Biosensors and Bioelectronics 24 (2009) 2598–2603

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Detecting and differentiating microbes by dendritic cells for the development of cell-based biosensors

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ARTICLE INFO

Article history:
Received 19 September 2008
Received in revised form 8 January 2009
Accepted 9 January 2009
Available online 23 January 2009

Keywords:
Immune cells
Lipopolysaccharide
Nitric oxide (NO)
Toll-like receptors

ABSTRACT

Dendritic cells (DCs) are a specialized family of antigen presenting cells. They play critical roles in sensing and processing microbial information through a series of pattern recognition receptors (PPRs), including the well-characterized toll-like receptors (TLRs). In this study, we demonstrated the utilization of a DC cell line, DC2.4, as a cell source for the detection and differentiation of microbes towards the development of cell-based biosensors. As a proof of principle, the Gram-negative bacteria *Escherichia coli* K12 strain D21 and its lipopolysaccharide (LPS) mutants were used as model targets. The stimulation of DCs by bacterial strains was monitored by the production of nitric oxide (NO), and the colorimetric Greiss assay was used to quantify the level of NO produced. Our results demonstrated that DCs could detect and differentiate microbes with subtle differences in the composition of specific cell surface components, i.e. LPS, within minutes. Though the current colorimetric-based NO assay limited the detection sensitivity, we showed that DCs were able to detect as low as 2–3 bacteria per cell. Furthermore, compared to macrophages, DCs were superior in discriminating LPS mutants. Our study demonstrates that DCs possess great potential as cell sources for the development of novel cell-based biosensors for detecting microbes with high selectivity and sensitivity and rapid responsiveness. In addition, when DCs are coupled with other biosensor platforms, higher sensitivity can be expected.

1. Introduction

Cell-based biosensors offer a promising alternative to conventional detection technologies. They typically employ live cells as the sensing component and convert cellular responses to environmental perturbations such as pathogens into signals conducive for processing. Many types of cells, such as bacteria, yeasts, and mammalian cells have been used for constructing cell-based biosensors (Pancrazio et al., 1999; Belkin, 2003). Compared to conventional chemical and biochemical biosensors, cell-based biosensors avoid the need for the extraction of enzymes or the development of antibodies, and yield more detailed and physiologically relevant information that can be used to categorize the specific nature of biological or chemical agents (Pancrazio et al., 1999; Daunert et al., 2000; Stenger et al., 2001).

Immune cells are thought as "Nature's biosensors" because they can specifically recognize and signal the presence of an environmental threat, and then establish corresponding innate and/or adaptive immune responses (Janeway, 2005). Several immune cells, such as mast cells (Pizziconi and Page, 1997; Curtis et al., 2008), B cells (Rider et al., 2003; Kim et al., 2004; Banerjee et al., 2008), T cells (Kim et al., 2006) and macrophages (Davies et al., 2005; Koh and Pishko, 2006; Veiseh et al., 2007), have been examined as resources for cell-based sensors. Dendritic cells (DCs), the most potent antigen presenting cells, represent a promising candidate for cell-based biosensors. Different from other immune cells, DCs act at the interface between the innate and adaptive-recognition systems and play a key role in coupling both arms of the immune systems (Banchereau and Steinman, 1998; Janeway and Medzhitov, 2002; Iwasaki and Medzhitov, 2004). Therefore, DC-based biosensors can potentially generate information on how an environmental threat would have an impact on the innate and/or adaptive immune system.

Immature DCs are distributed throughout the body, particularly at the portals where most pathogen encounter occurs. Through a series of pattern recognition receptors (PPRs), particularly toll-like receptors (TLRs), DCs can detect and signal the presence of pathogens or their products (Janeway and Medzhitov, 2002; Akira et al., 2006). Concomitantly, DCs undergo coordinated reprogramming of gene expression, leading to unique phenotypic and functional changes depending on pathogens or chemicals encountered (Banchereau and Steinman, 1998; Iwasaki and Medzhitov, 2004). Recently, genetically engineered DCs have been tested for their response to a variety of chemotherapeutic drugs (Mizumoto

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doi:10.1016/j.bios.2009.01.017

et al., 2005). It has been demonstrated that DCs exhibited distinct functional and phenotypic changes in response to drugs with different chemical structures.

TLRs are essential for DCs to accomplish their roles in sensing and processing microbial information and directing other cell types to respond accordingly (Medzhitov and Janeway, 1997; Iwasaki and Medzhitov, 2004). TLR family consists of 12 members in the human genome. TLR1, 2, 4, 5, and 11 are expressed on the cell surface and are involved in detecting extracellular pathogens or their cellular components (Akira et al., 2006). In comparison, TLR3, 7, 8 and 9 are expressed in endosomal compartments and involved in detecting viral pathogens or their cellular components.

In this study, to evaluate DCs as a source for cell-based biosensors, lipopolysaccharide (LPS) mutants derived from *Escherichia coli* K12 strain were chosen as model targets. LPS, clinically termed as endotoxin, is the outer cell wall of Gram-negative bacteria. It induces sepsis, which is one of the major health threats (Munford, 2006). About 750,000 people in the United States develop severe sepsis annually, and more than 200,000 people die of it (Martin et al., 2003). LPS of *E. coli* consists of an O-specific chain, a core oligosaccharide, and a lipid component (lipid A). The endotoxic activities of LPS, characterized by the production of diverse mediators of inflammation, such as nitric oxide (NO) and proinflammatory cytokines, have been shown to be closely related to the molecular structure of lipid A (Rietschel et al., 1994; Netea et al., 2002). However, few studies have demonstrated whether the activities of LPS are affected by the molecular structure of core oligosaccharides (Nowotny et al., 1975; Williamson et al., 1984). The LPS mutants used in this study contains progressively truncated core oligosaccharides of LPS (Eriksson-Grennberg et al., 1971; Boman and Monner, 1975; Havekes et al., 1977). DC-based biosensors would provide insightful information on the effect of the molecular structure of oligosaccharides on the activities of LPS mutants.

TLR2 and 4 have been reported to be responsible for the recognition of LPS (Kirschning et al., 1998; Poltorak et al., 1998; Hoshino et al., 1999; Qureshi, 1999; Werts et al., 2001). When LPS is engaged with TLR2 and/or TLR4, a cascade of signaling pathways is triggered, resulting in the production of proinflammatory cytokines and chemokines (Akira and Takeda, 2004; Akira et al., 2006). Inducible nitric oxide synthase (iNOS) is also up-regulated via the activation of the NF-kB pathway, leading to increased NO production (Palsson-McDermott and O'Neill, 2004). It has been shown that the level of NO production is closely associated with the molecular structures of LPS (Zughaier et al., 2005). Recent studies have suggested that the production of NO correlates with the secretion of cytokines as well (Kmonickova et al., 2007).

Therefore, we quantified the level of NO production by DCs as the readout by the well-established Griess chemical method. The Griess chemical method is not as sensitive as fluorescence and chemiluminescence-based methods (Fiddler, 1977; Nagano, 1999), but its ease of operation makes it attractive to prove our concept of characterizing DCs for the development of cell-based biosensors. We demonstrated that DCs were able to differentiate LPS mutants within minutes with a sensitivity as low as 2–3 bacteria per cell. In comparison to macrophages, DCs were superior in discriminating bacteria with subtle differences on the major component of their cell wall.

2. Materials and methods

2.1. Cell and growth conditions

The DC2.4 cell line was kindly provided by K.L. Rock (Dana Farber Cancer Institute, Boston, MA, USA). DC2.4 cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) with 1% L-glutamine (Invitrogen), 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Invitrogen), 50 μM 2-mercaptoethanol (Invitrogen) and 10% fetal bovine serum (FBS, Invitrogen) in an incubator with 5% CO_2 and 95% air at 37 °C. RAW264.7 murine macrophages were obtained from America Type Culture Collection (ATCC, USA), maintained at 37 °C in DMEM medium with 10% FBS, 4 mM L-glutamine, 1 mM sodium pyruvate (Invitrogen), 50 units/ml penicillin (Invitrogen), and 50 mg/ml streptomycin (Invitrogen).

2.2. Bacterial strains

E. coli K-12 mutants deficient in LPS synthesis were obtained from the *E. coli* Genetic Stock Center (Department of Biology, Yale University, New Haven, CT, USA). The parental strain D21 contains intact core LPS molecules while the isogenic mutants, including D21e7, D21f1, and D21f2, synthesize progressively truncated LPS, respectively (Boman and Monner, 1975; Havekes et al., 1977). D21e19 lacks a branched galactose residue (Eriksson-Grennberg et al., 1971). All the strains were grown in Luria broth at 37 °C.

2.3. Differentiation of LPS mutants by DCs or macrophage cells

DC2.4 or RAW264.7 cells were plated onto 96-well plates at a concentration of 10^5 cells/well and incubated at 37 °C in 5% CO_2 and 95% air. *E. coli* suspensions were washed with Dulbecco's phosphate buffered saline for three times and treated with 200 μg/ml gentamicin at 37 °C for 2 h. The viability of gentamicin-treated LPS mutants was confirmed by re-plating them onto LB agar and observing no growth of colonies. This insured that gentamicin-treated LPS mutants would not grow in the presence or absence of DCs or macrophages. At designated time points, 100 μl of gentamicin-treated *E. coli* strains at a given concentration were added to cells and centrifuged at 1500 rpm for 5 min to enhance the contact between bacteria and cells. At a given period of time, the supernatants were collected for NO assay. In addition, the viability of DC2.4 and Raw264.7 cells was examined by trypan blue exclusion method and there was no apparent cell death.

2.4. Griess assay

NO is very unstable, and under physiological conditions, it is readily oxidized to nitrite (NO_2^-) and nitrate (NO_3^-). The production of NO by DC2.4 or RAW264.7 cells was quantified with the Griess chemical reaction, which was based on the detection of nitrite. The Griess assay kit, which includes N-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilic acid and nitrite standard solution, was purchased from Invitrogen (Carlsbad, CA, USA). The supernatants from DC2.4 or RAW264.7 cells exposed to bacterial strains were mixed with the Griess Reagent in a 96-well plate. The absorbance of the resulting mixtures was measured by a VERSAmax tunable microplate reader (Molecular Devices Corporation, Sunnyvale, CA, USA) at 530 nm. Nitrite concentrations were derived from a standard plot constructed with known concentrations of sodium nitrite. Nitrate produced was determined by reducing the nitrate to nitrite with the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase (NR). The difference of measured nitrite before and after enzyme treatment was the quantity of nitrate in solution. The concentration of nitrate in supernatants collected from cells in the presence and absence of LPS mutants is the same (Supplemental Table 1). Therefore, only nitrite concentration was measured as the indication of the production of NO.

2.5. Real-time quantitative PCR

DC2.4 or RAW264.7 cells were plated onto 6-well plates at a concentration of 10^6 cells/well and incubated at 37 °C in 5% CO_2 and 95% air in the presence or absence of 10 nM of LPS derived from *E. coli* K12 D21. After 24 h, the total RNA of the DCs was extracted with RNeasy Mini Kit (Qiagen Corporation, Valencia, CA). All of the PCR primers were synthesized by Invitrogen (Carlsbad, CA, USA). Primers used in real-time PCR are shown in Supplemental Table 2. The mRNA was reverse-transcripted into cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) in 20 μl of reverse-transcription (RT) reaction solution. The reaction solution contained 1 μg of total RNA, 2 pmol of antisense primer, 5 mM dithiothreitol, 0.5 μM of the deoxynucleotide triphosphates, 1× first strand buffer and 1 μl of the reverse transcriptase. Real-time quantitative PCR was performed in the Chromo4 Real-Time PCR Detector (Bio-rad Corporation, Hercules, CA, USA). The reaction consisted of 12.5 μl Platinum SYBR Green qPCR SuperMix (Invitrogen, Carlsbad, CA, USA), 125 pM of primers and 10 ng of cDNA. Amplification was carried out by using the following program: (i) UDG incubation at 50 °C for 2 min to improve specificity; (ii) initial denaturation at 95 °C for 2 min; (iii) 50 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min and (iv) final extension at 72 °C for 7 min. After amplification, a melting curve was acquired by heating the product from 30 to 95 °C at 1 °C/min and holding at each temperature for 1 s. Based on the melting curve, the specificity of PCR products was determined. The number of template molecules present in the initial reaction mixture (X_0) was calculated basing on the formula $X_0 = X(1+E)^{-C_t}$, where X was the number of molecules synthesized at the threshold cycle (C_t) (Meijerink et al., 2001), E was the reaction efficiency. X was proportional to the fluorescence recorded. To take into account the differences in the amount of cDNA loaded between samples, the X_0 of the gene of interest was normalized against that of GAPDH, an endogenous housekeeping gene. The results were expressed as the relative mRNA level.

2.6. Microscopy analysis of the morphology of cells

A Nikon TEU 2000 microscope equipped with a Cool Snap CCD camera was used to examine the morphology of DCs or macrophages after being exposed to bacteria. Cells were co-cultured with bacteria for 24 h on flat-bottom 6-well plates (BD Biosciences, San Jose, CA, USA), and then phase-contrast images were acquired.

2.7. Statistical analysis

Three independent experiments were performed for each study. In each experiment, triplicates were used. Values were expressed as mean ± standard error of the mean.

3. Results and discussion

3.1. TLR2 and 4 expression in DC2.4 cells

Due to the difficulty of obtaining a large amount of primary DCs and maintaining *in vitro* cultures, a cell line, DC2.4, had been developed previously (Shen et al., 1997). This cell line has been widely used for testing the efficacy of vaccines (Cheadle et al., 2005; Vangasseri et al., 2006). However, the detailed characterization of the expression of TLRs has not been carried out. The major difference among the *E. coli* strains used in this study is in the synthesis of LPS molecules (Eriksson-Grennberg et al., 1971; Boman and Monner, 1975; Havekes et al., 1977). Both TLR2 and 4 have been shown to be responsible for the recognition of LPS (Kirschning et al., 1998; Poltorak et al., 1998; Hoshino et al., 1999; Qureshi, 1999; Werts et al., 2001). Therefore, we first analyzed the mRNA expression of TLR2 and 4 in DC2.4 cells by real-time quantitative PCR. As demonstrated in Fig. 1A, both TLR2 and 4 are constitutively expressed in DC2.4 and up-regulated upon the stimulation by LPS extracted from *E. coli* K12 D21. In addition, the levels of proinflammatory cytokines, IL-6 and TNFα, were up-regulated in response to the stimulation of LPS (Fig. 1B). These results demonstrate that TLR2 and 4 on DC2.4 cells are functionally active. Thus, DC2.4 cells can be used as a biosensor platform for detecting *E. coli* K-12 LPS mutants.

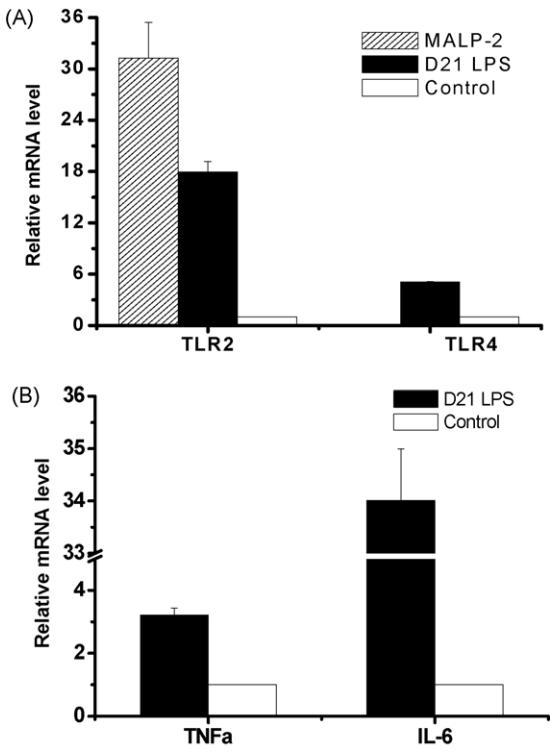

Fig. 1. (A) Relative mRNA level of TLR2 and 4. (B) Relative mRNA level of TNFα and IL-6 in the presence or absence of LPS and MALP-2. DC2.4 cells were plated onto 6-well plates at a concentration of 10^6 cells/well and incubated at 37 °C in 5% CO_2 and 95% air in the presence or absence of 10 nM of LPS and MALP-2.

3.2. Stability of DC2.4 cell lines

Mammalian cell-based biosensors have a distinct advantage in providing insight into physiological effects of analytes; yet, the signals obtained from them often vary due to the heterogeneity of cell populations and the variance between generations. We therefore tested whether DC2.4 cells from different generations would generate a reproducible level of NO in response to the same level of stimuli. Instead of bacteria, LPS extracted from *E. coli* K12 D21 was used as the stimuli so that the variation from the heterogeneity of bacterial populations can be excluded. DC2.4 cells, which had been in culture for 9, 12, 15 and 18 days, were stimulated with 10 nM of LPS extracted from *E. coli* K12 D21. As illustrated in Fig. 2, DC2.4 cells from different generations produced a relatively stable level of NO with a fluctuation within 3.5%. In addition, in the absence of LPS stimulation, DC2.4 cells produced insignificant levels of NO during the course of culture (data not shown). Therefore, DC2.4 cells are relatively stable in culture and are ideal as a cell resource for cell-based biosensors.

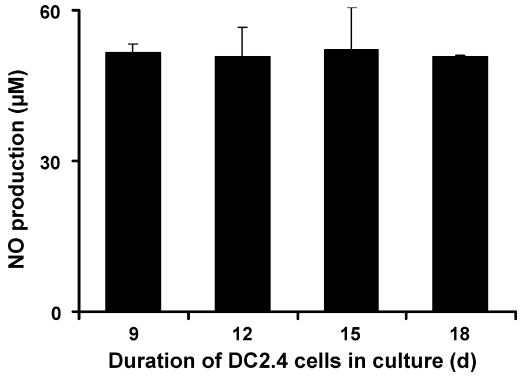

Fig. 2. NO production of the DC2.4 cells from different generations. DCs in culture for 9, 12, 15 and 18 days were stimulated with 10 nM of LPS extracted from *E. coli* K12 D21. 24 h later, the concentration of NO in the supernatants was analyzed by the Griess assay.

3.3. Time-resolved responses of DC2.4 cells

A set of isogenic *E. coli* K-12 strains, which carried mutations in the genes involved in LPS synthesis, was chosen to evaluate the ability of DC2.4 cells in differentiating bacteria with subtle differences. These mutants consisted of LPS molecules with progressively truncated core polysaccharide structures (Fig. 3A). Initially, the time-dependence of NO production by DC2.4 cells in response to *E. coli* K12 LPS mutants was measured. DC2.4 cells were exposed to bacteria for up to 24 h at the concentration of 100 bacteria/DC. As shown in Fig. 3B, for all the bacterial strains, a burst of NO production was observed within 3 h followed by a slow increase up to 24 h. However, distinct kinetics of NO production was observed for each bacterial strain. For the bacterial strain with shorter core oligosaccharides, such as D21f1, D21f2 and D21e7, a significant level of NO production was observed within 30 min after the exposure to DC cells. In contrast, for the bacteria with longer core oligosaccharides, such as D21e19 and D21, a 1-h lag phase was observed followed by a noticeable amount of NO production 1.5 h after the exposure. Within the first half an hour, the rate of NO production was inversely correlated to the length of the core oligosaccharide of LPS, in the order of D21f2 > D21f1> D21e7 > D21e19, D21 (Fig. 3C). These results demonstrate that the response-time of DCs is closely related to the molecular structure of LPS of bacteria, which can be used as a means of differentiating bacterial strains with distinct LPS structures and possibly various types of LPS (endotoxin).

3.4. Dose-responses of DC2.4 cells

DCs were exposed to the LPS mutants with varying concentrations ranging from 2.5 to 50 bacteria/DC for 24 h (Fig. 4). For different bacterial strains, DCs exhibited different dose-responses. For D21e19 and D21, NO production by DC2.4 cells increased with concentration up to 12.5 bacteria/DC and remained almost constant thereafter. For D21f1, D21f2 and D21e7, the level of NO production continuously increased within the range of concentration investigated. The difference between strains in terms of the level of NO production by DCs was insignificant when the concentration of bacteria was below 12.5 bacteria/DC. The difference, however, became evident when the concentration of bacteria was above 25 bacteria/DC. Interestingly, the level of NO production by DC2.4 cells was again inversely correlated with the length of core oligosaccharides of LPS on these strains, with the shorter core oligosaccharide eliciting higher level of NO production. To rule out that the amount of LPS on each LPS mutant may contribute to the differences of dose-response, we quantified the amount of LPS by

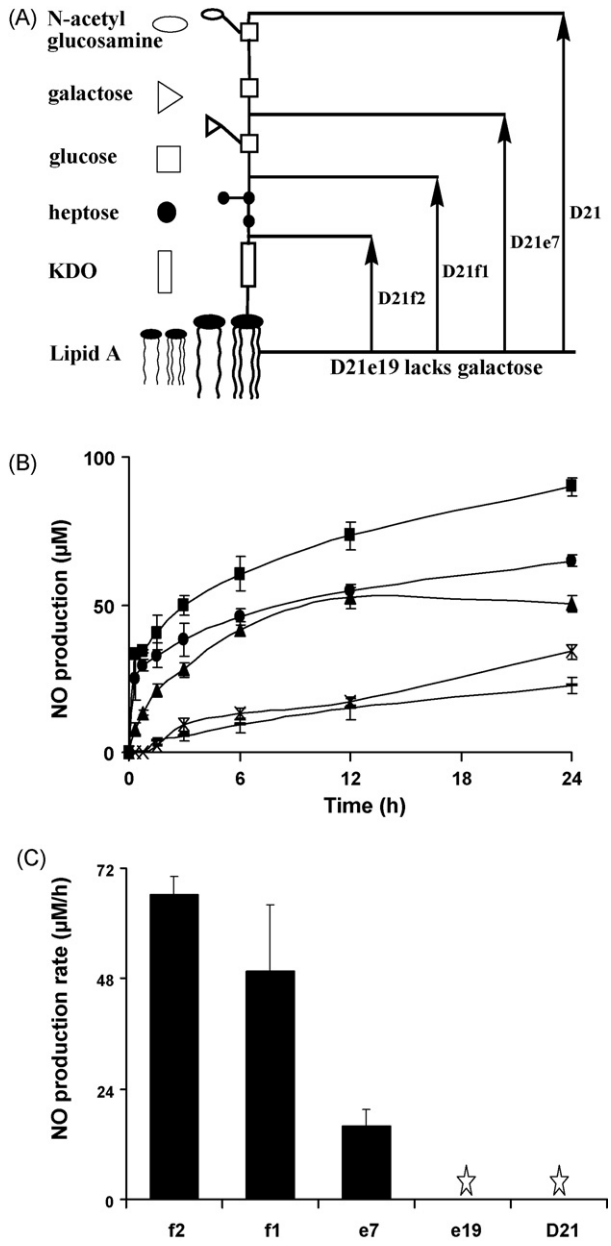

Fig. 3. (A) The LPS oligosaccharide structures of *E. coli* K12 strain D21 and its isogenic mutants D21f2, D21f1, D21e7 and D21e19. (B) Time-resolved NO production by DCs in response to *E. coli* strains. DCs were plated onto 96-well plates and treated with different strains of *E. coli* at 100 bacteria/DC for up to 24 h. At the given time points, the concentration of NO in supernatants of each well was assayed. ■, ●, ▲, *, and – denote D21f2, D21f1, D21e7, D21e19, D21, respectively. (C) The rate of NO production at the first half an hour upon exposure to bacteria.

either determining the 3-deoxy-D-manno-octulosonic acid (Kdo) content or using the Limulus Amoebocyte Lysate (LAL) method (Supplemental Table 3). There existed a slight difference, but this difference cannot completely explain the difference of NO production by DCs stimulated by LPS mutants. So far, our results have demonstrated that DC2.4 cells could distinguish these bacterial strains with subtle differences on one of the cell wall components, i.e. length and composition of LPS molecules. The ability of DCs to detect different LPS mutants was concentration-dependent, as no discrimination between different strains was observed below 12.5 bacteria/DC.

By correlating the time- and dose-response with their endotoxic activities, DCs can not only potentially distinguish pathogenic

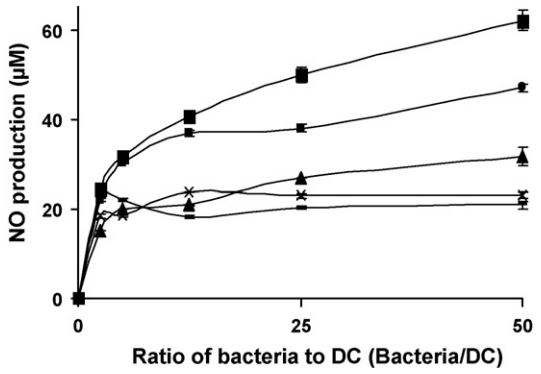

Fig. 4. Dose-response of DC2.4 cells to *E. coli* K12 D21 and its mutant strains: D21f2 (■), D21f1 (●), D21e7 (▲), D21e19 (*), D21 (–). DCs were stimulated with *E. coli* from 0 to 50 bacteria/DC for 24 h.

bacteria from non-pathogenic bacteria but also provide insight into the potential functional consequences of pathogenic bacteria.

3.5. Comparison of DCs and macrophages

We have demonstrated that DCs could be used as a cell source for cell-based biosensors for differentiating LPS mutants. Macrophage cells have previously been utilized as cell-based biosensors for bacterial detection (Davies et al., 2005; Koh and Pishko, 2006; Veiseh et al., 2007). Macrophage cells are well recognized as phagocytes that destroy invading pathogens, but are inefficient in processing antigens and stimulating T cells for the establishment of adaptive immune responses (Steinman, 2007). On the contrary, DCs are situated at the interface between the innate and adaptive immune system, and able to transmit the microbial information to establish potent adaptive immune responses against a specific pathogen (Banchereau and Steinman, 1998; Janeway and Medzhitov, 2002; Iwasaki and Medzhitov, 2004). We reason that DCs might be superior to macrophages as sources for cell-based biosensors in terms of discriminating bacteria strains. To test this hypothesis, we compared DC2.4 cells with the macrophage cell line RAW264.7 for discriminating LPS mutants. Initially, the expression of TLR2 and 4 on RAW264.7 was confirmed by RT-PCR, and the levels of TLR2 and 4 mRNA in RAW264.7 (Supplemental Fig. 1) were similar to those in DC2.4 cells (Fig. 1A). In the presence of LPS derived from D21, the levels of TNF-α and IL-6 were up-regulated as observed in DC2.4 cells (Supplemental Fig. 1). These results demonstrate that TLR2 and 4 on RAW264.7 cells are also functionally active.

RAW264.7 and DC2.4 cells were treated with an equivalent amount of LPS mutants. As demonstrated in Fig. 5A, within 1 h, both DC2.4 and RAW264.7 cells could produce a detectable level

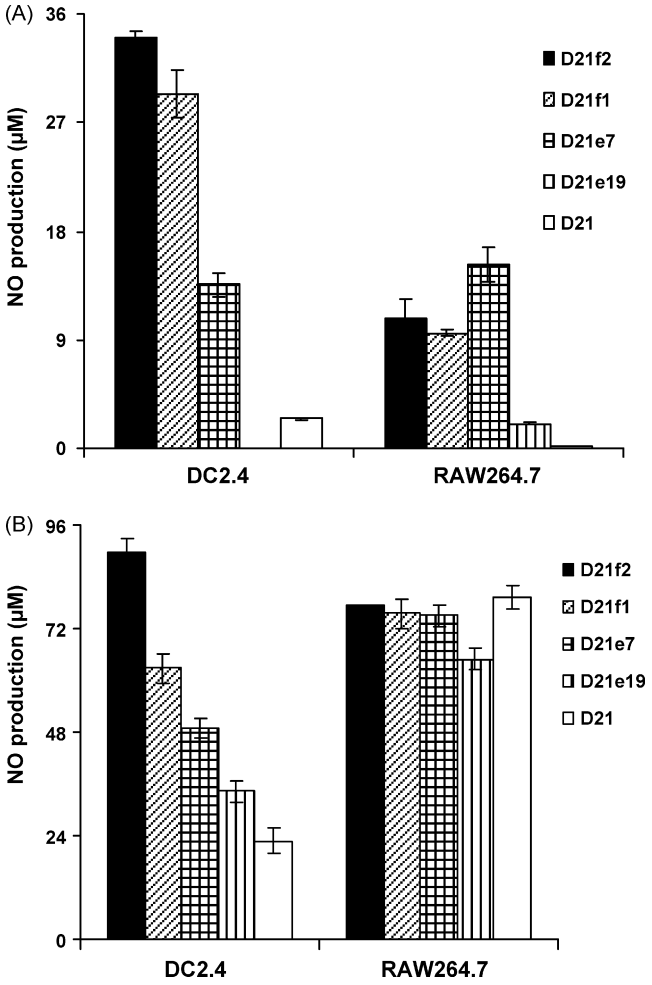

Fig. 5. (A and B) NO production by DC2.4 and RAW264.7 cells in response to *E. coli* strains at 1 and 24 h, respectively. Macrophage cells and DCs were treated with different strains of *E. coli* at a concentration of 100 bacteria/cell for up to 24 h. The NO concentration in supernatants was analyzed at 1 and 24 h.

of NO. However, in response to different LPS mutants, varying levels of NO were observed in DC2.4 cells while the difference was not apparent in RAW264.7 cells. The different selectivity exhibited by DC2.4 and RAW264.7 cells over LPS mutants was even more evident when both cells types were exposed to LPS mutants for 24 h (Fig. 5B). Since both DC2.4 and RAW264.7 cells expressed similar level of TLR2 and 4, the selectivity over different LPS mutants indicate that these two cell types may possess distinct mechanisms of processing microbial information.

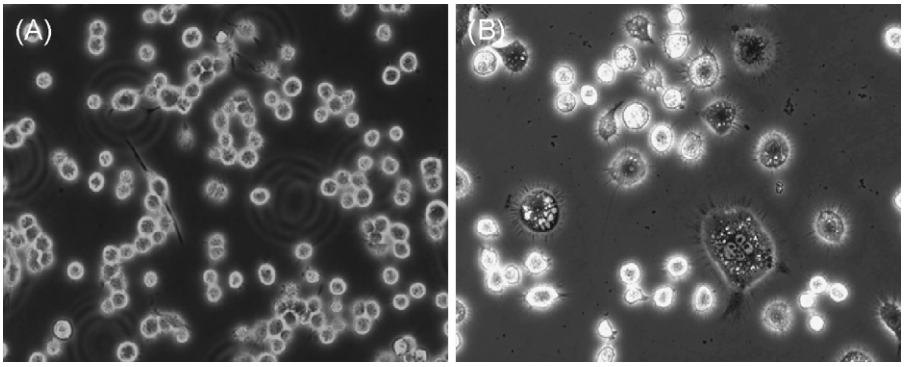

Fig. 6. (A and B) Phase-contrast images of DC2.4 and RAW264.7 cells, respectively. DC2.4 and RAW 264. 7 cells were exposed to *E. coli* D21f2 at a concentration of 100 bacteria/cell for 24 h before the acquisition of microscopy images. A 20× objective was used.

The difference between DC2.4 and RAW264.7 cells in response to bacteria was also examined by monitoring their morphological changes. As illustrated in Fig. 6, RAW264.7 cells displayed an enlarged cell volume while DC2.4 cells retained a relatively constant cell volume. In addition, more vacuoles were observed in RAW264.7 than in DC2.4 cells. These results further support the observations that these two cell types may have distinct roles in processing microbial information. The difference may be due to the intracellular signaling transduction but not the initial recognition through the TLRs since the expression levels of TLR2 and 4 on both cell types are similar (Fig. 1A and Supplemental Fig. 1).

Taken together, though both DCs and macrophages can detect bacteria through TLR2 and 4, DCs are able to discriminate bacterial strains with variations only on the core oligosaccharide of LPS molecules while macrophages cannot. Therefore, as a source for cell-based biosensor, DCs are superior to macrophages in terms of their ability of discriminating bacterial strains.

4. Conclusion

DCs were evaluated as the cell source for cell-based biosensors to detect microbes. The Gram-negative bacteria *E. coli* K12 strain D21 and its LPS mutants D21f1, D21f2, D21e7 and D21e19 were used as model organisms. Our results demonstrated that DCs could differentiate an *E. coli* K12 strain with mutations on LPS. The time- and dose-responses of DCs were dependent on the molecular structure of LPS, which could be used as a means of differentiating bacterial mutants. In comparison to macrophages, DCs demonstrated superior selectivity. Given the pivotal role of DCs in the establishment of both innate and adaptive immunity, DC-based biosensors can not only be used to detect pathogens in a variety of fields including clinical diagnostics, food analysis and environmental monitoring, but also provide critical information on the functional consequences of these pathogens.

Acknowledgements

This work is supported by the University of Washington Start-up fund and the National Science Foundation (NSF) Career Award to H.S.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.01.017.

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