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Antigen-specific T cell phenotyping microarrays using Grating Coupled Surface Plasmon Resonance Imaging and Surface Plasmon Coupled Emission

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Abstract

The circulating population of peripheral T lymphocytes obtained from a blood sample can provide a large amount of information about an individual's medical status and history. Recent evidence indicates that the detection and functional characterization of antigen-specific T cell subsets within the circulating population may provide a diagnostic indicator of disease and has the potential to predict an individual's response to therapy. In this report, a microarray detection platform that combines grating-coupled surface plasmon resonance imaging (GCSPRI) and grating-coupled surface plasmon coupled emission (SPCE) fluorescence detection modalities was used to detect and characterize CD4⁺ T cells. The microspot regions of interest (ROIs) printed on the array consisted of immobilized antibodies or peptide loaded MHC monomers (p/MHC) as T cell capture ligands mixed with additional antibodies as cytokine capture ligands covalently bound to the surface of a corrugated gold sensor chip. Using optimized parameters, an unlabelled influenza peptide reactive T cell clone could be detected at a frequency of 0.1% in a mixed T cell sample using GCSPRI. Additionally, after cell binding was quantified, differential TH1 cytokine secretion patterns from a T cell clone cultured under TH1 or TH2 inducing conditions was detected using an SPCE fluorescence based assay. Differences in the secretion patterns of 3 cytokines, characteristic of the inducing conditions, indicated that differences were a consequence of the functional status of the captured cells. A dual mode GCSPRI/SPCE assay can provide a rapid, high content T cell screening/characterization tool that is useful for diagnosing disease, evaluating vaccination efficacy, or assessing responses to immunotherapeutics.

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Keywords

T cell microarray; SPR; SPCE

Introduction

The circulating population of peripheral T lymphocytes rapidly and frequently traffics throughout the body and is exposed to many different tissue microenvironments. Functional and phenotypic characterization of these T cells can indicate prior exposure to vaccines (Whiteside et al. 2003), infectious agents (Lalvani et al. 2001), and toxicants (Raulf-Heimsoth et al. 2000). Furthermore, detection of certain self-antigen-specific T cells can help to confirm the presence of autoimmune disease (Cernea and Herold 2010) and may also reflect the propensity to develop autoimmune disease before the onset of symptoms. Detecting and characterizing antigen-specific T cells in peripheral blood, however, is a laborious, time-consuming, and costly activity. As a consequence, these types of analyses are usually done only after the appearance of clinically observable disease symptoms. At that point, damage to tissues may have progressed far enough to make treatment less effective than it might have been if started earlier.

The detection of antigen-specific T cells has previously been accomplished by specific labeling with fluorescently tagged oligomerized major histocompatibility complex (MHC) proteins that have been loaded with antigen-derived peptides (p/MHC). Cells that bind these complexes are subsequently identified by flow cytometry (Reijonen et al. 2003). The allelic form of the p/MHC complex must, in most cases, match the patient's MHC to promote a constructive interaction between the T cell receptor (TCR) and the fluorescently labelled p/MHC. Because MHC molecules in the human population are extremely polymorphic (Reche and Reinherz 2003), the patient's MHC must be determined before T cell analysis can proceed. In flow cytometry, this approach is limited by the number of different fluorescent labels that can be differentiated in a single assay, as well as significant labor and reagent requirements for screening assays. By using pre-determined spatial coordinates rather than fluorescent tags on a sensor chip to identify specific interactions, microarrays are not constrained to the same multiplexing limits as flow cytometry. However, traditional fluorescence based cellular microarrays still require a labeling step during sample preparation to generate signal and remain technically challenging (Deviren et al. 2007, Soen et al. 2003).

GCSPRI cellular microarrays retain the increased multiplexing capacity afforded by other microarray systems and do not require cells to be labeled. Cell binding to the sensor chip is quantified by measuring the change in SPR resonance angle at spatially defined regions of interest (ROIs) on the sensor chip (Chabot et al. 2009). Binding of a cell causes the resonance angle to shift, which results in a signal that is proportional to the number of cells captured (Unfricht et al. 2005). In a GCSPRI system, the evanescent wave vector extends only about 200nm from the chip surface into the dielectric medium above (Chabot et al. 2009) meaning that only cells directly in contact with the chip surface actually contribute to the GCSPRI measurement. This results in sufficient sensitivity to detect a single human T cell captured by an immobilized antibody ROI (Unfricht et al. 2005). The incorporation of microfluidics makes this an ideal assay platform because small volumes of sample can be recirculated over the sensor surface to facilitate increased sampling of the cell suspension. A GCSPRI sensor chip with 1 cm² active sensor surface area is capable of presenting more than 10³ ROIs for potential cell capture in a 50µl flow cell volume (Unfricht et al. 2005). The capacity to interrogate hundreds of peptide-MHC combinations in parallel in a single sample would allow screening for T cells that are specific for many putative disease-

associated peptides in combination with multiple allelic variations of the MHC class I and class II molecules.

Using the dual mode GCSPRI/SPCE based microarray described in this report, T cell subsets bound to the chip surface can be assessed for cytokine secretion by co-immobilizing cell capture ligands and anti-cytokine antibodies on the same ROI microspot. After cells are selectively immobilized and enumerated by GCSPRI and cultured on the microarray, ROIs can be probed for the presence of secreted cytokines using secondary antibodies conjugated to a fluorescent tag. Low levels of fluorescence generated in this assay can be detected because of the increased signal to noise ratio resulting from surface plasmon coupled emission (SPCE). By using the appropriate excitation wavelength, fluorophores within 200nm of the sensor chip surface are excited by both the excitation laser and the surface plasmon, rather than by the laser alone. Interaction of the fluorescent emission with the surface plasmon concentrates the fluorescence towards the path of the detector, increasing optical collection efficiency (Reilly et al. 2006). Together, increased excitation and collection efficiency improve the sensitivity of SPCE and allow for T cell activation status in response to stimulation to be characterized. By automating this process with programmed microfluidics, a dual mode GCSPRI/SPCE T cell detection/phenotyping assay is capable of assaying for the presence of hundreds of different antigen-specific T cells simultaneously and, when present on the ROI in sufficient numbers, determining the functional status of the immobilized cells.

Materials and Methods

Cell lines and culture conditions

The Jurkat E6 CD4⁺ T cell line was obtained from the American Type Culture Collection (ATCC #TIB-152, Manassas, VA). The CH7C17 CD4⁺ T cell line has been described previously (Wedderburn et al. 1995). Both cell lines were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, GA) and 4mM L-glutamine, Penicillin/Streptomycin, and 25mM HEPES buffer (Fisher Scientific Company, Morris Plains, NJ) at 37 °C and 5% CO₂. For secretion experiments, Jurkat E6 CD4⁺ T cells were stimulated with immobilized anti-CD3 (2 µg/ml) and soluble anti-CD28 (5 µg/ml) in the presence of IL-12 (5 ng/ml) and IFN-gamma (2 ng/ml) for 48 hours (TH1 inducing conditions), or antibody plus IL-4 (5 ng/ml) and IL-10 (10 ng/ml) for 48 hours (TH2 inducing conditions). Cells were then washed (3×) with media and resuspended in media without cytokines for 24 hours. Immediately prior to being assayed, cells were spun down and resuspended in media plus phytohemagglutinin (PHA) (5 µg/ml) (Roche, Indianapolis, IN) to a final concentration of 1×10^6 cells ml⁻¹ before testing for cytokine secretion. For cytokine secretion assays, 1.5 ml of cell suspension was added to wells of a 24 well tissue culture plate (BD Biosciences, San Diego, CA) and incubated at 37 °C for 4 hours before being spun down and collecting the supernatant. Supernatant was stored at -80 °C until analysis by ELISA.

GCSPRI instrumentation and sensor chips

The GCSPRI instrument used in these studies is an engineering prototype of the 8500 Flexchip Analyzer (HTS Biosystems, Hopkinton, MA) and has been described previously (Unfricht et al. 2005). GCSRPI sensor chips were provided by Ciencia, Inc. (East Hartford, CT). Assays requiring both GCSPRI and fluorescent measurements were made with a second generation GCSPRI / SPCE dual mode instrument designed and built by Ciencia, Inc. (East Hartford, CT). The second generation instrument is based on the same SPR principle as the 8500 Flexchip, but operates at a shorter wavelength that is compatible with

the excitation of Alexa fluor 647 (Invitrogen, Carlsbad, CA) and CellTrace Far Red DDAO-SE (Invitrogen, Carlsbad, CA).

GCSPRI sensor chip modification and printing

Gold sensor chips were washed with 70% ethanol and rinsed with water (18 MOhm) and dried under a stream of filtered air. For dithiobis-succinimidyl propionate (DSP) surface modifications, DSP (Thermo Fisher, Rockford, IL) was resuspended in dimethyl sulfoxide (DMSO) at 4 mg ml⁻¹ and contacted to the gold surface for 30 min. Chips were washed exhaustively with DMSO and water (18 MOhm) and immediately used for printing. 2-(pyridinyldithio) ethylcarbonyl dextran (PDEC dextran) surface modifications were performed as previously reported (Li et al. 2008). Before printing on PDEC dextran surfaces, inter-heavy chain disulfide bonds of antibodies were reduced with 1mM dithiothreitol (DTT) for 8 hours to expose sulfhydryl groups for covalent immobilization. For neutravidin surface modifications, cleaned sensor chips were incubated with a solution of 1mM 1,9 nonanedithiol (Sigma-Aldrich, St. Louis, MO) in ethanol for 2hrs and then washed extensively with ethanol. Chips were then incubated with 0.3 mg ml⁻¹ maleimide-activated Neutravidin (Thermo Fisher, Rockford, IL) in PBS for 1hr and then washed exhaustively with water (18 MOhm) and dried under a stream of filtered air. All surface modifications were made at 25 °C.

Microarrays were printed on sensor chips using the OmniGrid Micro robotic spotter (Genomic Solutions, Ann Arbor, MI) or a SpotBot II robotic spotter (TeleChem International, Inc., Sunnyvale, CA). and an ArrayIt Stealth micro spotting pin, size SMP7B (255 µm spot diameter, 3.1 nL delivery volume) (TeleChem International, Inc., Sunnyvale, CA). Chips were maintained in high humidity (75–85%) at 25 °C during spotting and allowed to incubate for an additional 1 hr in high humidity after spotting. Chips were then removed and fitted with a 0.02” thick, double-sided adhesive gasket and 1/8” thick glass window to form a 50µl flow cell over the printed array. Once printed, chips were stored desiccated at 4 °C for up to 4 days before use.

Antibodies and general reagents

Anti-human cytokine antibody matched pair sets for Interleukin-2 (IL-2), Interferon-gamma (IFN-gamma) and Tumor Necrosis Factor-alpha (TNF-alpha) were purchased as duosets from R&D systems (Minneapolis, MN). Mouse anti-human CD3ε and anti-human CD28 antibodies were purchased from BD Biosciences Pharmingen (San Diego, CA). Biotinylated mouse anti-human CD3 was purchased from Becton-Dickinson (Franklin Lakes, NJ). Superblock (in PBS) blocking agent was purchased from Thermo Scientific (Rockford, IL). Bovine serum albumin (BSA) was purchased from Fisher Scientific Company (Morris Plains, NJ). BSA was biotinylated using polyethylene oxide-maleimide-biotin from Pierce (Rockford, IL) and diluted to 500 µg ml⁻¹ in PBS for use on reference ROIs. CellTrace Far Red 9H-(1,3-dichloro-9, 9-dimethylacridin-2-one-7-yl) succinimidyl ester (DDAO-SE) was used for fluorescent cell labeling and streptavidin Alexa fluor 647 were purchased from Invitrogen (Carlsbad, CA).

Peptide-MHC monomer complexes

The influenza hemagglutinin peptide abbreviated HA (PKYVKQNTLKLAT), and the tetanus toxin control peptide abbreviated TT (QYIKANSKFIGITE), presented in the context of DR1 (DRA*0101 DRB1*0101) have been previously described (Stone et al. 2005; Zavala-Ruiz et al. 2004). Briefly, recombinant MHC proteins were prepared by standard methods (Cameron et al. 2002). Soluble HLA-DR1 including a uniquely reactive cysteine engineered at the C terminus of the alpha chain was produced in Schneider S2 insect cells,

and was chemically modified with polyethylene oxide-maleimide-biotin (Pierce, Rockford, IL).

GCSPRI assay procedure

Sensor chips were blocked by flushing 5ml of Superblock (Pierce, Rockford, IL) or 2% BSA in PBS over the chip and allowing the blocking agent to incubate for 10 min. This step was repeated once. Next, 5ml of complete RPMI 1640 with 10% FBS was flushed over the chip and allowed to incubate for 30 minutes. This step was repeated once. Cells were resuspended to 2×10^6 cells ml^{-1} in complete RPMI 1640 with 10% FBS before analysis. Complete RPMI 1640 10% FBS was passed over the chip surface for 5 to 15 min at $200 \mu\text{l min}^{-1}$ to establish a baseline SPR angle measurement. Three ml of sample was recirculated at $200 \mu\text{l min}^{-1}$ for 15–30 min to allow for cell binding. Non-specifically bound cells were minimized by following the cell suspension exposure with a wash step consisting of complete RPMI 1640 media 10% FBS at passed over the chip at 1ml min^{-1} for 10 min. For cytokine production assays, cells were allowed to incubate on the chip for 3 hours before exposure to lysis buffer (20mM Tris, 100mM NaCl, 1mM EDTA, 0.5% Triton $\times 100$, pH 7.4) for 5 min followed by a wash with PBS plus 0.0025% Tween20 plus .0002% NaN_3 (PBST) at $500 \mu\text{l min}^{-1}$ for 5 min. 2 ml of 0.1% BSA in PBST containing a cocktail of secondary matched-pair detection antibodies at recommended ELISA concentrations (R&D Systems, Minneapolis, MN) was recirculated over the chip at $500 \mu\text{l min}^{-1}$ for 60 min, followed by a wash with PBST at $500 \mu\text{l min}^{-1}$ for 5 min, followed by streptavidin-Alexa fluor 647 in PBST at 800ng ml^{-1} for 20 min and a final 10 minute wash step with PBST before measuring fluorescence using SPCE. Chips were kept at a constant 37°C for the duration of all experiments.

Data analysis

GCSPRI measurements of cell capture were analyzed with Flexchip 8500 Data Analysis Software (HTS Biosystems, Hopkinton, MA) for experiments using the Flexchip prototype and Microsoft Excel for experiments using the dual mode GCSPRI/SPCE prototype. ELISA measurements were made using a SpectraMax Plate Reader and SoftMaxPro software (Molecular Devices, Sunnyvale, CA). Linear regression and curve fitting was done with Graphpad Prism 4.0 (Graphpad Software Inc, San Diego, CA.) Significance analysis was done using a one-tailed T test ($p < .05$) or one-way ANOVA and Tukey's Test ($p < .05$ or $p < .002$).

Results and Discussion

In GCSPRI systems, the change in SPR angle over time is based on local refractive index changes that occur above the spatially defined ROIs on the biosensor chip (Jin et al. 2006) and is proportional to the density of cells bound to specific ROIs (Unfricht et al. 2005). To validate the utility of GCSPRI as a T cell detection platform, we compared two independent measurements of cell capture on the same GCSPRI chip. A CD4^+ T cell population was fluorescently labelled with the succinimidyl ester DDAO-SE, and then captured on 20 ROIs containing different concentrations of anti-CD3 antibody to facilitate a range of cell binding densities. Immobilization of antibodies for cell capture was mediated through a dithiobis-succinimidyl propionate (DSP) modified surface which acted as crosslinker between the gold chip and cell capture antibody. This surface was chosen for preliminary experiments because it is easily fabricated and results in uniform and reproducible immobilization of protein (Thampi et al. 2006). The resulting GCSPRI angle shift was compared to the fluorescent signal for each ROI (Supplementary Figure 1). The high degree of correlation ($r^2 = 0.9178$) between the two measurements indicates that SPR angle shift provides an

accurate measurement of captured cell populations that is comparable to direct detection of fluorescently labelled cells.

Non-specific binding (NSB) of protein and non-specific adhesion (NSA) of cells will influence the sensitivity of GCSPRI measurements by adding to background noise. Ideally, modifying the gold surface with a crosslinker will result in a more homogenous and reproducible deposition of the capture ligand. This should lead to more target molecules or cells bound per unit area (higher activity), more reproducible analyte capture (less inter-assay variation), and a surface that is less sensitive to temperature and redox changes.

To test the activity of capture ligands immobilized via different surface modifications, we compared changes in the SPR angle using the Flexchip 8500 GCSPRI instrument after recirculation of CH7C17 T cells that recognize the influenza peptide (HA 306-318) presented by HLA-DR1 (HA-DR1). Anti-CD3 antibodies or HADR1 p/MHC complexes with a C-terminal cysteine were immobilized via passive physical adsorption to unmodified gold as a control surface strategy. Alternatively, immobilization on the chip was facilitated using a 2-(pyridinyldithio) ethylcarbonyl dextran (PDEC dextran) which forms a covalent bond to the capture ligand through a free sulfhydryl group and represents the use of a hydrogel matrix modification. Biotinylated anti-CD3 antibodies or HA-DR1 p/MHC complexes with a C-terminal biotin were also immobilized via a neutravidin modified gold surface that utilizes a self assembled monolayer (SAM) of alkane-dithiols as a crosslinker (Supplementary Figure 2). Cell capture activity of the immobilized HA-DR1 p/MHC complex was similar for both the biotin-neutravidin surface and sulfhydryl-PDEC dextran surface immobilization strategies. However, the PDEC dextran coated surface showed significantly lower non-specific binding to bovine serum albumin (BSA) negative control ROIs compared with the other surfaces tested (Figure 1). Most non-specific cell and protein binding occurs through electrostatic or hydrophobic interactions with the surface (Andersson et al. 1971) and the neutral, hydrophilic PDEC dextran appeared to resist NSB which reduced background to less than 10% of signal. The coefficient of variation (CV) for p/MHC mediated cell capture on the PDEC dextran surface ($CV < 0.05$) was lower than unmodified gold ($CV < 0.20$) or neutravidin ($CV < 0.21$) and is an indirect indicator of reproducible ROI spotting of the capture ligands.

The effect of sample flow rate was tested to determine the rate that produces the highest specific signal to background ratio. It has been reported by other groups that the rate constant for cell adhesion (e.g. the number of cells captured per unit time) increases with velocity under the range of flow conditions used in cellular microarray assays (Pierres et al. 1994). As the flow rate increases under these conditions, the encounter rate between cell surface markers and capture ligands increases but the duration of the interactions decreases. At very high flow rates, the adhesion rate begins to plateau as the two effects counterbalance each other (Chang and Hammer 1999). We observed that faster sample flow rates increased the rate of cell adhesion up to a flow rate of $500 \mu\text{l min}^{-1}$ (Supplementary Figure 3) for our system. Flow rates below $100 \mu\text{l min}^{-1}$ resulted in higher background signals at control ROIs due to increased non-specific cell adhesion, which lowered the sensitivity of antigen-specific cell detection.

Optimal flow rate for the removal of non-specific cell adhesion by washing the chip surface with media was also investigated. At flow rates between 100 and $1000 \mu\text{l min}^{-1}$, removal of cells specifically bound to capture ligands was negligible, indicating that T cell capture via immobilized p/MHC or antibodies is a durable event. Removal of non-specifically bound cells was essentially equivalent for all flow rates above $1,000 \mu\text{l min}^{-1}$ after 15 min of wash (data not shown). For this reason, a flow rate of $1000 \mu\text{l min}^{-1}$ was used for removal of unbound cells.

Using these flow-rate parameters, we investigated the amount of time needed to generate a maximum SPR angle shift at a given ROI. Using a sample flow rate of $500\mu\text{l min}^{-1}$ we found that a maximum SPR angle shift of between 320 and 400 resonance change units (RCU) was generated within 30 minutes of sample exposure at anti-CD3 or anti-CD28 containing ROIs (Figure 2) on the dual mode GCSPRI/SPCE system. Variation in the rates of cell capture was observed when replicate ROIs were positioned near the center of the chip and near the flow cell edge, presumably due to laminar flow patterns. This positional effect disappeared when ROIs were placed more than 2mm from the flow cell edge and cell capture rates between 5 replicate ROIs placed at this minimum distance from the flow cell edge were similar ($\text{CV} < 0.05$) (data not shown). This spacing requirement was used in all cell capture microarray experiments reported here.

One application of GCSPRI technology is to interrogate a heterogeneous population of T cells and determine the presence of low-frequency T cells with specific antigen reactivity. To assess the limits of antigen-specific cell detection sensitivity, CH7C17 T cells were recirculated over a PDEC dextran biosensor chip either as a homogenous population, or in a mixed population with Jurkat T cells that do not recognize the HA-DR1 p/MHC construct. To test for non-specific binding to the DR1 MHC, we included a tetanus toxin derived control peptide also presented by DR1. There was a strong linear correlation ($r^2 = 0.93$) between antigen-specific T cell density and SPR angle shift (RCU). At the lower limit of detection, 0.1% HA-DR1 specific T cells in the pool of 6×10^6 total T cells resulted in cell capture signal that was significantly above signal from control peptide ROIs after 30 minutes of sample recirculation (Figure 3).

Because many autoimmune diseases are T cell mediated, detection and characterization of self-antigen reactive T cells is a more direct approach to specifically characterize an autoimmune disease and may provide better predictive indicator of patient risk (Trudeau et al. 2007). The frequency of a diabetes antigen-associated CD8^+ T cells in the non-obese diabetic (NOD) mouse model, for example, ranges from 0 – 0.76% of total CD8^+ T cells in peripheral blood (Trudeau et al. 2003) and correlates strongly with disease severity. The GCSPRI cellular microarray described here is sufficient to detect an antigen-specific T cell population in NOD mice after cells are positively selected for CD4 expression. The efficient screening of some T cell mediated autoimmune diseases, however, may require detection of very rare T cell populations and may have to overcome difficulties associated with very low avidity TCR – p/MHC interactions. To improve the detection sensitivity of a GCSPRI cellular microarray, strategies aimed at preventing non-specific cell binding or increasing the activity of the p/MHC reagents should be explored. A potential approach would be to improve the molecular flexibility of the immobilized p/MHC complex (Deviren et al. 2007) by tethering it to a more flexible crosslinking surface composed of mixed oligo-ethylene glycols. This could decrease non-specific adhesion of cells and increase cell capture efficiency which would result in more densely bound cells at p/MHC ROIs and a higher signal to noise ratio.

Another approach to further characterize T cells in a mixed population using SPR-based microarrays combines GCSPRI-based cell detection with an SPCE-based fluorescent measurement of cytokine secretion by captured cells. To test this application, we investigated whether T cell populations primed under different inducing conditions could be differentiated by a GCSPRI/SPCE cellular microarray. Jurkat T cells were exposed to TH1 inducing conditions (pre-cultured with anti-CD3, anti-CD28, IL-12 and IFN- γ), TH2 inducing conditions (pre-cultured with anti-CD3, anti-CD28, IL-10 and IL-4), or control conditions (no stimulation) for 24 hours, followed by culture in media without cytokines for 24 hours. T cells were then stimulated with phytohemagglutinin (PHA) and captured on a dual mode GCSPRI/SPCE microarray sensor chip or incubated in wells for supernatant

analysis by ELISA. For microarray experiments, antibodies against the T cell specific markers CD3 and CD28 were co-immobilized with a panel of anti-TH1 cytokine monoclonal antibodies to interrogate cell secretion. The fluorescent secretion signal was normalized to the density of captured cells at each ROI to determine a ratio of cytokine secretion per unit density of captured cells for each T cell sub population. In these experiments, cell capture signal for each of the three cell populations was roughly equivalent ($CV < 0.08$) because cells were passed over the chip surface until a monolayer of cells were immobilized on ROIs (Figure 4A). Adjacent ROIs comprised of anti-TH1 cytokine antibody alone were not fluorescent, indicating secretion signals are specific to cells immobilized at a particular ROI (data not shown).

In TH1 cytokine secretion experiments, IL-2 secretion was increased when cells were exposed to TH1 versus control or TH2 inducing conditions and measured by SPCE or ELISA (Figure 4). This observation shows that the measured increase in IL-2 after pre-incubation with TH1 inducing conditions is independent of the assay format used to detect it. Similarly, IFN-gamma secretion was increased after TH1 compared with TH2 conditions for both SPCE and ELISA based measurements. However, IFN-gamma levels were significantly higher after control versus TH2 conditions for both SPCE based assays but not in the supernatant used in the ELISA analysis. Increased secretion of TNF-alpha was observed after TH1 or control compared with TH2 conditions and measured by SPCE. This difference was also not observed in the ELISA supernatant, where TNF-alpha secretion was only higher in TH1 compared with control conditions. We believe the TH cytokine secretion profiles measured by SPCE may reflect an additional stimulatory effect of cell capture by surface immobilized antibodies in the presence of PHA which is absent from cell incubation in wells. Furthermore, if higher levels of CD3 or CD28 surface expression correlate with increased IFN-gamma and TNF-alpha secretion, selectively capturing and interrogating these cells could be responsible for the increased cytokine levels observed in SPCE assays but not in ELISA experiments. In the ELISA experiments, secretion from these higher secreting cells may be diluted out in the larger population which prevented differences in IFN-gamma or TNF-alpha being detected between TH2 or control conditions. Alternatively, differences could be due, in part, to the release and capture of intra-cellular cytokines during cell lysis in SPCE microarray experiments.

Cell secretion patterns also differed between similarly induced cells immobilized on anti-CD3 or anti-CD28 ROIs. Cells captured on anti-CD3 ROIs produced less IL-2 after TH1 or TH2 inducing conditions, and less TNF-alpha after control conditions compared to cells captured on anti-CD28 ROIs (Figure 4B and C). The significant differences in the secretion patterns of T cells exposed to different inducing and capture conditions demonstrates that this system can detect subtle changes in T cell programming induced by the cellular microenvironments. This programming contributes to a functional status that is distinct from the surface phenotype of the cell and results in a secretion pattern that depends on both the functional status and the capture ligand.

Detection of self-antigen reactive T cells alone may not be sufficient to predict or diagnose disease onset for all autoimmune diseases. CD4⁺ GAD65 reactive T cells, for example, can be found at equivalent frequencies in healthy and type 1 diabetic populations and it is their preferential activation in diabetic patients that is believed to be associated with disease (Danke et al. 2005). In these cases, characterizing the functional status of specific T cell subsets may be needed to overcome high false-positive rates that would be associated with a T cell screening assay that only measured the presence of autoreactive T cells. Similarly, the ability to differentiate T cells based on their functional status has shown more promise in identifying individuals at risk to develop rheumatoid arthritis than the detection of antigen-specificity of T cells or the presence of peripheral blood cytokines alone (Davis et al. 2010).

Furthermore, the diagnostic information gained by interrogating circulating populations of lymphocytes is not limited to autoimmune disease. For example, exposure to various environmental stressors such as toxic heavy metals has been demonstrated to affect T cell development and the response to activation signals (Mishra et al. 2003). By integrating information about the presence of antigen-specific T cells and the functional status of immune cell subsets, GCSPRI/SPCE may therefore be able to screen for exposure to different environmental stressors.

Conclusions

In this report, we have established the utility of a GCSPRI cellular microarray for the detection of antigen-specific T cells. Currently, our system is capable of detecting antigen-specific T cells at a frequency of 0.1% in a mixed T cell population. Further optimization of assay parameters including sensor surface modifications that are resistant to non-specific cell adhesion and increase cell capture ligand activity could lower this limit of detection even further. We have also established that selected populations of T cells can be interrogated for functional effector status using an SPCE based fluorescent microarray. Exposure to different microenvironments can alter the functional status of a population of T cells and these changes can be detected by comparing cytokine secretion patterns from captured cells. The functional status of T cells, based on cytokine secretion patterns, has been shown to be a better prognostic indicator of autoimmune disease and response to therapy than the detection of antigen-specific T cells or cytokines in peripheral blood. For these reasons, the broad application of this combination T cell detection / functional characterization platform could extend from early diagnosis of T cell mediated autoimmune disease to exposure to environmental stresses or the prediction and evaluation of therapeutic efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Bios-D-11-01119 Highlights

Circulating T lymphocytes from blood represent a cell population that can be interrogated for functions related to immune response to infection or vaccination, for indications of autoimmune disease, or for changes in immune capacity as a consequence of exposure to environmental toxicants. We describe a novel grating coupled surface plasmon-based microarray that can be employed for interrogation of T cell activities and antigen specificities. We conclude that T cells can be captured by cognate antigen complexed to an appropriate MHC monomer, and that function of these cells can be subsequently determined.

Figure 1. Effect of different biocompatible surfaces on cell capture ligand activity
 CH7C17 HA peptide specific T cells (1×10^6 cells ml^{-1}) in RPMI 1640 were recirculated over SPR sensor chips modified with neutravidin or PDEC dextran or left unmodified. The net SPR angle shift (RCU) after recirculation is shown. Bars are averages of 3 ROI $\pm$ S.D. #indicates a significant difference between groups ($p \leq .05$).

Figure 2. Kinetics of T cell capture on an antibody microarray

Anti-CD3 antibodies (circles), anti-CD28 antibodies (squares) or isotype matched negative control antibodies (triangles) were immobilized on a DSP modified GCSPRI sensor chip. Chips were washed with PBS (10 min) and a media baseline was established (5 min). Chips were exposed to a suspension of Jurkat E6 T cells (2×10^6 cells ml^{-1}) at $500 \mu\text{l min}^{-1}$ for 40 minutes. Each line represents one ROI on the microarray. Arrows indicate the duration of cell suspension exposure to the sensor chip surface.

Figure 3. Limits of HA-DR1 reactive T cell detection by GCSPRI microarray

CH7C17 HA-DR1 reactive T cells were mixed with Jurkat E6 T cells and the percentage of reactive cells in a cell sample of 6×10^6 total cells was varied from 100 to 0.01%. (A) A mixed T cell suspension was recirculated over a PDEC dextran modified SPR biosensor chip for 30 minutes and net GCSPRI angle shift (RCU) at ROIs containing cognate p/MHC (HA / DR1), negative control p/MHC (TT / DR1), or anti-CD3 antibody was determined. Bars are the average of 3 separate experiments $\pm$ S.D.. (B) Images of representative ROIs after T cell sample exposure from each condition are shown. # indicates a significant difference between groups ($p \leq .05$).

Figure 4. Cytokine release from T cells exposed to different inducing conditions can be differentiated by GCSPRI/SPCE microarray

Jurkat E6 T cells were stimulated under TH1 or TH2 inducing conditions or control conditions (no stimulation) for 48 hours and then cultured for 24 hours in media without cytokines. (A) Cells were then captured at ROIs containing anti-CD3 or anti-CD28 antibody and anti-TH1 cytokine antibodies and incubated on the chip in the presence of PHA for 3 hours to allow for cytokine secretion and capture. Cells were then lysed and ROIs were probed for the presence of secreted cytokines using fluorescently labeled secondary antibodies. (B,C) Fluorescence per unit of cell density was measured using a ratio of the surface plasmon coupled emission (SPCE) measurement (AFU) divided by the GCSPRI measurement (RCU). (D) Cells were incubated in wells of a 24 well tissue culture plate in the presence of PHA for 3 hours before cells were spun down and the supernatant was analyzed for the presence of 3 cytokines by ELISA. Bars are the average of 3 independent experiments $\pm$ S.D. # indicates a significant difference between groups ($p \leq .05$), ## ($p \leq .002$)