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Mycoplasma Infection Modifies the Proliferation and Attachment of Cal29 Bladder Cancer Cells, as Well as the Expression of Immune Checkpoint Antigen CD276

Nathalie E.Ch. Zenner[1], Simon Walz[2], Arnulf Stenzl[2], Niklas Harland[2]*, Wilhelm K. Aicher[1], Bastian Amend[2]

[1]Center for Medical Research, Dept. of Urology, University of Tuebingen Hospital, Tuebingen Germany
[2]Department of Urology, University of Tuebingen Hospital, Tuebingen, Germany

Abstract

Purpose

Bladder cancer is among the most prevalent malignancies. Despite intensive research, progress toward better diagnosis or therapy has been sparse in recent decades. However, many studies documented elevated expression of immune checkpoint antigens including CD276 on urothelial tumor cells, and novel therapies targeting immune checkpoint antigens are promising. At the same time, mycoplasma infections were reported in about 18% of sexually active adults. We, therefore, investigated if mycoplasma-contaminated urothelial carcinoma cells Cal29 cells differed in proliferation, attachment, appearance, and expression of immune checkpoint antigen CD276 from mycoplasma-free Cal29.

Methods

Mycoplasma-infected Cal29 cells (myCal29) and clean Cal29 cells (cCal29) were expanded separately and infection vs. sterility was monitored by PCR throughout the experiment. Mycoplasma-infected normal urothelial cells (myNUCs) and clean normal urothelial cells (cNUCs) served as controls. Cells were seeded and attachment, appearance, and size of the cells were observed by microscopy. Proliferation was enumerated in consecutive passages. Expression of CD276 was studied on the mRNA level by quantitative PCR of cDNA (RT-qPCR) and on protein levels by Western blot. CD276 amounts on cell surfaces were explored by flow cytometry (FC).

Results

Mycoplasma-infected Cal29 proliferated significantly faster than cCal29 (n=5, 132%, $p<0.001$). Upon seeding in flasks, cCal29 adhered faster than myCal29, and attached more firmly to the flasks as microscopy showed up to double diameters in comparison to myCal29. Expression of CD276 transcripts and total proteins in cCal29 ($p<0.001$) and cNUCs ($p<0.05$) was significantly higher when compared to the corresponding infected cells. By flow cytometry, myCal29 (MFI 4334) presented less than half of CD276 on the cell surface when compared to cCal29 (MFI 9729).

Conclusions

On one hand, urinary infection by mycoplasma may indirectly contribute to tumor spreading by accelerating the proliferation of bladder cancer cells and by reducing cell-cell and/or cell-matrix interactions as documented by our cell growth and attachment studies. On the other hand, on myCal29 and myNUCs cell surfaces less CD276 was recorded. Thus, mycoplasma infection of bladder cancer cells may facilitate T-cell-mediated immune responses by reducing the amounts of immune-checkpoint antigen CD276 on cell surfaces.

Keywords: Immune checkpoint ligands; CD276; B7-H3; Bladder cancer; Urothelial cells; Mycoplasma infection; TOLL-signaling

Highlights

Mycoplasma infection of bladder cancer cells Cal29 modulates cell proliferation, attachment, and expression of CD276. Mycoplasma may have an impact on bladder cancer development

Introduction

Nowadays, bladder cancer (BC) is among the most frequent malignancies worldwide [1-3]. Risk factors contributing to BC include compounds released from the combustion of organic materials and industrial processes [3-6]. In populations exposed to such chemicals, predisposition by genetic factors comes into play [7]. Besides these risk factors, infections may contribute to bladder cancer as well: Infection with *Schistosoma haematobium* is considered a causative agent [8], and recurrent infections of the urinary tract increase the risk of developing bladder cancer, albeit such infection by itself is not the cause but rather an enhancer of malignancy [9].

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Bladder infection encounters some individuals rather frequently. Enterobacteriaceae including *E. coli* are the main pathogens in women and men [10, 11]. Urinary symptoms were also associated with mycoplasma infections [12, 13], and urine samples of up to 50% of women tested positive for *Ureaplasma urealyticum*, while only 10% of samples were positive for *Mycoplasma hominis*. However, a correlation of mycoplasma infection with white blood cell counts in urine was not found [14]. Mycoplasma as a direct causative agent inducing urothelial carcinoma is a matter of debate [15]. However, persistent infection of human prostate cells with *Mycoplasma genitalium* or *Mycoplasma hyorinis* induced malignant transformation, suggesting that this pathology may act in certain cells [16]. *Mycoplasma penetrans* was detected in significantly higher numbers in patients diagnosed with muscle- invasive bladder cancer (MIBC) when compared to NMIBC samples, suggesting that mycoplasma infection might be associated with the development of transitional cell carcinoma [17].

Mycoplasma and mycoplasma-derived lipopeptides bind to toll-like receptors (TLR), frequently to TLR1/2 or TLR2/6 heterodimers on cell surfaces. In NUCs, transcripts encoding TLRs 1 to 6 were found and expression of TLR2, TRL4, and TLR5 receptors were recorded [18]. In bladder cancer cell lines comparable expression patterns were seen [18]. Engaging TLRs activates, among other intracellular pathways, MyD88, IRAK-4, NFκB, and MAP-kinases [18-20]. This may alter the release of prostaglandin E2 and cytokines [21-23]. Expression of membrane-anchored TLR2, TLR4, and TLR6 was upregulated on epithelial cells upon mycoplasma infections, and expression of IL-6, IL-8, and other interleukins was significantly enhanced [24]. In HeLa cells, increasing numbers of genes were altered in their expression depending on the time of infection with *Mycoplasma hominis* [25]. Mycoplasma inhibits p53-mediated responses, which may activate proliferation and inhibit apoptosis [26]. This confirmed a significant effect of mycoplasma on the metabolism and proliferation of epithelial cells. But effects of mycoplasma on the expression of immune checkpoint antigen CD276 on bladder cancer cells was not yet studied in detail.

CD276 is an immune checkpoint antigen also referred to as B7-H3. It is part of the immune globulin superfamily and is involved in the regulation of T-lymphocyte-mediated immune responses [27-29]. Tumors expressing elevated levels of CD276 inherit a worse prognosis, but immune checkpoint therapy yielded promising results [30]. Elevated expression of CD276 enhanced migration and invasiveness of bladder cancer cells [31] and facilitated the progression to muscle-invasive bladder cancer [32]. Decreasing its expression reduced tumor development [32]. A significant overexpression of CD276 in BC tissue samples across all BC stages and CD276 overexpression in a variety of BC cell lines were reported recently [33, 34]. We, therefore, investigated the effect of mycoplasma infection of human bladder cancer cells Cal29 and normal urothelial cells (NUC) on the expression of immune checkpoint antigen CD276 in a proof of principle study.

Materials and Methods

2.1 Cell culture and mycoplasma contamination

Cal29 tumor cells (purchased from Leibniz Institute DSMZ) [35], were seeded in cell culture flasks and expanded in cell expansion media (MEM; Biosell), complemented with 10% fetal bovine serum (FBS; Biosell) and 1% Penicillin/Streptomycin (Gibco). Upon reaching 90 % of confluence, expansion media was aspirated, cells were washed with PBS, detached by the aid of Trypsin-EDTA (Sigma-Aldrich), and counted using a hematocytometer and trypan blue dye exclusion as described [33]. To determine cell duplication rates, the yield of cells over time was counted in three consecutive passages [36]. Supernatants of cell cultures were monitored for mycoplasma contamination (see below). The myCal29 were maintained, processed, and analyzed throughout the whole study in a separate cell culture laboratory to avoid contaminations of the clean cells.

Clean normal urothelial cells (cNUCs) were isolated from fresh tissue samples of patients undergoing surgery after written and informed consent. The study was approved by the local ethics committee (project number 341/2002) and conducted in full compliance with the WMA Declaration of Helsinki and all other relevant guidelines and regulations. The NUCs were cultured as described before [37]. Cells were expanded upon reaching 90% confluence and passaged. After the first passage, an aliquot of cNUCs was transferred to the mycoplasma laboratory, expanded for one week, and infected with mycoplasma by adding 1ml of filtered mycoplasma-contaminated cell culture supernatant from a contaminated culture together with the fresh medium. Four days later, this infection procedure was repeated. The urothelial cells infected with mycoplasma (myNUCs) were maintained, monitored, and investigated in a separate cell culture lab to avoid contaminations of clean cells.

2.2 Detection of mycoplasma by polymerase chain reaction

The status of mycoplasma infection in cCal29, myCal29, cNUCs, and myNUCs was monitored in cell culture

supernatants by PCR. To this end 1 mL of supernatant was aspirated and denatured in a ThermoMixer with mild shaking (5 min., 95°C; Eppendorf). The insoluble debris was sedimented by centrifugation (2 min., 13 000 g, 20°C; Eppendorf). Mycoplasma was detected by polymerase chain reaction (PCR, LightCycler 480; Roche) in the supernatant using a specific detection kit as requested (MycoSPY kit, Bionex). The amplification was monitored by analysis of the melting point of the PCR products and by DNA agarose gel electrophoresis [38].

2.3 Analysis of CD276 transcript expression

Expression of CD276 transcripts was enumerated by reverse transcription (RT) of mRNA followed by quantitative PCR. For extraction of RNA, cells were washed with cold PBS, detached by Trypsin-EDTA, washed, counted, and transferred to microcentrifugation tubes. Total RNA was extracted using chemo-adsorption (RNeasy kit; Qiagen). Yield and purity of total RNA were determined by UV spectroscopy (NP80 NanoDrop; Implen). 1 µg of RNA was reverse transcribed using oligo-(dT) priming and AMV reverse transcriptase at 42°C (PrimeScript; Takara). For RT-qPCR, 2 µL of cDNA were amplified (hot start: 5 min 95°C; amplification by 39 cycles: 10 sec 95°C, 20 sec. 60°C, 30 sec. 72°C; melting point analysis: 5 sec. 95°C, 30 sec. 60°C, linear increase to 97°C; 30 sec cooling to 4°C, (LightCycler 480; Roche) [39]. The PCR product size was corroborated by DNA gel electrophoresis [38]. To compute CD267 transcript amounts, RT-PCR of β2-microglobulin (β2MG) and peptidopropyl-isomerase Aγ (PPIAγ) transcripts were employed as housekeeping standards. Normalized CD276 transcription rates were enumerated using the $2^{\Delta\Delta ct}$-method [39, 40].

2.4 Analysis of CD276 protein expression by Western blotting

For protein extraction and determination of protein expression, cells were expanded, harvested, washed, and counted as described above. 05E05 cells were sedimented in microtubes by centrifugation (7 min, 5000 g, 4°C; Eppendorf), the supernatant was aspirated, and the cells were resuspended in 200 µL cold lysis buffer (RIPA buffer; SigmaAldrich) complemented by 5 µL PMSF to block proteases. The lysed cells were further homogenized by aspirating the suspension through G20 needles. Insoluble debris was sedimented by centrifugation (20 min., 13,000 g, 4°C; Eppendorf). The protein yield was determined in cleared supernatants by colorimetry (DC protein assay kit II; BioRad) reading the absorption at λ = 600 nm in a microplate reader (GlowMax; Promega) by using serial dilutions of albumin as standards (DC protein assay kit II; BioRad). For gel electrophoresis, SDS-PAGE was employed and 10 µg of extracted proteins per lane were separated in a 10% separating gel (Protean Minigel System; BioRad) [38]. The proteins were transferred by submarine electrophoresis to nylon membranes (Protean Minigel System). To remove residual SDS, nylon membranes were washed three times with 0.1% Tween20 in PBS and blocked by incubation in blocking solution (= blotto; 60 min., 5% dry milk powder, 0.1% Tween20, PBS, 22°C, moderate shaking). Blotto was removed, membranes were washed twice (0.1% Tween20, PBS, 22°C, moderate shaking), rinsed in PBS again, and incubated with the primary antibody (overnight, mouse (mo) anti-CD276 monoclonal antibody clone MIH42, Abcam; 1:600, in blotto, 4°C, moderate shaking, humidified box). The primary antibody was aspirated, the membrane washed three times as described above, and incubated with detection antibody (2 hours, horse-radish peroxidase (HRP)-labeled polyclonal rabbit (rb) anti-mo IgG1; Dako, 1:5000 in blotto, 22°C, moderate shaking, in humidified box). The detection antibody was aspirated, and the membrane was washed three times as described above. The binding of the detection antibody was visualized by a blot scanner (C-Digit; Licor) and chemiluminescence reagents (Western Sure Premium; Licor). Detection of β-actin expression served as control. To this end, the antibodies binding to the nylon membrane were stripped off (0.2 M glycin, 0.1% SDS, 1% Tween20, pH 2.2, 30 min. vigorous shaking, 22°, three times). The membrane was washed twice with blotto (30 min., moderate shaking, 22°C) and incubated with the second primary antibody (rabbit (rb) serum anti-hu β-actin, Abcam; 1:1000 in blotto, 4°C, moderate shaking, humidified box). The binding of the anti-β- actin serum was detected by a specific detection antibody (2 hours, goat (gt) serum anti-rb IgG, HRP-labeled, Dako; 1:10000 in blotto, 22°C, moderate shaking, humidified box) and visualized as described above. Signal intensities produced on Western blots in the same sample by anti-CD276 versus anti-β-actin reagents were used to compute the normalized protein expression (ImageStudio Lite Software; Licor).

2.5 Analysis of CD276 expression on cell surfaces by flow cytometry

To explore the levels of CD276 expression on cell surfaces of cCal29 in comparison to myCal29, flow cytometry (FC) was employed (LRSII; BD Bioscience) [33, 41, 42]. Cells were harvested by mild proteolysis (Accutase; Sigma-Aldrich), washed in 1%FBS-PBS, and counted as described above. 05E05 cells were resuspended in PFEA sample buffer, incubated with pre-immune serum (Gammunex, 1:20 in PFEA, 30 min. on ice; Grifols) to avoid nonspecific antibody binding [41], washed again and incubated with PE-labelled anti-CD276 monoclonal antibody (clone MIH42, 1:20 in PFEA; BioLegend). The excess antibodies were washed off and expression of CD276 on Cal29 surfaces was explored by FC as described [33, 41, 42].

Isotype controls and compensation beads were employed to gate viable cells. For processing of FC data, FACS-

Diva and FlowJo software programs were used (BD Bioscience). The mean fluorescence intensity (MFI) of anti-CD276 staining is presented as a bold histogram, and controls as a thin histogram.

2.6 Statistics

Experimental data were processed by a spreadsheet program (Excel, Microsoft). Mean values and standard deviation of individual data sets were calculated. Significant differences were computed by unbiased, two-sided student's T-test, and p values below 0.05 (*), 0.01 (**), and 0.001 (***) were considered significant, very significant, and highly significant, respectively.

Results

3.1 Effects of mycoplasma infection on cell proliferation

To investigate the effects of mycoplasma infections, duplication rates over five consecutive passages were computed for cCal29 (mean DR = 0.74) and myCal29 (mean DR = 0.98), respectively **(Figure 1).**

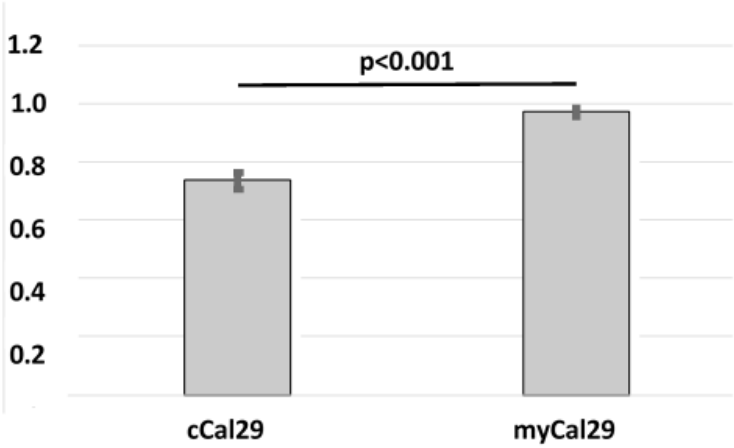

Figure 1: Duplication rates of Cal29 cells

Mycoplasma infection activated the proliferation of Cal29 cells significantly (p<0.001), but differences in cell appearance, shape, or size were not recorded by microscopy over a follow-up of up to 7 days in culture **(Figure 2).** Cultures of cCal29 reached confluence **(Figure 2A),** while confluence was not observed in myCal29 cultures **(Figure 2B)** despite enhanced proliferation **(Figure 1).** Comparably, infection of NUCs by mycoplasma failed to cause changes in the appearance of the cells, but fewer cells were attached in parallel cultures with identical seeding density and after the same time for follow-up **(Figure 2C, 2D).** Cell proliferation of cCal29 and myCal29 was determined over three consecutive passages and mean DR was calculated. myCal29 proliferate significantly faster than cCal29 (p<0.001).

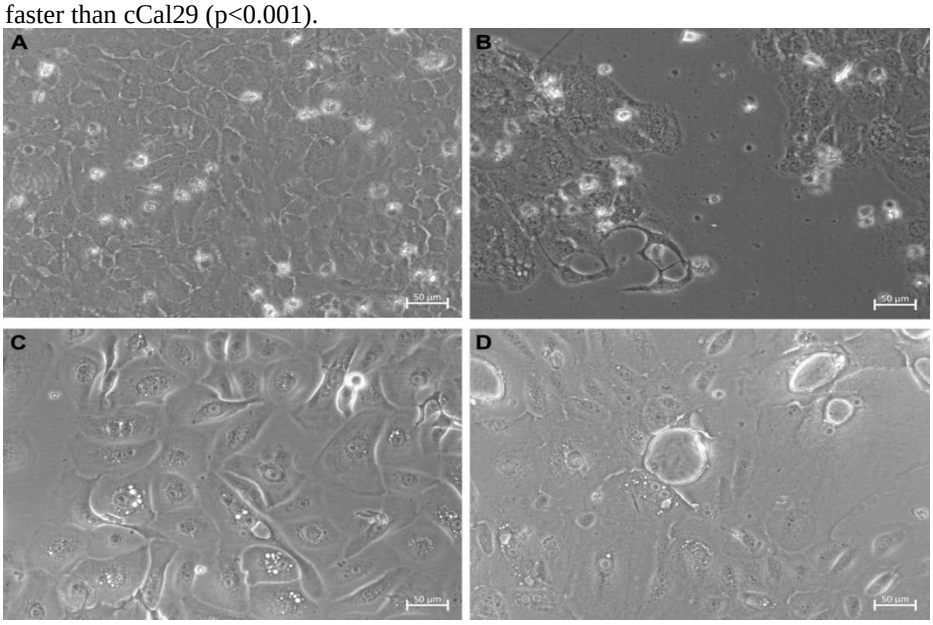

Figure 2: Cell appearance and culture density of Cal29 and NUC cells

Cells were seeded with identical inoculation density and expanded. Examples of expansions of cCal29 (2A) versus myCal29 for seven days (2B), and examples of expansions of cNUCs (2C) versus myNUCs (2D) for nine days as presented. The slower proliferation of NUCs vs Cal29 was compensated in part by longer follow-up. Size bars indicate 50 μm.

3.2 Effects of mycoplasma infection on expression of CD276

In the first set of experiments, the effect of mycoplasma contamination on CD276 transcription was **explored (Figure 3).** In myCal29, expression of CD276 mRNA was significantly below levels recorded for cCal29 ($p<0.001$; n=5 each; **Figure 3).** Stimulation of cCal29 by LPS served as control. Reduction of CD276 transcripts by LPS was not recorded (data not shown). In addition, NUCs were infected by mycoplasma as well. For myNUCs, variable effects on CD276 transcription were noted in comparison to cNUCs and significance was not reached ($p=0.14$, n=5 each; not shown). In a second set of experiments, the effects of mycoplasma infection were investigated on total protein levels **(Figure 4).** Protein extracts were generated from Cal29 and NUCs. The expression level of CD276 in cCal29 bladder cancer cells exceeded the level observed in NUCs **(Figure 4).** This is in line with our recent study [33]. Upon mycoplasma infection, a 66% reduction of CD276 protein expression was recorded in myCal29 when compared to cCal29, and a 55% reduction was observed in myNUCs when compared to cNUCs **(Figure 4).** The presentation receptors or ligands on cell surfaces is particularly important for the regulatory effects of immune checkpoint ligands on the immune system. Therefore, expression of CD276 on cell surfaces of cCal29 and myCal29 was studied as well **(Figure 5).** Investigating size and complexity of cCal29 **(Figure 5A)** versus myCal29 **(Figure 5C)** by comparing the side scatter (SSC) and forward scatter (FSC) by FC did not yield remarkable differences between these populations. This corroborated the observations by microscopy **(Figure 2).** Analysis of cell CD276 signal intensities on cCal29 vs. myCal29 provided evidence that the number of CD276 molecules on myCal29 cell surfaces (MFI = 4947; **Figure 5D)** was reduced to 42% of the signal intensity measured for cCal29 (MFI = 11738; **Figure 5B**). In addition, FC analyses were repeated over five consecutive weeks. The cCal29 (mean MFI = 5424 ± 1288, n = 5) expressed significantly more CD276 when compared to myCal29 (mean MFI = 2052 ± 666, n = 5, $p< 0.001$). This indicated that mycoplasma infection downregulates the expression of CD276 on Cal29 bladder cancer cells not only on transcript and total protein levels **(Figures 3, 4)** but significantly on cell surface levels.

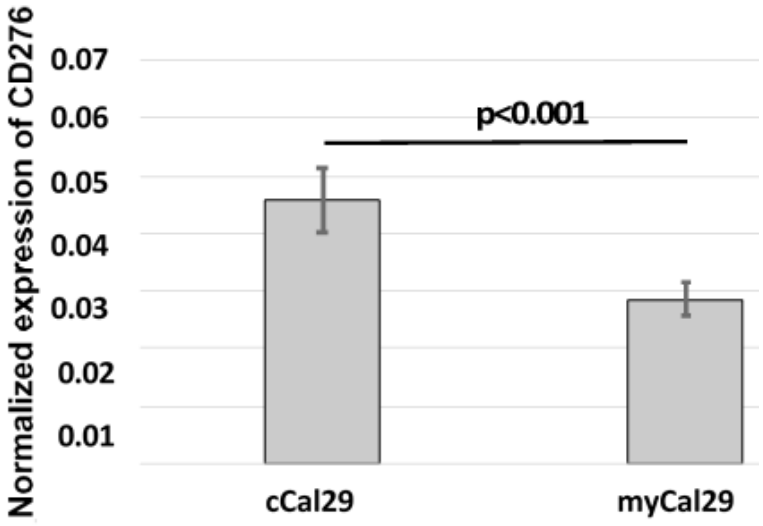

Figure 3: Enumeration of CD276 transcript expression

The steady-state transcript amounts encoding CD276 was investigated for cCal29 and myCal29 respectively from 5 individual samples each (x-axis) and normalized to the expression of β2MG and PPIAγ (mean expression ± standard deviation, y-axis). Clean Cal29 expressed significantly more CD276 than myCal29 ($p<0.001$).

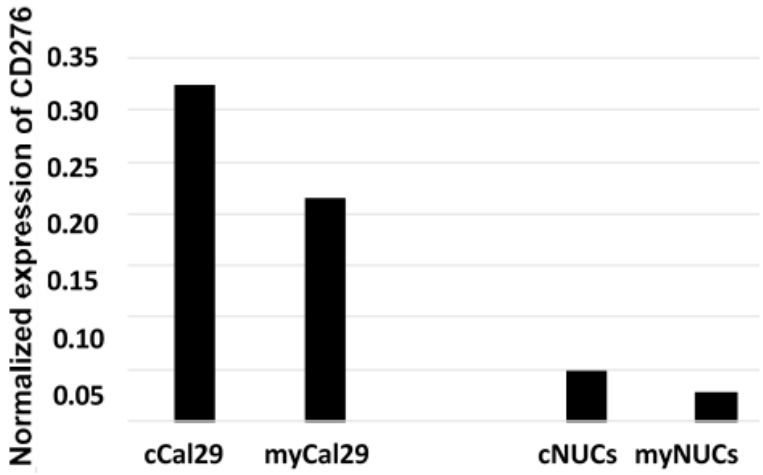

Figure 4: Expression of CD276 protein

Proteins were extracted in two independent experiments each and quantified by Western blot from cells as indicated (x-axis). The figure presents the mean of CD276 protein expression of the different cells normalized to expression of β-actin (y-axis). The cCal29 tumor cells express CD276 at higher levels when compared to NUCs. Upon mycoplasma infection CD276 expression is reduced.

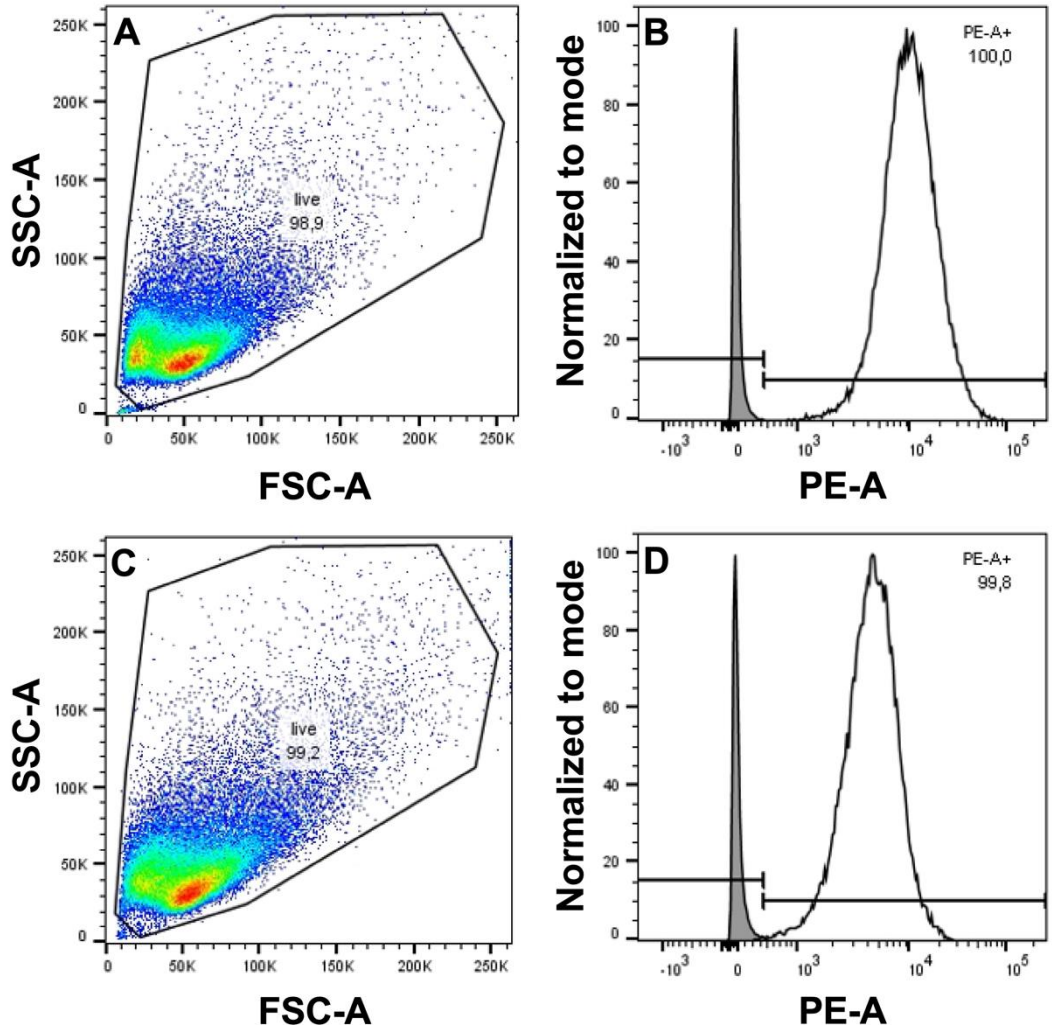

Figure 5: Analysis of the CD276 expression on cell surfaces by flowcytometry

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Cell size and complexity (FSC-A / SSC-A), and expression of CD276 (PE-A) on cell surfaces of cCal29 (A,B) and myCal29 (C,D) were investigated twice by flowcytometry. The figure shows a representative experiment. Size and complexity of cells did not change upon infection (A,C) but amounts of the immune checkpoint ligand CD276 were reduced in myCal29 when compared to cCal29 (clear histograms). Staining with isotype controls served as negative controls (grey histograms). The horizontal bars indicate the settings to calculate the percentage of positive cells.

Discussion

Different mycoplasma species are associated with inflammations of the urinary tract [43]. But they may escape proper diagnosis as mycoplasma infect cells in part as intracellular pathogen. Moreover, mycoplasma was found in asymptomatic as well as in symptomatic patients with sterile leukocyturia [43], and culture of mycoplasma is more complex when compared to most other microorganisms. But mycoplasma address the same pattern recognition receptors (PRRs) on cells as for instance eubacteria. Among them TLRs, as this type of PRRs recognizes conserved biochemicals structures of microorganisms which in turn act as external pathogen-associated molecular patterns (PAMPs) or internal, damage- associated molecular patterns (DAMPs) [44]. A meta study provided evidence that recurrent bladder infections inherited a significant risk of developing BC, with even higher evidence for muscle-invasive BC [9], and chronic infection by *E. coli* plays a synergistic role for bladder carcinogenesis [45]. Therapy with antibiotics significantly reduced the risk for BC, specifically in individuals with a history of smoking [9]. As chronic inflammation by mycoplasma is refractory to standard antibiotics, chronic and untreated infection with mycoplasma can be an additive factor towards BC as well [46]. However, mycoplasma as a candidate trigger contributing to BC is a matter of debate.

Low expression of TLR4 was associated with poor prognosis of BC patients. It correlated significantly with the tumor stage and the presence of metastases. Experimental depletion of TLR4 on BC cell lines augmented their invasiveness [47]. This suggested that TLR4 signaling ameliorates malignant development. However, single nucleotide polymorphism of TLR2, but not TLR4 was associated with an increased risk for BC [48]. Chronically imbalanced signaling by TLR2 and/or TLR4 contributes to BC. *Bacillus Calmette-Guerin* (BCG) instillation is a highly efficient therapy in early-stage BC. BCG acts through TLR2 heterodimeric and TLR4 homodimeric PRRs [18, 49]. The effect of BCG on the development of urothelial carcinoma has been studied. But based on our results, one aspect of TLR signaling merits discussion: Activation of urothelial cells via TLRs may reduce expression of CD276 on urothelial carcinoma cells and thus facilitate activation of cytotoxic CD8 positive cells with anti-tumor activity [27, 50]. Thus, TLR engagement not only addresses leukocytes directly to regulate immune responses, but it also may facilitate an indirect activation of cytotoxic immune responses by lowering the expression of the immune inhibitory checkpoint antigen CD276 on cancer cells.

We observed that myCal29 BC cells as well as myNUCs expressed less CD276 when compared to the corresponding clean cells. However, LPS did not affect the expression of CD276. The effects observed here are unlikely to be mediated by TLR4 alone, as LPS is a major ligand for TLR4 in concert with CD14, and the expression of CD14 on Cal29 is low. The reduction of CD276 expression by mycoplasma observed in this study is therefore rather mediated by TRL2 homodimeric or TLR2 heterodimeric receptors [51]. On NUCs, TLR 2 was found strongly overexpressed *in vitro*, TLR1 only in some samples, and TLR4 as well as TLR6 at or below the levels of the negative control [18]. This explains the low LPS responses seen here. Transcripts encoding the membrane-anchored TLR1, TLR4, TLR5, and TLR6 were detected in NUC cultures or normal bladder tissue samples [52, 53]. On protein levels, TLR4 was expressed strongly in normal bladder tissue, and TLR2 as well as TLR5 were expressed weaker [52]. The expression of TRLs was lower in non-muscle-invasive BC tissue samples when compared to normal tissue, and even less pronounced in muscle-invasive BC samples [52]. This indicated that expression levels of TLRs are modulated during the course of cancer. Therefore TLR-dependent cell responses of urothelial cells to infections may depend on BC staging [53]. This difference in TLR expression on UC may have consequences on the efficacy of BCG instillations in BC patients of different stages.

In addition, infection by mycoplasma yielded a time-dependent response in cells targeted [25]. The expression levels of different genes were altered in increasing numbers after four hours (i.e., 723) vs. two days (i.e., 1588) of infection, and in comparison to permanent infection (i.e., 1972) with *Mycoplasma hominis*, respectively [25]. This indicates that different results recorded upon experimental mycoplasma inoculation depend on the kinetics of the experiment.

As reported recently, NUCs expressed less CD276 when compared to the BC cell line Cal29 [33]. Elevated

expression of CD276 correlated with poor prognosis in BC [54]. The $CD276^{high}$ BC cells escape cytotoxic lysis by $CD8^{pos}$ T lymphocytes [27, 50]. As TLRs are increased in BC, one beneficial effect of enhanced TLR signaling may cause a reduction of CD276 expression, thus facilitating cancer elimination by $CD8^{pos}$ T lymphocytes.

We conclude that the expression of immune checkpoint antigen CD276 on normal urothelial cells and urothelial carcinoma cells Cal29 is altered by mycoplasma infection. As an elevated expression of immune checkpoint ligands protects cancer cells against lysis by $CD8^{pos}$ cytotoxic lymphocytes, a downregulation of CD276 on bladder cancer cells by mycoplasma infection may facilitate a more effective cytotoxic T-cell response.

Abbreviations

Bladder cancer (BC); Bacillus Calmette-Guerin (BCG); clean Cal29 (bladder cancer cells) (cCal29); clean (i.e., mycoplasma-free) normal urothelial cell (cNUC) ; Mycoplasma infected Cal29 (bladder cancer cells) (myCal29); Mycoplasma infected normal urothelial cell (myNUC); Normal urothelial cell (NUC); Lipopolysaccharide (LPS); Pattern recognition receptor (PRR); Toll-like receptor (TLR); Urothelial cells (UC)

Notes

Ethics Approval

The study was approved by the Ethics Committee of the University and Hospital (# 341/2002). The study was conducted in full compliance with the WMA Declaration of Helsinki on ethical principles for medical research and in accordance with all relevant guidelines and regulations.

Consent for publication ø not applicable Competing interests

The authors declare no conflict of interest for this study.

Availability of data and materials

Data and materials will be disclosed to colleagues in academia upon written, justified, and comprehendible request to the corresponding author.

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Author's contribution

Lab experiments, generation of experimental data, data evaluation, preparation of manuscript draft and artwork: N.E.Ch.Z., W.K.A.; Provision and preparation of clinical tissue samples: S.W., B.A.; Project management and supervision: W.K.A., A.S.; Revision of manuscript and artwork: all authors.

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