Dynamic and label-free monitoring of natural killer cell cytotoxic activity using electronic cell sensor arrays

Jenny Zhu, Xiaobo Wang, Xiao Xu, Yama A. Abassi *

ACEA Biosciences, 11585 Sorrento Valley Road, San Diego, CA 92121, United States

Received 10 June 2005; received in revised form 27 October 2005; accepted 27 October 2005
Available online 4 January 2006

Abstract

A microelectronic sensor-based platform, the RT-CES (real time electronic sensing) system, is introduced for label free assessment of natural killer (NK) cell-mediated cytotoxic activity. The RT-CES system was used to dynamically and quantitatively monitor NK-mediated cytotoxic activity towards 8 different adherent target cell lines, including cancer cell lines commonly used in laboratories. The cytotoxic activity monitored by RT-CES system was compared with standard techniques such as MTT measurement and shows good correlation and sensitivity. To test the specificity of the assay, pharmacological agents that inhibit NK cell degranulation and cytotoxic activity were employed and were shown to selectively and dose-dependently inhibit NK-mediated cytotoxic activity toward target cells. In summary, the RT-CES system offers fully automated measurement of cytotoxic activity in real time, which enables large-scale screening of chemical compounds or genes responsible for the regulation of NK-mediated cytotoxic activity.

Keywords: Natural killer; Cytolysis; Assay; Kinetic; Label-free

1. Introduction

Natural killer (NK) cells are an integral part of the innate immune response. NK cells have been endowed with the ability to recognize and annihilate cells with extreme stress load such as virus infected cells as well as tumor cells (Lanier, 2005). The death mediated by NK cells is rapid and multifaceted. In addition to their important role in innate immune response, NK cells also serve as key mediators for activation of the adaptive immune response, by secreting factors which serve to activate and propagate B and T lymphocytes (Djeu et al., 2002; Lanier, 2005; Smyth et al., 2005). By responding immediately to infected cells, NK cells keep pathogen infection at bay while giving the adaptive arm of the immune response time to mobilize and respond to pathogen challenge in a more specific manner.

NK cells express specific receptors on their surface that can recognize pathogen infected and tumor cells by their lack of expression or low expression of the major histocompatibility complex (MHC) at the membrane (Cerwenka and Lanier, 2001; Lanier, 2005). These receptors can be both inhibitory and stimulatory and the combined action of these receptors determines the extent of the dual nature of NK cell response to target cells; namely cytotoxicity and/or cytokine production (Lanier, 2005). When NK inhibitory receptors bind to MHC class I molecules, their effector func-
tions are blocked and therefore normal healthy cells which express adequate levels of these receptors are spared from the NK cell attack. On the other hand activating receptors such as NKG2D is engaged by ligands that are MHC-like and expressed by pathogen infected and transformed cells as well other stressed cells (Cerwenka and Lanier, 2001). The immediate effector function of NK cells results in the release of secretory granules which contain perforin and members of the granzyme family (Smyth et al., 2005). Perforin binds to the cell membrane and disrupts the integrity of the plasma membrane whereas granzymes are a special class of serine proteases with various substrate specificities, including caspases (Trapani and Smyth, 2002). The combined interplay of these proteins and enzymes ultimately result in target cell cytotoxicity and destruction. In addition to granzyme and perforin-mediated cytotoxicity, NK cells also express Trail and FasL, both of which can contribute to NK-mediated cytotoxicity towards target cells (Smyth et al., 2002).

A number of techniques have been devised for monitoring NK-mediated cytotoxicity towards target cells. The most popular method relies on chromium 51 labeling of target cells and measuring the release of chromium upon cytolysis (Brunner et al., 1968). Other label-based methods such as annexin-V staining of target cells and using fluorescence activated cell sorting (FACS) analysis to analyze apoptotic cells have also been described (Goldberg et al., 1999). Enzymatic assays which measure the activity of certain enzymes such as lactate dehydrogenase (LDH) or granzymes have also been described in the literature as a means to assess NK-mediated cytotoxicity (Korzenniewski and Callewaert, 1983; Ewen et al., 2003; Shafer-Weaver et al., 2003). While all of the above mentioned assays, especially chromium 51 release assay, have proven to be informative and are in routine use, they all have certain drawbacks which limit their utility. For example, chromium labeling involves the usage of a radioactive label which can be hazardous and difficult to dispose of. Furthermore, chromium 51 assay window is limited to a few hours beyond which the natural tendency of chromium to diffuse out of the cell may contribute to high background. In addition, all the mentioned assays are end-point assays which provide a “snapshot” of the NK-mediated cytotoxic activity and with respect to chromium 51 release assay, it would be difficult to measure NK-mediated target cell killing beyond a limited time window.

In order to address some of the limitations with current assay systems, in this report we describe a label-free and kinetic-based method for measuring NK-mediated cytotoxicity. The method is based on non-invasive measurement of the viability of target cell that have been seeded on microtiter plates integrated with microelectrodes at the bottom of the well (E-pitches™). The interaction of adherent target cells with the microelectrodes results in disruption of the ionic environment between the cell sensor electrode and the media in a very precise and specific way which is dependent on the number of target cells seeded, the morphology of the cells and the quality of cell adhesion (Abassi et al., 2004; Solly et al., 2004; Xing et al., 2005). The cellular status is continuously monitored using the real time cell electronic sensing (RT-CES™) system. Effector-mediated cytotoxicity results in target cell death which is accompanied by morphological changes such as a loss of the integrity of the actin cytoskeleton and cell rounding that is ultimately accompanied by deadhesion of the cells. All these morphological events leads to a loss of cell-substrate impedance signal over time.

Using the RT-CES system a human NK cell line (NK-92)-and a murine mouse cell line cytotoxic activity towards several target cells were monitored under label-free and dynamic conditions. The cytotoxicity as measured by RT-CES correlated directly with other standard method such as MTT measurement and crystal violet staining. Furthermore, specific inhibitors of NK-mediated signaling pathways were able to selectively and dose-dependently block NK-mediated cytotoxic effect. In summary the assay system we describe in this report provides a convenient, high throughput and label-free method which provides succinct information regarding the dynamics of NK-mediated cytotoxic activity.

2. Materials and methods

2.1. Cells

NK 92, NIH 3T3 and all the adherent cancer cell lines used in these experiments were purchased from ATCC. The mouse NK cell line (mNK) was provided by Dr. Hui Shao of University of Louisville. All the cell lines were maintained at 37 °C incubator with 5% CO2. The NK92 and mNK lines were maintained in Alpha MEM with 2 mM l-glutamine, 1.5 g/L sodium bicarbonate, supplemented with 0.2 m inositol, 0.1 mM 2-mercaptoethanol, 0.02 mM folic acid, 12.5% horse serum, 12.5% FBS, and 100–200 U/ml recombinant IL-2. Other cancer cell lines were maintained in RPMI media containing 5% FBS and 1% penicillin
and streptomycin (GIBCO). The NIH 3T3 cells were maintained in DMEM media containing 10% FBS and 1% penicillin and streptomycin.

2.2. RT-CES system

The system includes three components: the analyzer and E-plate station, the integrated software, and 16-well or 96-well E-plate (www.aceabio.com). The E-plate station is placed inside the incubator and connected to the analyzer outside the incubator through a thin cable. The E-plate containing the cells is placed onto the E-plate station inside the incubator and the experiment data are collected automatically by the analyzer under the control of an integrated software. The principles of RT-CES technology has been described previously (Abassi et al., 2004; Solly et al., 2004; Xing et al., 2005) (Fig. 1).

2.3. Assessment of NK-mediated cytotoxicity

Target cells were seeded into the wells of either 16-well or 96-well E-plate in 100 μl of media. Cell growth was dynamically monitored using the RT-CES system for a period of 24–34 h, depending on the experiment until cells reached log growth phase and formed a monolayer. Effector cells at different concentrations were then directly added into individual wells containing the target cells. For background control, effector cells were added to a well without target cells. After addition of the effector cells, the E-plate was returned to the E-plate station and the measurements were automatically collected by the analyzer every 15 min for up to 20 h.

2.4. Cell morphology analysis by microscopy

The effect of NK cell-mediated cytotoxicity on target cells was examined using a Nikon upright microscope. When Cell Index reached 50% of the control upon addition of effector cells, cells were fixed in 80% methanol for 5 min and stained with Giemsa blue. The morphology of the cells was examined by microscopy and photographed using an accompanying CCD camera.

2.5. Data analysis

The integrated software is able to display entire history of the experiment from seeding the cells to termination of cytotoxicity. The time and effector-to-target ratio (E/T)-dependent curves can be displayed in real time allowing for dynamic monitoring of NK cell cytotoxic activity. The electronic readout, cell-electrode impedance is displayed as an arbitrary unit called Cell Index. Cell Index at each time point is defined as $R_n/R_b$ where $R_n$ is defined as the cell-electrode impedance of the well with the cells and $R_b$ is defined as the background impedance of the well with the media only.

To quantify the lysis at specific time points, the data was exported to excel and percentage cytotoxic activity at specific E/T ratio at a given time was determined by using the following equation: Percent cytotoxicity = $(1-\text{CI at given E/T ratio/CI without NK}) \times 100$.

2.6. Determination of cytotoxic activity using MTT assay and crystal violet staining

Target cells growing on E-plates were incubated with different ratios of effector cells and incubated
overnight. The cytotoxic activity was dynamically monitored using the RT-CES system. At the end of the experiment, the wells were washed with PBS to remove unbound cells and the viable cells attached to the bottom of the E-plate were quantified using MTT reagents according to manufacturers protocols (Sigma) or by fixing the cells with 4% paraformaldehyde followed by staining with 0.1% crystal violet solution in H₂O for 20 minutes. The cells were washed five times to remove the non-specific stain and the stained cells were solubilized in the presence of 0.5% TX-100 overnight. The solubilized stain was measured at wavelength of 590 nm using a plate reader.

3. Results

3.1. Dynamic monitoring of NK-mediated cytotoxic activity using the RT-CES system

To assess Effector-mediated cytotoxic activity towards target cells we employed NK-92 cell line. NK-92 is a human IL-2 dependent cell line derived from a non-Hodgkin’s lymphoma patient that can be easily maintained and propagated in culture and has a very robust cytotoxic activity towards different target cell lines (Gong et al., 1994). NK-92 cell line and some of its derivatives are currently in Phase I clinical trials for the treatment of various cancers (Tam et al., 2003). In order to assess NK-92 mediated cytotoxicity, A549 lung carcinoma cells were seeded in ACEA E-plates at a density of 10,000 cells/well and cell growth and proliferation was dynamically monitored using the RT-CES system. Twenty-four hours after seeding, NK-cells were added at the indicated effector-to-target (E/T) ratios (Fig. 2) and the extent of A549 cell viability was dynamically monitored. As shown in Fig. 2A, NK-92 cell addition to A549 cells leads to a density-dependent decrease in A549 cell index over time, indicating that NK-92 cells are eliciting a cytotoxic effect upon A549 cells. NK-92 addition to wells which do not contain any A549 cells did not have any appreciable change relative to background (data not shown). Also, A549 cells which were treated with the media alone continue to grow and proliferate (Fig. 2A). In order to compare the cell viability measured by the RT-CES system with a standard assay, at the end of the experiment, the wells of the E-plate were washed to remove the NK cells and any dead cells and the extent of target cell viability was determined by MTT assay. Fig. 2B shows a comparison of the

![Fig. 2. RT-CES monitoring of NK-92 mediated cytotoxicity. (A) 10,000 A549 target cells were seeded in the wells of E-plates. At the indicated time point (arrow), NK-92 cells were added at different E/T ratios and the viability of A549 cells were dynamically monitored using the RT-CES system. (B) Comparison of RT-CES readout with MTT at 25 h after addition of NK-92 cells. The A549 cells described in (A) were washed and cell viability was assessed using MTT. For each E/T ratio the normalized CI value obtained by RT-CES is plotted together with the absorbance value obtained using MTT assay. (C) For both the RT-CES readout and MTT assay the percent cytolytic activity of NK-92 cells at different E/T ratios was calculated as described in Materials and methods.](image-url)
cell index reading to MTT at 50 h when the experiment was terminated. Overall, the MTT readout correlates very well with RT-CES measurement of cell impedance as displayed by Cell Index. In addition to MTT, crystal violet staining of the target cells was also conducted in parallel which confirmed the MTT results (data not shown). The relative cytotoxic activity of NK-92 cells towards A549 target cells as measured by both RT-CES system and MTT at different E/T ratios is shown in Fig. 2C.

In addition to NK-92 cells, the cytotoxic activity of a mouse NK cell line (mNK) was also assessed using the RT-CES system (Fig. 3A). NIH3T3 mouse fibroblast cell lines were seeded in E-plates and allowed to grow overnight. Twenty-four hours after seeding, NK cells were added to the wells at different E/T ratio and the viability of NIH3T3 cells were continually monitored (Fig. 3A). As a control, YAC cells or media alone was added to NIH3T3 cell growing in the wells of the E-plate. The mNK cells induced a progressive decrease in cell index where as addition of YAC cells or media alone had very little effect on the Cell Index recording of NIH3T3 cells (Fig. 3A). To ascertain that the progressive decrease in cell index correlates with cytolysis, the cells in the bottom of the E-plate were washed, fixed, stained with Giemsa dye and photographed using a CCD camera attached to a microscope (Fig. 3B). As shown in Fig. 3B, addition of YAC cells to NIH3T3 cells growing on the bottom of the E-plates had no detectable effect on the target cells, whereas addition of mNK cells leads to large gaps and areas on the sensors in the bottom of the well that are devoid of target cells, indicating that cell deadhesion due to cytotoxicity has taken place. In summary, The RT-CES system allows for a non-invasive, label-free and dynamic way of monitoring effector-mediated cytotoxic activity towards adherent target cells.

Fig. 3. Dynamic monitoring of mouse NK cell cytolysis of target cells using the RT-CES system. (A) NIH3T3 cells were seeded in the wells of E-plate and mouse NK cells, YAC cells, or media alone were added at the indicated time point (arrow) at different E/T ratios. The viability of NIH3T3 cells were continually monitored using the RT-CES system. (B) At the end of the experiment described in (A), the wells of the E-plates were washed, fixed in methanol and stained with Giemsa dye as described in Materials and methods. The wells were visualized and photographed using a light microscope connected to CCD camera.
3.2. Dynamic monitoring of NK-mediated cytotoxic activity toward different target cells using the RT-CES system

Cytotoxic activity of NK92 and mNK cells were tested using 7 different human cancer cell lines (for NK92) and mouse NIH3T3 cells (for mNK). The susceptibility of different target cells lines to NK-mediated cytotoxic activity is summarized in Table 1. NK92 shows a broad spectrum of cytotoxic activity towards cancer cell lines. The cytotoxic activity mediated by NK92 occurs fast and reaches the maximum killing activity prior to 8 h after addition of NK92 cells. Among 7 cancer target lines tested, over 90% cytotoxicity of 4 target lines was achieved, including H460, HepG2, MCF7 and MDA-MB231. In contrast, mNK cell-mediated cytolysis appears to have much slower kinetics when compared to NK92, reaching maximum cytotoxic activity 12 h after addition of mNK cells.

3.3. Inhibition of NK-mediated cytotoxic activity by agents which block the signaling pathways leading to cytotoxicity

The engagement of NK receptors at the cell membrane by target cells leads to the activation of signal transduction machinery which results in cytolysis of target cells (Vivier et al., 2004). One of the key signaling cascades which regulate the fusion of perforin and granzyme containing secretory granules at the NK/target cell synaptic junction is the phosphatidyl-inositol-3-kinase (PI3K), Rac and mitogen activated protein kinase 1 (Erk) pathway (Djeu et al., 2002; Vivier et al., 2004). In order to determine the specificity of the signaling pathways in regulating NK-mediated cytotoxicity, we therefore sought to utilize specific inhibitors of the PI3K and Erk pathways. NK-92 cells were first incubated with both the PI3K inhibitor Wortmannin and the Erk pathway inhibitor PD98059 or DMSO as a control and then added to A549 target cells. As shown in Fig. 4, both Wortmannin and PD98059 dose-dependently inhibited NK-92-mediated cytotoxic activity towards A549 cells as monitored by the RT-CES system. As an additional control, A549 target cells were also incubated with both Wortmannin and PD 98059 alone which had minimal effect on the baseline. We calculated percent inhibition of cytotoxic activity for both inhibitors at different time points after NK addition. While the Erk pathway inhibitor resulted in consistent inhibition of cytotoxicity at all the indicated time points (Fig. 4B), the PI3K inhibitor-mediated inhibition of cytotoxicity clearly depends on the time point at which the analysis is performed (Fig. 4D). In summary, the experiments shown here clearly establish that the RT-CES system can be used to monitor NK-mediated cytotoxic activity against a variety of different cell lines. Furthermore, interfering with the NK-mediated cytotoxicity response by using inhibitors of the signaling cascade regulating granule fusion significantly attenuates the cytotoxicity response as measured by the RT-CES system. The data also shows that the extent of inhibition can primarily depend on the length of NK incubation with target cells and provides a good rationale for dynamic monitoring of effector-mediated cytotoxicity.

4. Discussion

NK cells play a central role in innate immune response as well as an intermediary role in bridging the gap between innate immunity and adaptive immunity (Lanier, 2005). NK cells have specialized organelles or granules which house cytolytic enzymes and proteins that are responsible for its cytolytic activity upon encountering pathogen infected cells or tumors (Smyth et al., 2002, 2005). Recently a number of receptors have been identified on the surface of NK-cells which regulate NK-mediated cytotoxicity in a positive or negative manner depending on the identity of the target cell (Lanier, 2005).

In this report we have described a novel label-free and real-time assay for monitoring NK-mediated cytotoxicity of different target cells lines (for NK92) and mouse NIH3T3 cells (for mNK). The susceptibility of different target cells lines to NK-mediated cytotoxic activity is summarized in Table 1. NK92 shows a broad spectrum of cytotoxic activity towards cancer cell lines. The cytotoxic activity mediated by NK92 occurs fast and reaches the maximum killing activity prior to 8 h after addition of NK92 cells. Among 7 cancer target lines tested, over 90% cytotoxicity of 4 target lines was achieved, including H460, HepG2, MCF7 and MDA-MB231. In contrast, mNK cell-mediated cytolysis appears to have much slower kinetics when compared to NK92, reaching maximum cytotoxic activity 12 h after addition of mNK cells.

Table 1

<table>
<thead>
<tr>
<th>Target cell line</th>
<th>Origin</th>
<th>Effector cell line</th>
<th>Percent cytotoxicity^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT1080</td>
<td>Fibrosarcoma</td>
<td>NK-92</td>
<td>42.17</td>
</tr>
<tr>
<td>H460</td>
<td>Lung cancer</td>
<td>NK-92</td>
<td>95.4</td>
</tr>
<tr>
<td>HepG2</td>
<td>Hepatoma</td>
<td>NK-92</td>
<td>94.1</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Breast cancer</td>
<td>NK-92</td>
<td>96.5</td>
</tr>
<tr>
<td>A549</td>
<td>Lung cancer</td>
<td>NK-92</td>
<td>52.2</td>
</tr>
<tr>
<td>HELA</td>
<td>Cervical cancer</td>
<td>NK-92</td>
<td>51</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast cancer</td>
<td>NK-92</td>
<td>97</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>Mouse fibroblast</td>
<td>Mouse NK cell line</td>
<td>58.8</td>
</tr>
</tbody>
</table>

Target cells were seeded in E-plates and continuously monitored by the RT-CES system. Approximately 24 h after seeding, NK cells were added at E/T ratio of 15:1. Cytotoxicity was dynamically monitored and percent cytotoxicity was calculated as described in Materials and methods.

^a NK-92 mediated cytotoxicity was calculated at 8 h after addition to the target cells, while mouse NK cytotoxicity was calculated at 12 h after addition to NIH3T3 cells.
toxicity towards different adherent target cells. The assay is based on monitoring the viability of adherent target cells growing on electronic cell sensor arrays fabricated in the bottom well of microtiter plates (E-plates). The interaction of the cells with sensors generates an impedance response which is indicative of the general health of the cell. We have used both human and mouse NK-cells to demonstrate that NK-mediated cytotoxic activity can be monitored using the RT-CES system, which is composed of a monitoring system as well as E-plates which contains the micro-electrodes for detection of target cells (Abassi et al., 2004; Solly et al., 2004; Xing et al., 2005). NK-mediated cytotoxicity as monitored on the RT-CES system correlates with standard assays such as MTT and crystal violet staining of target cells to assess effector-mediated cytotoxicity (Fig. 2). Both of these assays have been used to monitor effector-mediated cytotoxic activity towards target cells (Wahlberg et al., 2001; Peng et al., 2004). The release of granules containing the lytic proteins and proteases is regulated by receptor-mediated signal transduction cascade. Specifically, it has been shown that the PI3K and ERK pathways play a major role in granule exocytosis and cytotoxicity in addition to cytokine secretion (Djeu et al., 2002; Vivier et al., 2004). Using the RT-CES system and confirming earlier data we were able to demonstrate that interfering with the PI3K and Erk pathways by means of small molecular inhibitors blocks NK-mediated cytotoxic activity (Fig. 4). The extent of the inhibition of cytotoxicity with different pathway blockers depends on the duration of incubation of NK cells with target cells (Fig. 4B and D). Utilizing standard single point assays such as chromi-

---

Fig. 4. Specificity of NK-92 mediated cytolysis of target cells. (A) A549 cells were seeded in the wells of E-plates. Twenty-four hours after seeding NK-cells at a density of 16:1 (E/T ratio) were pre-incubated with the different concentrations of the MEK inhibitor PD 98059 for thirty minutes and then added to A549 cells at the indicated time point (arrow). The viability of A549 cells were continually monitored using the RT-CES system. (B) The extent of NK-92 mediated cytotoxic activity in the presence of MEK inhibitor was calculated at the indicated times post-NK addition as described in Materials and methods section. (C) The effect of PI3 Kinase inhibitor Wortmannin on NK-92 mediated cytolysis of A549 cells. The cells were treated with the inhibitor as described in (A). (D) Percent cytotoxic activity of NK-92 cells was calculated at the end of the experiment described in (C).
um release assay (CRA), it would've been easy to miss the time-dependent effect of the inhibitors upon cytolysis.

The standard assays monitoring NK-mediated cytotoxicity are label-based and end-point assays requiring significant investment in terms of labor and time. The standard assays can be subdivided into 6 different categories which include radioactive labeling method, enzymatic methods, fluorometric and spectrophotometric methods, ELISA-based methods and morphometric methods. CRA remains the most popular and widely used methods in most research settings for assessing NK and cytotoxic T lymphocyte (CTL) cytotoxicity activity towards target cells (Brunner et al., 1968). However, despite its popularity the CRA does pose certain problems the most formidable of which is the fact that it is a radioactive material with environmental and health hazards. Secondly, the CRA assay has a limited assay window beyond which the natural tendency of chromium 51 to diffuse out of the cell may contribute to background noise in the assay. Several enzymatic methods based on measuring the activity of enzymes such as lactate dehydrogenase (LDH) and granzyme B has been described (Korzeniewski and Callewaert, 1983; Shafer-Weaver et al., 2003). LDH is a common enzyme that is released from target cells upon cytolysis and its activity is quantified in a coupled enzymatic assay which results in the conversion of tetrazolium salt into a red formazan product (Korzeniewski and Callewaert, 1983). There are several major drawbacks to using the LDH assay for NK-mediated cytotoxicity studies. Primarily, serum in the media which is used to culture both the target and effector cells contains significant amounts of LDH which can contribute to the background. Also, the amount of LDH released from target cells upon cytolysis can vary dramatically from cell type to cell type and therefore optimization studies need to be performed for each target cell used. In addition, LDH can be released from effector cells upon cytolysis and in combination with LDH activity in the media can greatly obscure the amount of LDH that is released from target cells upon cytolysis. A granzyme activity assay in combination with a colorimetric peptide substrate has also been described as a specific measure of effector-mediated cytolysis of target cells (Ewen et al., 2003).

Flow cytometry is another way of assessing effector-mediated cytolysis of target cells (Zimmermann et al., 2005; Hatam et al., 1994). Typically, effector cells are incubated with the target cells and the target cells are labeled with fluorescent-labeled annexin V which recognizes cells that are undergoing cell death. The cells can also be labeled with propidium iodide to discriminate between cells undergoing early apoptotic or necrotic cell death. Alternatively, the effector cells may also be labeled with an antibody specific for membrane marker to discriminate between target cells and effector cells. Taken together, flow cytometry may be a more quantitative and specific way of assessing effector-mediated cytolysis of target cells. However, as described it entails numerous labeling and optimization procedures and combined with relatively limited throughput it may not warrant wide applicability. An ELISA method based on the detection and quantification of granzyme B has been described in the literature as an alternative to CRA for measuring cytotoxic activity of effector cells (Shafer-Weaver et al., 2003, 2004). In summary, all the standard assays described here for assessing effector-mediated cytotoxic activity towards target cells involve extensive labeling of target cells. The assays are typically endpoint assays which only provide a “snapshot” of the effector-mediated cytotoxicity process.

Unlike traditional end-point assays the RT-CES readout of impedance is non-invasive and continuous providing a dynamic measure of the cytotoxic activity of effector cells. The time resolution in the assay processes provides high content information regarding the extent of the cytotoxicity in addition to the exact time the cytotoxicity takes place at different effector to target ratio. In addition to effector-mediated cytotoxicity, cell sensor impedance technology can also be used to measure cytotoxicity due to other agents such as anti-mitotic and anti-cancer compounds (Solly et al., 2004; Xing et al., 2005). In fact Solly et al. (2004) have compared data generated for cell proliferation, cytotoxicity, cytoprotection, cell growth inhibition and apoptosis on the RT-CES system and classical methods such as CellTiter-Glo. They have concluded that the data generated by the RT-CES system is comparable to traditional methods with the added advantage of being non-invasive and providing kinetic parameters (Solly et al., 2004).

In summary, in this report we have described a new method based on cell-substrate impedance technology for assessing cytotoxic activity of effector cells which does not require any labels and allows for dynamic monitoring of the cytotoxicity process. The assay has been validated by using traditional methods such as MTT and crystal violet staining of target cells.

Acknowledgements

The authors wish to thank Dr. Hui Shao (University of Louisville) for her generous gift of mouse NK cells.
and YAC cells. The authors also would like to acknowledge the significant contribution of the Engineering Department of ACEA Biosciences towards the development of the RT-CES system.

References


