



Development of a mast cell-based biosensor

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Abstract

A mast cell-based biosensor has been developed to enable the use of these cells in numerous applications including pharmaceutical screening, environmental monitoring, clinical diagnosis and homeland security. Rat basophilic leukemia (RBL) mast cells offer excellent potential for biosensor applications because they are robust and undergo a dramatic exocytotic response within minutes of antigen addition. To monitor mast cell activation, fluorescent dyes were loaded into the cells and used as indicators of alkalinization of secretory granules, calcium fluxes or generation of reactive oxygen species. These fluorescence assays efficiently measure activation of antigen-stimulated RBL mast cells, detecting the antigen with picomolar sensitivity. To demonstrate the utility of this mast cell-based biosensor for detection of microbial pathogens, an IgE chimeric protein was created by fusing the Fc region of the IgE antibody to CD14, a receptor for lipopolysaccharide. This chimeric protein has the capacity to bind to *Escherichia coli* and *Listeria monocytogenes* and also to IgE receptors on the mast cells, thereby stimulating a signaling response to bacteria. RBL mast cells labeled with the calcium indicator Fluo-4 are shown to be responsive to *E. coli*, only when sensitized with the chimeric protein, thus demonstrating a highly versatile biosensor for bacterial contamination.

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1. Introduction

Pathogen detection has vital importance in the food industry, water and environmental quality control and clinical diagnosis for health and safety reasons. Traditionally, pathogen detection has been based on cultural, biochemical or other methods such as genetic and immunological-based (Lazcka et al., 2007). Culture methods can take days to perform, and PCR and ELISAs usually require hours to perform. Even though these assays have high sensitivity and specificity, the extended analysis time is an obvious inconvenience in many industrial and environmental applications.

Noninvasive label-free cell-based assays represent a growing area for *in vitro* diagnostics because they offer important advantages over traditional label-based endpoint assays. In label-free cell-based assays the cellular machinery maintains the physiological status of the receptors involved in detection. In addition, these assays allow real-time monitoring of live cells with the preclusion of a labeling step. As such, cell-based biosensors constitute an emerging field with numerous applications including pharmaceutical screening, environmental monitoring, clinical diagnosis and homeland security (Chen et al., 2004; Olivier et al., 2006; Pancraccio et al., 1999; Stenger et al., 2001).

Mast cells offer an excellent potential for biosensor applications because they are robust and undergo a dramatic exocytotic response within minutes of antigen addition. The signal transduction system amplifies the input signal greatly making the system responsive to very small quantities of analyte. Soluble IgE antibodies bind very tightly to receptors (FcεRI) on mast cells, and crosslinking of two or more receptor/IgE complexes by oligovalent ligands, including protein antigens, initiate a sequence of biochemical events that activates the mast cell, causing cellular degranulation and release of inflammatory

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molecules (Kinet, 1999). Some of the cellular events that occur during antigen-induced mast cell activation include alkalinization of the secretory granules (Williams and Webb, 2000), increase of intracellular calcium concentrations (Beaven et al., 1984) and generation of reactive oxygen species in the cytoplasm (Brubacher and Bols, 2001; Suzuki et al., 2003). We previously showed that an in situ assay for degranulation could detect as little as 0.1 ng/mL of antigen in a microtiter-based array (Naal et al., 2004).

The focus of the present study was to determine whether the RBL mast cell response could be engineered to respond to *Escherichia coli* contamination. To monitor RBL mast cell activation, fluorescent dyes were loaded into the cells and used as indicators of alkalinization of secretory granules, calcium fluxes or generation of reactive oxygen radicals. To initiate activation, a chimeric protein, CD14-Fc ϵ , was engineered to bind to IgE receptors at surface of mast cells and to a variety of bacteria. Using dye-loaded RBL mast cells sensitized with the CD14-Fc ϵ protein; the presence of *E. coli* could be detected within minutes of addition. The results of this study support the feasibility and potential versatility of mast cells for the selective detection of a wide range of pathogens and other antigens of interest.

2. Material and methods

2.1. Reagents

Fluo-4 AM, 2',7'-dichlorodihydrofluorescein-diacetate (DCDHF) and Lysotracker were purchased from Molecular Probes Inc. (Eugene, OR, USA). Acridine Orange was purchased from Sigma–Aldrich (St. Louis, MO, USA). Mouse monoclonal anti-2,4-dinitrophenyl (DNP) IgE (Liu et al., 1980) was purified from ascites cells as previously described (Posner et al., 1992). BSA conjugated with an average of 15 DNP groups (DNP–BSA) was prepared as previously described (Hardy, 1986).

2.2. Mast cell preparation

RBL-2H3 cells (Barsumian et al., 1981) were maintained in monolayer culture in minimum essential medium supplemented with 20% fetal bovine serum (Atlanta Biologicals, Norcross, GA, USA) and 10 μ g/mL gentamicin sulfate. Cells were harvested with trypsin/EDTA and resuspended at $\sim 5 \times 10^6$ cells/mL in buffered saline solution (BSS; 135 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 1 mg/mL BSA and 20 mM Hepes, pH 7.4). For confocal fluorescence experiments, 2.0 mL of this cell suspension was plated into glass bottom Petri dishes (MatTek Corp., Natick, MA, USA). The cells were sensitized by addition of three to five-fold molar excess of anti-DNP–IgE over Fc ϵ RI for either 1 h or overnight incubation at 37 °C. For Fluo-4 biosensor-experiments, RBL mast cells were seeded at 0.25×10^6 cells/mL in a 96-well plate (12 $\times$ 8-well strip format) and cultured overnight.

2.3. Bacterial cell preparation

E. coli (DH5 α ; Invitrogen, Carlsbad, CA, USA) were grown in Luria Bertani broth (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl) shaking overnight at 37 °C. The *Listeria monocytogenes* (IHE/90/1104/62-24; American Type Culture Collection (ATCC), Manassas, VA, USA) were grown in ATCC medium 44 shaking overnight at 37 °C. Both bacteria were spun at $2500 \times g$ for 10 min, resuspended in BSS and heat killed at 95 °C for 10 min before use with mast cells. The bacterial concentration in all preparations was determined by dilution and plate counting.

2.4. Confocal fluorescence microscopy

Adherent cells were either loaded with acridine orange (4.5 μ M) for 5 min, Fluo-4-AM (2.5 μ M) or dihydrodichlorofluorescein (DHDCF)/lysotracker (5 μ M and 50 nM, respectively) for 20 min, at 37 °C. Fluo-4 loaded cells were washed with BSS that was supplemented with 0.25 mM sulfinpyrazone to inhibit the dye from leaking out of the cell. Adherent cells in 2 mL of BSS in MatTek wells, were imaged with a BioRad MRC 600 confocal apparatus (BioRad, Cambridge, MA, USA) in conjunction with a Zeiss Axiovert 10 microscope (Thornwood, NY, USA). During measurements, the cells were maintained at 37 °C using a microscope objective heater from Biophtechs (www.biophtechs.com; Butler, PA, USA). All solutions added to the cells were pre-warmed to 37 °C to reduce movement of the focal plane induced by changes in temperature. Either an $\times 63$ or $\times 100$ achromatically corrected objective (NA 1.4 for each) was used to collect simultaneous green and red images of adherent labeled cells. Images were collected every 5 s for 5 min, before and after addition of multivalent antigen, DNP–BSA. The 488 nm line of the krypton–argon ion laser (Ion laser Technology, Salt Lake City, UT, USA), was selected for excitation of Fluo-4, acridine orange or DHDCF, and 510 nm dichroic and 515 nm long pass filters were used to monitor green fluorescence emission. The 568 nm line of a krypton–argon ion laser was selected for excitation of acridine orange (or Lysotracker), and 568 nm dichroic and 585 nm long pass filters were used to monitor red emission. Confocal fluorescence images collected with BioRad Comos software were analyzed with Adobe Photoshop image analysis software (Adobe Systems Inc., San Jose, CA, USA). The fluorescence intensity for each image was quantified using the Scion Image software (Scion Corporation, Frederick, MD, USA) and data from representative experiments are shown in Section 3.

To calculate the cumulative number of Fluo-4 loaded cells activated after *E. coli* addition, confocal images were automatically analyzed using a scripting language within IDL (ITT Visual Information Solutions, Boulder, CO, USA). Regions of fluorescence change were located by calculating a difference image series in which each image is subtracted by the one preceding it. A calcium oscillation recognition protocol was adopted in which each difference image was smoothed and thresholded by an amount given by the average plus 3 standard deviations of the difference image. The resulting regions of fluorescence change were selected according to size. Those smaller than 36 μ m²

were assumed to be isolated noise islands; those greater than $36 \mu\text{m}^2$ were counted. The data from a representative experiment is shown in Section 3.

2.5. Cloning of CD14-Fc ϵ

For the cloning of CD14, total RNA was isolated from the mouse macrophage cell line, P388D (ATCC). For the cloning of the IgE Fc domain, total RNA was isolated from a hybridoma cell line which produces a functional IgE antibody directed against DNP (Liu et al., 1980). In both cases, total RNA was isolated (RNeasy Protect Cell Mini Kit; Qiagen, Valencia, CA, USA), reverse transcribed, and used as templates in gene-specific PCR reactions.

CD14 was initially amplified using primers that remove the N-terminal signal sequence (5'-tctccgccccaccagag-3') and the termination codon (5'-aacaagaggcgatctcctaggag-3'). This amplification product was cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA). The pGEM-T-CD14 clone was confirmed by DNA sequencing. The Fc ϵ gene fragment was amplified using PCR and primers that were designed to incorporate a NotI restriction site at the 5'-end (5'-acgtcagtcggcgccgcatctatcaggaaccctcagctc-3') and a XhoI site at the 3'-end, and to remove the Fc ϵ termination codon (5'-gtactctagagcgccgctcagatggaggaggtgttaccag-3'). This PCR product was cloned into pGEM-T Easy and sequenced.

CD14 was then fused to Fc ϵ in the pET22b(+) bacterial expression vector (Novagen, San Diego, CA, USA). The CD14 gene was PCR amplified from the pGEM-T-CD14 clone using primers that incorporate an NdeI restriction site in the 5'-end of the gene (5'-agtgtataaacatgaagctgggactggacc-3') and a NotI site at the 3'-end, along with a 15-base segment that encodes five glycine residues as a linker between the CD14 domain and the Fc ϵ (5'-agcataattagcgccgcacctccaccctcgcactctatatactggacattg-3'). Because the mouse CD14 gene contains an internal NotI site, the PCR product was partially digested and the appropriate sized fragment was purified. The NdeI/NotI CD14 fragment and NotI/XhoI Fc ϵ fragment derived from pGEM-T-Fc ϵ were ligated into an NdeI/XhoI-digested pET22b(+) vector. After repeated problems expressing a functional bacterial CD14-Fc ϵ protein, the fusion was transferred into a baculovirus expression system to allow for glycosylation of the chimeric-gene product.

Construction of recombinant baculovirus was accomplished using the Bac-to-Bac system (Invitrogen). The CD14-Fc ϵ gene fusion was PCR amplified from the pET22 vector using a 5'-primer (5'-agtgtataaacgatcccatatgtctccgccccaccagag-3') that introduced a BamHI restriction site and a 3'-primer (5'-ttgaatatagcatgctcagtggtggtggtggtggtgctc-3') that created a SphI site. The amplified fragment was digested with BamHI and SphI and cloned into the pFastBac vector, which was then used to generate recombinant baculovirus for expression.

2.6. Expression of CD14-Fc ϵ

Culture supernatant containing baculovirus isolated from an initial transfection of Sf9 insect cells was found to con-

tain protein of the correct size by Western blot analysis. To obtain a higher protein yield, the viral stock was used to infect *Trichoplusia ni* caterpillars (Entopath Inc., Easton, PA, USA). To isolate the protein, five frozen caterpillars were ground with mortar and pestle on dry ice to yield fine powder. The powder was resuspended in cold phosphate-buffered saline (PBS; 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) with protease inhibitors (0.25 mM serine protease inhibitor Pefabloc SC (Sigma–Aldrich), and complete protease tablet (Roche, Indianapolis, IN, USA), sonicated for 2 h, and spun at $25,000 \times g$ for 2 min. Resuspended proteins were loaded at 1 mL/min onto a sulfopropyl-Sepharose cation exchange column (Sigma–Aldrich) equilibrated with PBS (containing protease inhibitors), connected to a Bio-Rad EP-1 pump and fraction collector (Bio-Rad Laboratories, Hercules, CA, USA). Bound proteins were eluted with PBS containing 1 M NaCl, and dialyzed in PBS before use.

2.7. Fluo-4 biosensor assay

Adherent cells were loaded with Fluo-4 (2.5 μM) in BSS containing 0.25 mM sulfinpyrazone for 20 min at 37 °C. The cells were then washed twice in BSS plus sulfinpyrazone to remove excess dye, and a baseline fluorescence reading was obtained. Immune complexes containing anti-DNP IgE (1 $\mu\text{g/mL}$) and DNP-BSA (50 ng/mL) or CD14-Fc ϵ (10 $\mu\text{g/mL}$) and *E. coli* (1×10^7 cfu/mL) were then added to the cells and fluorescence was analyzed every 30 s in each well. The data are presented as F/F_0 (fluorescence value at each time point divided by the initial fluorescence value), which corrects for the starting fluorescence value. The values are represented as the mean from 2–4 wells of cells. The data points were fitted to a regression line using least squares analysis.

For portable field measurements such as those illustrated in Fig. 6, an in-house portable fluorimeter was built that excites Fluo-4 in mast cells seeded in 8-well strips. The system was constructed based on an epifluorescence module consisting of a Nichia NBP500A Light Emitting Diode (LED; Nichia, Detroit, MI, USA) having a peak wavelength of 465 nm. A Hamamatsu 5784 metal can PMT (Hamamatsu Corporation, Bridgewater, NJ, USA) with a built-in amplifier (20 kHz bandwidth) was utilized to detect the fluorescence emission. The signal from the PMT is sent to an additional programmable gain module for increased dynamic range and this is processed by a computer. This fluorimeter was also equipped with a custom built TEC (Thermo Electric Cooler; FerroTec, Bedford, NH, USA) heated 8-well strip holder to run mast cell experiments in a portable format.

3. Results and discussion

3.1. Monitoring mast cells with fluorescent dyes

To develop a robust, sensitive cell-based biosensor, it is necessary for the experimental system to produce a readily detectable, rapid response to challenge with appropriate target antigens. Our first objective toward this goal was to optimize detection of

responses in RBL mast cells using fluorescent dyes in order to generate a real-time signal in response to antigen stimulation. For this purpose, several different fluorescent dyes were loaded into the RBL cells sensitized with anti-dinitrophenyl (DNP) IgE, then monitored with confocal fluorescence microscopy before and after stimulation by multivalent DNP conjugated to BSA.

Alkalinization of the secretory granules is one of the early cellular events that occurs during antigen-induced mast cell activation, and acridine orange (AO) has been demonstrated to be a sensitive dye to monitor this process (Williams and Webb, 2000). AO is a weakly acidic amine dye that diffuses across lipid membranes and enters acidic intracellular compartments, such as the mast cell secretory granules. Upon entering acidic environments, the dye becomes protonated, cannot escape the compartment and accumulates to a very high concentration. At these high concentrations, AO undergoes a spectral shift to longer wavelength excitation and emission maxima. Under these conditions, the fluorescence of AO in the cytoplasm is green, whereas AO emission in the secretory granules is red. During cell activation, as the granule becomes alkaline, AO is deprotonated and dif-

fuses rapidly out of the granule into the cell cytoplasm. This rapid redistribution of the dye can be monitored as a decrease in red fluorescence and a concomitant increase in green fluorescence. [Supplemental movie I](#) shows this stimulated process, as AO released from individual granules (red) appears as local bursts of green fluorescence, often occurring at multiple sites from the same stimulated cell.

To determine if this system could be adapted for a mast cell-based biosensor, confocal microscopy was used to image and quantify changes following the addition of antigen to AO-labeled RBL mast cells. [Fig. 1A](#) shows images of the green channel of cytoplasmic AO fluorescence in cells challenged with 100 ng/mL DNP–BSA as a function of stimulation time. It can be seen from these images that activation of mast cells with antigen leads to a rapid and robust increase in cytoplasmic AO fluorescence. Quantification of confocal images is shown in [Fig. 1B](#). The data shows that measurable increases in mast cell AO fluorescence can be observed after addition of DNP–BSA as low as 10 ng/mL. At the lowest concentration tested (0.1 ng/mL), the cells show a slow decline in fluorescence, indicating that no

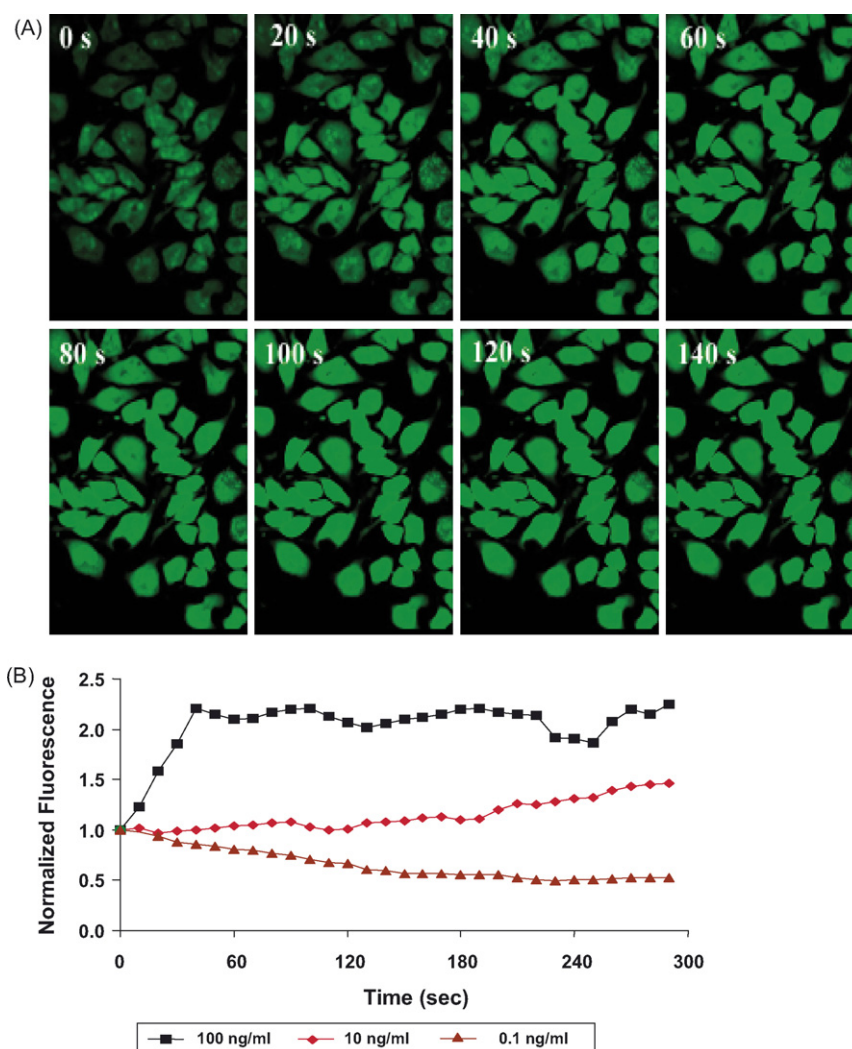


Fig. 1. Time-dependent increases in stimulated acridine orange cytoplasmic fluorescence. Fluorescence confocal images of RBL mast cells loaded with acridine orange (AO). (A) Cells were sensitized with anti-DNP IgE, loaded with 3 μ M AO and imaged every 20 s after activation after addition of 100 ng/mL DNP–BSA. (B) Quantification of confocal images in AO loaded RBL cells activated with various concentrations of DNP–BSA.

detectable stimulation above the time-dependent photobleaching is observed. A similar time course is observed in the absence of antigen (data not shown). Although the AO response is rapid and dramatic at optimal receptor activation, the overall response is limited by a modest signal to noise ratio at low doses of antigen and by photobleaching which further reduces sensitivity.

Reactive oxygen species (ROS) are commonly produced in a variety of immune cells when they become activated (Bland et al., 2001; Brubacher and Bols, 2001). To develop a mast cell biosensor with broad applicability, a fluorescent dye sensitive to production of ROS was used. RBL mast cells were loaded with 2',7'-dichlorodihydrofluorescein-diacetate (DCFDA), a dye that is nonfluorescent in the absence of reactive oxygen or other free radical species. When the cells are stimulated by antigen, the released ROS oxidize DCFDA, which then fluoresces green. In these experiments, the cells were also loaded with LysoTracker, which labels low pH organelles in the cells, to provide a red fluorescence signal, so the cells can be focused before activation by antigen. Fig. 2 shows images of the green DCFDA fluorescence and red LysoTracker fluorescence in cells stimulated with 100 ng/mL DNP-BSA (Fig. 2A) or with unmodified BSA alone as a negative control (Fig. 2B). As seen in Fig. 2, cells treated with antigen steadily increase their green fluorescence. Quantification of these confocal images is shown in Fig. 2C. At the highest concentrations of DNP-BSA (100 ng/mL), increases in fluorescence can be seen almost immediately, and these plateau after 1–2 min due to a desensitization mechanism (Weetall et al., 1993). As the concentration of antigen is decreased, a lag is observed in the initiation of activation, but changes in cell fluorescence can clearly be measured at concentrations as low as 1 ng/mL DNP-BSA.

Whereas degranulation is not a universal response for activation of receptors on immune cells, calcium mobilization is a ubiquitous event in immune cell signaling. Thus, we evaluated the applicability of fluorescent dyes that detect stimulated increases in cytoplasmic Ca^{2+} in order to monitor activation of RBL mast cells. In this approach, the calcium-responsive fluorescent dyes, Fluo-4, Fluo-3 and Calcium green were investigated. Initial experiments indicated that Fluo-4, a fluorogenic calcium dye, showed the largest and most reproducible increases in fluorescence when dye-loaded, IgE-sensitized RBL cells were challenged with antigen. Fig. 3A shows Fluo-4 fluorescence of RBL mast cells during stimulation by 100 ng/mL DNP-BSA. It can be seen from the images that Fluo-4 fluorescence increases strongly in response to antigen stimulation. Close inspection of Fig. 3A shows that individual RBL mast cells show oscillations in fluorescence over time, consistent with previous observations (Millard et al., 1989). Quantification of confocal images similar to these is shown in Fig. 3B. The data shows that a robust response could be measured at target concentrations as low as 10 ng/mL. At 1 ng/mL, stimulated fluorescent oscillations were observed, but averaged fluorescent changes were only marginally greater than in unstimulated cells because of the asynchronous nature of these responses.

Collectively, these data represented in Figs. 1–3 show that RBL mast cells labeled with fluorescent dyes that monitor several different processes produce a real-time signal in response to

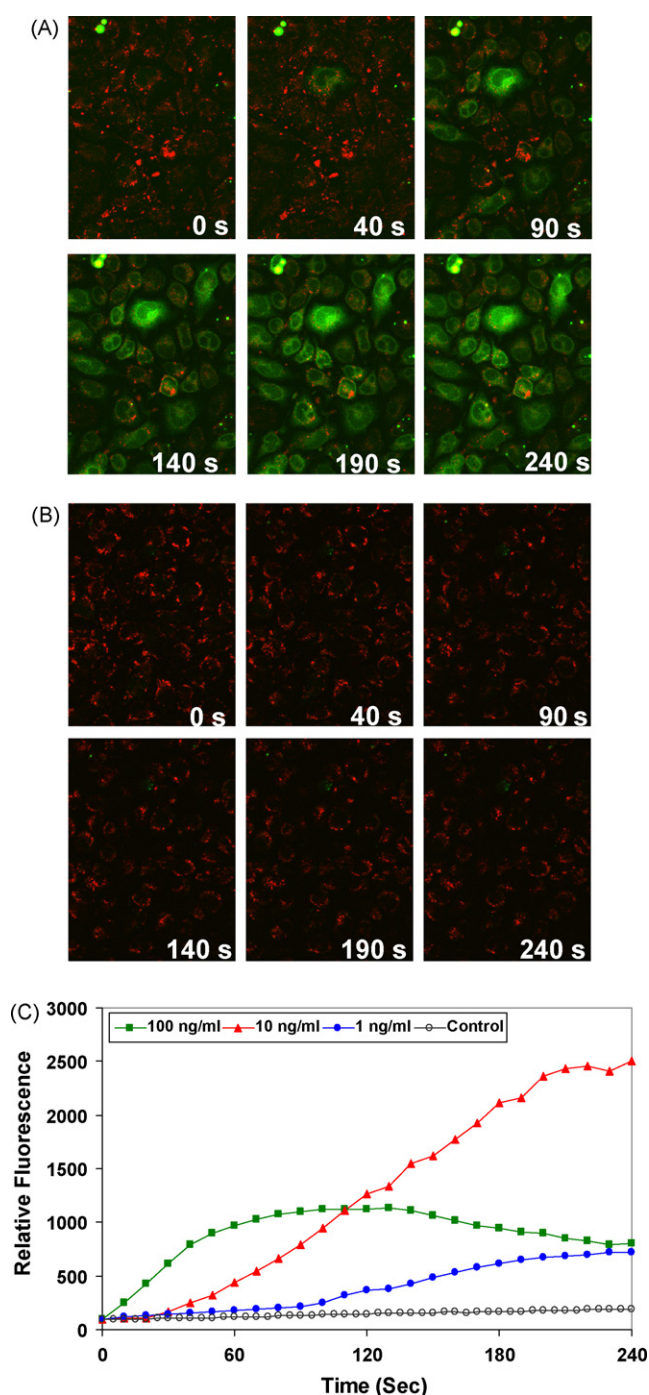


Fig. 2. Time-dependent increases in stimulated production of reactive oxygen detected by DCFDA cytoplasmic fluorescence. (A) Fluorescence confocal images of RBL mast cells loaded with DCFDA that detects ROS. Cells were sensitized with anti-DNP IgE, loaded with DCFDA and stimulated with 100 ng/mL DNP-BSA at $t=0$ s. (B) Unstimulated cells. (C) Quantification of fluorescent changes in DCFDA-loaded RBL cells activated with various concentrations of DNP-BSA. Each plot shows the integrated fluorescence response for cells from a single representative field similar to those in (A) and (B).

antigen stimulation. The data using all three dyes supports the existence of a quantitative relationship between antigen concentration and fluorescence changes measured. Based on these data using DNP-BSA as a model target, detection of 10 pM concentrations of target is achievable. Although DCFDA and Fluo-4

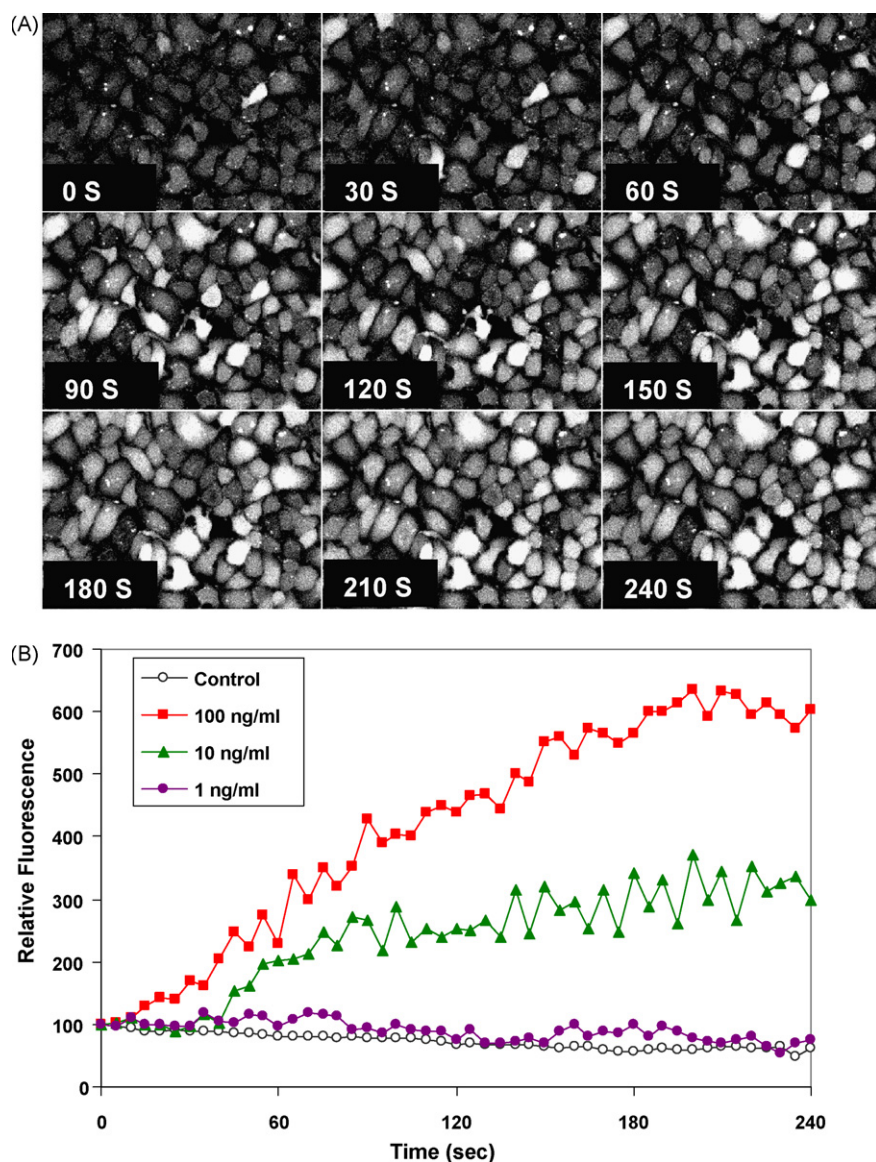


Fig. 3. Time-dependent antigen-stimulated increases in cytoplasmic Ca²⁺ detected by Fluo-4 fluorescence. (A) Fluorescent confocal images of RBL mast cells labeled with the Ca²⁺ indicator dye, Fluo-4. RBL cells were sensitized with anti-DNP IgE, loaded with Fluo-4 and imaged every 30 s after the addition of 100 ng/mL DNP-BSA. (B) Quantification of fluorescence changes in Fluo-4-loaded RBL cells activated with various concentrations of DNP-BSA.

exhibit similar high sensitivity in this regard, the response to 1 ng/mL of DNP-BSA detected by DCDHF has a long (~2 min) lag phase, whereas oscillatory spikes in [Ca²⁺]_i detected by Fluo-4 are observed in a significant percentage of cells stimulated by this concentration of antigen within less than 1 min of addition.

3.2. Development of a functional chimeric CD14-Fcε protein

In principle, the mast cell biosensor can be customized to react to virtually any target by producing IgE antibodies to the antigen of interest. Monoclonal IgE antibodies specific for a variety of antigens have been prepared previously (Böttcher et al., 1978; Eshhar et al., 1980; Liu et al., 1980; Rudolph et al., 1981) by using modifications to the clas-

sic hybridoma production technique of Köhler and Milstein (1975).

The mast cell biosensor can also be tailored to detect a wide range of antigens by engineering chimeric proteins that contain the Fc domain from an IgE antibody and an antigen binding domain to the target of interest. For this approach to be valid, the antigen must be multivalent to induce clustering of the Fc domains of IgE on the surface of the mast cell (Holowka et al., 2007). For this purpose, a chimeric IgE protein was created that contains the Fc domain from an IgE molecule and the bacterial binding protein, CD14 that mediates innate immune responses. The CD14 protein is expressed on many immune cells and has the unique capacity to recognize and bind a variety of cell wall components derived from an evolutionarily diverse array of bacteria (Wright et al., 1990; Yu et al., 1997). This novel chimeric protein, CD14-Fcε, binds to FcεRI on the surface of RBL mast

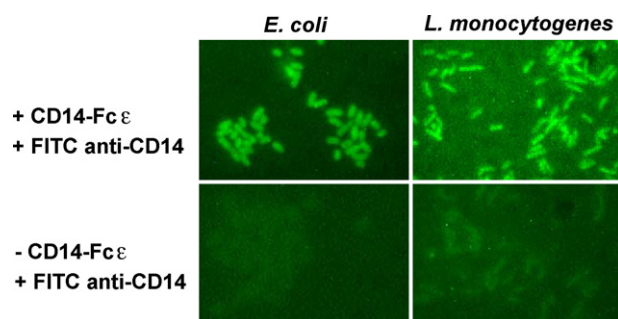


Fig. 4. Immunofluorescence images of CD14-Fc ϵ bound to *Escherichia coli* and *Listeria monocytogenes*. The bacteria were heat-killed and then incubated with or without CD14-Fc ϵ (10 μ g/mL) followed by fluorescein-anti-CD14 (1 μ g/mL).

cells and also to a variety of bacterial cell walls. By labeling RBL mast cells with Fluo-4, and binding CD14-Fc ϵ to the mast cell surface, a uniquely sensitive and highly versatile biosensor for bacterial contamination was created.

The CD14-Fc ϵ protein was expressed in *T. ni* caterpillars because complex glycosylation of the Fc domain is required for proper tertiary structure and binding to Fc ϵ RI. After purification of the chimeric protein, immunofluorescence microscopy was used to confirm that it binds to various types of bacteria. Either 1×10^7 cfu/mL of *E. coli* (Gram-negative bacterium) or *L. monocytogenes* (Gram-positive bacterium), were incubated with the CD14-Fc ϵ (10 μ g/mL), followed by incubation with fluorescein-labeled rat anti-mouse CD14 monoclonal antibody (PharMingen San Diego, CA, USA; 1 μ g/mL). Fig. 4 shows that the fusion protein can bind to both Gram-negative and Gram-positive bacteria.

3.3. Detection of bacteria by the mast cell biosensor

To evaluate the mast cell biosensor, *E. coli* was chosen as the model target because of its importance in food and water safety. *E. coli* is often used as an indicator organism in water sample analysis because it most reliably reflects fecal origin as a natural inhabitant of the intestinal tract of humans and other warm-blooded animals (Gauthier and Archibald, 2001).

To test whether RBL mast cells sensitized with CD14-Fc ϵ are activated by the addition of *E. coli*, confocal microscopy was used to image calcium changes in Fluo-4 loaded cells. Fig. 5 shows the cumulative percentage of cells activated after *E. coli* addition. This quantitation shows that there is a rapid burst of activation during the first 60 s in cells treated with *E. coli*, and this Ca $^{2+}$ response is sustained for >4 min in more than 50% of the cells. Mock-activated cells show only a marginal increase in intracellular Ca $^{2+}$ over the same time course.

For purposes of developing a field device, RBL mast cell Fluo-4 fluorescence was examined in a portable fluorimeter after antigen stimulation. As a positive control, Fluo-4 loaded mast cells were treated with immune complexes containing anti-DNP IgE (1 μ g/mL) and DNP-BSA (50 ng/mL). Fig. 6A shows a large, time-dependent increase in Fluo-4 fluorescence after these immune complexes were added, whereas a small increase in fluorescence was observed when either the IgE or DNP-BSA was

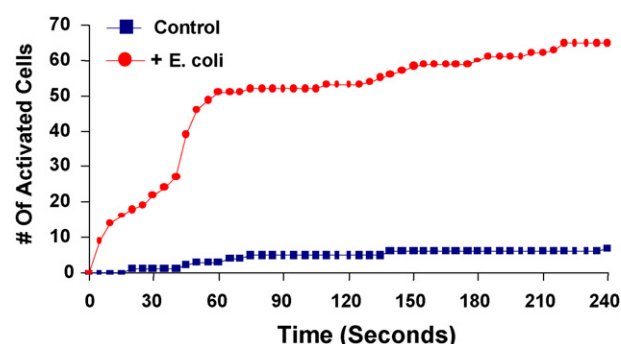


Fig. 5. Stimulated intracellular Ca $^{2+}$ mobilization in Fluo-4-loaded RBL cells sensitized with CD14-Fc ϵ (10 μ g/mL) and treated with *E. coli* (1×10^7 cfu/mL) or buffer only (control). The cells were imaged by confocal microscopy, and the cumulative number of activated cells from a single representative field is shown.

added alone. The slope of the response is determined by least squares regression analysis to quantify the change in fluorescence over time. The slope of the fitted line for IgE or DNA-BSA when added alone is $0.092F/F_0$ per min or $0.095F/F_0$ per min, respectively. The slope increases to $0.60F/F_0$ per min when immune complexes are added to activate the cells.

To test the activity of the CD14-Fc ϵ protein, immune complexes containing CD14-Fc ϵ (10 μ g/mL) and *E. coli* (1×10^7 cfu/mL) were added to Fluo-4-labeled RBL mast cells.

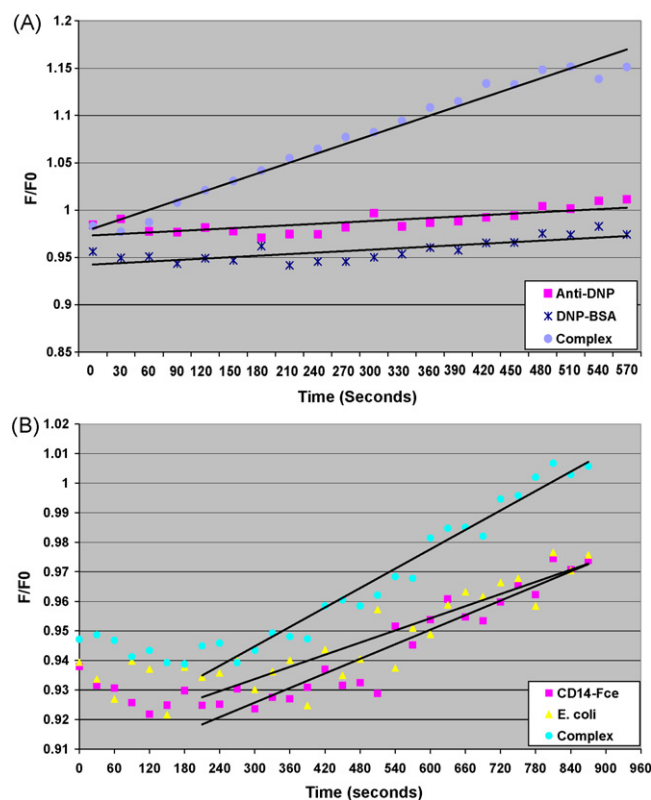


Fig. 6. Fluorescence changes in Fluo-4-loaded RBL cells activated with immune complexes containing (A) anti-DNP IgE (1 μ g/mL) and DNP-BSA (50 ng/mL) or (B) CD14-Fc ϵ (10 μ g/mL) and *E. coli* (1×10^7 cfu/mL). Immune complexes were added to dye-loaded cells and the fluorescence was monitored in a portable fluorimeter. The data points represent the mean from two to four wells and the black lines represent linear fitted regression analyses.

The CD14-Fc ϵ protein or the *E. coli* were added alone as controls. The data shown in Fig. 6B indicates that when the CD14-Fc ϵ and bacteria are added together, a larger time-dependent increase in Fluo-4 fluorescence is observed than for either component alone. The time courses for these responses exhibit an initial lag period that probably results from slow binding to Fc ϵ RI on the mast cell surface by these complexes. Following this initial lag phase (210 s), the time course was adequately fit by a linear regression to yield a rate of 0.0049F/F₀ per min or 0.0041F/F₀ per min, for addition of CD14-Fc ϵ or *E. coli*, respectively. The rate increased to 0.0066F/F₀ per min when immune complexes were formed between the two components. The fluorescence of the controls increases slowly over time chiefly because the dye leaks out of the cell over the 15 min measurement time, causing an increase in background fluorescence. These results demonstrate that it is feasible to engineer chimeric IgE class antibodies that can sensitize and activate mast cells.

4. Conclusions

A key finding of this study is that RBL mast cells can be used as a convenient and sensitive biosensor by monitoring intracellular signal transduction events, such as alkalization of secretory granules, increases in intracellular calcium and the generation of reactive oxygen species in the cytoplasm after stimulation by antigen. These responses are rapid, within seconds of antigen addition, and they are extremely sensitive to small amounts of antigen with detectable amounts as low as 1 ng/mL. Different laboratory methods were used to monitor the RBL mast cell stimulation including confocal microscopy (Figs. 1–5) and fluorimetry (Fig. 6), pointing to the utility of this cell-based biosensor in a number of monitoring schemes, from environmental toxicology monitoring to high-throughput drug screening.

This study also shows that RBL mast cells can be activated by *E. coli* using a novel chimeric protein. The sensitivity of *E. coli* detection is relatively low with this construct, but current efforts are aimed at improving the binding of this and other chimeric constructs. The results support the feasibility and potential versatility of mast cell biosensors for the selective detection of a variety of analytes of interest. This new class of biosensors goes beyond current immunological systems that utilize the specificity of antibodies for detection. With the mast cell system, antibody specificity is amplified by the natural biochemical cascade of mast cell degranulation. In the same manner, other chimeric IgE antibodies could be developed towards a wide array of antigen targets. Engineered chimeric IgE antibodies could be used to detect any oligovalent antigen including airborne and foodborne allergens in the environment, food pathogens and biological and chemical warfare agents. Single chain Camelid antibodies are particularly exciting tools for this purpose (Rothbauer et al., 2006). Combining fluorescently loaded mast cells, engineered IgE antibodies and

a compact portable fluorimeter will create a low-cost, highly sensitive, specific and flexible biosensor for a variety of antigen targets.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2007.10.007.

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