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 with the parasite merozoites 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 HLA-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

Introduction

The human immune response to malaria is multifaceted and complex since the malaria parasite, Plasmodium, has many life stages and infect several organs in man. During the blood stage, infected erythrocyte releases the parasite merozoites together with erythrocyte and malaria specific content that elicit a strong innate pro-inflammatory response, which leads to activation of T-cells and to the clearance of the parasite (reviewed in 1). Co-infections with viruses or bacteria usually accelerate both the malaria and the infection, in particular in children and naïve infected individuals (2, 3).

The erythrocyte release contains several Plasmodium and host molecules (reviewed in 3 - 5), which all contribute to the immune response to malaria and maybe to the more sever disease progression in co-infections. Isolated macrophages or dendritic cells (DC) incubated with infected red blood cell lysate (iRBC) or purified hemozoin (HZ), a crystalline pigment from Plasmodium digested haem, do not elicit a pro-inflammatory response (6, 7) unless at high concentration (8). Other stimuli are also required and isolated cells require external ROS to mount a pro-inflammatory response (9, 10). Nevertheless, iRBC or HZ affects innate cells in other ways, as iRBC and HZ impairs DC function at several stages; the differentiation of monocytes to DC (moDC) *in vitro* (11, 12), moDC maturation upon exposure to stimuli (8, 11-15), and impaired T-cell activation (8, 13-16). However, the mechanism behind the interplay between pathways is not known.

Pre-incubation of DCs with DC for 24 hours or more affects subsequent responses to LPS, with an impaired maturation (8, 11). These effects may be a result of priming of innate cells to establish a trained or tolerance memory by epigenetic (histone modifications) and metabolic long-term reprogramming (18, 19). iRBC and HZ exposure induce a trained phenotype in monocytes from naïve blood donors, with a higher level of H3K4me3 at immune genes (19, 20). These changes work long-term by priming innate cells to subsequent infections, however, whether short-term exposure with HZ also has modulatory effects and alters the response to co-infections is poorly understood. iRBC

65 have been shown to induce early responses in the acute phase of blood-derived DC, including
66 secretion of chemokines but more prominently by altering the transcription of genes within a few
67 hours (7). An early effect on transcription is observed in HZ exposed monocyte derived DC (moDC),
68 and the early exposure time inhibited LPS induced maturation (14). The receptor for HZ is not
69 identified or which pathways are activated. Nevertheless, when phagocytosed, HZ activates NFκB
70 signalling in a MYD88 independent way (21-23).
71
72 Here, we show that exposure to HZ modulated the acute transcriptional response to LPS; co-
73 exposure with HZ specifically prevented the upregulation by LPS of HLA-DR and PD-L1 gene
74 expression, in contrast to inflammatory genes, where HZ had no effect on the LPS-induced activation.
75 Many immune genes are regulated by NFκB and IRFs in an SWI/SNF dependent manner with specific
76 constellations operating on different gene promoters (24, 25). We show that the HLA -DR and PD-L1
77 associated with the BAF180-PBAF variant upon LPS stimulation, and this complex was replaced by the
78 silencing NuRD complex in co-exposed cells. NuRD antagonise the SWI/SNF on immune genes (26).
79 Despite the association of chromatin remodelling factors, the regulation of these genes was not
80 dependent on chromatin alterations. The genes in the DC response either established an open
81 configuration during differentiation from monocytes to moDC, or was already pre-set in a very open
82 state at an even earlier stage. Furthermore, HZ kept moDC in an immature state with maintained
83 transcription of DC-SIGN and MMR genes (27). We propose that HZ primes specific gene promoters
84 to respond differently to subsequent or simultaneous stimulation by bacteria through the
85 recruitment of SWI/SNF complexes and NuRD, with a capacity to transiently alter a following T-cell
86 response. At the same time transcription is actively maintained on immaturity genes important for
87 homeostasis.
88
89

90 ## Material and Methods

91 **Human subjects**

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

94

95 **Natural Hz preparation**

96 Natural HZ was prepared as previously described (14, 15). Briefly, 12 h after schizogony of
97 synchronized FCR3 parasite cultures, the supernatant was collected. A 10%/40% interphase of a 6%
98 mannitol-containing Percoll gradient was used to collect natural HZ, which was then extensively
99 washed with 10 mM phosphate buffer followed by washing with PBS. HZ was opsonized with equal
100 volume of human AB serum for 30 min at 37°C and then it was quantified according to its haem
101 content. HZ preparation with 4 nmol/µl haem content was used in the study. Prior to use, HZ was
102 resuspended by passage through a 0.4 mm needle fitted to a 1 ml syringe.

103

104 **Cell culture and stimulations**

105 Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats using Ficoll-Hypaque
106 (Cytiva) gradient centrifugation. Monocytes were enriched from PBMC by negative selection using
107 EasySep™ human monocyte kit (STEMCELL Technologies) according to the manufacturer´s
108 instructions. Monocytes were resuspended at $1x10^6$ cells/ml and were seeded in 96-well plates (with
109 200 µl/well) or 6-well plates (with 2 ml/well) using RPMI-1640 culture medium which was
110 supplemented with 20 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin
111 (all from Cytiva), 5% human AB serum (Sigma-Aldrich), 50 µM 2-mercaptoethanol, 2% sodium
112 pyruvate (both from Gibco), 35 ng/ml IL-4 and 50 ng/ml GM-CSF (both from PeproTech). On day 3,
113 half of the medium volume was replaced with the fresh medium containing IL-4 and GM-CSF. On day
114 5, cells were pre-exposed to HZ (5 µl for 96-well plates and 50 µl for 6-well plates) and the plates
115 were centrifuged at 700 r.p.m. for 20 s in order to initiate the contact with Hz. After 2 h, 50 ng/ml of

116 LPS from *Escherichia coli* O26:B6 (Sigma-Aldrich) was added to appropriate wells for additional 2 h or
117 24 h. Cells assigned to HZ treatment alone were exposed to HZ at the same time as was added LPS.
118 Cells kept in complete culture medium served as the control. Supernatants were collected and stored
119 at -20°C until further analysis, while cells were collected either for western blot, chromatin
120 immunoprecipitation (ChIP), mRNA expression analysis, ATAC-qPCR or flow cytometry. Throughout
121 the experiment, cells were incubated at 37°C with 5% CO_2.
122
123 **Immunoblotting**
124 MoDC were harvested in 4xLameli buffer, separated on a 12% PAGE and transferred to a PVDF
125 (Miilipore) membrane. The membrane was blocked in 5% milk and incubated separately with primary
126 antibodies: anti-Phospho-IRF3 (Ser 396) (D601M, Cell Signaling), anti-IRF3 (D6I4C, Cell Signaling),
127 anti-Lamin B1 (ab65986, Abcam), anti-Caspase-1 (ab207802, Abcam), anti-beta Tubulin (ab6046,
128 Abcam). Immunoblots were developed using ECL Western Blot Substrate (BioRad). The quantification
129 of protein signals was performed by ImageLab™ (ChemiDoc™ Imaging system, BioRad) software by
130 comparing the signals from each target proteins to control proteins.
131
132 **Chromatin immunoprecipitation**
133 Chromatin from different treatment groups was cross-linked using 1% formaldehyde (Sigma-Aldrich).
134 Chromatin from $1x10^6$ cells was incubated with antibodies against histone H3 (ab1791), histone
135 H3K27Ac (ab4729), histone H3K4me3 (ab8580), RelA (NFκB p65, ab7970), IRF3 (ab76409) IgG
136 (ab171870) (all from Abcam), or BRG1 antibody (generated as previously described, 28). Protein A/G
137 magnetic beads (ThermoScientific™) were used to precipitate. The target DNA was amplified by
138 qPCR using KAPA SYBR FAST qPCR Master mix (Kappa Biosystems Inc). The results are presented as
139 percentage of input, with the IgG control subtracted from the Ct values obtained for samples.
140 Primers are listed in the Table S1.
141

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

143 Total RNA was extracted using TRI reagent (ThermoScientific™), and reverse transcription of RNA
144 was performed using SuperScript VILO cDNA synthesis kit (ThermoScientific™), according to the
145 manufacturers' instructions. qPCR was performed using KAPA SYBR FAST qPCR Master mix. mRNA
146 expression levels were calculated using $2^{-\Delta\Delta Ct}$ method and PP1A was used for normalization. Primer
147 pair list can be found in the Table S1.

148

149 **ATAC-qPCR**

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

155

156 **Flow cytometry**

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

164

165 **Cytometric bead array (CBA)**

166 Soluble levels of TNF-α, IL-10, IL-6 and IL-1β cytokines were measured in the cell culture supernatant
167 after 2 h of exposure using CBA human Inflammatory cytokine kit (BD Biosciences), while chemokines

168 CXCL8/IL-8, CCL5/RANTES, CXCL9/MIG, CCL2/MCP-1 and CXCL10/IP-10 were quantified using CBA
169 human chemokine kit (BD Biosciences), according to the instructions from the manufacturer.
170 Samples were acquired using FACSVerse flow cytometer and FlowJo Software was used to analyse
171 the data.
172
173 **Enzyme-linked immunosorbent assay**
174 Soluble levels of TNF-α, IL-6, IL-1β, IL-23 and IL-10 (MabTech AB), as well as IL-RA (R&D systems, Bio-
175 Techne), were measured in the cell culture supernatant after 2 h and/or 24 h of co-exposure
176 according to the instructions from the manufacturer. The results were analysed using SoftMax Pro
177 5.2 rev C (Molecular Devices Corp.).
178
179 **Statistical analysis**
180 Statistical analysis was carried out using GraphPad Prism 10 (GraphPad Prism Inc.). Non-parametric
181 Friedman ANOVA test or non-parametric Kruskal-Wallis ANOVA test, followed by Dunn´s Multiple
182 Comparison Test was applied to determine the differences between multiple treatment conditions.
183 Non-parametric Wilcoxon matched-pairs signed rank test or non-parametric Mann-Whitney test was
184 used to determine the differences between two treatment conditions. Statistical differences were
185 considered significant if p-values were <0.05.
186
187

188 ## Results

189 **HZ compromises LPS-induced upregulation of HLA-DR and PD-L1 gene expression**

190 To address the question whether HZ has an impact on the early moDC phenotype and functional
191 response to an unrelated microbial compound such as the bacterial-derived LPS, we pre-incubated
192 cells with HZ for 2 h to allow its phagocytosis before adding LPS as depicted in Figure 1A. To mimic
193 the state of HZ after its release in bloodstream, we used an opsonized preparation in which the HZ
194 crystal contained plasma components. HZ was phagocytosed by the cells already after 2 h, as shown
195 by the increased granulation of cells observed by flow cytometry (Supplementary Figure S1A). The
196 exposure to HZ for 2 h did not affect the surface expression of HLA-DR and it did not hamper LPS-
197 induced expression of this marker after 24 h (Figure 1B). As expected, the expression of the
198 maturation marker CD83 was significantly upregulated by LPS after 2 h and 24 h (Figure 1B). Since HZ
199 mainly impacts the early transcriptional response (14), we investigated mRNA levels of these DC
200 markers upon LPS-stimulation. Interestingly, the LPS-triggered increase in HLA-DR transcripts was
201 markedly reduced when the cells was co-exposed to HZ (Figure 1C). Transcription of CD83, however,
202 was not reduced by co-exposure of HZ and LPS compared to LPS alone. This was even more
203 pronounced for the gene expression of the maturity marker CCR7, which was induced by LPS and
204 further increased when the cells were co-exposed to HZ (Figure 1C).

205

206 To further assess if moDC remained immature with poor T-cell activation capacity upon HZ addition,
207 we investigated the surface expression of the C-type lectin receptors DC-SIGN (CD209) and MMR
208 (CD206), which are associated with an immature phenotype (27). The surface expression of both
209 receptors in HZ exposed cells was high and comparable to the unstimulated cells, while LPS caused a
210 downregulation of these receptors after 24h, which was not prevented by HZ pre-exposure (Figure
211 1D). Further, HZ did not block the LPS-mediated upregulation of co-stimulatory receptor CD86 or the
212 surface expression of PD-L1, which is a negative co-stimulatory receptor important for T-cell
213 inhibition, following 24 h (Figure 1E). To assess the early transcriptional level of these factors, we

214 examined the mRNA levels of DC SIGN and MMR. The mRNA levels were reduced by LPS compared
215 to the cells only exposed to HZ (Figure 1F). The mRNA levels of PD-L1 displayed a similar pattern to
216 HLA-DR; co-exposure with HZ strongly reduced the expression of LPS-induced PD-L1 (Figure 1G).
217 CD86 did not differ among samples at this early time point (Figure 1G). Taken together, our results
218 suggest that HZ interferes with the stimulation by bacterial antigen during the early induction of gene
219 expression specifically of genes important in antigen presentation and activation of T-cells.
220
221 **HZ does not influence the secretion of LPS-induced inflammatory and anti-inflammatory factors**
222 **early during the response**
223 We next investigated whether HZ had an impact on the LPS-induced inflammatory and anti-
224 inflammatory protein production. As expected, LPS induced TNFα and IL-6 release already after 2 h,
225 while IL-23 secretion was induced by LPS after 24 h, regardless whether HZ was present or not
226 (Figure 2A and Supplementary Figure S2A). LPS also induced a strong transcriptional response of the
227 TNFα, IL-6, and 1L-23 genes after 2 hours, which was not interfered with by HZ (Figure 2B and
228 Supplementary S2A, lower panel). Furthermore, HZ did not interfere with the LPS-induced secretion
229 of CXCL8, CXCL9 or CXCL10 chemokines (Figure 2C). CCL2 was not induced above background, while
230 CCL5 was slightly upregulated in co-exposed cells (Supplementary Figure S2B). At the transcriptional
231 level co-exposure resulted in enhanced CXCL9 expression, while CXCL10 resembled a similar pattern
232 to that of HLA-DR and PD-L1; the increase was only detected in LPS exposed cells but not in LPS and
233 HZ co-exposed cells (Figure 2D). For the other chemokines, mRNA expression varied substantially at 2
234 h (Figure 2D and Supplementary Figure S2B, lower panel).
235
236 HZ did not interfere with the anti-inflammatory response as LPS-induced IL-10 production both at the
237 protein and transcriptional levels 2 h post exposure (Figure 2E). Further, HZ did not have clear effects
238 on the expression of the immunosuppressive or tolerance markers SOCS2 and IDO1, or reduced the
239 LPS induction in co-exposed cells (Supplementary Figure S2C). We also investigated the protective

240 enzyme Haem oxygenase (HO-1), as it might be induced upon haem release and dampen the
241 response (29). HZ exposure did not trigger the gene expression of HO-1, regardless of LPS presence
242 (Supplementary Figure S2C). In summary, HZ did not induce an inflammatory response or interfere
243 with the response by LPS regarding secretion of cytokines and chemokines, nor with the
244 transcriptional induction of the genes.
245
246 **HZ accounts for the release of inflammasome-derived IL-1β during co-exposure with LPS**
247 Since HZ exposure has been associated with NLRP3-inflammasome activation (30, 31), we measured
248 the inflammasome-dependent cytokine IL-1β secretion. The cytokine was detected only from cells
249 co-exposed to HZ and LPS after 24 h (Figure 2F), preceded by enhanced levels of transcription by LPS
250 stimulation as detected at 2 h (Figure 2G). In addition, transcriptional induction of the inflammasome
251 component NLRP3 was upregulated by HZ and LPS co-exposure and by LPS stimulation alone, while
252 the pro-caspase-I exhibited inductions by LPS alone (Supplementary Figure S2D). This suggests that
253 the release of the IL-1β requires HZ to be co-exposed with a pathogen which induces a transcriptional
254 response of inflammasome associated genes. The inflammasome activation involves the cleavage of
255 pro-caspase 1 and we could not detect any cleavage as early as 2 h after stimulation, showing that 2
256 h is not sufficient for the full activation of the inflammasome to release IL-1β (Supplementary Figure
257 S2E). During inflammation, the production of IL-1β is balanced by the IL-1 receptor antagonist (IL-
258 1RA) (32). IL-1RA release was lower in LPS exposed and co-exposed cells after 2 h (Figure 2H), similar
259 to IL-1RA mRNA expression at 2 h (Figure 2I). Still, regardless of HZ-exposure LPS-stimulation resulted
260 in an increase of IL-1RA secretion after 24 h (Figure 2H). Our results indicate that HZ is responsible for
261 the IL-1β inflammasome response during co-stimulation with LPS, and LPS responsible for the
262 required induction of IL-1β and NLRP3 gene expression and the IL-1β secretion this is accompanied
263 by a higher IL-1RA release.
264
265 **HZ does not interfere with the LPS activation by TLR4**

266 To investigate the mechanism behind the dampening effect of HZ on the transcriptional response of
267 HLA-DR and PD-L1 but not on inflammatory genes, we examined whether HZ changed the level of
268 LPS activation through TLR4. The percentage of TLR4 expressing cells significantly decreased after 2 h
269 both in cells stimulated with LPS and cells exposed to LPS and HZ (Figure 3A), showing that HZ did not
270 affect receptor internalisation. The receptor level was restored at 24 hours, even significantly
271 upregulated in co-exposed cells (Figure 3A). The receptor for HZ is still not known, but CLEC12A, the
272 receptor for uric acid has been suggested to be the receptor for phagocytosis of HZ (33). The surface
273 level of CLEC12A did not change in any treatments (Supplementary Figure S3A).
274
275 **HZ phagocytosis changes the transcription factor recruitment to promoters**
276 TLR4 ligation activates several transcription factors including the NFκB factor RELA and IRF3. To
277 investigate the differentiated gene response to HZ in co-exposed cells, we examined the binding of
278 these factors to the promoters of HLA-DR and PD-L1 genes. These promoters bound significant levels
279 of RELA in cells stimulated with only LPS, while HZ and LPS co-exposed cells displayed markedly lower
280 levels of binding (Figure 3B, upper panel). IRF3 was only recruited at low levels in all samples (Figure
281 3B, lower panel). In contrast, HZ did not hamper the binding of RELA at the promoters of
282 inflammatory genes in LPS stimulated and HZ and LPS co-exposed cells, as it was significantly
283 recruited to the promoters of the primary response gene TNFα and the secondary response genes IL-
284 6 and IL-1β genes (Figure 3C, upper panels). A similar trend for RELA was also observed for IL-23 and
285 CCL5 genes (Supplementary Figure S3B). IRF3 did only bind at low levels to the promoters of the
286 inflammatory genes (Figure 3C, lower panels), even to the IRF3 target gene CCL5 after 2 hours
287 (Supplementary Figure S3B). The RELA and IRF3 recruitment on the CD83 promoter resembled that
288 of HLA-DR and PD-L1 with RELA occupancy in LPS stimulated cells, and low levels of IRF3, although no
289 compromised transcription was observed in co-stimulated cells (Supplementary Figure S3C).
290

291 The slightly lower mRNA levels of DC-SIGN and MMR in LPS stimulated cells compared to HZ exposed
292 cells (Figure 1F), prompted us to also assess transcription factor binding at these promoters. The
293 difference in expression depended on transcription factor recruitment to DC-SIGN and MMR and
294 both RELA and IRF3 were bound to the promoters of DC-SIGN and MMR in HZ stimulated cells (Figure
295 3D), a binding that was counteracted in LPS-stimulated cells (Figure 3D). To further examined the
296 transcriptional effect on these genes, we explored published RNA seq data from DCs after 3h of
297 exposure (7). This data clearly shows an induction of DC-SIGN by iRBC, whereas the transcript level in
298 LPS-exposed cells remained at the unstimulated level. Taken together, this indicates that HZ not only
299 impairs the recruitment of activating factor at certain genes, but also activates other groups of gene
300 to maintain the level of transcription without hampering the regulation by other stimuli.
301
302 Since IRF3 was bound to the promoters of DC-SIGN and MMR in HZ exposed cells after 2 h, we
303 examined the activation of IRF3 in the different treatments. IRF3 was activated, as shown with the
304 presence of phosphorylated IRF3, in cells exposed to HZ alone and cells co-exposed with LPS and HZ
305 (Figure 3E), showing that HZ exposure had an immune modulatory effect by activating both RELA and
306 IRF3.
307
308 **Exposure to HZ does not alter chromatin states**
309 The differential binding of RELA and IRF3 to gene promoters in exposed cells motivated us to
310 investigate the chromatin states at the different gene promoters. No significant changes were
311 detected in the chromatin accessibility analysed by ATAC-qPCR; HLA-DR and PD-L1 displayed no
312 altered accessibility at the gene promoters or at gene enhancers by any exposure (Figure 4A). Nor did
313 changes occur at the promoters, enhancers or gene bodies of inflammatory genes or lectin receptor
314 genes, despite the large transcriptional induction in inflammatory genes (Supplementary Figures S4A
315 and S4B). Many of these genes are also expressed in monocytes and may therefore already have the
316 chromatin accessibility state established. To test this, the accessibility of the response genes and

317 moDC marker genes were examined in monocytes and in moDC. The monocyte marker CD14 was
318 accessible in monocytes and closed in moDC (Figure 4B), whereas the DC marker DC-SIGN as well as
319 HLA-DR, PD-L1 and TNFα exhibited a higher accessibility in moDC compared to the more closed
320 configuration in monocytes (Figure 4C). The promoters of IL-6, IL-1β, and MMR had similar open
321 accessibility in monocytes and moDC (Figure 4C). Chromatin changes are also formed by histone
322 modifications and histone H3K4me3 and histone H3K27Ac have been associated with an active
323 chromatin state coupled to the establishment of a trained or tolerised immune phenotype (17). HLA-
324 DR and PD-L1 exhibited a trend of higher H3K27Ac at the promoter in cells stimulated by LPS alone
325 (Figure 4D, upper panel), whereas no change was observed H3K4me3 (Figure 4D, lower panel). No
326 such trends in the histone modifications were observed in other groups of gene (Supplementary
327 Figures S4C and S4D). This suggests that no trained phenotype was induced at this short time-span
328 and the H3K27Ac at the promoter of HLA-DR and PD-L1 may be associated with the transcriptional
329 activation by LPS. Taken together, the genes important for a DC response already have a hyper-
330 accessibility chromatin structure, established in differentiation steps prior to the activation of the
331 genes upon stimulation.
332
333 **Exposure to HZ changes the recruitment of the SWI/SNF complex**
334 Next, we examined the association of chromatin remodelling complexes to the immune genes;
335 SWI/SNF complexes are required for transcriptional activation of many immune genes and NuRD or
336 EZH2 in PCR2-polycomb for the repression (26, 33, 34). The ATPase BRG1/SMARCA4 was recruited to
337 the promoters of HLA-DR and PD-L1 in LPS stimulated cells, while co-exposure with HZ and of LPS had
338 reduced levels of BRG1 associated with the promoters, which is in agreement with the reduced levels
339 of transcript (Figure 4E, upper panel). Co-exposure of HZ and LPS led instead to recruitment of the
340 ATPase CHD4 in NuRD, but not the methyltransferase EZH2, to the promoters (Figure 4E, lower panel
341 and Supplementary S4E). The promoters of the inflammatory genes, TNFα, IL-6, IL-1β, and CCL5,
342 displayed higher BRG1 occupancy in cells stimulated with LPS and most genes retained this high level

343 in cells co-exposed with HZ and LPS (Figure 4F, upper panel and Supplementary Figure S4F, upper
344 panel). CHD4 was only associated with the TNFα promoter in LPS stimulated cells, indicating that
345 repression of the transcription had started on early primary response genes (Figure 4F, lower panel
346 and Supplementary Figures S4F, lower panel). The promoters of IL-23, IL-10, and CD83 did not display
347 any changes in the association with BRG1 or CHD4 (Supplementary Figure S4G), suggesting that other
348 factors are operating on these gene promoters. In contrast to the other groups of gene, BRG1 levels
349 associated to the promoters of DC-SIGN and MMR were higher in cells exposed to HZ alone, which
350 maintained a higher transcriptional level than in LPS-stimulated cells, and CHD4 associated with the
351 promoters in cells co-exposed with HZ and LPS (Figures 4G), possible to allow for a reduction in
352 transcription.
353
354 **SWI/SNF variants operating on the genes decide transcription factor preference**
355 To further investigate the specificity of the binding of factors to the different types of gene, we
356 determined the expression of ncRNAs regulating the binding of BRG1-SWI/SNF to immune genes. The
357 recruitment of SWI/SNF complexes to immune genes is affected by two LPS-regulated lncRNAs, IL-7-
358 as and FIRRE, in opposite ways; IL-7-as enhances, and FIRRE reduces the association (36, 37). Both
359 RNA levels were significantly induced by LPS stimulation and co-exposure to LPS and HZ for 2 h
360 compared to HZ exposed and unstimulated cells (Figure 5A), suggesting that the expression of these
361 RNAs does not explain the differential recruitment of factors to the promoters. Instead, we examined
362 whether the promoters associated with different SWI/SNF complex constellations and used BRD9 as
363 a marker for the non-canonical SWI/SNF (ncBAF complex), BAF180/PBRM1 for PBAF and
364 BAF250/ARID1 for the conventual BAF (cBAF) complex. BAF180 was recruited to the HLA-DR and PD-
365 L1 promoters upon LPS stimulation for 2 h (Figure 5B), something that HZ exposure clearly prevented
366 at the PD-L1 promoter. This demonstrates that these genes worked with the PBAF constellation to
367 activate transcription and associated with NuRD in co-exposed cells to decrease transcription. The
368 promoters of inflammatory genes TNFα, IL-6 and IL-1β showed a preference for BRD9, which

369 associated upon LPS stimulation and co-exposure (Figure 5C). BAF250 was only found associated with
370 CCL5 in cells stimulated with LPS and co-exposed cells (Figure 5D), which indicates that the cBAF
371 complex is not involved in the HZ affected gene in the early response. HZ-exposure to DC-SIGN and
372 MMR led to both BRD9 and BAF180 associating at their promoters (Figure 5E), both subunits were
373 lost in the co-exposed cells together with BRG1 and replaced by CHD4. In conclusion, the specificity
374 in the transcriptional response to HZ is achieved by the recruitment of specific SWI/SNF complexes,
375 with different features, to the various promoter.

376 ## Discussion

377 Malaria co-infection with bacteria accelerate both the malaria progression and the bacterial infection
378 (38, 39). The cellular response to malaria antigens and its response during co-infections are
379 multifactorial and contradictory results have been reported (2). Here, we focused on the immune
380 modulatory effects imposed by HZ during co-infections with bacteria and to answer the question, we
381 investigated the early transcriptional events in DCs. The transcriptome analysis of the early gene
382 expression response to iRBC in blood-derived DC shows that the main group of gene affected is
383 associated with lipid synthesis and only a few immune genes were upregulated (7). Here, we show
384 that although HZ had no direct effects on gene expression of immune genes, it modulated the
385 response to bacterial stimuli. HZ did not induce the maturation of moDC and the cells did not secrete
386 inflammatory cytokines and chemokines early in the acute phase after exposure. Instead, HZ
387 exposure hampered the early transcriptional activation induced by LPS of HLA-DR, PD-L1 and CXCL10
388 and at the same time maintained the expression of the immaturity markers DC-SIGN and MMR,
389 possibly to dampen or skew a subsequent T-cell response. The transcriptional response is regulated
390 by activated transcription factors and in addition to activating NFκB signalling (21-23), we show that
391 HZ activated IRF3 signalling after 2 h. Even if activated, the factors were differentially recruited to the
392 different genes; activated IRF3 was differentially recruited to the promoters of DC-SIGN and MMR
393 and not to other IRF3 targets, such as CCL5. Differential recruitment may occur in several ways, and
394 HZ is a molecule able to modulate the binding of transcription factors by replacing activating
395 SWI/SNF complexes by the NuRD complex during co-infection on specific genes such as HLA-DR and
396 PD-L1 (Figure 6).

397 The specific response of genes depends on their promoter and enhancer architecture as well as on
398 their chromatin state. Immune genes are divided into groups depending on the type of response
399 elements in regulatory regions and HLA-DR, the PD-L1 as well as the CXCL10 are interferon
400 stimulated genes (IGS) (40-42). The promoters of these genes harbour interferon-sensitive response
401 elements that are specifically responsive to IFNγ activation of the transcription factor STAT1 and IRFs

402 (43, 44). The gene promoters also harbour NFκB binding sites and are regulated by TLR signalling,
403 through the NF-κB pathway in response to pathogens. Inflammatory and maturation genes are also
404 induced early by NFκB transcription factors RELA and later by IRF3/IRF7 (45, 46). While LPS
405 stimulation recruited RELA to both IGS- and inflammatory genes, co-exposure with HZ reduced the
406 recruitment of RELA to the IGS genes. This may be attributed to different signalling codons, such as
407 stimulus specific activation of pathways, and scRNA transcription analyses have shown that although
408 genes respond to the same transcription factors, they are activated at different time points and at
409 different amplitudes (41, 45, 46). This is caused by specific responses to the type and quality of signal
410 triggered by the stimulus, to the signals secreted by neighbouring cells and the chromatin states of
411 individual cells (42, 46-48).

412 Only very small changes in chromatin accessibility or histone modification histone H3K4me3 and
413 histone H3K27A were detected at the promoters of the genes upon activation compared to
414 unstimulated cells after 2 h. Accessible chromatin and active histone modifications are often
415 correlated with active transcription, but these can be set at different stages. Exposure of either iRBC
416 or HZ for 24 hours induces a trained phenotype with increased enrichment of H3K4me3 at specific
417 genes in monocytes (19. 20), which suggests that longer exposure times than 2 hours are required to
418 establish training or tolerance. Furthermore, the differentiation also see changes in histone
419 modifications and differentiation of monocytes to moDC involves loss of H3K4me3 at the promoter
420 of the monocyte marker CD14 gene and a gain at the DC-SIGN gene (49). Similarly, the accessibility of
421 gene promoter is pre-set during differentiation (50), which includes immune genes genomically
422 primed at an earlier step in oligodendrolia development (51). We investigated the accessibility during
423 development and show that the CD14 promoter changed from a more open configuration in
424 monocytes to a closed one in moDC, and the DC-SIGN showed the opposite pattern and obtained a
425 more accessible configuration. HLA-DR, PD-L1 and the inflammatory genes, which are expressed
426 upon maturation either gained an open configuration during the differentiation to moDCs or already
427 had an open configuration established in an even earlier step. Our results show that maturation of

428 DCs does not require further alteration of accessibility at the promoters in the early acute phase and
429 the transcriptional induction of DC depends on the activation and binding of transcription factor.
430
431 Many immune genes require SWI/SNF complexes for transcriptional activation. This can be
432 counteracted in different ways, either through the NuRD complex that antagonises SWI/SNF
433 activation (26, 52) or through EZH2-PCR2 that can silence the genes (34, 35, 52). We observed that
434 the promoters of several of the investigated genes, including HLA-DR and PD-L1, associated with
435 BRG1 when activated by LPS, and that BRG1 was replaced by the CHD4 when transcription was
436 reduced following HZ and LPS co-exposure. These complexes are tightly correlated with chromatin
437 changes, something we did not observe in our study. However, studies with perturbed SWI/SNF and
438 NuRD factors show that the transcriptional effect is not always the expected (52-56). It has been
439 shown that SWI/SNF and NuRD are involved in transcriptional changes by regulating RNA pol II
440 occupancy at the gene promoter and in the gene body of highly expressed genes instead of
441 chromatin remodelling (52). We did not detect EZH2 on HLA-DR and PD-L1 promoters, which confer
442 Polycomb silencing in cancer cells (34, 35, 56). EZH2 has also been associated with silencing of
443 SWI/SNF activated bivalent genes expressed at a low level by reducing the chromatin accessibility
444 (52). In connection with our finding, we propose that many genes in the acute phase are induced by
445 activated RELA and IRF3 and regulated by the SWI/SNF and the NuRD complexes through alteration
446 of RNA pol II kinetics rather than changing chromatin configuration. In this context, HZ exposure
447 establishes a different balance of the complexes at the HLA-DR and PD-L1 genes as well as the C-
448 lectin genes which results in a compromised gene expression in response to concomitant LPS
449 stimulation.

450 The specificity of the response was achieved by recruiting different SW/SNF constellations. These
451 constellations operate on different regulatory elements and regulate cell type specific expression
452 (57, 58). Our results show that these constellations worked with different gene promoters, and the
453 BAF180-PBAF was associated with the promoters of HLA-DR and PD-L1 upon LPS stimulation. It was

454 replaced by the NuRD complex in co-exposed cells resulting in reduced transcription. PBAF
455 association to promoters often lead to repression, such as repression of genes in T-regs and IFNγ
456 response genes (24, 25, 59-61) In contrast to the PBAF operating on HLA-DR and PD-L10, we found
457 that a number of the inflammatory genes associated with the BRD9-ncBAF upon LPS stimulation, and
458 this complex was not antagonised by NuRD upon co-exposure with HZ. We propose that the gene
459 architecture determines what type of SWI/SNF complex that associate with the promoter and this
460 determines the regulatory events. PBAF is associated with the NuRD antagonistic activity which may
461 be responsible for the more repressive activity associated with the PBAF complex.

462 IL-1β was the only cytokine secreted from co-exposed cells after 24 h of stimulation and not from HZ
463 and LPS alone. This suggest that neither HZ or LPS alone could activate the inflammasome, which
464 requires the transcriptional activation of the IL-1β gene and other inflammasome genes, like NLPR3
465 and caspases, as well as the activation and assembly of the inflammasome (62). The activation of the
466 inflammasome has been attributed to HZ by activating the STAT pathway through LYN/SYK kinases
467 (30) or to induce the production of ROS (23, 30). The release of IL-1β after 24 h co-exposure with HZ
468 and LPS may have implications in malaria co-infections with bacteria and contribute to an excessive
469 inflammatory reaction. Equally important is the early modulation of the response, in which HZ-
470 exposure of DCs maintains the cells in a partially immature state, which might lead to a skewed T-cell
471 activation and fine-tune a homeostatic response to avoid random maturation. The modulation
472 imposed of certain promoters by short-term HZ exposure primes the cells for subsequent or
473 simultaneous bacterial infections and these effects may lead to a more severe disease during co-
474 infections.

475

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.

Funding

This research was funded by The Swedish Cancer Society (19 0453 Pj and 22 2310 Pj to AÖF and 20 1117 Pj and 23 2985 Pj to ESE), The Swedish Research Council (2020-01839 and 2023-02616 to ESE), and Stockholm University.

Conflict of Interest

The authors declare no conflict of interests.

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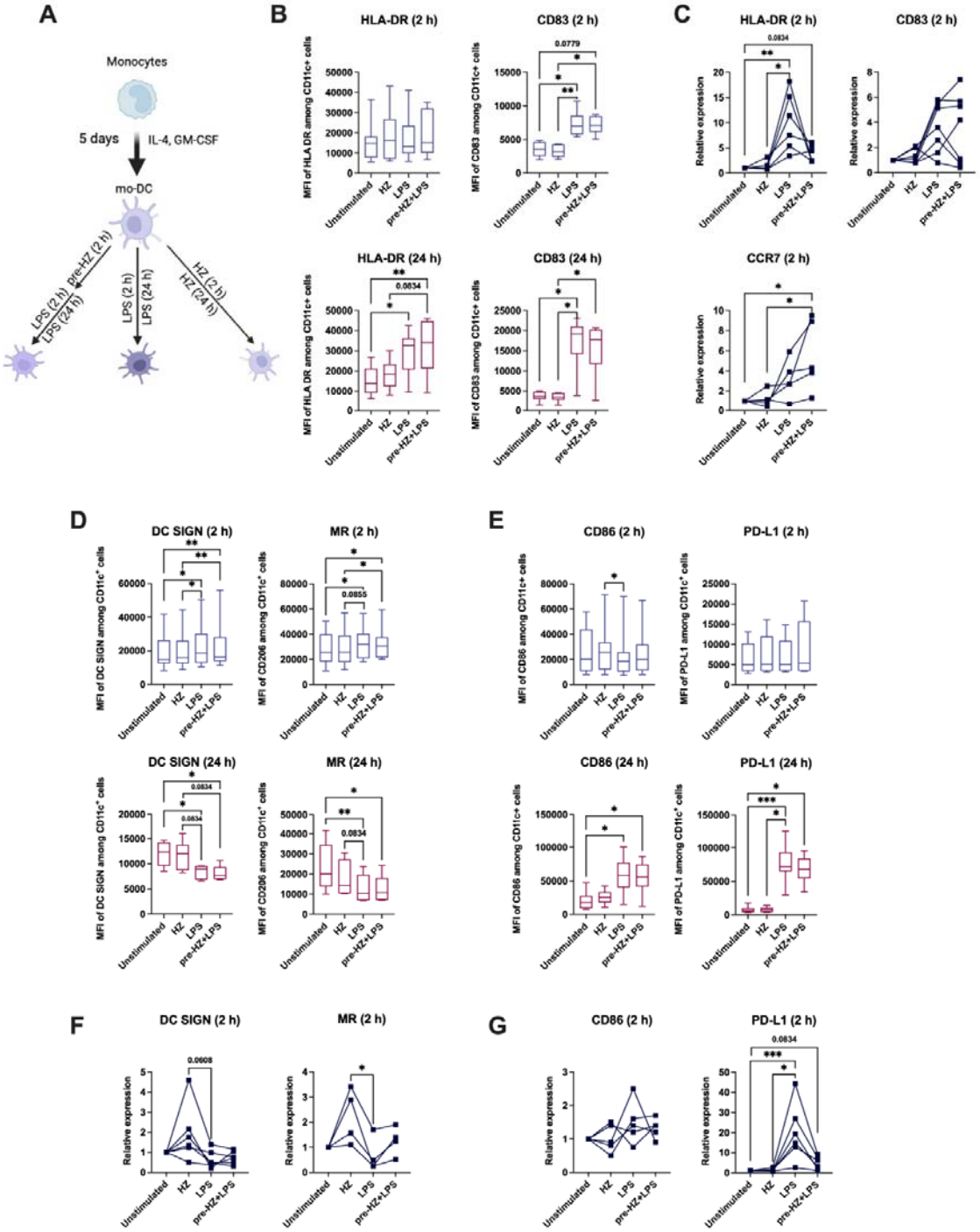
708

709

710 **FIGURES**

711 **Figure 1**



712

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

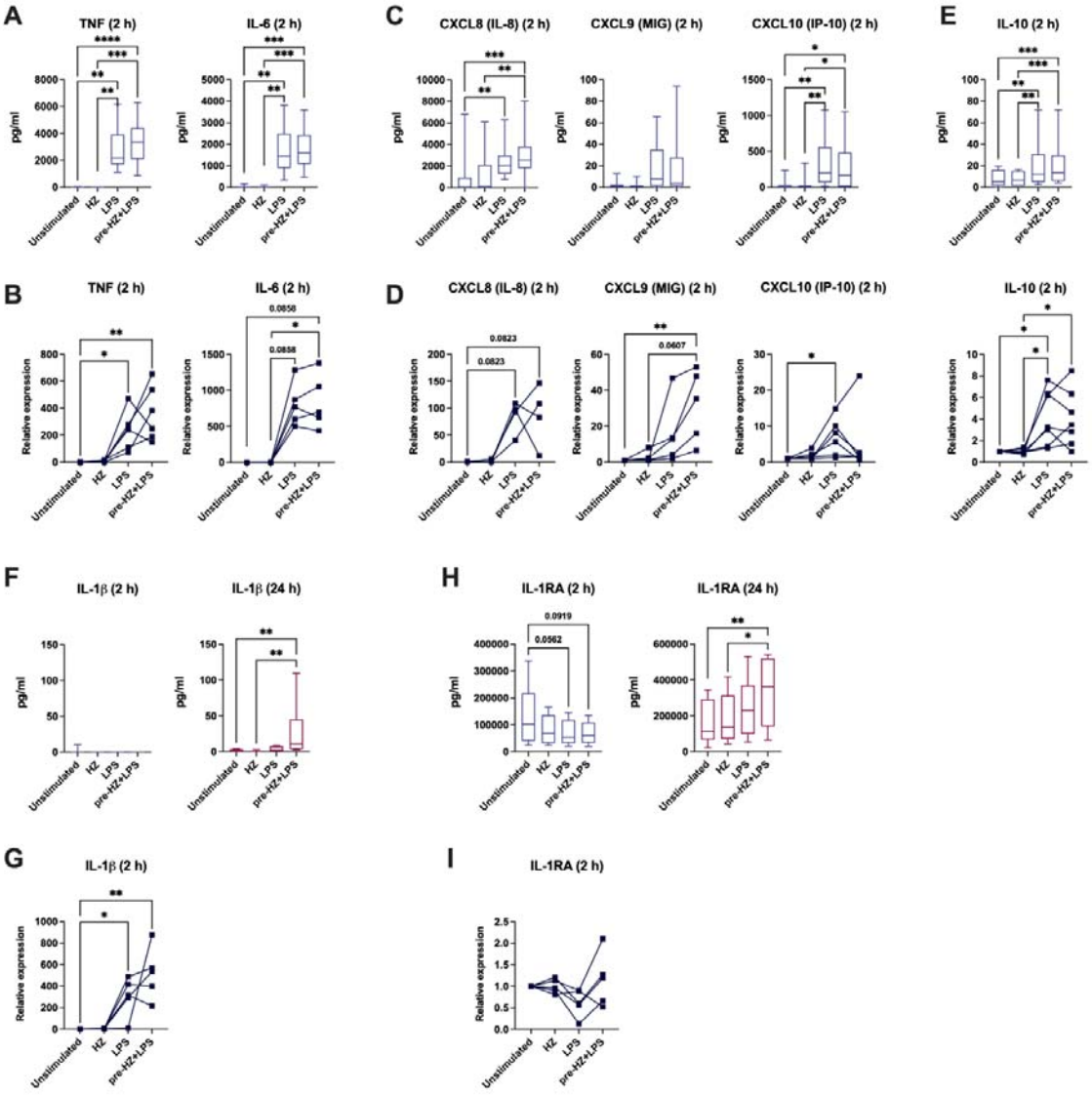
714 **LPS. (A)** Experimental model of monocytes differentiation to mo-DC during 5 days culture and

715 exposure to different stimuli. MoDC were pre-exposed to HZ for 2 h, then LPS was added for

716 additional 2 h or 24 h (co-exposure condition). Cells kept in complete culture medium served as the
717 control. **(B)** The mean fluorescent intensity (MFI) of HLA-DR and CD83 in moDC after 2 h exposure
718 (upper graphs) and 24 h exposure (lower graphs). **(C)** The relative mRNA expression of HLA-DR, CD83
719 and CCR7 in moDCs after short-term exposure. The mean fluorescent intensity (MFI) **(D)** DC SIGN and
720 MMR, **(C)** CD86 and PD-L1, expression in moDC after 2 h exposure (upper graphs) and 24 h exposure
721 (lower graphs). The relative mRNA expression of **(F)** DC SIGN and MMR, **(G)** CD86 and PD-L1, in moDC
722 after short-term exposure. (B-G) Paired Friedman test followed by Dunn´s multiple comparison was
723 used to determine statistical difference, n.s.=$p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, n=4-12.

724

725 Figure 2



726

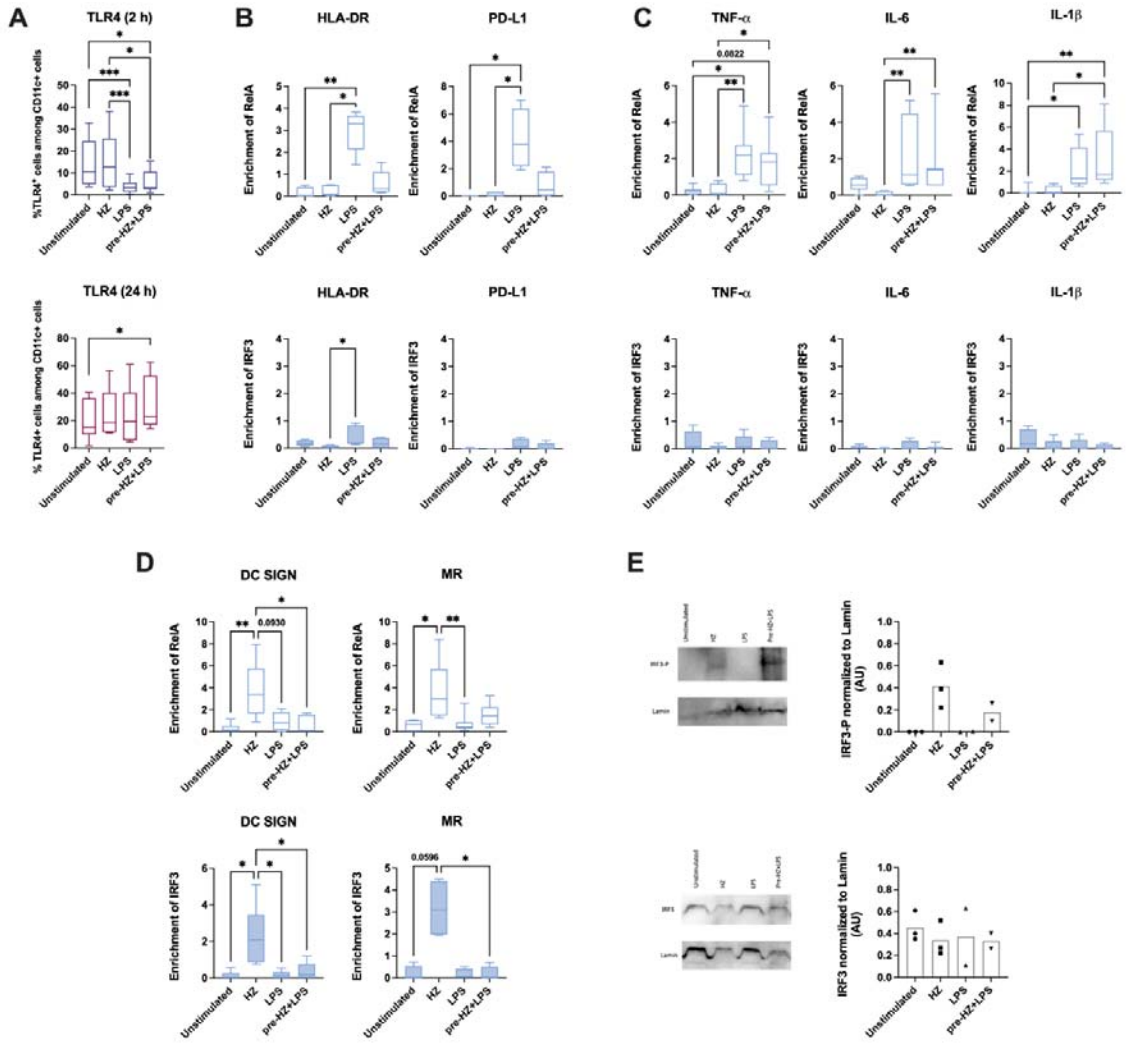
727 **Figure 2. HZ does not interfere with the upregulation of inflammatory gene transcripts, but both**
728 **HZ and LPS are required for the release of inflammasome-derived IL-1β**. **(A)** Soluble levels of TNF-α
729 and IL-6 in moDC after 2 h exposure. **(B)** Relative mRNA expression of TNF-α and IL-6. **(C)** Soluble
730 levels of CXCL8, CXCL9, CXCL10 after 2 h exposure. **(D)** Relative mRNA expression of CXCL8, CXCL9,
731 CXCL10. **(E)** Soluble levels and relative mRNA expression of IL-10 after 2 h exposure. **(F, H)** Soluble
732 levels of IL-1β and IL-1RA after 2 h and 24 h exposure. **(G, I)** Relative mRNA expression of IL-1β and
733 IL-1RA. mRNA levels were measured in all samples after 2 h of exposure. (A-I) Paired Friedman test

734 followed by Dunn´s multiple comparison was used to determine statistical difference, n.s.=$p>0.05$,

735 *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, n=4-14.

736

737 **Figure 3**



738

739 **Figure 3. HZ influences transcription factor recruitment to the promoters of moDC genes after**

740 **short term exposure. (A)** The percentage of TLR4 expressing cells among CD11c^{+} moDC population.

741 Paired Friedman test followed by Dunn´s multiple comparison was used to determine statistical

742 difference, *p<0.05, **p<0.01, ***p<0.001. The enrichment of RelA and IRF3 at the promoters of **(B)**

743 HLA-DR and PD-L1, **(C)** TNF-α, IL-6 and IL-1β, **(D)** DC SIGN and MMR. (B-D) Non-parametric Kruskal-

744 Wallis ANOVA test, followed by Dunn´s multiple was comparison was applied to determine significant

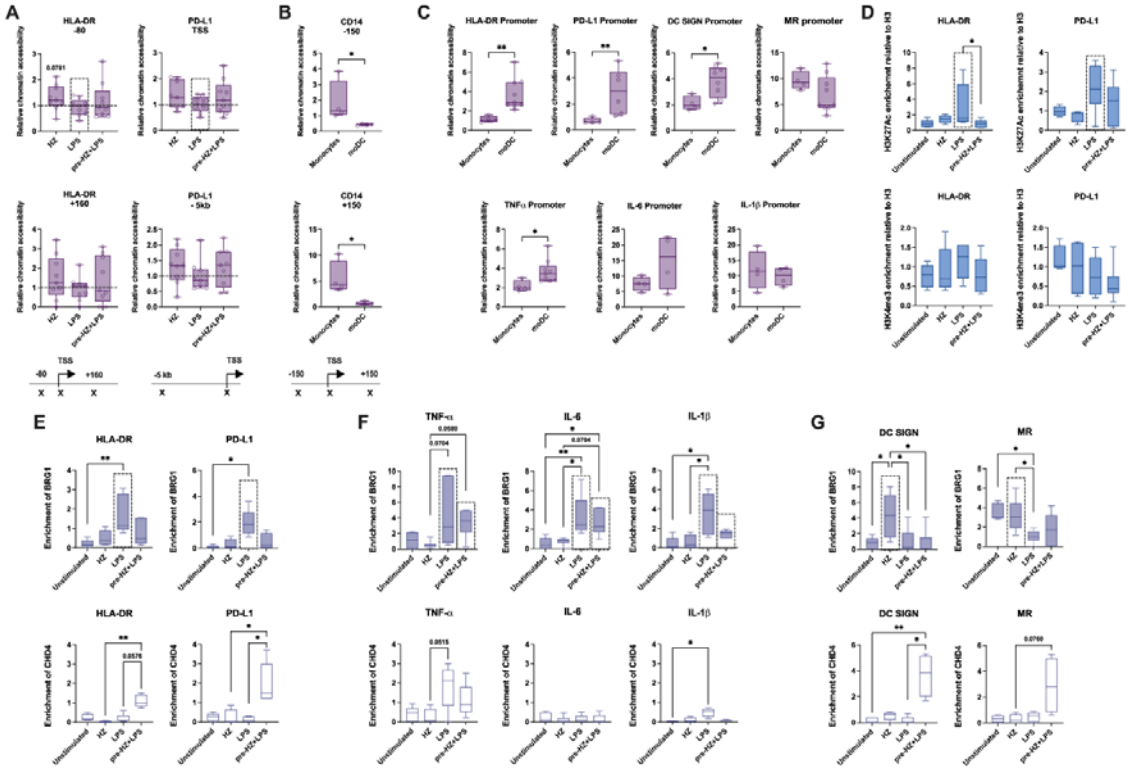
745 differences, n.s.=p>0.05,*p<0.05, **p<0.01. The data are present as median with interquartile range.

746 N=4-12 **(E)** Representative immunoblot of the IRF3 protein and its phosphorylated form and

747 quantitative analysis of each to the right. IRF3 levels were normalized to lamin levels (n=2-3).

748

749 Figure 4



750

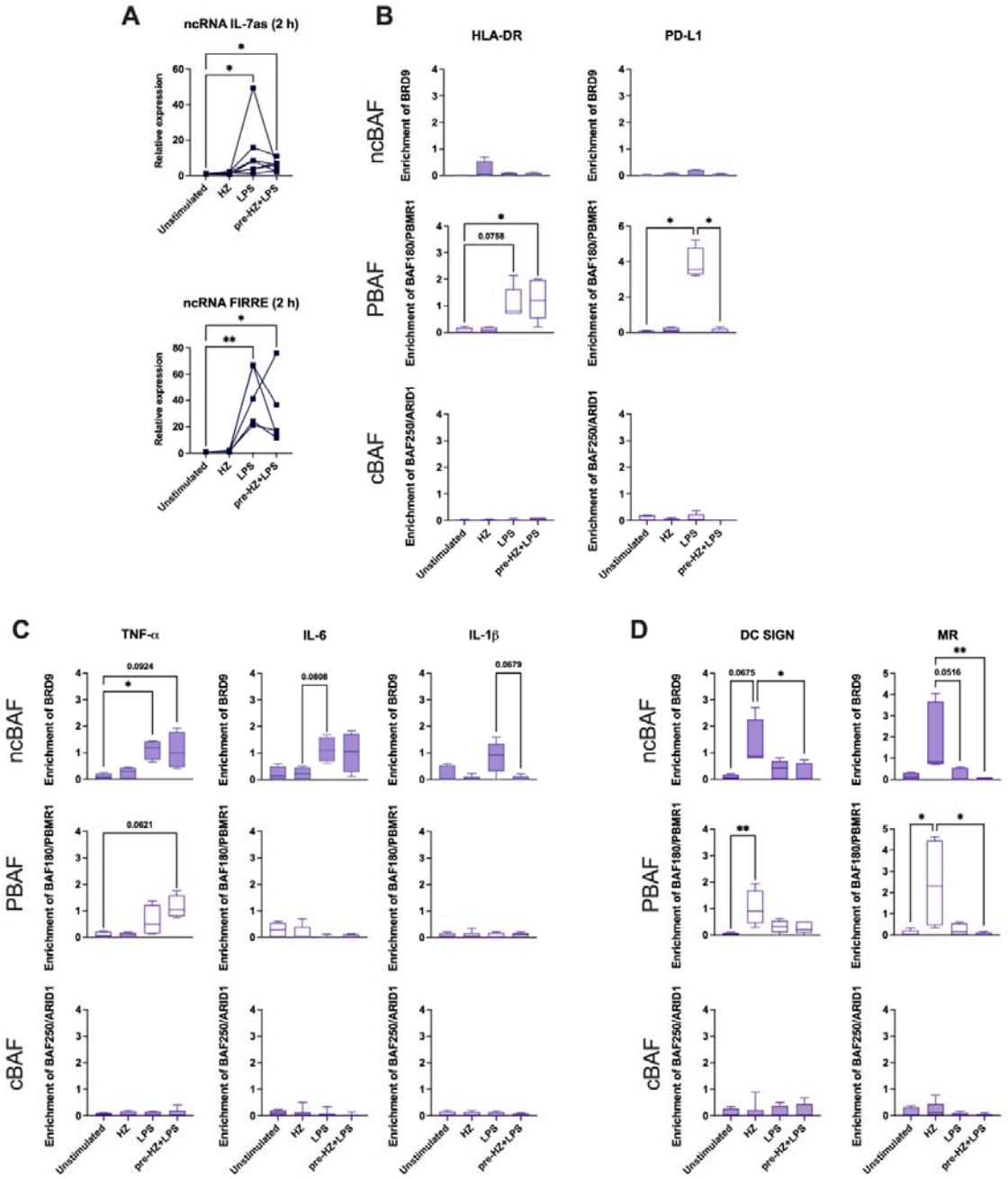
751 **Figure 4. Exposure to HZ does not alter chromatin state but it changes the recruitment of BRG1**
752 **(SWI/SNF complexes) and CHD4 (NuRD) to the promoters of moDC genes. (A)** Relative chromatin
753 accessibility at the promoters of HLA-DR and PD-L1 genes. The primer positions are depicted under
754 the graphs for each gene. Non-parametric Wilcoxon matched-pairs signed rank test was applied to
755 determine significant differences, n.s.=p>0.05. Relative chromatin accessibility at the promoters of
756 **(B)** CD14 and **(C)** HLA-DR, PD-L1, DC SIGN, MMR, TNF-α, IL-6 and IL-1β, in isolated monocytes and
757 differentiated moDC on day 5. Non-parametric Mann-Whitney test was used to determine the
758 differences, *p<0.05, **p<0.01. **(D)** The recruitment of histone H3K27Ac (upper graphs) and
759 H3K4me3 (lower graphs), at the promoters of HLA-DR and PD-L1 genes. H3K27Ac and H3K4me3 were
760 normalised to the enrichment of H3. The enrichment of BRG1 and CHD4 at the promoters of **(E)** HLA-
761 DR and PD-L1, **(F)** TNF-α, IL-6 and IL-1β, **(G)** DC SIGN and MMR in moDC. (D-G) Non-parametric

762 Kruskal-Wallis ANOVA test, followed by Dunn´s multiple was comparison was applied to determine

763 significant differences, n.s.=p>0.05, *p<0.05, **p<0.01. N=3-9.

764

765 Figure 5



766

767 **Figure 5. Differential recruitment of SWI/SNF complex signature proteins BRD9, BAF180 /PBMR1**
768 **and BAF250/ARID1 at the promoters of moDC genes. (A)** Relative expression of ncRNA IL-7as and
769 ncRNA FIRRE in moDC after 2 h exposure. The enrichment of BRD9 (ncBAF), BAF180/PBMR1 (PBAF)
770 and BAF250/ARID1 (cBAF) proteins at the promoters of **(B)** HLA-DR and PD-L1, **(C)** TNF-α, IL-6 and IL-

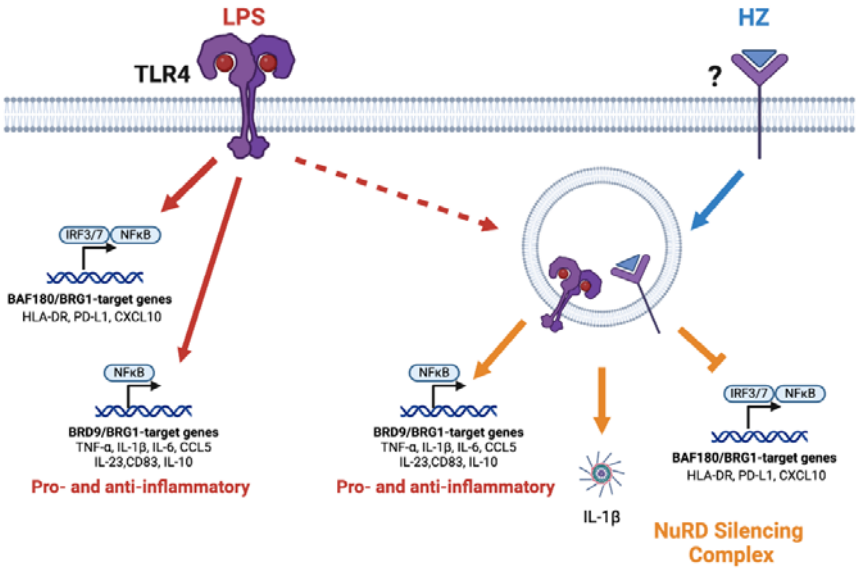
771 1β, **(D)** DC SIGN and MMR. (A-D) Non-parametric Kruskal-Wallis ANOVA test, followed by Dunn´s

772 multiple was comparison was applied to determine significant differences, n.s.=p>0.05, *p<0.05,

773 **p<0.01, n=3-7.

774

775 Figure 6



776

777 Figure 6. Model of the induction of LPS through TLR4 alone to the right and co-exposure with LPS and

778 opsonized HZ leading to NuRD dampening the LPS response on HLA-DR and PD-L1 to the left.

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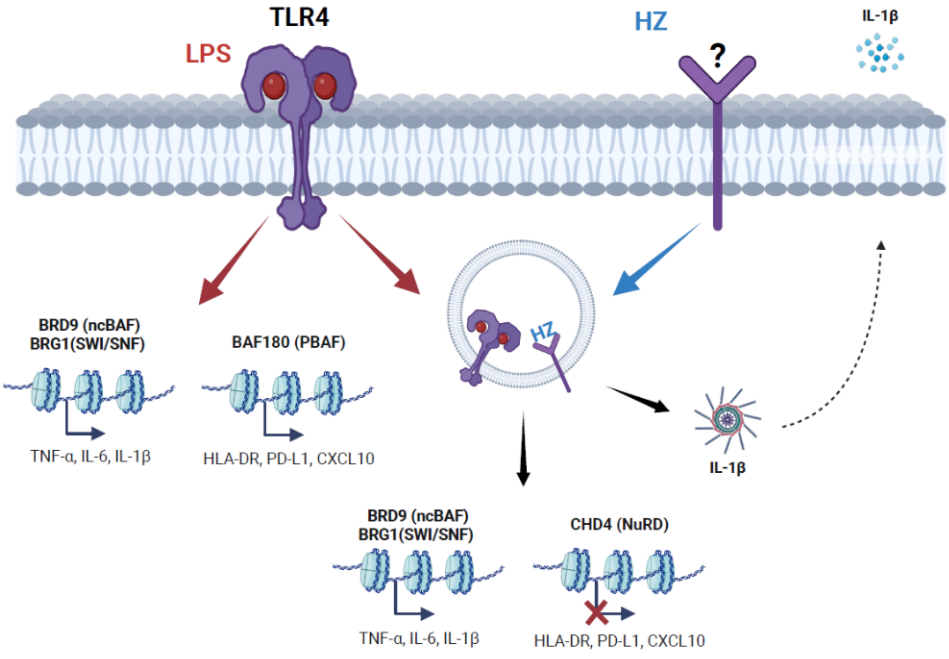
780

781 Graphical abstract



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