Original article

European Journal of Microbiology and Immunology 6 (2016) 4, pp. 306–311
DOI: 10.1556/1886.2016.00031

USAGE OF MURINE T-CELL HYBRIDOMA CELLS AS RESPONDER CELLS REVEALS INTERFERENCE OF *HELICOBACTER PYLORI* WITH HUMAN DENDRITIC CELL-MEDIATED ANTIGEN PRESENTATION

Michael Fehlings[1,*,,+], Lea Drobbe[1,+], Macarena Beigier-Bompadre[1], Pablo Renner Viveros[4], Verena Moos[2], Thomas Schneider[2], Thomas F. Meyer[1], Toni Aebischer[3], Ralf Ignatius[4]**

[1]Department of Molecular Biology, Max Planck Institute for Infection Biology, Charitéplatz 1, 10117 Berlin, Germany
[2]Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany
[3]Mycotic and Parasitic Agents and Mycobacteria, Department of Infectious Diseases, Robert Koch-Institute, Berlin, Germany
[4]Institute for Microbiology and Hygiene, Charité – Universitätsmedizin Berlin, 12203 Berlin, Germany

Received: October 9, 2016; Accepted: October 18, 2016

Direct effects of *Helicobacter pylori (H. pylori)* on human $CD4^+$ T-cells hamper disentangling a possible bacterial-mediated interference with major histocompatibility complex class II (MHC-II)-dependent antigen presentation to these cells. To overcome this limitation, we employed a previously described assay, which enables assessing human antigen-processing cell function by using murine T-cell hybridoma cells restricted by human leukocyte antigen (HLA) alleles. HLA-$DR1^+$ monocyte-derived dendritic cells were exposed to *H. pylori* and pulsed with the antigen 85B from *Mycobacterium tuberculosis (M. tuberculosis)*. Interleukin-2 (IL-2) secretion by $AG85B_{aa97-112}$-specific hybridoma cells was then evaluated as an integral reporter of cognate antigen presentation. This methodology enabled revealing of interference of *H. pylori* with the antigen-presenting capacity of human dendritic cells.

Keywords: *Helicobacter pylori,* dendritic cells, antigen presentation, T-cells, hybridoma cells

Introduction

Helicobacter pylori (H. pylori) is considered as type I carcinogen by the World Health Organization (WHO) since 1994. In most cases, *H. pylori*-infected individuals remain asymptomatic. Chronic infection, however, is a major risk factor for the development of gastric cancer or lymphoma. Major histocompatibility complex class II (MHC-II)-restricted antigen presentation to $CD4^+$ T-cells is a prerequisite for protection against *H. pylori* [1, 2], and persistent colonization might result from deficient antigen presentation and $CD4^+$ T-cell activation. The induction of human T-cell immunity by antigen-presenting cells (APCs) involving *H. pylori* stimulation has been studied before [3–5]. Bacterial virulence factors, however, were shown to negatively affect human $CD4^+$ T-cell activation by exerting direct anti-proliferative effects as well as by inhibiting T-cell cytokine production, such as interleukin-2 (IL-2) or interferon-γ (IFN-γ) [6–9]. In addition, the bacteria may directly modulate functions of human APCs [10]. To study the functional consequences of a possible modulation of antigen-processing and presentation in professional APCs by *H. pylori,* confounders such as direct bacterial effects on T-cell functions have to be eliminated. Here, we investigated antigen presentation by human monocyte-derived dendritic cells (DCs) to a murine $CD4^+$ T-cell hybridoma. Murine T-cell functions are not affected by *H. pylori* stimulation and $CD4^+$ T-cell-derived hybridoma cells respond to cognate presentation of T-cell epitopes relatively independent of additional costimulatory interactions by APCs.

* Corresponding author: Michael Fehlings; 8A Biomedical Grove, #0306 Immunos, 138648 Singapore, Singapore;
Phone: +65 64070668; E-mail: m.fehlings@googlemail.com

** Current address: Agency for Science, Technology and Research (A*STAR), Singapore Immunology Network (SIgN), Singapore

+ These authors contributed equally to this work.

The use of T-cell hybridoma cells from human leukocyte antigen (HLA)-transgenic mice as responder cells has previously been shown to enable quantitative detection of antigen processing by human APCs [11]. By applying this methodology to the *H. pylori* system, we show that *H. pylori* exposure hampers MHC-II-restricted antigen presentation by human DCs. This effect may rely on bacterial factors shared at least within additional Gram-negative bacteria as it can partly be mimicked when substituting *H. pylori* with lipopolysaccharide (LPS) from *Escherichia coli (E. coli)*.

Materials and methods

Preparation of H. pylori

H. pylori P12 wild type strain was grown and prepared as described elsewhere [10]. For stimulation, *H. pylori* was adjusted to a multiplicity of infection (MOI) of 10.

T-cell hybridoma cell culturing

$AG85B_{aa97-112}$-specific, HLA-DR1-restricted F9A6 cells [11] were grown in 75 cm^2 flasks (TPP, Trasadingen, Switzerland) in DMEM medium (Gibco/Invitrogen, Life Technologies, Darmstadt, Germany), supplemented with 2 mM L-glutamine (Gibco), penicillin (100 U/ml), streptomycin (100 µg/ml), gentamicin (30 µg/ml), ciprofloxacin (10 µg/ml), 0.05 mM 2-mercaptoethanol (all from Sigma, Taufkirchen, Germany), and 10% fetal calf serum (Biochrom, Berlin, Germany) at 37 °C and 5% CO_2. Cells were passaged every second day and harvested from day 3 to day 10.

Generation of human HLA-DR1$^+$ DCs

Healthy blood donors that volunteered were HLA-typed by the tissue typing laboratory of the Charité, Campus Virchow Klinikum (Berlin, Germany). DCs were generated as described previously [10]. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated via Ficoll density gradient centrifugation. $CD14^+$ monocytes were enriched by using magnetic microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany), and 3×10^6 cells/well were cultured in six-well culture plates (TPP) in complete RPMI medium, containing 2 mM L-glutamine, 10 mM HEPES (Gibco), penicillin (100 U/ml), streptomycin (100 µg/ml), 10% FCS, 1000 U/ml of granulocyte-macrophage colony-stimulating factor (GM-CSF) (sargramostim, Leukine®, Berlex, Richmond, CA), and 100 U/ml recombinant IL-4 (R&D Systems, Wiesbaden-Nordenstadt, Germany). Fresh medium supplemented with cytokines was added every second day to the wells, and cells were harvested between day 6 and day 8. Immature DCs displayed a down-regulated CD14 expression and were further defined by the expression of high levels of CD11b and low levels of CD80 [10].

Stimulation of cells with H. pylori

A total of 1×10^5 F9A6 cells was stimulated for 1 h at 37 °C with *H. pylori* in RPMI medium supplemented with 10% FCS. The cells were washed twice and subsequently transferred to one well of a 96-well plate (TPP) precoated with 3 µg/ml anti-CD3 mAbs (BD Pharmingen) for another 12 h of incubation. For the stimulation of DCs with *H. pylori,* 2×10^6 cells were incubated in 500 µl RPMI medium containing 10% FCS, 1000 U/ml of GM-CSF, and 100 U/ml recombinant IL-4 for 1 h at 37 °C in the presence of *H. pylori*. LPS derived from *E. coli* (Invivogen, Toulouse, France) (100 ng/ml) was used as a control stimulus while cells maintained in medium alone served as negative control. After 1 h of incubation, cells were washed twice with medium and resuspended in RPMI medium supplemented with 10% FCS, 1000 U/ml GM-CSF, 100 U/ml recombinant IL-4, penicillin (100 U/ml), streptomycin (100 µg/ml), and 100 µg/ml gentamicin (Sigma) for subsequent F9A6 cell activation.

T-cell hybridoma cell activation by DCs

After stimulation, DCs were incubated in the presence or absence of 12 µg/ml recombinant antigen 85B from *Mycobacterium tuberculosis (M. tuberculosis)* (AG85B; Abcam, Cambridge) for 6 h at 37 °C. A total of 8×10^4 cells/well of a 96-well flat-bottom plate (TPP) in 100 µl of this suspension were incubated with 1×10^5 F9A6 cells in 100 µl of RPMI medium containing 10% FCS, 1000 U/ml GM-CSF, 100 U/ml IL-4, penicillin (100 U/ml), streptomycin (100 µg/ml), and gentamicin (100 µg/ml) for a further 12 h at 37 °C. For kinetic experiments, 12 µl/ml AG85B was added to the DCs 24 and 48 h prior to or at the time of exposure to *H. pylori*. Incubation with F9A6 cells followed immediately or either 24 or 48 h later.

Analysis of IL-2 secretion by T-cell hybridoma cells

Twelve hours after the addition of the F9A6 cells, cell-free supernatants were collected and stored at –80 °C until monitoring for IL-2 by sandwich ELISA (R&D) according to the manufacturer's instructions.

Cell viability assay

After 12 h of incubation, cells were tested for viability by using a colorimetric cell viability assay according to the manufacturer's protocol (Colorimetric Cell Viability Kit I; PromoKine, Heidelberg, Germany).

Statistics

Statistical analyses were performed using the nonparametric Wilcoxon matched-pairs signed rank test. Differences were considered statistically significant for $p < 0.05$.

Ethics statement

The study was approved by the ethical committee of the Charité and informed consent was obtained from all donors.

Results

H. pylori has been shown to inhibit human but not murine $CD4^+$ T-cell proliferation through direct interaction [6, 7, 9]. We first confirmed that *H. pylori* did not affect relevant murine F9A6 T-cell properties, such as viability or IL-2 secretion, by investigating F9A6 T-cell responses using anti-CD3 stimulation. F9A6 cells were incubated with *H. pylori* for 1 h, the bacteria were washed out, and the cells were transferred to plates coated with anti-CD3 mAbs. Control cells were not exposed to *H. pylori*. Twelve hours later, supernatants were harvested in order to determine murine IL-2 concentrations by ELISA, while the cells were subjected to a cell viability assay. F9A6 T-cells incubated with *H. pylori* did not differ from unstimulated control cells regarding secretion of IL-2 *(Fig. 1)* or cell viability (data not shown). Therefore, F9A6 cells were suitable read-out T cells in the cell culture assays involving *H. pylori*.

Next, human immature monocyte-derived DCs were generated from HLA-$DR1^+$ donors as previously described [10]. Cells were stimulated with *H. pylori* for 1 h while control cells were maintained in medium alone. Cells were washed and subsequently incubated for 6 h in the presence of a model recombinant antigen of *M. tuberculosis* (AG85B). Cells were washed to remove free antigen and $AG85B_{aa97\text{-}112}$-specific, HLA-DR1-restricted murine F9A6 T-cell hybridoma cells were added. Twelve hours later, supernatants were harvested and IL-2 concentrations were determined by ELISA as an integral surrogate of cognate AG85B presentation by DCs. Supernatants from AG85B-exposed, uninfected DCs cocultured with F9A6 cells contained elevated concentrations of IL-2. In contrast, prior exposure of DCs to *H. pylori* significantly impaired IL-2 production by F9A6 cells *(Fig. 2)*. This effect was not due to reduced viability of the antigen-presenting cells owing to the bacteria since we did not detect cell death in the DC populations in response to *H. pylori* stimulation (data not shown). Thus, incubation of immature DCs with *H. pylori* prior to AG85B addition reduces IL-2 secretion of F9A6 cells incubated together with such DCs.

Exposure of DCs to *H. pylori* induces the expression of molecules associated with DC activation, such as CD25,

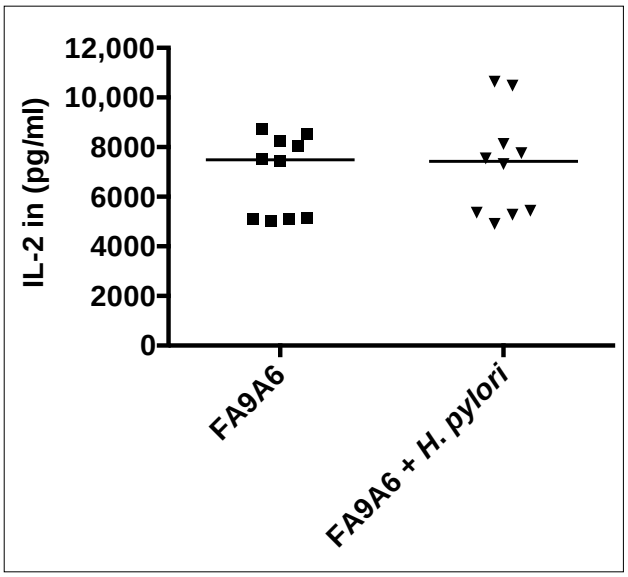

Fig. 1. IL-2 secretion by F9A6 T-cell hybridoma cells is not affected after stimulation with *H. pylori*. F9A6 cells were incubated with *H. pylori* (MOI of 10) or medium alone for 1 h followed by anti-CD3 stimulation (3 µg/ml) for 12 h. Afterwards, cell-free supernatants were collected, and concentrations of murine IL-2 were determined by ELISA. Each symbol per condition represents data obtained from one independent experiment (one donor). Horizontal lines show the median values of 10 experiments (donors)

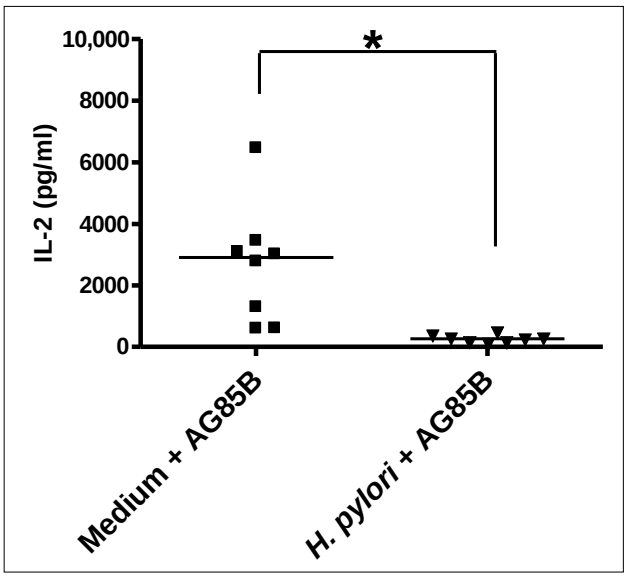

Fig. 2. Stimulation of human immature DCs with *H. pylori* inhibits IL-2 production by T cells. Immature HLA-$DR1^+$ DCs were incubated with *H. pylori* (MOI of 10) or medium alone for 1 h and afterwards cultured for 6 h in the presence of recombinant antigen 85B from *M. tuberculosis* (AG85B, 12 µg/ml). HLA-$DR1^+$ $Ag85B_{aa97\text{-}112}$-restricted murine F9A6 T-cell hybridoma cells were added and 12 h later, cell-free supernatants were collected and concentrations of murine IL-2 were determined by ELISA. Data represent the mean values ± SD of eight independent experiments (eight donors). *: $p < 0.05$ compared to DCs incubated in the absence of *H. pylori* (Wilcoxon matched-pairs signed rank test)

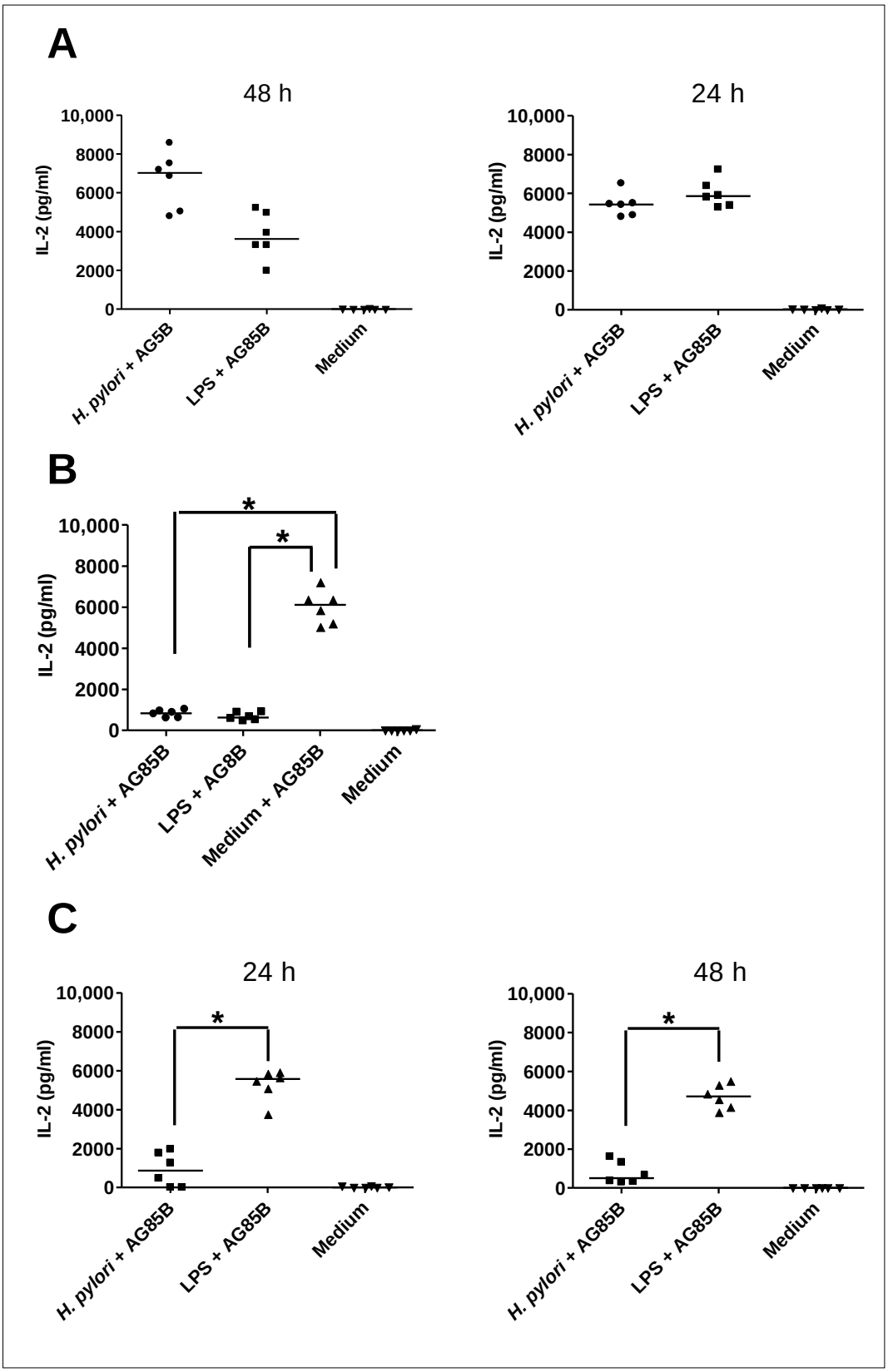

Fig. 3. *H. pylori* but not LPS suppressed antigen presentation by human DCs for up to 48 h. Immature HLA-DR1$^+$ DCs were incubated in the presence or absence of recombinant antigen 85B from *M. tuberculosis* (AG85B, 12 µg/ml) (A) 24 or 48 h prior to or (B, C) at the time of stimulation for 1 h with *H. pylori* (MOI of 10). Cells incubated in the presence of LPS (100 ng/ml) or maintained in medium alone served as controls. F9A6 cells were added (A, B) immediately after stimulation or (C) 24 or 48 h later. Twelve hours after the addition of F9A6 cells, cell-free supernatants were collected and concentrations of murine IL-2 were determined by ELISA. Each symbol per condition represents the data obtained from one independent experiment (one donor). Horizontal lines show the median values of six experiments (donors). *: $p < 0.05$ (Wilcoxon matched-pairs signed rank test)

CD80, and CD83, and results in a reduced capability of the cells to take up exogenous particles [10]. Hence, we were interested in how the kinetics of the *H. pylori* addition may influence the inhibitory effect of the bacteria on antigen presentation by DCs. We therefore altered the time interval between addition of antigen and infection by adding AG85B to immature HLA-DR1$^+$ DCs 24 and 48 h prior to or at the time of *H. pylori* stimulation. Bacteria were washed out after 1 h and F9A6 cells were added to the DCs immediately. In similar experiments, DCs were exposed to antigen and simultaneously stimulated with *H. pylori,* but the time of F9A6 cell addition varied, i.e., they were added immediately or either 24 or 48 hours later. As controls we used LPS-stimulated DCs and cells kept in medium alone. The latter therefore did not receive a maturation stimulus. Supernatants were always collected 12 h after the addition of F9A6 cells and the concentrations of murine IL-2 secretion were measured.

In contrast to DCs kept in medium alone, we detected substantial amounts of IL-2 when DCs were incubated with *H. pylori* or LPS 24 or 48 h after AG85B addition *(Fig. 3A)*. Comparable to our previous observation, IL-2 production by F9A6 cells was significantly reduced when AG85B was added at the time of *H. pylori* or LPS stimulation *(Fig. 3B)*.

Similarly, we detected low levels of IL-2 in supernatants of *H. pylori*-treated DCs when F9A6 cells were added 24 or 48 h after stimulation *(Fig. 3C)*. Notably, in comparison to *H. pylori*-stimulated DCs, IL-2 levels were significantly increased in supernatants of LPS-treated DCs, when F9A6 cells were added 24 or 48 h after stimulation *(Fig. 3C)*. Thus, both LPS and *H. pylori* interfere with antigen presentation by DCs when the antigen is added around the time of stimulation. A substantial time span between antigen uptake and stimulation, in our experiments 24–48 h, is required for optimal antigen presentation. In contrast to LPS activation of DCs, however, *H. pylori* interferes for a prolonged period of time with presentation of exogenously added antigens by human DCs.

Discussion

Our approach offers a method to study antigen presentation to CD4$^+$ helper type T cells by *H. pylori*-stimulated human DCs, irrespectively of immunomodulatory effects of bacterial virulence factors that directly affect human T-cell activation. We have previously shown that incubation of DCs with *H. pylori* or LPS does not affect the expression of HLA-DR but reduces the capability of engulfing exogenous particles following incubation of the cells for 1 h with either stimulus [10]. By adding antigen to cells simultaneously with bacterial stimulation, we excluded this maturation/activation-induced incapacity of the cells to pick up further antigen. The fact that LPS-treatment induced a similar although more transient inhibition of antigen presentation indicates that at least Gram-negative bacteria associated Toll-like receptor (TLR) ligands may be involved. Indeed, this offers a parsimonious explanation of similar observations that were reported for *Salmonella enterica* serovar Typhimurium-stimulated murine DCs where stimulation with *Salmonella* resulted in a reduced MHC-II presentation of antigenic peptides [12]. At a first glance, it appears paradoxical and counter-intuitive that antigens in the course of DC activation by multicomponent-immunostimulatory agents are not efficiently presented. The reported observation, however, may highlight a physiologically important aspect of antigen-processing and presentation by DCs, i.e., focusing T-cell responses to antigens of the stimulating pathogen while limiting presentation of other exogenous or – physiologically more relevant – autoantigens that may inevitably be sampled by the DC at the same time. These effects may also be relevant for subunit vaccine formulation strategies since vaccines that physically link vaccine antigens to APC stimulating molecules seem to be more effective [13, 14].

Competing interests

The authors declare no conflict of interest.

Funding sources

This study was supported by the DFG (SFB 633).

Acknowledgements

We thank David H. Canaday for providing the AG85B$_{aa97\text{-}112}$-specific, HLA-DR1-restricted F9A6 cells.

References

1. Aebischer T, Bumann D, Epple HJ, Metzger W, Schneider T, Cherepnev G, Walduck AK, Kunkel D, Moos V, Loddenkemper C, Jiadze I, Panasyuk M, Stolte M, Graham DY, Zeitz M, Meyer TF: Correlation of T cell response and bacterial clearance in human volunteers challenged with *Helicobacter pylori* revealed by randomised controlled vaccination with Ty21a-based Salmonella vaccines. Gut 57(8), 1065–1072 (2008)
2. Ermak TH, Giannasca PJ, Nichols R, Myers GA, Nedrud J, Weltzin R, Lee CK, Kleanthous H, Monath TP: Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. J Exp Med 188(12), 2277–2288 (1998)
3. Bimczok D, Clements RH, Waites KB, Novak L, Eckhoff DE, Mannon PJ, Smith PD, Smythies LE: Human primary gastric dendritic cells induce a Th1 response to *H. pylori*. Mucosal Immunol 3(3), 260–269 (2010)
4. Khamri W, Walker MM, Clark P, Atherton JC, Thursz MR, Bamford KB, Lechler RI, Lombardi G: *Helicobacter pylori* stimulates dendritic cells to induce interleukin-17 expression from CD4+ T lymphocytes. Infect Immun 78(2), 845–853 (2010)

5. Mitchell PJ, Afzali B, Fazekasova H, Chen D, Ali N, Powell N, Lord GM, Lechler RI, Lombardi G: *Helicobacter pylori* induces *in-vivo* expansion of human regulatory T cells through stimulating interleukin-1β production by dendritic cells. Clin Exp Immunol 170(3), 300–309 (2012)
6. Beigier-Bompadre M, Moos V, Belogolova E, Allers K, Schneider T, Churin Y, Ignatius R, Meyer TF, Aebischer T: Modulation of the CD4+ T-cell response by *Helicobacter pylori* depends on known virulence factors and bacterial cholesterol and cholesterol α-glucoside content. J Infect Dis 204(9), 1339–1348 (2011)
7. Gebert B, Fischer W, Weiss E, Hoffmann R, Haas R: *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. Science 301(5636), 1099–1102 (2003)
8. Schmees C, Prinz C, Treptau T, Rad R, Hengst L, Voland P, Bauer S, Brenner L, Schmid RM, Gerhard M: Inhibition of T-cell proliferation by *Helicobacter pylori* gamma-glutamyl transpeptidase. Gastroenterology 132(5), 1820–1833 (2007)
9. Sundrud MS, Torres VJ, Unutmaz D, Cover TL: Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc Natl Acad Sci U S A 101(20), 7727–7732 (2004)
10. Fehlings M, Drobbe L, Moos V, Renner Viveros P, Hagen J, Beigier-Bompadre M, Pang E, Belogolova E, Churin Y, Schneider T, Meyer TF, Aebischer T, Ignatius R: Comparative analysis of the interaction of *Helicobacter pylori* with human dendritic cells, macrophages, and monocytes. Infect Immun 80(8), 2724–2734 (2010)
11. Canaday DH, Gehring A, Leonard EG, Eilertson B, Schreiber JR, Harding CV, Boom WH: T-cell hybridomas from HLA-transgenic mice as tools for analysis of human antigen processing. J Immunol Methods 281(1–2), 129–142 (2003)
12. Cheminay C, Möhlenbrink A, Hensel M: Intracellular Salmonella inhibit antigen presentation by dendritic cells. J Immunol 174(5), 2892–2899 (2005)
13. Husseiny MI, Hensel M: Evaluation of Salmonella live vaccines with chromosomal expression cassettes for translocated fusion proteins. Vaccine 27(28), 3780–3787 (2009)
14. Xu X, Husseiny MI, Goldwich A, Hensel M: Efficacy of intracellular activated promoters for generation of Salmonella-based vaccines. Infect Immun 78(11), 4828–4838 (2010)