ADP-heptose enables *Helicobacter pylori* to exploit macrophages as a survival niche by suppressing antigen-presenting HLA-II expression

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The persistence of *Helicobacter pylori* in the human gastric mucosa implies that the immune response fails to clear the infection. We found that *H. pylori* compromises the antigen presentation ability of macrophages, because of the decline of the presenting molecules HLA-II. Here, we reveal that the main bacterial factor responsible for this effect is ADP-heptose, an intermediate metabolite in the biosynthetic pathway of lipopolysaccharide (LPS) that elicits a pro-inflammatory response in gastric epithelial cells. In macrophages, it upregulates the expression of miR146b which, in turn, would downmodulate CIITA, the master regulator for HLA-II genes. Hence, *H. pylori*, utilizing ADP-heptose, exploits a specific arm of macrophage response to establish its survival niche in the face of the immune defense elicited in the gastric mucosa.

Keywords: antigen-presentation; Helicobacter pylori; HLA-II; immunoevasion; macrophages

Helicobacter pylori is an exceptionally successful pathogen because of its ability to survive for a lifetime in the human host even in the face of a robust innate and adaptive immune response which is elicited by the infection. This capacity relies on several strategies that *H. pylori* has evolved to avoid detection and to dampen the immune responses [1]. T helper (Th) 1 cells accumulate abundantly in the gastric mucosa of infected patients where they are supposed to activate the killing potential of macrophages, following the specific recognition of bacterial antigens-HLA class II complexes by T-cell receptors. Nevertheless, the fact that *H. pylori* establishes a persistent infection suggests that macrophages and/or Th1 cells might ultimately be ineffective to a certain extent. In accordance, faulty bacterial degradation and nonsuccessful HLA-II-dependent antigen presentation occur in macrophages exposed to bacteria [2,3]. In the same line of evidence, the reduced exposure of HLA-II-bacterial antigen complexes on the plasma membrane of macrophages, under the influence of *H. pylori*, independent on whether cells are activated or not activated by IFN-γ, renders these cells less efficacious in forming productive immune synapses with Th1 lymphocytes [2,4].

Own previous studies have shown that the bacterial interference with the antigen presentation machinery in macrophages is attributable to the upregulation of some miRNAs, predominantly miR146b, targeting CIITA, the master regulator for the expression of HLA-II genes [2].

Abbreviations

ETL, Enzymatically treated lysates; LPS, lipopolysaccharide; MAMPs, microbe-associated molecular patterns; Th, T helper; TIVSS, type-4 secretion system; TT, Tetanus toxoid.

In the present study, we aimed to identify the bacterial factor(s) responsible for the impairment of HLA-II expression and increase of miR146b. Starting from the evidence that the effect does not involve the main virulence factors, CagA, VacA, or HP-NAP [2], we reasoned on the possibility that the factor accountable for the action of *H. pylori* on macrophages was not a protein. The idea stemmed from the evidence that *H. pylori* modulates innate immune responses in gastric epithelial cells through the *cag*PAI-dependent injection of small metabolites which act as microbe-associated molecular patterns (MAMPs), namely, peptidoglycan, which is recognized by NOD1 [5], and intermediate metabolites in the biosynthetic pathway of lipopolysaccharide (LPS) [6,7]. The latter metabolites include d-glycero-β-d-manno-heptose 1,7-bisphosphate (HBP), which is probably not the only genuine heptose MAMP. Indeed, HBP is the precursor of ADP-glycero-β-D-manno-heptose (ADP-heptose) which, following translocation into the cell cytosol or uptake by human cells through yet unknown mechanisms, activates the ALPK1-TIFA signaling pathway, leading to NF-κB activation [8,9]. We demonstrate here that bacterial heptose metabolites, exemplified by pure ADP-heptose and recapitulated by heptose metabolite-containing *H. pylori* cleared lysates, are necessary and sufficient to induce miR146b upregulation in primary human macrophages and subsequently lead to CIITA and HLA-II reduction.

Methods

Ethics statement

The investigation has been conducted following the ethical standards, the Declaration of Helsinki, and national and international guidelines.

Peripheral blood mononuclear cells utilized in this study derived from buffy coats obtained from healthy blood donors, as anonymously provided by the Transfusion Centre of the Hospital of Padova. Written informed consent for the use of buffy coats for research purposes was obtained from blood donors by the Transfusion Centre. Data related to human samples were all analyzed anonymously.

Bacterial culture and preparation of conditioned media

H. pylori strain N6 was maintained in 5% CO_2 at 37 °C on Columbia agar plates supplemented with 5% horse blood (Thermo Fisher Scientific, Waltham, MA, USA). Colonies were taken directly from plates and resuspended in PBS (Euroclone, Pero, Italy). Bacterial counting was performed

by determining the optical density (OD) at 600 nm (1 $OD_{600} = 10^9$ $CFU \cdot mL^{-1}$). Before proceeding with the infection experiments, bacteria motility was verified at the optical microscope. Cells were infected with bacteria (5×10^6 $CFU \cdot mL^{-1}$, MOI = 10) in RPMI 1640 (Euroclone) 10% FBS (BioWest, Riverside, MO, USA).

H. pylori-conditioned media were obtained from 12-h liquid culture of bacteria in RPMI 1640 10% FBS . Proliferation of bacteria was verified by comparing OD_{600} at 12 h with that at T_0. Bacteria suspension was centrifuged; supernatant was harvested and subsequently filtered through 0.2-μm membranes (Merck-Millipore, Burlington, MA, USA). Bacteria pellet was resuspended in RPMI 1640 10% FBS and quantified by determining the OD_{600}. Macrophages were infected with bacteria harvested from the liquid culture (MOI = 10) or exposed to the amount of conditioned medium corresponding to an MOI of 10. Finally, conditioned medium was treated with a cocktail of proteinase K (QIAGEN, Hilden, Germany), RNase (Sigma, St. Louise, MO, USA), and DNase (QIAGEN), before administrating to macrophages.

Production of heat-killed bacteria, water extract, and ETL

Enzymatically treated lysates (ETL) were prepared starting from *H. pylori* grown for 2 d on agar plates. *H. pylori* colonies were taken directly from plates and resuspended in sterile PBS. Bacterial suspension ($OD_{600} = 2$) was boiled for 10 min to kill the bacteria. The suspension was centrifuged at 10 000 x *g*, 4 °C. The supernatant, named water extract, was transferred to a new tube. Proteins, RNA, and DNA were degraded as reported previously [6], and the ETL obtained were stored at −20 °C. To make comparable the results obtained from the experiments with heat-killed bacteria, water extract, and ETL treatments with those obtained from infection experiments with live bacteria, we exposed macrophages to an amount of heat-killed bacteria, water extract, and ETL corresponding to an MOI of 10 bacteria. All treatments and supplementations were performed in RPMI 1640 10% FBS. ETL were prepared from *H. pylori* strain N6 wild type and from an isogenic insertion mutant of the same strain in the gene *hldE*, unable to produce ADP-heptose (HP0858) [6].

Monocyte isolation, macrophage differentiation, and cell treatment

Monocytes were purified from buffy coats by density gradient protocol, as described previously [10].

After isolation, monocytes were seeded at the cell density of 0.5×10^6 per well in 24-well plates, in differentiating medium, RPMI 1640 medium, 20% FBS, 4 mM HEPES (Euroclone), and 50 μg·mL^{-1} gentamycin (Euroclone) in

the presence of 100 ng·mL^{-1} M-CSF (Miltenyi Biotec, Bergisch Gladbach, North Rhine-Westphalia, Germany) for 3 d. On the third day, the exhausted medium was removed and replaced with fresh differentiating medium. After 6 d, macrophages are fully differentiated.

Macrophages were infected with *H. pylori* (5×10^6 CFU·mL^{-1}, MOI = 10) or exposed to water extract, ETL, or pure ADP-heptose (2 µg·mL^{-1}, InvivoGen, San Diego, CA, USA). Cells were processed after 6, 12, and 24 h for qRT-PCR analysis or after 24 and 48 h for flow cytometry. The expression of miR146b was evaluated after 12 h.

To block phagocytosis in macrophages, the latter were pre-treated for 30 min with cytochalasin D (20 µM, Sigma) and then infected with *H. pylori* (MOI = 10). After 3 h, medium containing bacteria and drug were changed with fresh medium. Cells were harvested after 6 and 48 h, for qRT-PCR and flow cytometry, respectively.

To assess the release of ADP-heptose by bacteria, we exposed macrophages to *H. pylori* culture medium, treated or not with protease, DNase, and RNase. Cells were harvested after 6 h for qRT-PCR and after 48 h for flow cytometry analysis.

For the experiments including NF-κB inhibition, macrophages were pre-treated with BAY 11-7082 (15 µM, Calbiochem, Merck-Millipore) or with DMSO. After 30 min, medium was changed with a fresh medium containing 2 µg·mL^{-1} ADP-heptose. After 6 or 12 h, cells were processed for qRT-PCR. HLA-II exposure was evaluated after 24 h by flow cytometry and the expression miR146b was determined after 12 h of treatment.

Flow cytometry

Macrophages were harvested from culture plates using 5 mM Na–EDTA in PBS, pH 7.5, and incubated for 15 min at RT with 10% human serum to saturate Fc receptors. 5×10^5 cells were stained with a monoclonal antibody anti-HLA-II (clone L243, Ebiosciences, San Diego, CA, USA). We used fixable cell viability dye eFluor780 (Ebiosciences) to exclude dead cells from the analysis. Cells were resuspended in FACS buffer (PBS, 1% BSA) and analyzed by BD Fortessa X-20 (Becton Dickinson, Franklin Lakes, NJ, USA). Forward and side scatter light was used to identify cell populations. Values were expressed as *n*-fold change of median fluorescence intensity (MFI) of live cells positive for HLA-II vs control cells. All data were analyzed using FlowJo software, version 10.3 (Tree Star Inc., Ashland, OR, USA).

RNA extraction

Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. RNA was quantitated using NanoDrop 1000 spectrophotometer (Nanodrop, Wilmington, DE, USA).

qRT-PCR

mRNAs

One microgram of total RNA was retrotranscribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA), following the manufacturer's instructions. The cDNA obtained from the retro-transcription was directly used in real-time PCR reaction performed in QuantStudio 5 Real-Time PCR System (Applied Biosystem). qRT-PCR was performed in 8 µL using SYBR Green master mix (Thermo Fisher Scientific), according to the following cycle: 95°C for 5 min; 95 °C for 15 s, 60 °C for 1 min, for 40 cycles. For each sample, data were normalized to the endogenous reference gene β-actin. Expression levels of treated cells are relative to values of control cells, at each time point, set as 1. The pairs of primers used for real-time PCR analysis were the following:

β-actin forward 5′-TGAGATGCGTTGTTACAGGA-3′, reverse 5′-ACGAAAGCAATGCTATCA-3′; CIITA forward 5′-GGTCCAGGGTTTGAGTTCAT-3′, reverse 5′-TGATTTGGGGTGGCTTGTTA-3′; IL-8 forward 5′-TT GGCAGCCTTCCTGATT-3′, reverse 5′-AACTTCTCCA CAACCCTCTG −3′; HLA-II α chain forward 5′-GCC CTGTGGAACTGAGAGAG-3′ reverse 5′-CTGGTGGGG TGAACTTGTCT-3′; HLA-II β chain forward 5′-AGG CAGCATTGAAGTCAGGT-3′, reverse 5′-ATTCTGAAT CAGGCCTGTGG-3′.

miRNA

Retro-transcription of miRNA was performed using TaqMan R MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific) with 10 ng of total RNA following the manufacturer's instructions. RT-PCR was then performed using TaqMan R Universal PCR Master Mix II, no UNG (Thermo Fisher Scientific), and the specific primers from TaqMan R MicroRNA Assay. At least three replicates were performed for each reaction. The expression of miR146b (Thermo Fisher Scientific—Assay ID 001097) was normalized with that of U6 small nuclear RNA (U6 snRNA, Thermo Fisher Scientific—Assay ID 001973). Expression levels of treated cells were relative to values of control cells, set as 1.

Reactions were run in 96 CFX (Bio-Rad, Hercules, CA, USA) with the following program: 95 °C for 10 min; 35 cycles of 95 °C for 15 s; and 60 °C for 1 min. Data analysis was carried on according to ΔΔCt method for both mRNAs and miRNAs.

Generation of tetanus toxoid-specific cell clones

Tetanus toxoid (TT)-specific T-cell clones were obtained from peripheral blood of two healthy donors, as described previously [11]. Ten TT-specific T-cell clones from each donor were selected for this study.

CFSE-based proliferation assay of T cells

1×10^5 macrophages were seeded in 96-well plates and treated with ETL or ADP-heptose for 24 h. After this time, 3×10^5 CFSE-stained T-cell clones were added into each well in medium plus 0.5 μg·mL^{-1} TT. After 24 h of coculture, the percentage of proliferating T-cell clones in response to TT was determined by measuring the CFSE (Celltrace CFSE cell proliferation kit, Thermo Fisher Scientific) fluorescence by flow cytometry; 5000 events were acquired for each sample.

Results

Our main aim in this study was to identify the bacterial factors which mediated the dampening effect of *H. pylori* on HLA-II expression and reduced antigen presentation functions in human macrophages. Once we excluded the contribution of most of the well-known *H. pylori* virulence factors [2], we argued that the molecule responsible for the down-modulation of HLA-II in primary human macrophages infected with *H. pylori* was indeed not a protein. To verify this hypothesis, we evaluated the expression of the antigen-presenting molecules in macrophages exposed to boiled bacteria, in which heat-sensible components, mainly proteins, were effectively destroyed. As shown in Fig. 1A, the level of HLA-II in macrophages exposed to heat-killed bacteria was as low as in human macrophages treated with live bacteria and the effect was recapitulated by applying the soluble fraction of a whole-cell lysate obtained from boiled bacteria (water extract). By administrating to the macrophages bacterial lysates treated with a cocktail of protease, DNAse, and RNAse (enzymatically treated lysate, ETL [6]), we obtained the final confirmation that neither proteins nor DNA/RNA were responsible for the downregulation of the antigen-presenting molecules. All treatments derived from wild-type *H. pylori* bacteria reduced the expression of the mRNA for CIITA to a very low level (Fig. 1B). Conversely, the administration of ETL obtained from an insertion, allelic-exchange mutant strain of *H. pylori*, unable to produce ADP-heptose (HP0858 [6]), did clearly not result in the suppression of HLA-II and CIITA expression (Fig. 2A,B). The final confirmation that the down-modulation of HLA-II was due mainly to *H. pylori*-produced heptose metabolites, most likely ADP-heptose, was obtained by applying the pure molecule to the cells. Indeed, in macrophages exposed to pure ADP-heptose, the expression of HLA-II on the plasma membrane and that of mRNA for CIITA were significantly lower than in control cells (Fig. 2C,D). In
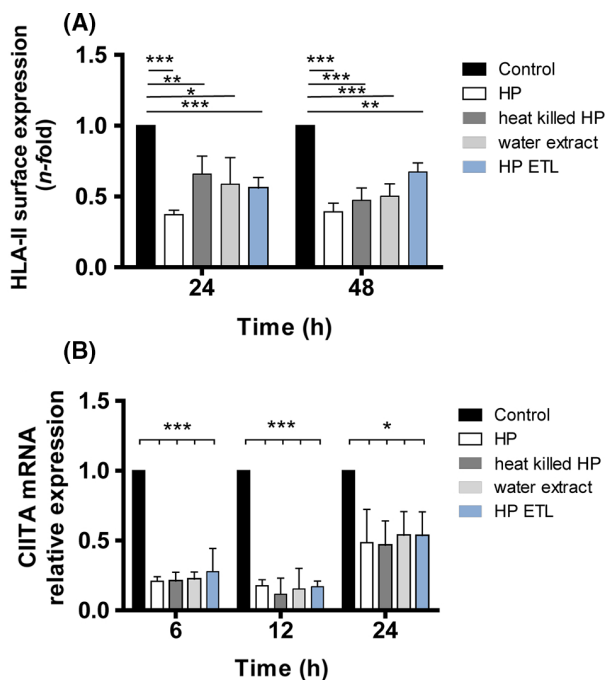

Fig. 1. Accountable for the decline of HLA-II in macrophages exposed to *H. pylori* is not a protein. Macrophages were infected with *H. pylori* (HP) or exposed to heat-killed bacteria (heat-killed HP), water extract, or to enzymatically treated lysates (HP ETL). (A) After 24 and 48 h cells were harvested, and the surface expression of HLA-II was analyzed by flow cytometry. MFI of HLA-II ± SEM of 6 independent experiments was calculated and expressed as *n*-fold change relative to control cells set as 1. (B) After 6, 12, and 24 h, cells were processed for mRNA extraction and the expression of mRNA for CIITA was determined by qRT-PCR. Data were normalized to β-actin. Expression levels of treated cells are relative to values of control cells set as 1. Data are shown as mean ± SEM of 6 independent experiments. Significance was determined by two-way ANOVA with Bonferroni's *post hoc* test. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

accordance with the direct role of CIITA in promoting transcription of *HLA-II* genes, the expression level of mRNAs for HLA-II α- and β-chains was also very low in ADP-heptose-treated primary human macrophages (Fig. S1).

In contrast with the prior evidence that the activation of NF-κB in gastric epithelial cells by *H. pylori* itself depends on its functional *cag* pathogenicity island type-4 secretion system (TIVSS) [6], we reported that the TIVSS is dispensable for the suppression of HLA-II in human macrophages [2]. We considered the possibility that this discrepancy might partially be explained by the different human cell systems used, macrophages in our study, and epithelial cells in the previous one. It is plausible that in macrophages the release of ADP-heptose can follow the destruction of
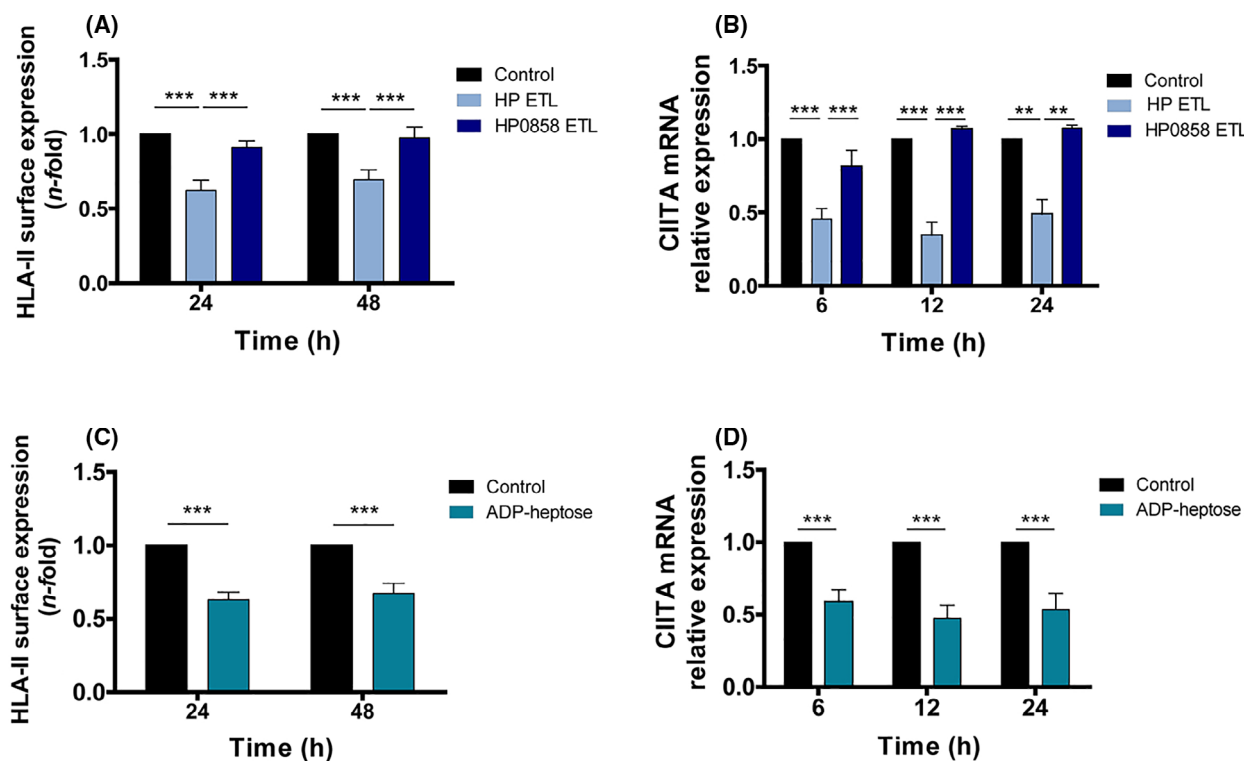
Fig. 2. ADP-heptose is the main responsible for the decrease of HLA-II in macrophages. (A, B) Macrophages were exposed to HP ETL or to ETL obtained from HP0858 (HP0858 ETL). (C, D) Macrophages were treated or not with ADP-heptose. (A, C) After 24 and 48 h cells were harvested, and the surface expression of HLA-II was analyzed by flow cytometry. MFI of HLA-II ± SEM of 6 independent experiments was calculated and expressed as *n*-fold change relative to control cells set as 1. (B, D) After 6, 12, and 24 h, cells were processed for mRNA extraction and the expression of mRNA for CIITA was determined by qRT-PCR. Data were normalized to β-actin. Expression levels of treated cells are relative to values of control cells set as 1. Data are shown as mean ± SEM of 6 independent experiments. (A–B) Significance was determined by two-way ANOVA with Bonferroni's *post hoc* test. **$P \leq 0.01$; ***$P \leq 0.001$. (C–D) Significance was determined by Student's *t*-test. ***$P \leq 0.001$.

bacteria inside phagolysosomes. To explore this possibility, we infected macrophages with wild-type *H. pylori* bacteria after blocking the phagocytic machinery with cytochalasin D. Surprisingly, the inhibition of bacteria engulfment did not compromise the ability of *H. pylori* to dampen the expression of HLA-II and CIITA (Fig. 3A,B).

It has been suggested that small amounts of ADP-heptose may be released, by experimental or spontaneous lysis of bacteria [9]. Consistently with this possibility, we found that the addition of the *H. pylori* culture medium to macrophages recapitulated the suppressive effect of live bacteria, water extract, and ETL. The treatment of culture medium with protease cocktail, DNase, and RNase maintained unaltered the effect on the expression of both HLA-II and CIITA (Fig. 3C,D). Taken together, these data support the notion proposed by others that small amounts of ADP-heptose or possibly other

heptose metabolites released by *H. pylori* can enter the cells without requiring any perturbance of the cell membrane integrity [9]. The exact mechanism of uptake or translocation of extracellular heptose metabolites into host cells requires further investigation, but, at least for primary human macrophages in our settings, we exclude that it occurs *via* endocytosis/phagocytosis.

Finally, we tested macrophages exposed to ETL from wild-type bacteria or ETL from the HP0858 mutant strain, or to pure ADP-heptose, in antigen-specific T-cell proliferation assays. The results showed that wild-type ETL and pure ADP-heptose do suppress the capacity of macrophages to activate T cells, while ETL from HP0858 mutants, which do not produce several heptose metabolites [6,9], do not (Fig. 4A).

In a previous study, we evidenced that *H. pylori* dampens HLA-II expression on macrophages *via* the
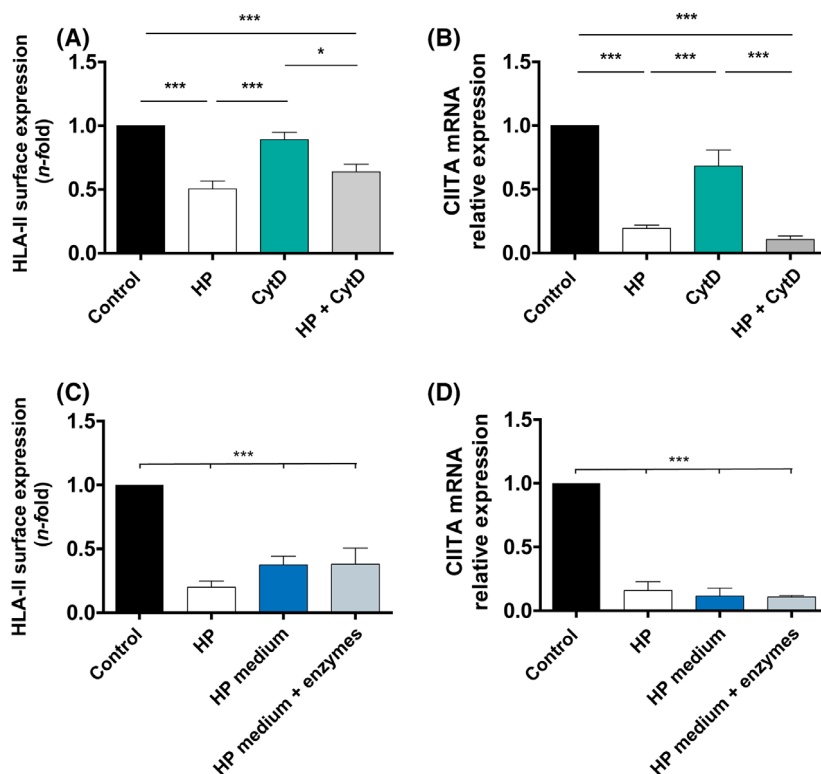
Fig. 3. Phagocytosis is dispensable for the drop-down of HLA-II in infected macrophages and the culture medium recapitulates the effect of bacteria on the antigen-presenting complex. (A, B) Macrophages were pre-treated or not for 30 min with cytochalasin D (CytD) and then infected with HP. After 3 h, medium containing bacteria and drug was changed with fresh medium. (C, D) Macrophages were infected with HP or exposed to HP-conditioned medium, treated, or not with the cocktail of proteinase K, RNase, and DNase (enzymes). (A, C) After 48 h, cells were harvested, and the surface expression of HLA-II was analyzed by flow cytometry. MFI of HLA-II ± SEM of 5 independent experiments was calculated and expressed as n-fold change relative to control cells set as 1. (B, D) After 6 h, cells were processed for mRNA extraction. The expression of mRNA for CIITA was determined by qRT-PCR. Data were normalized to β-actin. Expression levels of treated cells are relative to values of control cells set as 1. Data are shown as mean ± SEM of 4 independent experiments. Significance was determined by one-way ANOVA with Bonferroni's *post hoc* test. $*P \leq 0.05$; $***P \leq 0.001$.

upregulation of miRNAs targeting CIITA, in particular miR146b [2]. Accordingly, we found miR146b upregulated only in macrophages exposed to ETL from wild-type bacteria and to pure ADP-heptose, while it remained unaltered in macrophages treated with ETL of the mutant (Fig. 4B).

ADP-heptose has been recognized recently as a potent NF-κB -activating MAMP that, by acting *via* the ALPK1-TIFA signaling axis, leads to the expression and secretion of IL-8 [12–14]. To provide proof that the pathway leading to the downregulation of HLA-II expression was not distinct from that responsible for the transcription of IL-8, we administered ADP-heptose to macrophages in which NF-κB was inhibited. The expression of HLA-II and CIITA was rescued by the inhibition of the transcription factor, while that of IL-8 was depressed (Fig. 5A,B and S2). Moreover, macrophages inhibited for NF-κB activity

did not increase miR146b following the exposure to the pure MAMP (Fig. 5C).

Discussion

In this study, we demonstrated that ADP-heptose is a major player for the negative impact of *H. pylori* on the expression of the antigen-presenting molecules in macrophages. Recently [15], we found that the bacterial heptose metabolite ADP-heptose is required and sufficient as a novel MAMP to induce NF-κB activation in human macrophages. This specific transcriptional activation program, on the one hand, leads to the expression of pro-inflammatory cytokines but, on the other hand, as shown here and by others [16], is sufficient to upregulate miR146b. The upregulation of the miRNA in turn, as we could show before [2], negatively affects the expression of CIITA and therefore
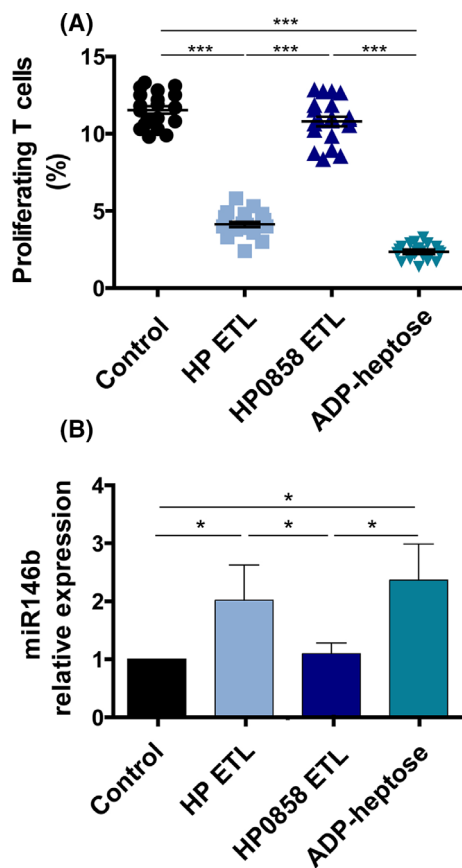
Fig. 4. ADP-heptose affects the antigen presentation capacity of macrophages toward T lymphocytes and upregulates the expression of miR146b. (A) Macrophages exposed to HP ETL, HP0858 ETL, and ADP-heptose were engaged in an antigen presentation assay with T lymphocytes. Data are expressed as percentage of proliferating cells as determined by CFSE-based staining (mean ± SEM). Two independent experiments were performed with 10 different T-cell clones for each experiment. (B) Relative expression of miR146b in macrophages treated or not for 12 h with HP ETL, HP0858 ETL, or with ADP-heptose. Data were normalized to the endogenous reference gene U6. Expression levels of treated cells are relative to values of control cells set as 1. Data are shown as mean ± SEM of 4 independent experiments. Significance was determined by one-way ANOVA with Bonferroni's *post hoc* test. $*P \le 0.05$; $***P \le 0.001$.

that of HLA-II, ultimately compromising the possibility for infected macrophages to be recognized by T cells. Notably, the involvement of miR146 a/b in a negative feedback regulation loop has been already shown to occur in THP-1 (a human monocyte leukemia cell line) activated by LPS [16]. As we can elucidate here, the dampening activity of heptose metabolite on antigen-presenting functions is clearly NF-κB -dependent, probably *via* the heptose-triggered ALPK-1 TIFA-axis [8,14], since NF-κB inhibition in the setting of ADP-heptose-dependent activation
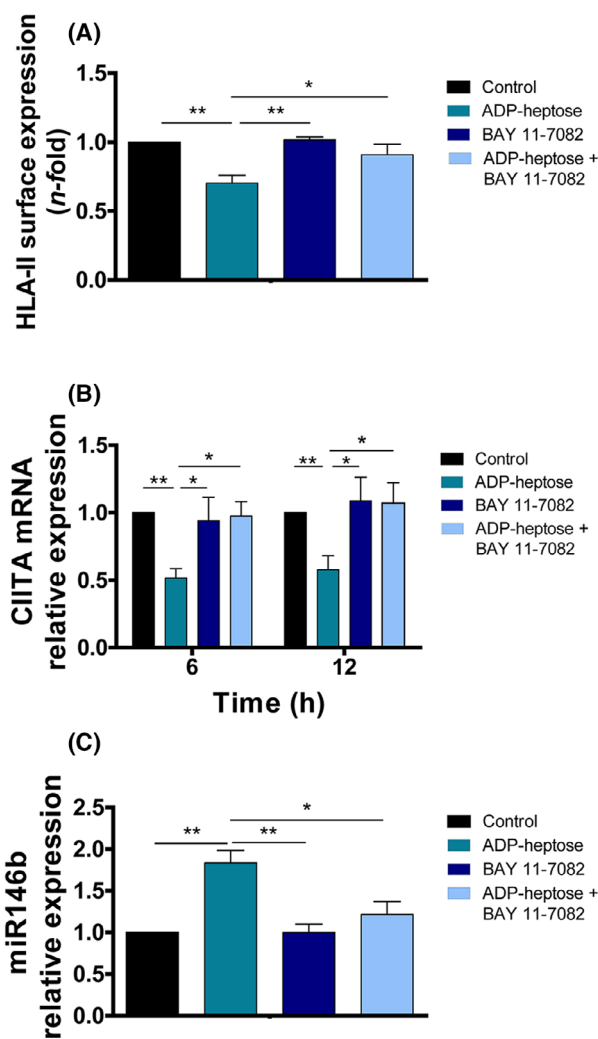

Fig. 5. Active NF-κB is required for the expression of miR146b and for the downregulation of CIITA. (A–C) Macrophages were pre-treated with the NF-κB inhibitor BAY 11-7082. After 30 min, medium was changed with fresh one containing ADP-heptose. After 24 h, the expression of HLA-II was evaluated by flow cytometry (A). After 6 and 12 h, cells were processed for qRT-PCR to quantify the mRNA for CIITA (B), and, after 12 h of treatment, to quantify miR146b (C). (A, C) Significance was determined by one-way ANOVA with Bonferroni's *post hoc* test. $*P \le 0.05$; $**P \le 0.01$. (B) Significance was determined by two-way ANOVA with Bonferroni's *post hoc* test. $*P \le 0.05$; $**P \le 0.01$.

restored CIITA expression. The mechanism by which the bacterial ADP-heptose or other heptose metabolites access the cytosol of phagocytic cells to modulate the activity of NF-κB remains to be clarified. In contrast with the prior evidence that the activation of NF-κB in gastric epithelial cells by *H. pylori* itself almost fully depends on its functional TIVSS [6], we reported before that in our experimental setting, the TIVSS is virtually dispensable for the suppression of HLA-II in

human macrophages [2]. Likewise, phagocytosis of bacteria is dispensable for the phenotype. These findings differ from those obtained before by Faass and colleagues [15]. In this work, it has been provided evidence that in THP-1 cells IL-8 release induced by *H. pylori* was partially associated with an active *cag*-PAI and that NF-κB activation induced by *H. pylori* ETL or live bacteria were partially inhibited by CytD [15]. These small discrepancies are most likely due to the use of different cell models, since some differences in the cell responses exist between the immortalized and the human primary cells [17,18]. As to the mechanism by which pure ADP-heptose or possibly other heptose metabolites, available in the bacterial culture medium, can be taken up by macrophages, our present study and that of Faass *et al.* [15] are fully concordant that it can easily occur in the absence of a dedicated bacterial secretion/injection system. Glut6, a monosaccharide transporter of unknown specificity, was found strongly upregulated in macrophages by ADP-heptose exposure, thus suggesting that it might be directly involved in the import of heptoses into macrophages. It has been reported before that human epithelial cells can also take up free heptose metabolites from the medium or environment [9,12,19].

ADP-heptose and other heptose metabolites are essential intermediates of the lipopolysaccharide heptose core biosynthetic pathway in all Gram-negative bacteria [12,14,20–23] and can also be incorporated into bacterial surface layers [24,25] or produced by some Gram-positive bacteria [26]. Therefore, our results strongly suggest that the mechanism of adaptive immune response modulation and effects on antigen presentation based on this specific MAMP might also be adopted by other pathogenic bacteria.

Acknowledgements

Conflict of interest

Authors declare that no conflict of interest exists.

Author contributions

SC designed and performed experiments and analyzed and interpreted the data; GB designed and performed experiments and analyzed the data; CDB performed experiments and analyzed and interpreted the data; MF performed experiments; MH prepared and quality-tested materials and reagents; LF prepared and quality-tested materials and reagents; MMD helped with data analysis and interpretation; CJ provided materials and helped with experimental design, data analysis, and interpretation; and MDB designed the research, interpreted the data, and wrote the paper. All authors read, edited, and approved the final version of the manuscript.

Data accessibility

The data that support the findings of this study are available in Figs 1–5 and in the Figs S1 and S2 of this article.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. mRNA expression of HLA-II α and β chains in macrophages exposed to ADP-heptose.

Fig. S2. Active NF-κB is required for the expression of IL-8 in macrophages exposed to ADP-heptose.