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Escherichia coli-induced productions of pro-inflammatory cytokines are regulated by MAP kinases and G-protein but not by Akt: Relationship with phylogenetic groups and resistance patterns

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ABSTRACT

Introduction: We investigated the role of PI3-K, MAP kinases, and heterotrimeric G proteins in inducing cytokines production in human whole blood cultures stimulated by viable *Escherichia coli* (*E. coli*) clinical strains.

Materials and methods: We used eight *E. coli* strains that belong to different phylogenetic groups and presented by different antibiotic resistance patterns. Whole blood from healthy volunteers was incubated at 37 °C for 150 min, with lipopolysaccharide (LPS) from *E. coli* O111:B4 or selected viable *E. coli* clinical strains, with or without SB202190 (p38 inhibitor), PD98059 (ERK inhibitor), PTX (pertussis toxin; heterotrimeric G proteins inhibitor), wortmaninn (PI3-K inhibitor). The TNF-α, IL-1β, IL-10 and IFN-γ concentrations were measured in culture supernatants (ELISA).

Results: IL-10 and IFN-γ were not detectable. Susceptible strains induced higher TNF-α and IL-1β productions than β-lactam resistant strains ($p < 0.05$), with no difference between phylogenetic groups. A transformed strain carrying a plasmid-mediated AmpC-β-lactamase gene (CMY-2) induced lower TNF-α and IL-1β production than the parent wild type strain ($p < 0.05$). SB202190 (p38 inhibitor) and PD98059 (ERK inhibitor) reduced TNF-α concentrations by, respectively, 80% ($p < 0.05$) and 50% ($p < 0.05$). Wortmaninn (PI3-K inhibitor) had no significant effect. PTX (heterotrimeric G proteins inhibitor) altered TNF-α production after viable bacteria stimulation (1.7-fold increase; $p < 0.05$) but not after LPS (TLR-4) stimulation. Regarding IL-1β, wortmaninn, SB202190 and PTX had no significant effect whereas PD98059 significantly decreased production in whole cell cultures ($p < 0.05$).

Conclusion: Susceptible strains induce greater TNF-α and IL-1β productions than resistant strains. ERK kinase plays a major role in viable *E. coli* strains inducing TNF-α and IL-1β production. *E. coli* exerts an effect on the pertussis toxin-sensitive G-protein through a TLR-4-independent mechanism.

1. Introduction

Escherichia coli (*E. coli*) is a commensal bacteria from the human digestive flora, but some strains can cause infections, such as diarrhea, urinary tract infections, septicemia or neonatal meningitis [1]. Phylogenetic analyses have shown that commensal strains belong mainly to phylogenetic groups A and B1, whereas the most virulent strains belong to group B2 and, to a lesser extent, to group D [2]. Strains of the B2 phylogenetic group frequently possess virulence determinants, such as adhesins, hemolysin or iron acquisition systems. The innate immune system, which is the first line of defense against pathogens [3], has to prevent infections by pathogen organisms, but must also allow commensal flora to persist. Toll-like Receptors (TLRs) recognize Pathogen-Associated Molecular Patterns (PAMPs). Among these, the Gram-negative endotoxin lipopolysaccharide (LPS), is known to bind to TLR4, and to engage in the downstream signaling pathway [4]. The recognition of PAMPs leads to activation of nuclear factor-kappa B (NF-κB) and the production of chemokines and proinflammatory cytokines. This signaling pathway is regulated by phosphatidyl-inositol 3-ki-

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nase (PI3-K) [5], mitogen-activated protein kinases (MAPK) [6,7] or by heterotrimeric G proteins [8] in human monocytes or in macrophage-differentiated THP-1 cells.

Mechanisms that enable immunity to respond differently to commensal and virulent strains of *E. coli* are poorly understood. Bristianou et al. recently reported that, in 20 *E. coli* isolates incubated with human monocytes, antibiotic susceptible strains induced less release of TNF-α than multidrug-resistant (MDR) and extended-spectrum β-lactamase producing strains [9].

In this *in vitro* study, we investigated the role of PI3-K, MAPK (p38, ERK), and heterotrimeric G proteins in inducing cytokine production in human whole-blood cultures stimulated by previously characterized viable *E. coli* clinical strains [10]. We also addressed the potential relationship between phylogenetic group, antibiotic-resistance pattern and cytokine production.

2. Materials and methods

2.1. Bacterial strains

Eight *E. coli* clinical strains isolated from urine or blood culture were used. Determination of the phylogenetic group, antimicrobial susceptibility pattern and *ampC* promoter sequencing have been already described [10]. The phylogenetic group was determined using triplex-PCR targeting *chuA*, *yja* and TSPE4 [11]. The pattern of PCR products obtained allowed classification of the *E. coli* strains into one of the four main phylogenetic groups : A, B1, B2 and D. Six virulence-associated genes, *papGII*, *papGIII*, *sfa/foc*, *hly*, *aer* and *fimH* were identified by multiplex PCR, as previously described [12,13]. The characteristics are shown in Tables 1 and 2.

For human-cell stimulation, a single colony of each strain was dispensed in Brain–Heart Infusion (Becton Dickinson, Sparks, MD, USA) and incubated for 18 h at 37 °C. The culture was washed two times. The cultures were diluted in sterile isotonic saline to be calibrated by nephelometry. The resulting inoculum was adjusted to obtain 10^6 CFU/mL in the culture plates. Bacterial concentration (CFU) was controlled by quantitative culture.

2.2. Preparation and transformation of competent E. coli [14]

An overnight culture of a single colony of the strain C1 was transferred into 100 ml of tryptic soja (TS) medium and incubated at 37 °C for 3 h under vigorous agitation. When the bacterial culture reached an OD_{600} of 0.5, bacterial cells were pelleted (2700 *g* for 10 min at 4 °C) and resuspended in an ice-cold Mg–Ca solution (80 mM $MgCl_2$, 20 mM $CaCl_2$). After 10 min on ice, bacterial cells were pelleted and the competent cells were resuspended in the Mg–Ca solution. An aliquot of the competent bacteria suspension was incubated with a plasmid-containing the CMY-2 *ampC* β-lactamase gene (10 ng) for 30 min on ice. The tubes were quickly heated at 42 °C for 90 s then transferred on ice for 2 min. Transformed *E. coli* strain C1 cells were then incubated in TS medium for 45 min at 37 °C to allow plasmid-gene expression. Finally, transformed *E. coli* strain C1 was transferred onto agar TS medium containing amoxicillin (64 mg/L).

Table 2
Virulence-associated genes. As previously described (3, 32), the virulence-associated genes, *papGII*, *papGIII*, *sfa/foc*, *hly*, *aer* and *fimH*, were identified by multiplex PCR.

Strains	Virulence-associated genes					
	pap GII	*papGIII*	*hly*	*sfa/foc*	*aer*	*fimH*
C1	–	–	–	–	–	+
C2	–	–	–	–	+	+
C3	–	–	–	–	–	+
C4	–	–	–	–	–	+
C5	–	–	–	–	–	+
C6	–	–	–	–	+	+
C7	–	–	–	–	+	+
C8	–	–	–	–	–	+

2.3. Reagents

E. coli LPS O111:B4, wortmannin (PI3-K inhibitor), SB202190 (p38 inhibitor), PD98059 (ERK inhibitor), pertussis toxin (PTX, heteromeric-G protein inhibitor) were purchased from Sigma Chemicals Co (St Louis, MO, USA). TLR-4-blocking antibody was purchased from R&D system (Mineapolis, MN, USA). The plasmid-mediated CMY-2 *ampC* β-lactamase gene was kindly provided by Dr. Hedi Mammeri (Amiens University Hospital, France).

2.4. Human cell stimulation

After approval from the Institutional review board, blood samples (20 ml) from 51 healthy donors (20 women and 31 men) were collected.

The whole blood culture technique has been described elsewhere [15]. Briefly, the blood was diluted to one fifth in RPMI-1640 medium (Institut de Biotechnologies Jacques Boy, Reims, France) and pretreated for 30 min at 37 °C in a shaking incubator with or without a specific inhibitor (wortmannin 1 μM [5], SB202190 15 μM [16], PD98059 30 μM [17] or PTX 0.4 μM [8]), TLR-4-blocking antibody 10 μg/mL or RPMI (control), prior to stimulation. Cultures were then incubated in 24-well plates for 150 min at 37 °C with either RPMI (control), or LPS O111:B4 (10 μg/mL) or one *E. coli* viable strain (10^6 UFC/mL). After stimulation, supernatants were collected, centrifuged (at 5000 *g* for 5 min at 4 °C) and stored at −80 °C until determination of TNF-α concentration. The viability of leukocytes after performance of whole blood cell cultures was checked by the trypan blue method exclusion and exceeded 90% with each bacterial strain. At the end of

Table 1
Characteristics of *E. coli* strains. Commensal strains belong to A and B1 phylogenetic groups and virulent strains belong to B2 and D phylogenetic groups. Strain origin : H = blood culture, U = urine sample. β-lactamases : CMY = plasmidic cephalosporinase, CTX-M = extended-spectrum β-lactamase, AmpC chr = overproduced chromosomal AmpC cephalosporinase. Amox: Amoxicillin, Tic Clav: Ticarcillin clavulanate; Cefo: Cefotaxime; Cefta: ceftazidime.

Strains	Origin	Phylogenetic group	Main mutations in *ampC* gene promoter	β-Lactam resistance pattern	Minimal inhibitory concentration (mg/ml)			
					Amox	Tic Clav	Cefo	Cefta
C1	H	A	−88 −82 −18 −1 +58	Wild type	⩽2	⩽2	⩽1	⩽1
C2	H	D	70	Wild type	⩽2	⩽2	⩽1	⩽1
C3	H	B2	−73 −28 +81	Wild type	⩽2	⩽2	⩽1	⩽1
C4	H	B1	−88 −82 −18 −1 +58	Wild type	⩽2	⩽2	⩽1	⩽1
C5	U	B1	−88 −82 −18 −1 +58	CMY-2	⩾32	⩾128	16	⩾64
C6	H	B2	−73 −28	CTX-M1	⩾32	32	⩾64	16
C7	U	A	−88 −82 −42 −18 −1 +58	AmpC chr	⩾32	32	2	16
C8	U	A	−88 −82 −18 −1 +58	CTX-M9	⩾32	⩽8	⩾64	⩽1

each whole blood cells experiment homogenous bacterial growth was checked for each strain by assessing final bacterial count.

2.5. Bacterial count

Culture cell supernatants were subjected to serial 10-fold dilution and cultured at 37 °C on selective medium. After a 48-h incubation, colonies were counted and results expressed as log10 CFU.

2.6. Cytokine measurements

Immunoreactive TNF-α, IL-1β, Interferon (IFN)-γ and IL-10 were quantified using commercially available ELISA kits (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions.

2.7. Statistical analysis

Comparisons were done using analysis of variance (ANOVA). Inter-group comparison was performed by a Newman–Keuls for *post hoc* analysis. The inhibitor effect was expressed as the concentration ratio [with/without] inhibitor, and was compared with the theoretical ratio (equal to one, traducing no apparent effect of inhibitor) (Wilcoxon Signed Rank test). Results of cytokine concentration in supernatants were expressed as their median [25thpercentile; 75thpercentile]. A *p*-value <0.05 was considered statistically significant.

3. Results

3.1. Bacterial count

Comparison of final bacterial count after cultures did not show any differences between the strains, except for the strain number 8 (6×10^3 UFC/mL *vs.* 1×10^7 UFC/mL for the other strains, $p < 0.05$). During culture, bacterial growth was unchanged with and without inhibitors.

3.2. Influence of phylogenetic group and antibiotic-resistance phenotype on cytokine productions

IFN-γ and IL-10 were not detectable. TNF-α and IL-1β concentrations in control wells (without stimulation) were lower than the sensitivity threshold (60 pg/mL), indicating that the experimental conditions had not interfered with TNF-α production (Fig. 1a–b). TNF-α and IL-1β concentrations after stimulation by LPS or by each *E. coli* strain were measured in the supernatant. LPS induced less TNF-α production than three out of four susceptible strains ($p < 0.01$ for all comparisons, Fig 1a). LPS challenge of whole blood cell cultures induced less IL-1β production than susceptible strains ($p < 0.01$ for all comparisons, Fig 1b).

TNF-α and IL-1β productions induced by commensal *E. coli* strains was not different to that of the virulent strains (NS; Fig. 1c–d). Conversely, stimulation by susceptible *E. coli* strains induced higher concentrations of TNF-α and of IL-1β ($p < 0.05$ for both comparisons, Fig. 1c–d). Stimulation by transformed *E. coli* strain C 1 induced a lower concentration of TNF-α and IL-1β than stimulation by wild type strain C 1 ($p < 0.05$; Fig. 1e–f).

3.3. Determination of virulence-associated genes

As no difference was found in the studied signaling pathway, we assessed the presence of virulence-associated genes for each *E. coli* strain. All *E. coli* strains were found to possess the gene *fimH* (Table 2). The gene *aer* was only detected in strains C2, C6 and C7. None of the other virulence-associated genes was detected (Table 2).

3.4. Effects of inhibitors on TNF-α and IL-1β production

To investigate the specific intracellular events that led to TNF-α and IL-1β productions, selective inhibitors of specific intracellular pathways were used. Wortmannin 1 μM (PI3-K inhibitor) did affect neither TNF-α nor IL-1β productions after stimulation by LPS or by groups of viable *E. coli* strains (Figs. 2a and 3a). SB202190 15 μM (p38 inhibitor) significantly reduced both TNF-α and IL-1β production in cultures stimulated with LPS and with all groups of *E. coli* strains ($p < 0.05$ for both comparisons, Figs. 2 and 3b). PD98059 30 μM (ERK inhibitor) significantly reduced TNF-α production ($p < 0.05$, Fig. 2c) but did not alter IL-1β production (NS, Fig. 3c) in cultures stimulated with LPS and with all groups of *E. coli* strains. In contrast, treatment of blood cultures with PTX 0.4 μg/mL (heterodimeric-G protein inhibitor) dramatically increased TNF-α production for all groups of strains but PTX had no effect on TNF-α production after LPS stimulation ($p < 0.05$, Fig. 2d). PTX did not alter IL-1β concentrations (Fig. 3d). TLR-4-blocking antibody decreased both TNF-α and IL-1β production after stimulation with LPS and *E. coli* clinical strains (Figs. 2 and 3e). After TLR-4 inhibition, the concentrations of TNF-α and of IL-1β were significantly lower in LPS group compared with all groups of *E. coli* strains (Figs. 2 and 3e).

3.5. Influence of sex on TNF-α production

TNF-α concentrations in blood cultures incubated with viable E. coli strains without inhibitor were significantly lower for female donors (2356 [954 ; 5836] pg/mL) than for male donors (3309 [1839 ; 5924] pg/mL, $p < 0.05$).

4. Discussion

TLR-4 signaling pathways after LPS stimulation are now well described mainly in monocytes and monocytic cells [4]. The signaling pathways involved after stimulation by viable *E. coli* was described for epithelial cell infections [18–20], and remain poorly studied in whole blood cell cultures. We chose to stimulate whole blood cells rather than isolated monocytes to keep the immune cells in their natural environment, and also because TNF-α production in blood is mainly due to monocytes [15,21].

Great variations of cytokine productions between volunteers have been observed, reflecting its heterogeneity within the population, which is partly due to genetic polymorphisms [22]. The differences observed between men and women in terms of cytokine production have already been reported [23]. This feature may explain the higher rate of sepsis described in men compared to women [24].

Regarding *E. coli* phylogenetic groups, most *E. coli* strains involved in "human pathology" belong to group B2 and, to a lesser extent, to group D, whereas commensal strains belong mainly to phylogenetic groups A and B1 [2]. In the current study, A and B1 *E. coli* strains did not induce different TNF-α production patterns compared to B2 and D *E. coli* strains. In contrast, we demonstrated that stimulation of whole blood samples by susceptible strains led to significantly higher TNF-α production than resistant strains. It has been demonstrated that an early burst of pro-inflammatory cytokines is deleterious to the host in the setting of a polymicrobial septic model in mice involving susceptible strains of bacteria [25]. Also, Bristianou et al., in a rabbit model of acute pyelonephritis, demonstrated that survival was prolonged after infection by esistant, as compared to susceptible, strains of *E. coli* [9]. Giamarel-

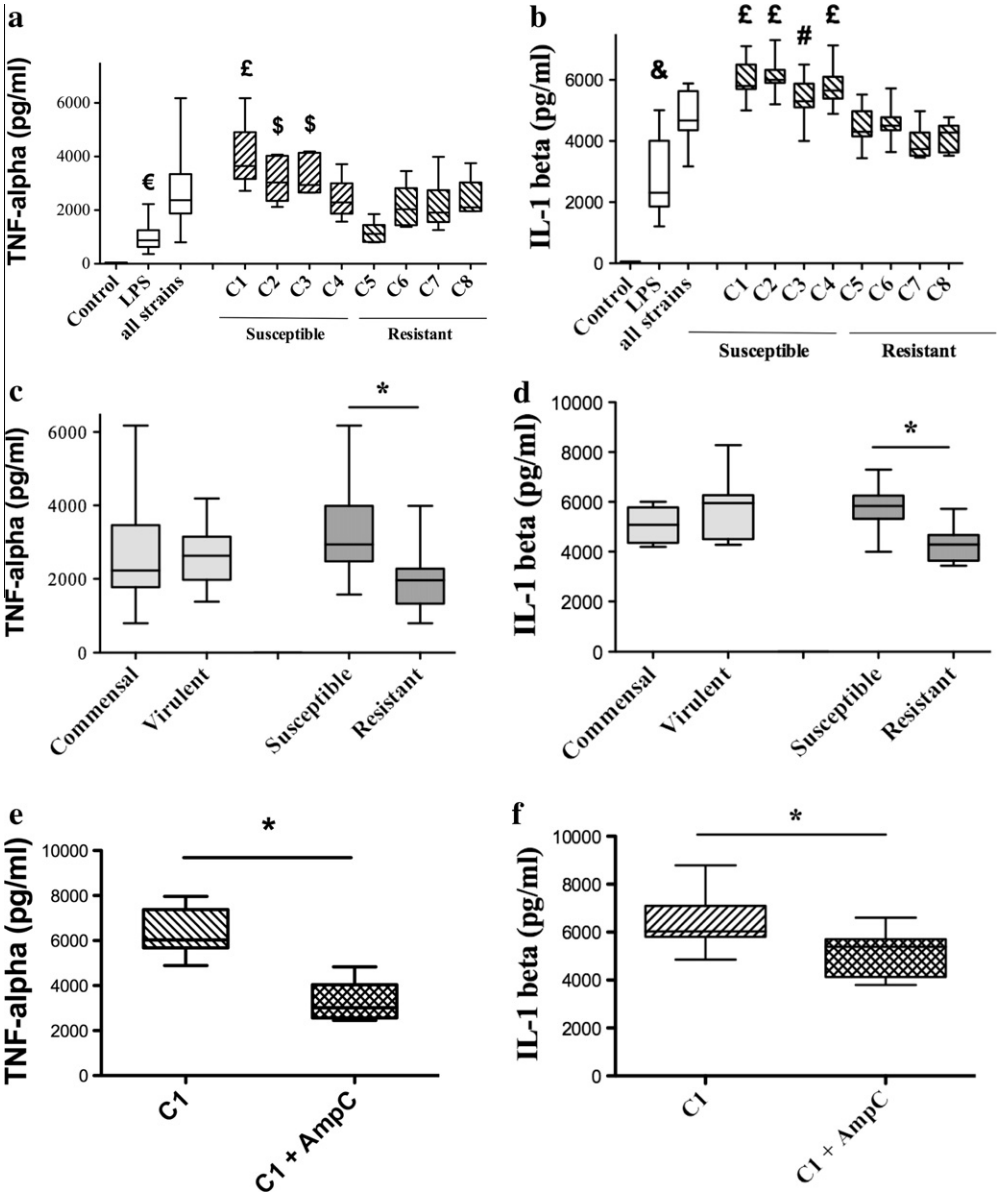

Fig. 1. Influence of phylogenetic group and antibiotic resistance phenotype on TNF-α and IL-1β production TNF-α and IL-1β productions analyzed by ELISA. (a–b) Diluted whole blood from healthy donors was incubated 150 min at 37 °C with medium alone (control), or medium containing LPS O111:B4 (10 μg/mL) or with eight *Escherichia coli* clinical strains (C1–C8; 10^6 CFU/mL). (c–d) Commensal (phylogenetic groups A and B1) and virulent (B2 or D) strains as well as susceptible and resistant strains were compared. (e–f) The transformed *E. coli* strain C1 (with a plasmid-mediated CMY-2 AmpC-β-lactamase gene) was compared to the wild type C1 strain. Cytokine concentrations in the absence of stimulation were always below the detection threshold (60 pg/ml). Data are representative of two independent experiments (each group, $n = 6$). Boxes represent median (interquartile range). € $p < 0.01$ *versus* All strains group and *versus* Strains C1, C2 and C3; £ $p < 0.01$ *versus* resistant strains; $ $p < 0.01$ *versus* C5; & $p < 0.01$ *versus* All strains group and *versus* susceptible Strains; # $p < 0.05$ *versus* C5, C7 and C8, $^*p < 0.05$.

los-Bourboulis et al. compared cytokine production by human monocytes, after stimulation with susceptible and resistant *Pseudomonas aeruginosa* strains [26]. Although they did not highlight any difference regarding TNF-α production, susceptible strains did induce a greater production of two other pro-inflammatory cytokines, IL-1β and IL-6, compared to resistant strains. The same researchers obtained the opposite results with *E. coli* strains: concentrations of TNF-α, IL-6, and IL-8 were higher after stimulation by MDR strains than with susceptible strains [9]. In the latter study, the authors stimulated isolated monocytes rather than whole blood cells, and the monocytes were therefore not kept in their natural environment.

By using specific inhibitors, we explored the signaling pathways that may differentially affect TNF-α production. TNF-α production by monocyte/macrophage or whole blood after LPS or Gram-negative bacteria stimulation involves different intracellular signaling pathways downstream of TLR-4. These pathways include NF-κB, MAPK or PI3 K that cooperatively modulate the inflammatory response to pathogens or to LPS and other constituents of Gram-negative bacteria [4,30]. Considering all *E. coli* strains, the present results show that either p38 or ERK inhibitor dramatically decreased the TNF-α production. This indicates that the viable *E. coli* strains studied here require p38 and ERK kinases for induction of TNF production. Stimulation of human monocytes or THP-1 cells with LPS (*E. coli*) led to similar results regarding the ERK and p38 pathways [6,16]. Despite the clinical importance of gram negative infections, only limited attention has been paid to the role of PI3-K in cellular activation induced by *E. coli*. The present results obtained with viable *E. coli* strains, did not reveal any effect in spite of the use of a high concentration of wortmannin. Most studies,

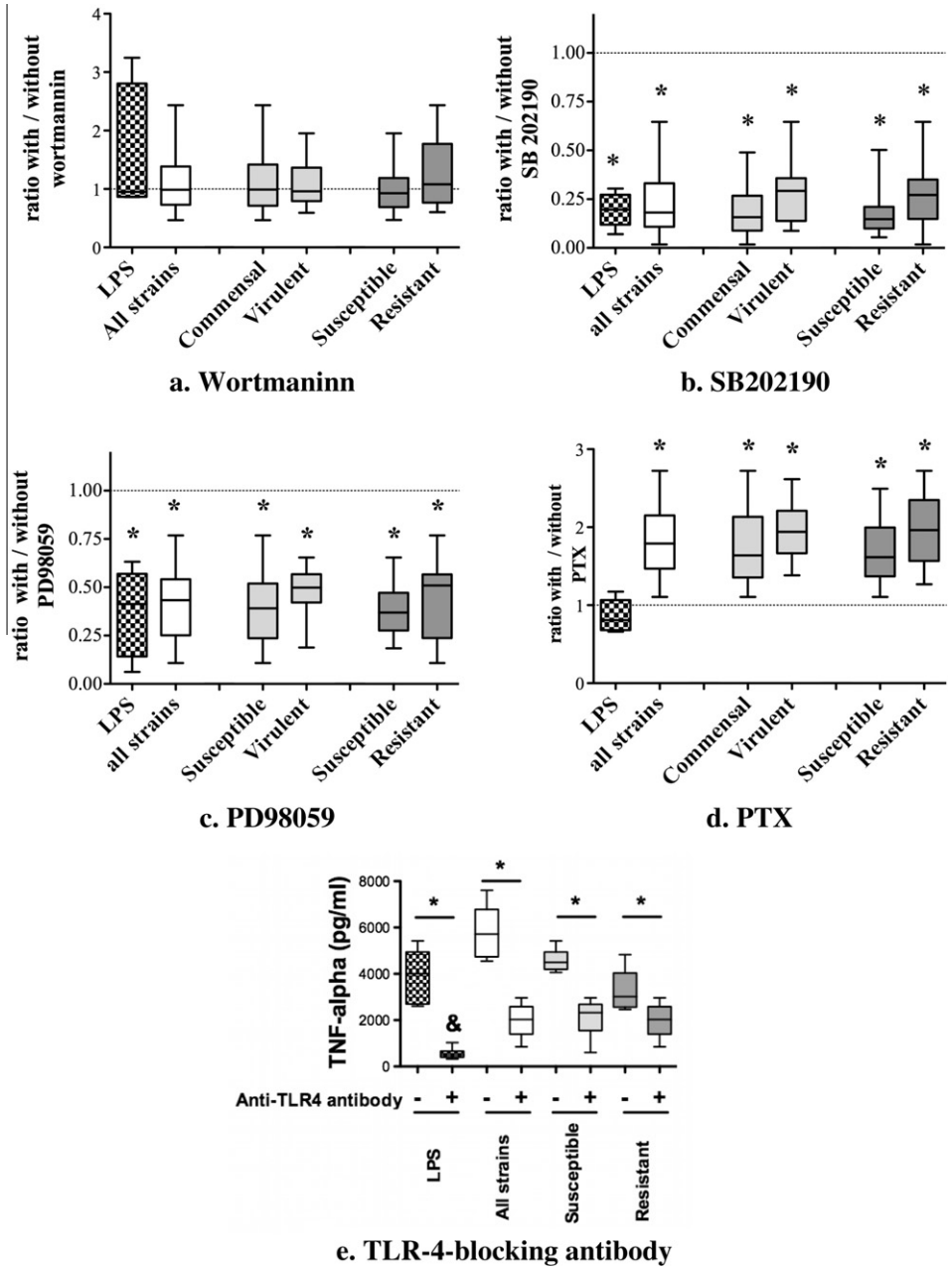

Fig. 2. Influence of phylogenetic group and antibiotic resistance phenotype on signaling pathways of TNF-α. TNF-α production analyzed by ELISA. Diluted whole blood from healthy donors was pretreated for 30 min prior to stimulation at 37 °C with the following inhibitors: (a) wortmannin 1 μM, (b) SB202190 15 μM, (c) PD98059 30 μM, (d) PTX 0.4 μM, (e) TLR-4-blocking antibody 10 μg/mL. The human blood cells were then incubated for 150 min at 37 °C with LPS O111:B4 (10 μg/mL) or with eight *Escherichia coli* clinical strains (10^6 UFC/mL). Data are representative of two independent experiments (each group, $n = 6$). The condition with inhibitor was compared to the condition without inhibitor through a calculated ratio. The calculated ratio was compared to the theoretical ratio (= 1, no effect of inhibitor, dotted line). Graphs represent the median (interquartile range) of the calculated ratio. Cytokine concentrations in the absence of stimulation were always below the detection threshold (60 pg/mL). *$p < 0.05$ *versus* theoretical ratio. & = $p < 0.05$ *versus* all strains.

using LPS, revealed that PI3-K activation in monocytes after TLR4 stimulation, decreased pro-inflammatory cytokines production [30,31]. The discrepancies between our results and previous reports indicate that results obtained with LPS are different from those obtained when viable bacteria are used. The signaling pathway, which activates PI3 K downstream of TLRs, is not completely characterized and remains highly controversial. Moreover, some pathogen can affect NF-κB signaling pathways. In particular, inhibition of NF-kB allows the pathogen to interfere with the immune response in a way that promotes its growth and dissemination [27]. Multiple Drug Resistant *Pseudomonas aeruginosa* was demonstrated to induce an early apoptosis of blood monocytes [28]. It was also demonstrated that *E. Coli* may induce apoptosis, decreasing therefore pro-inflammatory cytokines production, via quorum-sensing (QS) signal molecules activation [29]. An early activation of QS molecules may be an explanation regarding the lower production of IL-1β and TNF α in resistant bacteria.

Studies have shown that the response to LPS may involve a pertussis toxin-sensitive G-protein [32]. It was also demonstrated that blocking the pertussis toxin-sensitive G-protein with PTX exerts negative effects on LPS-induced TLR-4 activation. In particular, TNF-α induction by *E. coli* LPS was suppressed by 0.1 or 1 μg/mL

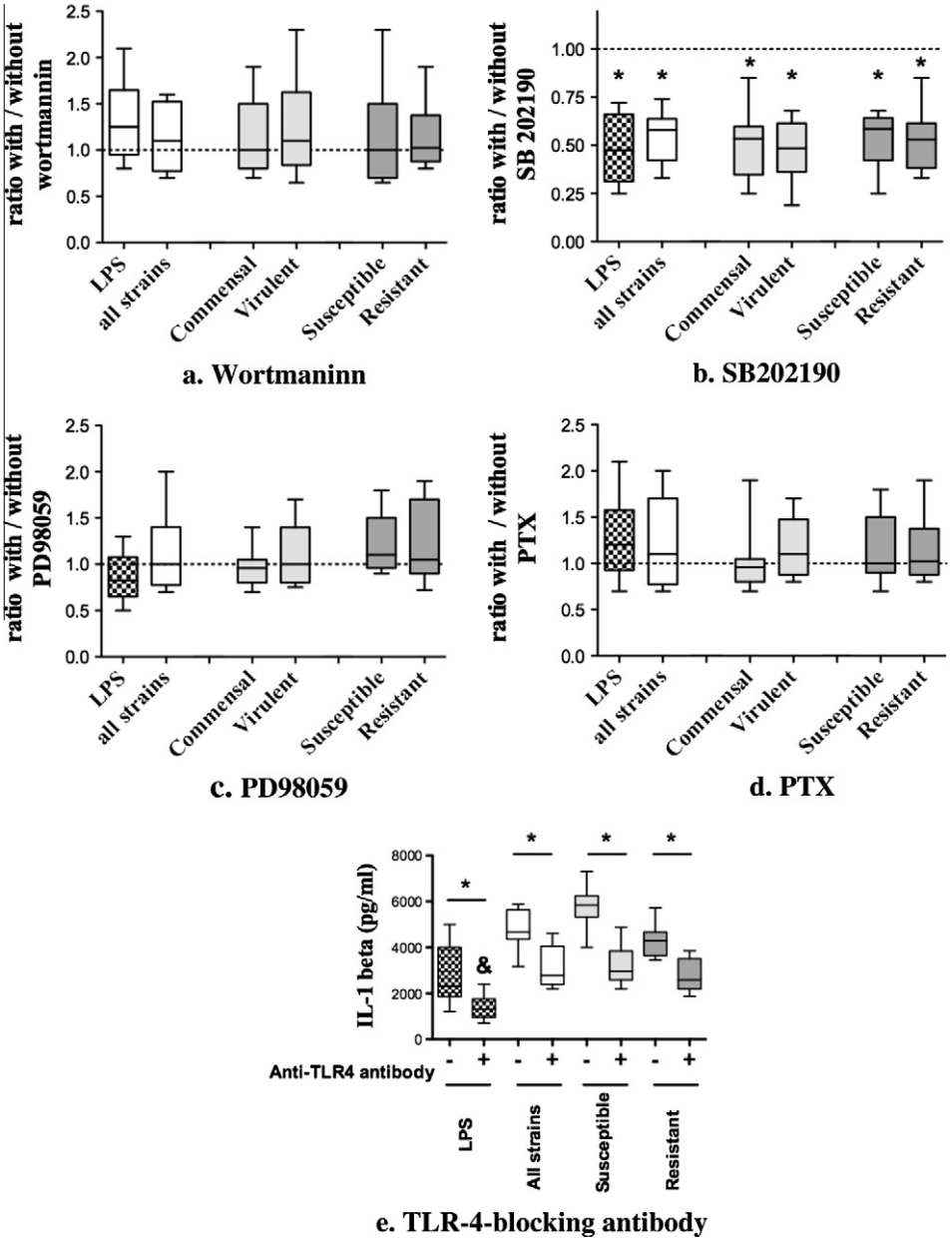

Fig. 3. Influence of phylogenetic group and antibiotic resistance phenotype on signaling pathways of IL1-β. IL1-β production analyzed by ELISA. Diluted whole blood from healthy donors was pretreated for 30 min prior to stimulation at 37 °C with the following inhibitors: (a) wortmannin 1 μM, (b) SB202190 15 μM, (c) PD98059 30 μM, (d) PTX 0.4 μM or (e) TLR-4-blocking antibody 10 μg/mL. The human blood cells were then incubated for 150 min at 37 °C with LPS O111:B4 (10 μg/mL) or with eight *Escherichia coli* clinical strains (10^6 UFC/mL). Data are representative of two independent experiments (each group, $n = 6$). The condition with inhibitor was compared to the condition without inhibitor through a calculated ratio. The calculated ratio was compared to the theoretical ratio (= 1, no effect of inhibitor, dotted line). Graphs represent the median (interquartile range) of the calculated ratio. Cytokine concentrations in the absence of stimulation were always below the detection threshold (60 pg/mL). $^*p < 0.05$ *versus* theorical ratio. & = $p < 0.05$ *versus* all strains.

of PTX, in THP-1 cells [19]. To the best of our knowledge, no data are available regarding viable bacteria. The present results show that, for the first time, inhibition of pertussis toxin-sensitive G-protein with PTX dramatically increases the TNF-α production after stimulation of whole blood cultures with viable *E. coli* strains: this indicates that toxin-sensitive G-protein is a negative regulator of *E. coli*-induced TNF-α production. PTX alters TNF-α production after viable bacteria stimulation, but not after LPS (TLR-4) stimulation. This indicates that viable *E. coli* exerts an effect on the pertussis toxin-sensitive G-protein through a TLR-4 independent mechanism.

Isogenic strains with and without β-lactamase define the resistant and susceptible strains respectively. When using inhibitors of signaling pathways, the differences observed in terms of TNF-α production between susceptible and resistant strains (Fig. 1b) were abolished (Fig. 2). This result suggests that PI3-K, p38, ERK and G proteins differentially regulate susceptible or resistant strains in terms of TNF-α production. In an attempt to explain the differences observed between susceptible and resistant strains regarding signaling pathways engagement, the main virulence-associated genes [33–35] were determined in each strain but no major differences were apparent. It has been reported that the genetic acquisition of resistance occurs frequently through genes involved in bacterial survival [36], for example, factors that allow immunity escape. TNF production is essentially driven by the NF-kB pathway [37]. The results obtained with IL-1β confirm that other pathways are involved in host defense against virulent/resistant bacteria. Future experiments will aim to identify such genes in an attempt to ex-

plain this difference between susceptible and resistant strains regarding the inflammatory response and signaling pathways.

The main limitation of our study relies on the number of strains used. However, our main goal was to mimic, *ex vivo*, a clinical scenario. We therefore used clinical strains isolated from patients. These strains were identified in terms of phylogenetic group as well as virulent factors. Also, the resistance patterns of resistant strains were characterized and described by our group as well as by other groups [10,38]. Another limitation may rely on the short time used to stimulate the blood cultures. This technical limitation is because *E. coli* is a rapidly multiplying bacteria in growth media: its growth after 3 h may, therefore, alter the function of white blood cells in culture. Regarding both Interferon γ and IL-10 the values remained below the detection threshold for all groups mainly for technical reasons. Indeed, accounting for the fast proliferation of living bacteria in whole blood cell cultures, we couldn't challenge the cultures for a longer time without disturbing cell cultures conditions. We [39] and others [9] have already reported these restrictions.

5. Conclusion

In conclusion, p38, and ERK kinases as well as G proteins play a major role in viable *E. coli* strains inducing cytokine production in whole blood cells cultures. Also, these signaling pathways differentially regulate cytokine productions with regard to susceptible or resistant strains: susceptible strains induce higher TNF-α and IL-1β productions than strains producing chromosomal or plasmidic-acquired β-lactamases. Finally, *E. coli* exerts an effect on the pertussis toxin-sensitive G-protein through a TLR-4 independent mechanism.

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GA, ARo, JPS performed the experiments. GA, SC, ARo, JPS, ARe, KA participated in data interpretation. GA, SC, ARo, JPS, DL, ARe, KA participated to redaction of the manuscript.

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