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Research paper

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

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**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 histocompatability 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-

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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 (Korzeniewski 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-plates™). 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% $CO_2$. 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-mercaptoenthanol, 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