plates and 50 μl for 6-well plates) and the plates were centrifuged
131 at 700 r.p.m. for 20 s in order to initiate the contact with Hz. After 2 h, 50 ng/ml of LPS from *Escherichia*

132 *coli* O26:B6 (Sigma-Aldrich) was added to appropriate wells for additional 2 h or 24 h. Cells assigned to
133 HZ treatment alone were exposed to HZ at the same time as was added LPS. Cells kept in complete
134 culture medium served as the control. Supernatants were collected and stored at -20°C until further
135 analysis, while cells were collected either for western blot, chromatin immunoprecipitation (ChIP),
136 mRNA expression analysis, ATAC-qPCR or flow cytometry. Throughout the experiment, cells were
137 incubated at 37°C with 5% CO_2.
138
139 **Immunoblotting**
140 MoDC were harvested in 4xLameli buffer, separated on a 12% PAGE and transferred to a PVDF
141 (Miilipore) membrane. The membrane was blocked in 5% milk and incubated with primary antibody:
142 anti-Phospho-IRF3 (Ser 396) (D601M, Cell Signaling), anti-IRF3 (D6I4C, Cell Signaling), anti-Lamin B1
143 (ab65986, Abcam), anti-Caspase-1 (ab207802, Abcam), anti-beta Tubulin (ab6046, Abcam).
144 Immunoblots were developed using ECL Western Blot Substrate (BioRad). The quantification of protein
145 signals was performed by ImageLab™ (ChemiDoc™ Imaging system, BioRad) software by comparing
146 the signals from each target proteins to control proteins.
147
148 **Chromatin immunoprecipitation**
149 ChIP assays were performed as described previously[4]. Briefly, chromatin from different treatment
150 groups was cross-linked using 1% formaldehyde (Sigma-Aldrich). Chromatin from $1x10^6$ cells was
151 incubated with IgG (ab171870), H3 (ab1791), H3K27Ac (ab4729), H3K27me3 (ab6002), H3K4me3
152 (ab8580), RelA (NFκB p65, ab7970), IRF3 (ab76409) (all from Abcam), or BRG1 antibody (generated as
153 previously described[5]). Protein A/G magnetic beads (ThermoScientific™) were used to precipitate. The
154 target DNA was amplified by qPCR using KAPA SYBR FAST qPCR Master mix (Kappa Biosystems Inc. The
155 results are presented as percentage of input, with the IgG control subtracted from the Ct values
156 obtained for samples. Primers are listed in the Table S1.
157

158 **RNA extraction and real-time quantitative polymerase chain reaction**

159 Total RNA was extracted using TRIzol reagent (Invivogen), and reverse transcription of RNA was
160 performed using SuperScript VILO cDNA synthesis kit (Invitrogen), according to the manufacturers´
161 instructions. qPCR was performed using KAPA SYBR FAST qPCR Master mix. mRNA expression levels
162 were calculated using $2^{-\Delta\Delta Ct}$ method and PP1A was used for normalization. Primer pair list can be found
163 in the Table S1.

164

165 **ATAC-qPCR**

166 Cells were collected and tagged with Tn5 transposase (Illumina) according to the manufacturer's
167 instructions. DNA was purified using MinElute PCR purification kit (Qiagen) and it was stored at -20°C
168 until further analysis by qPCR. Tagmentation-specific forward primer and gene-specific reverse primer
169 was used for amplification. IL-2 was used for normalization. Primer pair list can be found in the Table
170 S1.

171

172 **Flow cytometry**

173 Following cell collection on day 5 or day 6, mo-DC were stained with alternating combination of
174 antibodies: CD11c APC (clone 3.9), MMR PerCP-Cy5.5 (clone 15-2), TLR4 PE (clone HTA125), CLEC12A
175 FITC (clone 50C1), CD83 PeCy-7 (clone HB15e), HLA-DR PerCP (clone L243), PD-L1 PE (clone 29E.2A3)
176 (all from BioLegend), CD14 PeCy7 (clone MϕP9), DC SIGN BV421 (clone DCN46) and CD86 FITC (clone
177 FUN-1) (all from BD Biosciences). A fixable viability stain 780 (BD Biosciences) was used to exclude non-
178 viable cells. Samples were acquired using FACSVerse flow cytometer (BD Biosciences) and FlowJo
179 Software (TreeStar) was used to analyse the data.

180

181 **Cytometric bead array (CBA)**

182 Soluble levels of IL-12p70, TNF-α, IL-10, IL-6 and IL-1β cytokines were measured in the cell culture
183 supernatant after 2 h of co-exposure using CBA human Inflammatory cytokine kit (BD Biosciences),

184 while chemokines CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1 and CXCL10/IP-10 were
185 quantified using CBA human chemokine kit (BD Biosciences), according to the instructions from the
186 manufacturer. Samples were acquired using FACSVerse flow cytometer and FlowJo Software was used
187 to analyse the data.
188
189 **Enzyme-linked immunosorbent assay**
190 Soluble levels of TNF-α, IL-6, IL-1β, IL-23 and IL-10 (MabTech AB), as well as IL-18, IL-18BP and IL-RA
191 (R&D systems, Bio-Techne), were measured in the cell culture supernatant after 2 h and/or 24 h of co-
192 exposure according to the instructions from the manufacturer. The results were analysed using
193 SoftMax Pro 5.2 rev C (Molecular Devices Corp.).
194
195 **Statistical analysis**
196 Statistical analysis was carried out using GraphPad Prism 10 (GraphPad Prism Inc.). Non-parametric
197 Friedman ANOVA test or non-parametric Kruskal-Wallis ANOVA test, followed by Dunn´s multiple was
198 comparison was applied to determine the differences between multiple treatment conditions. Non-
199 parametric Wilcoxon matched-pairs signed rank test or non-parametric Mann-Whitney test was used
200 to determine the differences between two treatment conditions. Statistical differences were
201 considered significant if p-values were <0.05.
202
203

204 ## Results

205 **HZ prevents LPS-induced upregulation of HLA-DR and PD-L1 gene expression**

206 To address the question whether HZ has an impact on the early moDC phenotype and functional
207 response to an unrelated microbial compound such as the bacterial-derived LPS, we pre-incubated
208 cells with HZ for 2 h to allow its phagocytosis before adding LPS as depicted in Figure 1A. To mimic the
209 state of HZ shortly after rupture, we used a crude preparation in which the HZ crystal contained cellular
210 component. HZ was phagocytosed by the cells already after 2 h, as shown by the increased granulation
211 of cells observed by flow cytometry (Supplementary Figure S1A). The exposure to HZ for 2 h did not
212 affect the surface expression of HLA-DR and it did not hamper LPS-induced expression of this marker
213 after 24 h (Figure 1B). As expected, the expression of the maturation marker CD83 was significantly
214 upregulated by LPS after 2 h and 24 h, while HZ exposure slightly delayed this expression at the later
215 time point during co-exposure (Figure 1B). To further assess if moDC remain immature upon HZ
216 addition, we investigated the surface expression of the C-type lectin receptors DC-SIGN (CD209) and
217 MMR (CD206), which are associated with an immature phenotype. The surface expression of both
218 receptors in HZ exposed cells was high and comparable to the unstimulated cells, while LPS caused a
219 downregulation of these receptors after 24h, which was not prevented by HZ pre-exposure (Figure 1C).
220 Further, HZ did not block the LPS-mediated upregulation of co-stimulatory receptor CD86 following 24
221 h (Figure 1D). The surface expression of PD-L1, which is a negative co-stimulatory receptor important
222 for T-cell inhibition, was significantly upregulated by LPS after 24 h, while HZ co-exposure slightly
223 delayed this expression (Figure 1D).

224 To focus on the early moDC response, we further studied the mRNA levels of aforementioned markers
225 together with two chemokine receptors CCR7 and CCR5, which are associated with a mature and
226 immature moDC phenotype, respectively (Sallusto et al., 1998). Interestingly, the LPS-triggered
227 increase in HLA-DR transcripts was markedly prevented when the cells had been pre-exposed to HZ
228 (Figure 1E). DC SIGN and MMR mRNA levels were reduced by LPS compared to the cells only exposed
229 to HZ, and co-exposure to HZ partly abolished this drop in transcription for MMR (Figure 1F). The mRNA

230 levels of PD-L1 displayed a similar pattern to HLR-DR; pre-treatment with HZ strongly reduced the
231 expression of LPS-induced PD-L1 (Figure 1G). CCR7 gene expression was induced by LPS and further
232 increased when the cells were co-exposed to HZ (Figure 1H). The expression of CD83 (Figure 1E), CD86
233 (Figure 1F), and CCR5 (Supplementary Figure S1B), did not differ among samples. Taken together, our
234 results suggest that HZ interferes with the stimulation with bacterial antigen during the early induction
235 of gene expression of genes important in antigen presentation and activation of T-cells.
236
237 **HZ does not influence the secretion of LPS-induced inflammatory and anti-inflammatory factors**
238 **early during the response**
239 We next investigated whether HZ had an impact on the LPS-induced inflammatory and anti-
240 inflammatory protein production. As expected, LPS induced TNFα and IL-6 release already after 2 h,
241 while IL-23 secretion was induced by LPS after 24 h, regardless if HZ was present or not (Figure 2A-B
242 and Supplementary Figure S2A-B). Further HZ did not interfere with the LPS-induced secretion of
243 CXCL8, CXCL9 or CXCL10 chemokines (Figure 2C). CCL2 was not induced above background, while CCL5
244 was slightly upregulated in co-exposed cells (Figure 2C). At the transcriptional level co-exposure
245 resulted in enhanced CXCL9 expression, while CXCL10 resembled a similar pattern to that of HLA-DR
246 and PD-L1; the increase was only detected in LPS exposed cells but not in HZ co-exposed cells (Figure
247 2D). For the other chemokines, mRNA expression varied substantially at 2 h (Figure 2D).
248 Regarding the anti-inflammatory response, HZ exposure did not interfere with the LPS-induced IL-10
249 production, neither at the protein nor transcriptional levels 2 h post exposure (Figure 2E). Further, HZ
250 did not have clear effects on the expression of the immunosuppressive markers SOCS2 and IDO1
251 (Supplementary Figure S2C). We also investigated the scavenger protein Haem oxidase (HO-1) as it
252 might be induced upon haem release. HZ exposure did not trigger the scavenger protein haem oxidase
253 HO-1 mRNA expression, regardless of LPS presence (Supplementary Figure S2C).
254
255 **HZ accounts for the release of inflammasome-derived IL-1β during co-exposure with LPS**

256 Next, we measured the inflammasome-dependent cytokine IL-1β, which was detectable only in the
257 LPS and HZ co-exposed cells after 24 h (Figure 2F), preceded by enhanced levels of transcription at 2
258 h, seen for both LPS stimulation and co-exposed cells (Figure 2G). As the response to HZ has previously
259 been associated with the activation of NLRP3-inflammasome (Shio et al. 2009; Pack et al. 2021), we
260 examined the transcriptional induction of other inflammasome components, such as NLRP3, pro-
261 caspase-I, pro-caspase 4 and pro-caspase-8. The expression of NLRP3 was clearly upregulated by HZ
262 and LPS co-exposure and by LPS stimulation alone, while the caspases exhibited a varied response to
263 the treatments (Supplementary Figure S2D). We could not detect any cleavage of pro-caspase I as early
264 as 2 h after stimulation, showing that 2 h is not sufficient for the two-step reactions during IL-1β release
265 (Supplementary Figure S2E). During inflammation, the production of IL-1β is balanced by the IL-1
266 receptor antagonist (IL-1RA) (Gabay et al., 2010). IL-1RA release was lower in LPS exposed and co-
267 exposed cells after 2 h (Figure 2H), similar to IL-1RA mRNA expression at 2 h (Figure 2I). Still, regardless
268 of HZ-exposure LPS-stimulation resulted in an increase of IL-1RA secretion after 24 h (Figure 2H). Our
269 results indicate that HZ is responsible for the IL-1β inflammasome response during co-stimulation with
270 LPS, which is accompanied by a higher IL-1RA release.
271
272 **HZ does not interfere with the LPS activation by TLR4**
273 To investigate the mechanism behind the dampening effect of HZ on the transcriptional response of
274 HLR-DR and PD-L1 but not on inflammatory genes, we assessed whether HZ interfered with the
275 activation by LPS. The percentage of TLR4 expressing cells significantly decreased after 2 h - both in
276 cells stimulated with LPS and exposed to LPS and HZ (Figure 3A), showing that HZ did not affect
277 receptor internalisation. The receptor level was restored at 24 hours, even significantly upregulated in
278 co-exposed cells (Figure 3A). The receptor for HZ is still not known, but CLEC12A, the receptor for uric
279 acid (Neumann et al. 2014) has been suggested to be the receptor for phagocytosis of HZ (Raulf et al.
280 2019). The surface level of CLEC12A did not change in any treatments (Supplementary Figure S3A).
281

282 **HZ phagocytosis changes the transcription factor recruitment to promoters**

283 TLR4 ligation activates several transcription factors including RELA and IRF3. To investigate the
284 differentiated gene response to HZ in co-exposed cells, we examined the binding of these factors to
285 the promoters of HLA-DR and PD-L1 genes. These promoters bound significant levels of RELA in cells
286 stimulated with only LPS, while HZ and LPS co-exposed cells displayed markedly lower levels of binding
287 (Figure 3B, upper panel). IRF3 was also significantly recruited at the promoter of HLA-DR gene in LPS
288 stimulated cells, but the levels bound was low in all samples (Figure 3B, lower panel). In contrast, HZ
289 did not hamper the binding of RELA at the promoters of inflammatory genes, as it was significantly
290 recruited to the promoters of the primary response gene TNFα and the secondary response genes IL-
291 6 and IL-1β genes in LPS stimulated and HZ and LPS co-exposed cells (Figure 3C, upper panels). A similar
292 trend for RELA was also seen for IL-23 and CCL5 genes (Supplementary Figure S3B). IRF3 did only bind
293 at low levels to the promoters of the inflammatory genes (Figure 3C, lower panels), even to the IRF3
294 target CCL5 after 2 hours (Supplementary Figure S3B).

295 We observed that DC-SIGN and MMR were differentially regulated by HZ and LPS with a slight decrease
296 in LPS stimulated cells (Figure 1C, 1F). Published RNA seq data from DCs after 3 h show that DC-SIGN
297 is one of the genes induced by iRBC, whereas the transcript level in LPS-exposed cells remained at the
298 unstimulated level (Götz et al., 2017). Accordingly, we further examined the transcription factor
299 recruitment to DC-SIGN and MMR. Both RELA and IRF3 were bound to the promoters of DC-SIGN and
300 MMR in HZ stimulated cells (Figure 3D), a binding that was counteracted after LPS was added to the
301 cultures (Figure 3D). The RELA and IRF3 recruitment on the CD83 promoter resembled that of HLR-DR
302 and PD-L1 with RELA occupancy in LPS stimulated cells, and low levels of IRF3 (Figure 3E). This indicates
303 that HZ not only impairs the recruitment of activating factor at certain genes, but also activates other
304 groups of gene to maintain the level of transcription without hampering the regulation to other stimuli.
305 Since IRF3 bound to the promoters of DC-SIGN and MMR in HZ exposed cells after 2 h, we examined
306 the activation of IRF3 in the different treatments. IRF3 was activated, as shown with phosphorylated

307 IRF3, in cells exposed to HZ alone and cells co-exposed with LPS and HZ (Figure 3F), showing that HZ
308 exposure had an immune modulatory effect by activating both RELA and IRF3.
309
310 **Exposure to HZ does not alter chromatin states**
311 The differential binding of RELA and IRF3 to gene promoters in exposed cells prompted us to
312 investigate the chromatin states at the different gene promoters. No significant changes were detected
313 in the chromatin accessibility analysed by ATAC-qPCR at the gene promoters or gene enhancers by any
314 exposure. Nevertheless, a trend to more accessible chromatin state at the promoter of HLR-DR and
315 PD-L1, was observed in HZ stimulated cells (Figure 4A) whereas no such pattern was observed in the
316 other groups of gene, despite the large transcriptional induction of inflammatory genes
317 (Supplementary Figures S4A and S4B). Many of these genes are also expressed in monocytes and may
318 therefore already have the chromatin accessibility state established. To test this, the accessibility of
319 the response genes and moDC marker genes were examined in monocytes and in moDC. The monocyte
320 marker CD14 was accessible in monocytes and closed in moDC (Supplementary Figure S4C), whereas
321 DC-SIGN, HLR DR, PD-L1 and TNFα exhibited a higher accessibility in moDC compared to the more
322 closed configuration in monocytes (Figure 4B). The promoters of IL-6 and IL-1β, as well as MMR had
323 similar accessibility in monocytes and moDC (Figure 4B). Chromatin changes are also formed by histone
324 modifications and histone H3K27Ac as well as H3K4me3 have been associated with an active chromatin
325 state coupled to the development of a trained or tolerised immune phenotype (Netea et al.). HLA DR
326 and PD-L1 exhibited a trend of higher H3K27Ac at the promoter in cells stimulated by LPS alone (Figure
327 4C, upper panel), whereas no change was observed H3K4me3 (Figure 4C, lower panel). No changes in
328 the histone modifications were observed in other groups of genes, except for a higher H3K27Ac level
329 at the IL-6 promoter compared to unstimulated cells (Supplementary Figures S4D and S4E). We
330 conclude that the genes important for a DC response already had a hyper-accessibility, established in
331 differentiation steps prior to the activation of the genes upon stimulation.
332

333 **Exposure to HZ changes the recruitment of the SWI/SNF complex**

334 Next, we examined the association of SWI/SNF complexes, required for transcriptional activation of
335 many immune genes. The ATPase BRG1/SMARCA4 was recruited to the promoter of HLR DR and PD-
336 L1 in LPS stimulated cells, while co-exposure with HZ and of LPS had reduced levels of BRG1 associated
337 with the promoters, which is in agreement with the reduced levels of transcript (Figure 4D, upper
338 panel). Co-exposure of HZ and LPs led instead to recruitment of CHD4, but not the methyltransferase
339 EZH2 in PCR2, to the promoters (Figure 4D, lower panel and Supplementary S4F). The promoters of
340 the inflammatory genes, TNFα, IL-6, IL-1β, and CCL5, displayed higher BRG1 occupancy in cells
341 stimulated with LPS and most genes retained this high level in cells co-exposed with HZ and LPS (Figure
342 4E, upper panel and Supplementary Figure S4G, upper panel). CHD4 was only associated with the TNFα
343 promoter in LPS stimulated cells, indicating that repression of the transcription had started on early
344 primary response genes (Figure 4E, lower panel and Supplementary Figures S4G, lower). The IL-23
345 promoter and IL-10 did not display any changes in the association with BRG1 or CHD4 (Supplementary
346 Figure S4H), suggesting that other factors are operating on these gene promoters. In contrast to the
347 other groups of genes, BRG1 levels associated to the promoters of DC-SIGN and MMR were higher in
348 cells exposed to HZ alone and CHD4 was not detected (Figures 4F). However, co-exposure of HZ and
349 LPS had CHD4 associated with the promoters, suggesting that NuRD antagonises the BRG1 association
350 induced by the pre-treatment for 2 h of HZ. The CD83 gene promoter did not show an enrichment of
351 BRG1 or CHD4 in any exposure, indicating that this gene is not dependent on SWI/SNF complexes and
352 NuRD activities (Figure 4F).

353

354 **SWI/SNF variants operating on the genes decide transcription factor preference**

355 Different SWI/SNF complex constellations are recruited to specific genes by ncRNAs. To further
356 investigate the specificity of the binding of factors to the different types of genes, we determined the
357 expression of ncRNA regulating the binding of BRG1-SWI/SNF to immune genes. Two LPS-regulated
358 lncRNAs, IL-7-as and FIRRE, affect the association of BRG1 to immune response genes in opposite ways;

359 IL-7-as enhances, and FIRRE reduces the association (Lu et al., 2018; Liu et al. 2019). Both RNA levels
360 were significantly induced by LPS stimulation and co-exposure to LPS and HZ for 2 h compared to HZ
361 exposed and unstimulated cells (Figure 5A), suggesting that the expression of these RNAs does not
362 explain the differential recruitment of factors to the promoters. Instead, we examined whether the
363 promoters associated with different SWI/SNF constellations; and used BRD9 as a marker for the non-
364 canonical SWI/SNF (ncBAF complex), BAF180/PBRM1 for PBAF and BAF250/ARID1 for the common
365 conventual BAF (cBAF) complex. BAF180 was recruited to the HLA DR and PD-L1 promoters upon LPS
366 stimulation for 2 h (Figure 5B and Supplementary Figure S5A), something that HZ exposure prevented
367 for the PD-L1 promoter. This demonstrates that these genes worked with the PBAF constellation,
368 which associated simultaneously with CHD4 in co-exposed cells; indicating a dynamic balance between
369 the PBAF and NuRD complexes as also previously reported (Ramirez-Carrozzi et al., 2006; Pundhir et
370 al., 2023). The inflammatory genes TNFα, IL-6 and IL-1β promoters showed a preference for BRD9,
371 which associated upon LPS stimulation and co-exposure (Figure 5C and Supplementary Figure S5A). IL-
372 1β, which had low levels of BRG1 associated in co-exposure cells, did not have any of the specific
373 subunits at the promoter. ARID1 was only found associated with CCL5 in cells stimulated with LPS and
374 co-exposed cells (Supplementary Figure S5B), which indicates that the cBAF complex is not involved in
375 the early acute response. IL-23 and IL-10 did not display significant association of any of the subunits,
376 in agreement with the lack of BRG1 binding (Supplementary Figures S5B and S5C). HZ-exposure to DC-
377 SIGN and MMR led to both BRD9 and BAF180 associating at their promoters (Figure 5D), both subunits
378 were lost in the co-stimulated cells together with BRG1. In conclusion, the specificity in the
379 transcriptional response to HZ is achieved by the recruitment of specific SWI/SNF complexes, with
380 different features, to the various promoter.

381 ## Discussion

382 Malaria co-infection with bacteria accelerate both the malaria progression and the bacterial infection
383 (Church and Maitland, 2014; Gomez-Perez et al., 2014). The cellular response to malaria antigens and
384 its response during co-infections are multifactorial and contradictory results have been reported
385 (Harding et al. 2017). Here we show that although HZ had only subtle direct effects on gene expression,
386 it modulated the response to bacterial stimuli. HZ did not induce the maturation of moDC and the cells
387 did not secrete inflammatory cytokines and chemokines early in the acute phase after exposure.
388 Instead, HZ exposure hampered early transcriptional activation induced by LPS for HLR-DR, PD-L1 and
389 CXCL10 and at the same time maintained the expression of immaturity markers DC-SIGN and MMR,
390 possibly to dampen or skew a subsequent T-cell response.

391 The specific response of genes depends on their promoter and enhancer architecture as well as on
392 their chromatin state. Immune genes are divided into groups depending on the type of response
393 elements in regulatory regions. HLR-DR, the PD-L1 as well as the CXCL10 are interferon inducible genes
394 (IGS) (Ivashkiv, 2018; Schoggins, 2019; Naigles et al., 2023) with promoters harbouring interferon-
395 sensitive response elements, that are specifically responsive to IFNγ activation of the transcription
396 factor STAT1 and IRFs (Padmanabhan et al., 2022; Sekrecka et al., 2023). The genes are also regulated
397 by TLR signalling, through the NF-κB pathway in response to pathogens and the promoters harbour
398 NFκB binding. Inflammatory and maturation genes are also induced early by NFκB transcription factors
399 RELA and later by IRF3/IRF7 (Ahmed et al., 2015; Adelaja et al., 2021). While LPS stimulation recruited
400 RELA to both IGS- and inflammatory genes, co-exposure with HZ rather reduced the recruitment of
401 RELA to the IGS genes. This may be attributed to stimulus specific responses and interference between
402 NFκB signals (Adelaja et al. 2021; Cheng et al., 2021). scRNA transcription has shown that although
403 genes respond to the same transcription factors, they are activated at different time points and at
404 different amplitudes. This is caused by specific responses to the type of signal triggered by the stimulus,
405 to the signals secreted by neighbouring cells and the chromatin states of individual cells (Shalek et al.,
406 2016; Adelaja et al., 2021; Cheng et al., 2021; Naigles et al., 2023). In contrast to LPS, HZ activated IRF3

407 after 2 h but the factor was differentially recruited and it bound to the promoters of DC-SIGN and MMR
408 and not to other IRF3 targets, such as CCL5. Similar to HLR-DR and PD-L1, co-exposure with HZ and LPS
409 abolished the binding of both RELA and IRF3, further showing that HZ is modulating the early
410 transcriptional response.

411 The differential binding of transcription factors to promoters and enhancers on the different types of
412 genes relies on an accessible chromatin state with active histone modification. It is known that
413 enrichment of H3K4me3 and H3K27Ac at specific genes in monocytes is induced after 24 h following
414 exposure of either iRBC or HZ (Schrum et al. 2018). We only detected very small changes in the
415 accessibility or these histone modification at the promoters of the genes upon activation compared to
416 unstimulated cells after 2 h. Accessible chromatin and establishment of active histone modification is
417 often correlated with active transcription, but these can be set at different stages. The differentiation
418 of monocytes to moDC involves loss of H3K4me3 at the promoter of the monocyte marker CD14 gene
419 and a gain at the DC-SIGN gene (Bullwinkel et al., 2011). Similarly, the accessibility of gene promoter is
420 pre-set during differentiation (Mansisidor and Risca, 2022), which includes immune genes genomically
421 primed at an earlier step in oligodendrolia development (Meijer et al., 2022). We investigated the
422 accessibility during development and show that the CD14 promoter changed from a more open
423 configuration in monocytes to a closed one in moDC, and the DC-SIGN showed the opposite pattern
424 and obtained a more accessible configuration. HLR-DR and PD-L1 or the inflammatory genes, which
425 are expressed upon maturation either gained an open configuration during the differentiation or
426 already had an open configuration established in an even earlier step. Our results show that
427 maturation of DCs does not require further alteration of accessibility at the promoters in the early
428 acute phase and the transcriptional induction of DC depends on the activation and binding of
429 transcription factor.

430

431 Many immune genes require SWI/SNF complexes for transcriptional activation. This can be
432 counteracted in different ways – either via the NuRD complex that antagonises SWI/SNF activation

433 (Ramirez-Caross et al 2006; Pundhir et al., 2023) or via EZH2-PCR2 that can silence the genes (Pundhir
434 et al., 2023). We observed that the promoters of several of the investigated genes, including HLR-DR
435 and PD-L1, associated with BRG1 when activated by LPS, and that BRG1 was replaced by the CHD4
436 when transcription was reduced following HZ and LPS co-exposure. These complexes are tightly
437 correlated with chromatin changes, something we did not observe in our study. However, studies with
438 perturbed SWI/SNF and NuRD factors show that the transcriptional effect is not always the expected
439 (Yildirim et al. 2011; de Dieuleveult et al. 2016; Bornelöv et al. 2018; Lu et al. 2019; Pundhir et al. 2023).
440 It has been shown that SWI/SNF and NuRD are involved in transcriptional changes by regulating RNA
441 pol II occupancy at the gene promoter and in the gene body of highly expressed genes instead of
442 chromatin remodelling (Pundhir et al 2023). We did not detect EZH2 on HLR-DR and PD-L1 promoters,
443 which are commonly silenced by the EZH2-PCR2 Polycomb complexes in cancer cells (Dersh et al.,
444 2021; Xiao et al., 2019; Morel et al. 2021). EZH2 has also been associated with silencing of SWI/SNF
445 activated bivalent genes expressed at a low level by reducing the chromatin accessibility (Pundhir et
446 al. 2023). In connection with our finding, we propose that many genes in the acute phase are induced
447 by activated RELA and IRF3 and regulated by the SWI/SNF and the NuRD complexes through alteration
448 of RNA pol II kinetics rather than chromatin configuration. HZ exposure pre-sets a different balance of
449 the complexes at the HLR-DR and PD-L1 genes as well as the C-lectin genes which results in a
450 compromised gene expression in response to concomitant LPS stimulation.

451 The specificity of the response was achieved by recruiting different SW/SNF constellations. These
452 constellations operate on different gene elements and regulate cell type specific expression (Innis and
453 Cobot, 2020; Otto et al., 2023). Our results show that these constellations worked with different gene
454 promoters, and the PBAF was associated with the promoters of HLR-DR and PD-L1 upon LPS
455 stimulation and it was replaced by the NuRD complex in co-exposed cells which reduced transcription.
456 PBAF association to promoters often lead to repression, such as repression of genes in T-regs and IFNγ
457 response genes (Miao et al, 2018; Pan et al., 2018; Ahmed et al., 2022; Loo et al. 2020. In contrast to
458 the PBAF operating on HLR-DR and PD-L10, we found that a number of the inflammatory genes

459 associated with the BRD9-ncBAF upon LPS stimulation, and this complex was not antagonised by NuRD
460 upon co-exposure with HZ. We propose that the gene architecture determines what type of SWI/SNF
461 complex that associate with the promoter and sets the regulatory way. PBAF is associated with the
462 NuRD antagonistic activity which is responsible for the more repressive activity of the PBAF complex.

463 IL-1β was only secreted from co-exposed cells after 24 h of stimulation, which suggest that neither HZ
464 or LPS alone could activate the inflammasome. Isolated blood DCs from healthy malaria-naïve
465 individuals exposed to iRBC do not induce a pro-inflammatory response, but require the co-exposure
466 of external ROS for a pro-inflammatory response in DCs and macrophages, including IL-1β (Götz et al.
467 2017). Activation of the inflammasome requires the transcriptional activation of the IL-1β gene and
468 other inflammasome genes, like NLPR3 and caspases, as well as the activation and assembly of the
469 inflammasome which has been attributed to HZ (Matikainen et al. 2020). Although the mechanism
470 behind this is not fully elucidated, HZ is known to activate the STAT pathway by activating LYN/SYK
471 kinases (Shio et al., 2009) or to produce ROS (Shio et al., 2009; Velagapudi et al., 2019). The release of
472 IL-1β after 24 h co-exposure with HZ and LPS may have implications in malaria co-infections with
473 bacteria and contribute to an excessive inflammatory reaction. Equally important is the modulation of
474 the response, in which HZ-exposure of DCs maintains the cells in a partially immature state, which
475 might lead to a skewed T-cell activation and fine-tune a homeostatic response to avoid random
476 maturation. The modulation imposed of certain promoters by short-term HZ exposure primes the cells
477 for subsequent or simultaneous bacterial infections and these effects may lead to a more severe
478 disease during co-infections

479

Author Contributions

AÖF, ESE, MTB designed the study. GL, KT, AS, JQ, AO, IB performed lab work. GL, KT, AS, JQ, IB, ESE and AÖF analysed and/or finalized the data. GL performed statistical analyses. AÖF, GL and ESE wrote the paper.

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Conflict of Interest

The authors declare no conflict of interests.

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699 **FIGURES**

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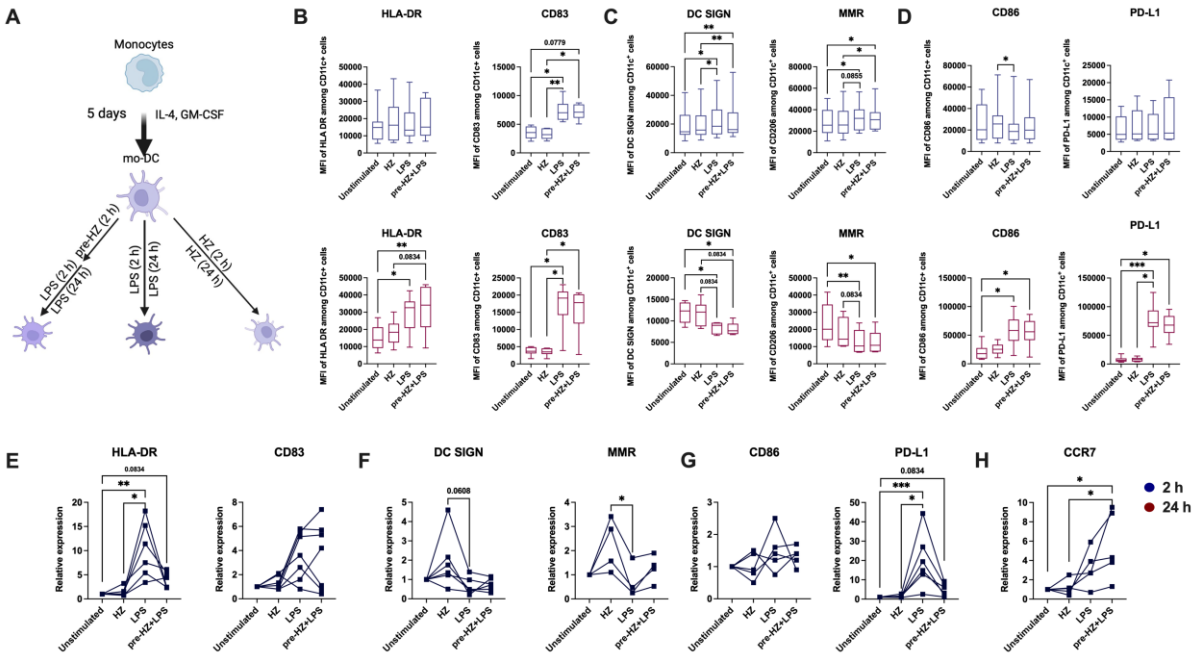

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703 **Figure 1. HZ prevents the upregulation of HLA-DR and PD-L1 transcripts during co-exposure with LPS.**

704 **(A)** Experimental model of monocytes differentiation to mo-DC during 5 days culture and exposure to

705 different stimuli. MoDC were pre-exposed to HZ for 2 h, then LPS was added for additional 2 h or 24 h

706 (co-exposure condition). Cells assigned to HZ treatment alone were exposed to HZ at the same time as

707 was added LPS, while cells kept in complete culture medium served as the control. The mean

708 fluorescent intensity (MFI) of **(B)** HLA-DR and CD83, **(C)** DC SIGN and MMR, **(D)** CD86 and PD-L1,

709 expression in moDC after 2 h exposure (upper graphs) and 24 h exposure (lower graphs). The relative

710 mRNA expression of **(E)** HLA-DR and CD83, **(F)** DC SIGN and MMR, **(G)** CD86 and PD-L1, and **(H)** CCR7,

711 in moDC after short-term exposure. (B-H) Paired Friedman test followed by Dunn´s multiple

712 comparison was used to determine statistical difference, n.s.=p>0.05, *p<0.05, **p<0.01, ***p<0.001,

713 n=4-12.

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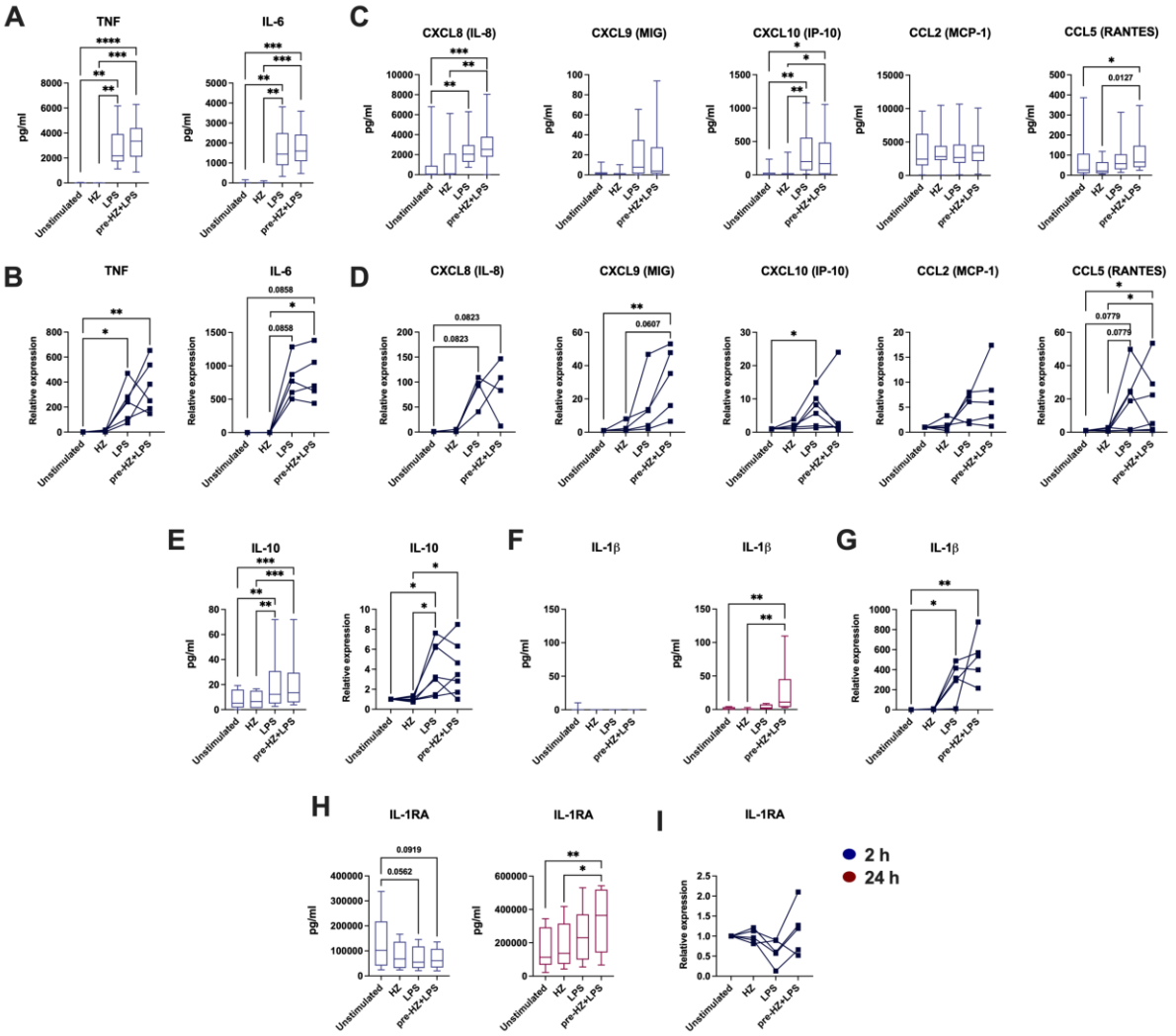

Figure 2. Both HZ and LPS are required for the release of inflammasome-derived IL-1β. **(A)** Soluble levels of TNF-α and IL-6 in moDC after 2 h exposure. **(B)** Relative mRNA expression of TNF-α and IL-6. **(C)** Soluble levels of CXCL8, CXCL9, CXCL10, CCL2 and CCL5 after 2 h exposure. **(D)** Relative mRNA expression of CXCL8, CXCL9, CXCL10, CCL2 and CCL5. **(E)** Soluble levels and relative mRNA expression of IL-10 after short term exposure. **(F, H)** Soluble levels of IL-1β and IL-1RA after 2 h and 24 h exposure. **(G, I)** Relative mRNA expression of IL-1β and IL-1RA. mRNA levels were measured in all samples after 2 h of exposure. (A-I) Paired Friedman test followed by Dunn´s multiple comparison was used to determine statistical difference, n.s.=p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=4-14.

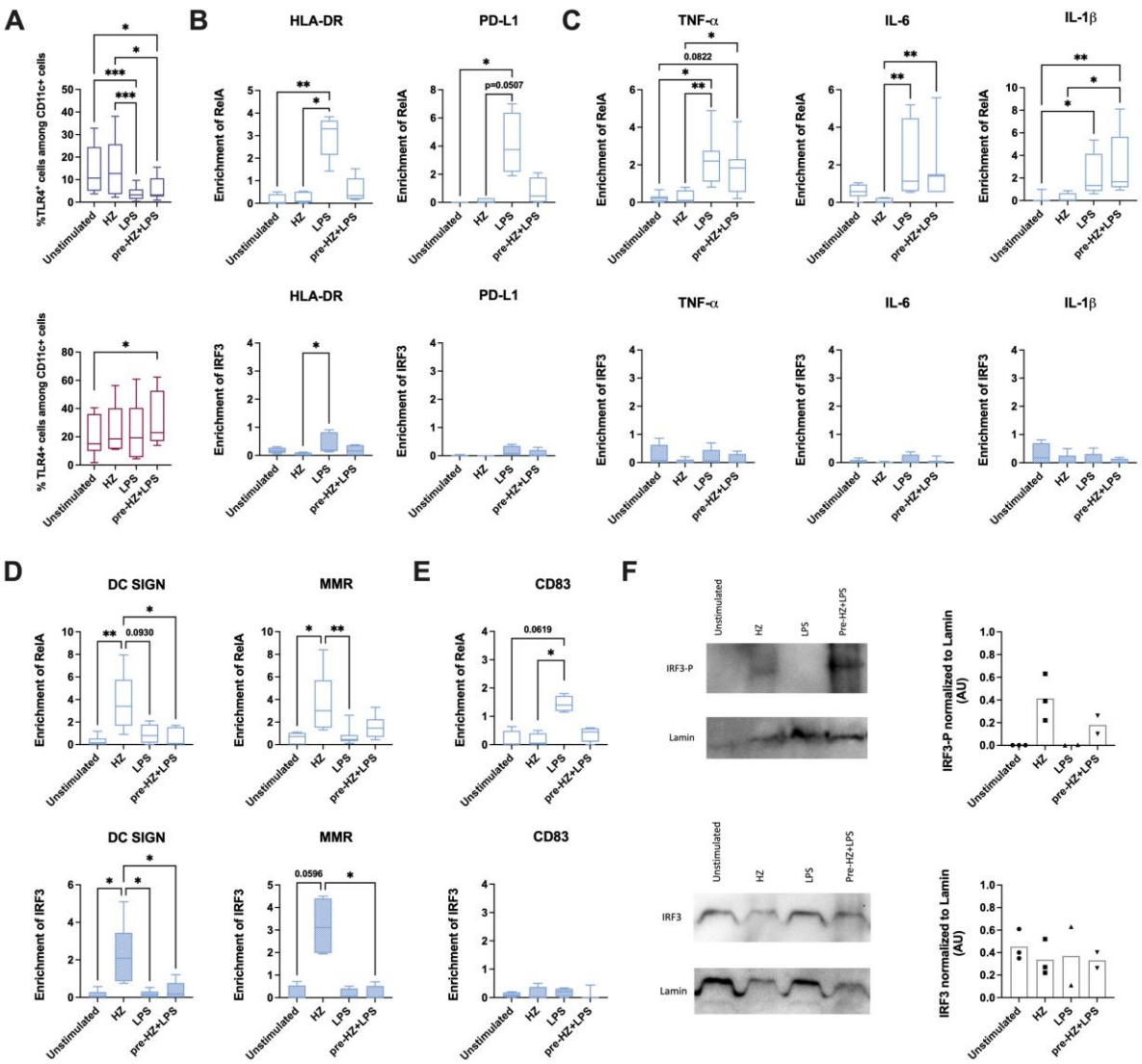

Figure 3. HZ influences transcription factor recruitment to the promoters of moDC genes after short term exposure. (A) The percentage of TLR4 expressing cells among CD11c^{+} moDC population. Paired Friedman test followed by Dunn´s multiple comparison was used to determine statistical difference, *p<0.05, **p<0.01, ***p<0.001. The enrichment of RelA and IRF3 at the promoters of **(B)** HLA-DR and PD-L1, **(C)** TNF-α, IL-6 and IL-1β, **(D)** DC SIGN and MMR, and **(E)** CD83, genes. (B-E) Non-parametric Kruskal-Wallis ANOVA test, followed by Dunn´s multiple was comparison was applied to determine significant differences, n.s.=p>0.05,*p<0.05, **p<0.01. **(F)** The representative picture and quantitative analysis of IRF3 protein and its phosphorylated form measured by western blot. IRF3 levels were normalized to Lamin levels. The data are present as median with interquartile range. N=2-12.

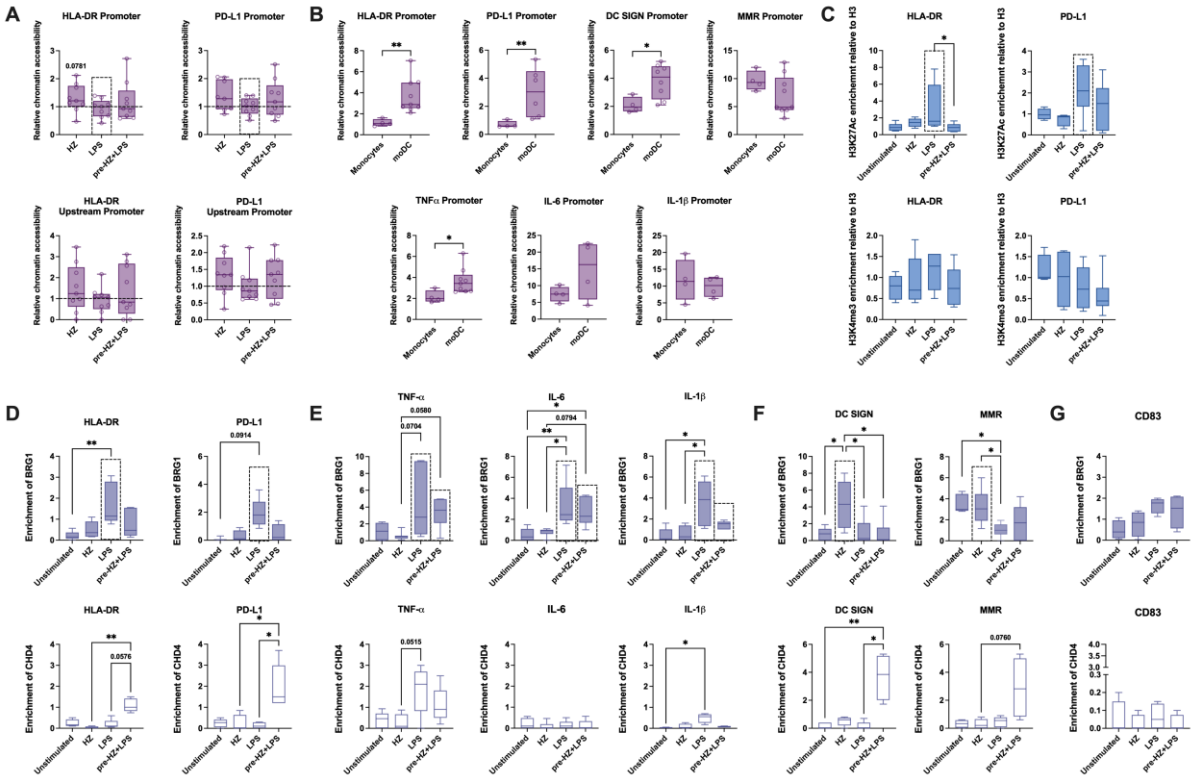

Figure 4. Exposure to HZ does not alter chromatin state but it changes the recruitment of SWI/SNF complex proteins to the promoters of moDC genes. (A) Relative chromatin accessibility at the promoters of HLA-DR and PD-L1 genes. Non-parametric Wilcoxon matched-pairs signed rank test was applied to determine significant differences, n.s.=p>0.05. **(B)** Relative chromatin accessibility at the promoters of HLA-DR, PD-L1, DC SIGN, MMR, TNF-α, IL-6 and IL-1β, in isolated monocytes and differentiated moDC on day 5. Non-parametric Mann-Whitney test was used to determine the differences, *p<0.05, **p<0.01. **(C)** The recruitment of HS27Ac (upper graphs) and H3K4me3 (lower graphs), at the promoters of HLA-DR and PD-L1 genes. The enrichment of BRG1 and CHD4 at the promoters of **(D)** HLA-DR and PD-L1, **(E)** TNF-α, IL-6 and IL-1β, **(F)** DC SIGN and MMR, and **(G)** CD83, genes in moDC. (C-G) Non-parametric Kruskal-Wallis ANOVA test, followed by Dunn´s multiple was comparison was applied to determine significant differences, n.s.=p>0.05, *p<0.05, **p<0.01. N=3-9.

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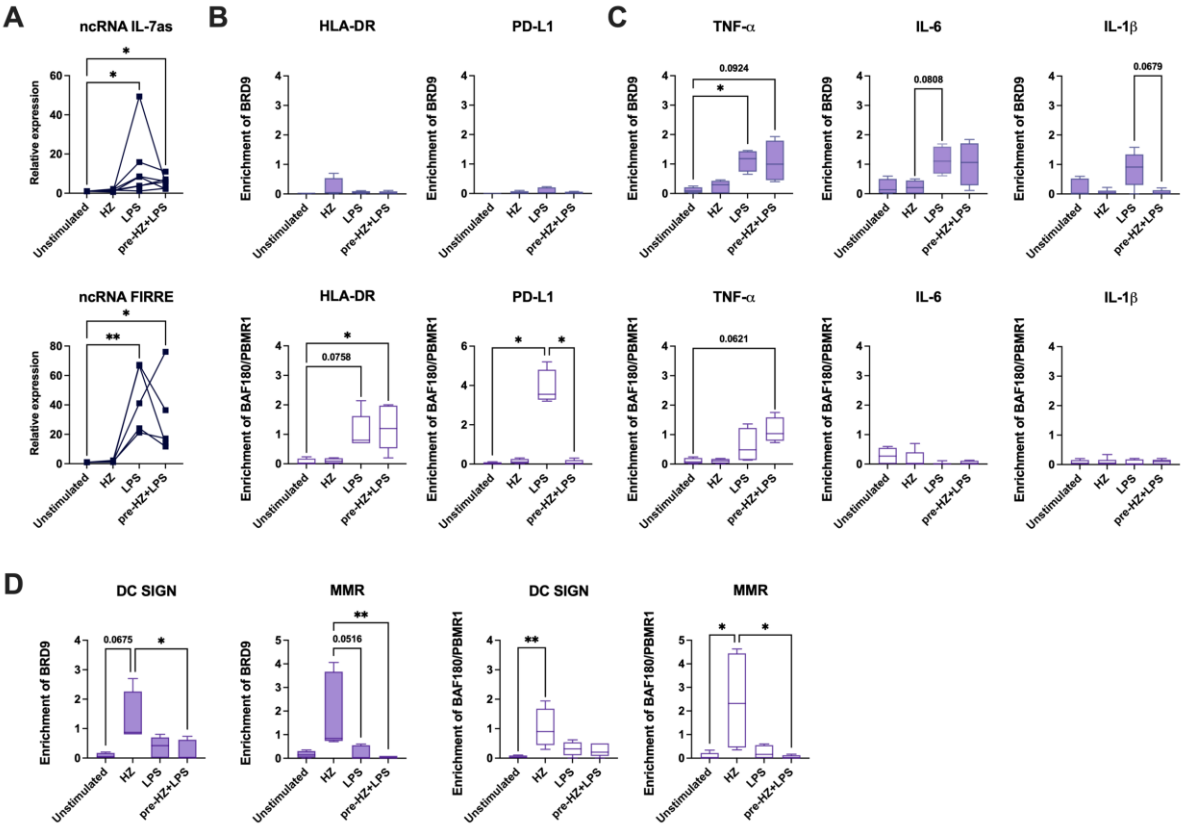

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752 **Figure 5. Differential recruitment of BRD9 and BAF180 proteins at the promoters of moDC genes. (A)**

753 Relative expression of ncRNA IL-7as and ncRNA FIRRE in moDC after 2 h exposure. The enrichment of

754 BRD9 and BAF180/PBMR1 proteins at the promoters of **(B)** HLA-DR and PD-L1, **(C)** TNF-α, IL-6 and IL-

755 1β, **(D)** DC SIGN and MMR, genes. (A-D) Non-parametric Kruskal-Wallis ANOVA test, followed by

756 Dunn´s multiple was comparison was applied to determine significant differences, n.s.=p>0.05,

757 *p<0.05, **p<0.01, n=3-7.