Immunology

Kinetics of CpG internalization and sub-cellular organelle co-localization within circulating human plasmcytoid dendritic cells

ImageStream Capabilities Featured: Internalization / Cell Signaling & Molecular Translocation / Cell–Cell Interaction / Morphology/ Cell Cycle & Mitosis / Co-localization / Spot Counting / DNA Damage & Repair / Cell Death & Autophagy / Immunology / Oncology / Biochemistry / Virology / Microbiology / Parisitology / Hematology / Stem Cell Biology / Oceanography / Toxicology / Drug Discovery /

Abstract

Plasmacytoid dendritic cells are principal players in the innate immune response to viruses. Although rare (less than 0.2% of circulating peripheral blood cells), they secrete over 3 pg of the potent antiviral cytokine IFN-α per cell in response to HSV, mainly via TLR-mediated signaling upon recognition of viral DNA motifs. As ligand-bound TLR9 enters the endosome, MyD88 dependent IRF-7 and NF-kB signaling is promoted, resulting in the sustained anti-viral responses mediated by the pDC. The lack of available cell lines and the poor adaptability of pDC to culture have rendered the study of TLR-mediated internalization and sub-cellular trafficking of viral particles, as well as TLR-mediated signaling within pDC difficult by tradition biochemical or microscopic techniques. We have recently shown that nuclear translocation events within non-adherent primary cells can be quantified by correlating transcription factor and nuclear images collected in flow using the ImageStream imaging flow cytometer. We have also demonstrated the ability to quantify sub-cellular trafficking of internalized molecules in tumor cells using the ImageStream. This instrument automatically acquires up to six different spatially registered images (brightfield, darkfield, and four fluorescent) per cell at very high rates using a digital CCD camera. The digital imagery obtained is analyzed using the IDEAS statistical image analysis program which provides tools for the objective numerical scoring and discrimination of cells based on the characteristics of their imagery. The ability to numerically score large numbers of automatically acquired images is ideally suited to the analysis of internalization, sub-cellular trafficking and nuclear translocation within rare primary immune system cells such as pDC. In this study, we use ImageStream cytometry to measure a) CpGB-induced nuclear translocation of NF-κB and b) the internalization and intracellular trafficking of CpGB to endosomes and lysosomes within pDC using image-based quantitative metrics.

Results

Figure 1.

Identification of T cell: APC doublets

To limit analysis to intact single pDC, we gated events with intermediate BF area and high aspect ratio, which distinguishes single cells (R1) from debris (low BF area) and multi-cellular (high DRAQ5 intensity and low aspect ratio) events (A). BDCA+ cells were gated in (B). The percentages of single cells and single cells that are pDC are indicated in the upper right corner of each plot. Out-of-focus cells (low BDCA contrast) were eliminated from further analysis (data not shown). Because the CpG stain is concentrated in small areas, we used the CpGB Max Pixel feature to identify those pDC with associated CpG (C).

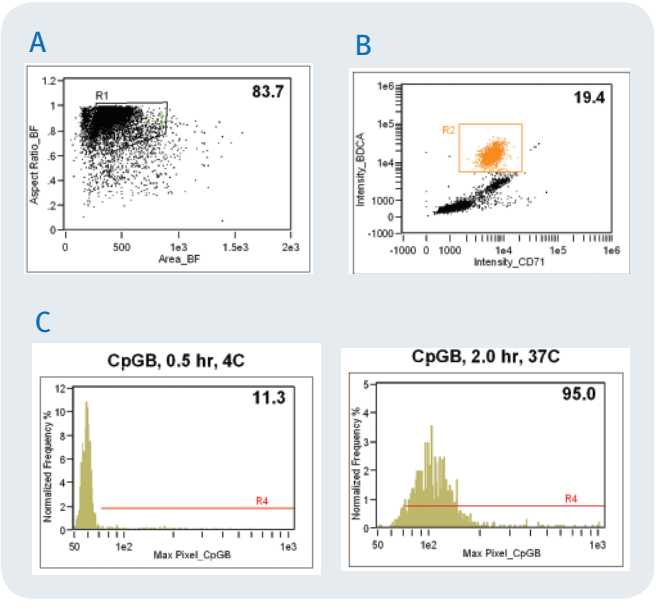

Figure 2.

Correlation between CpGB and CD71

To measure the relative co-localization of pDC-associated CpGB to organelles, we calculated the correlation between the bright details of the CpGB and CD71 or CD107a (Figure 3) image pair for each pDC using the Bright Detail Similarity feature (X-axis). This feature correlates only the portion of each image in the optimal plane of focus, and ignores data contributed by background pixels within the cells. As CpGB molecules internalize and co-localize to the endosome, the similarity between the CpGB and CD71 images increases.

The region R7 was drawn to include those cells with clear visual evidence of co-localization. The internalization feature (y-axis) is derived from the ratio of CpGB bright detail intensity inside the cell to the total CpGB bright detail intensity. The internalization score is conditioned such that a value greater than zero indicates that more CpGB is found inside the cell compared to outside the cell. Representative images are shown here.

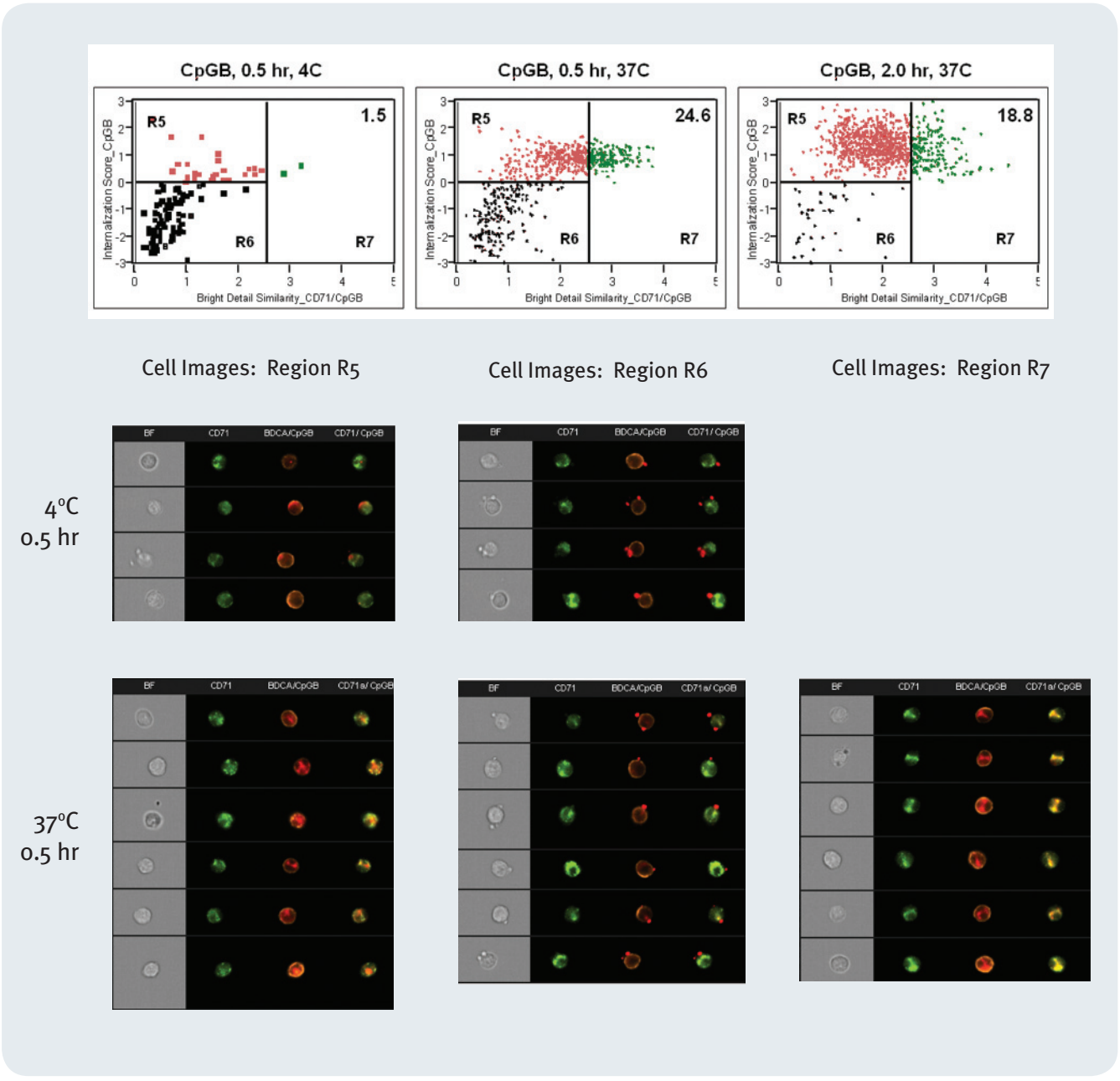

Figure 3.

Correlation between CpGB and CD107a

As in Figure 2, the relative co-localization of pDC-associated CpGB to organelles was measured using a calculated correlation between the bright details of the CpGB /organelle (CD107a) image pair for each pDC using the Bright Detail Similarity feature. As CpGB molecules internalize and co-localize to the endosome, the similarity between the CpGB and CD71 images increases. The region R7 was drawn to include those cells with clear visual evidence of co-localization.

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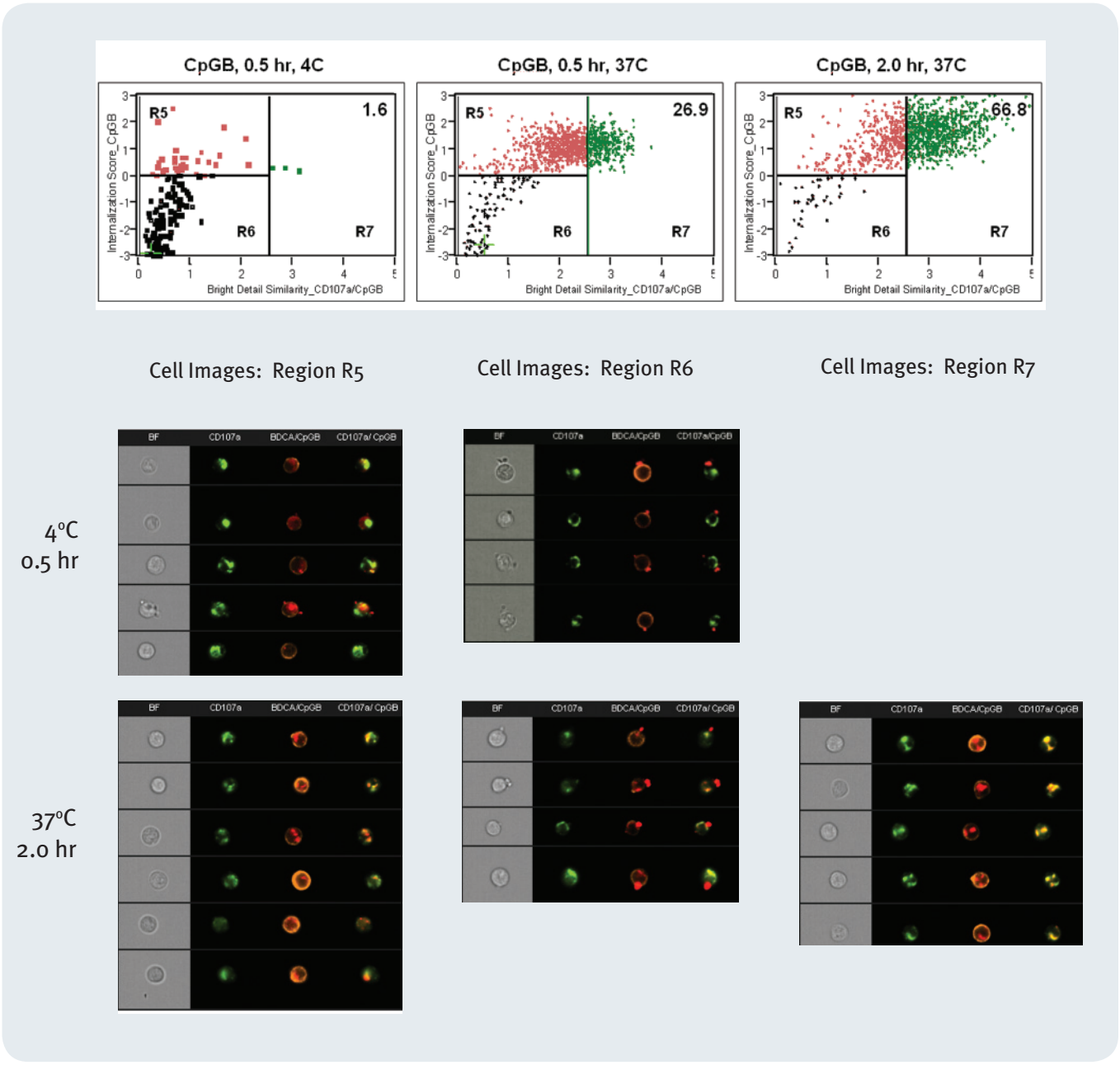
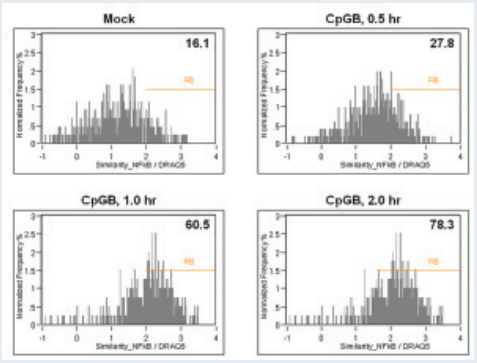
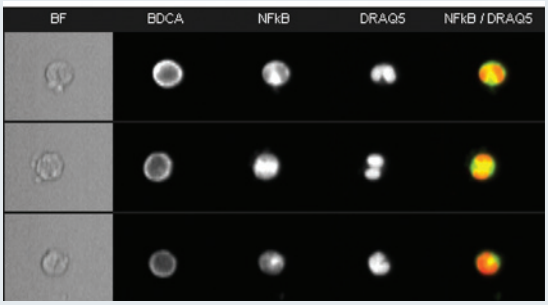


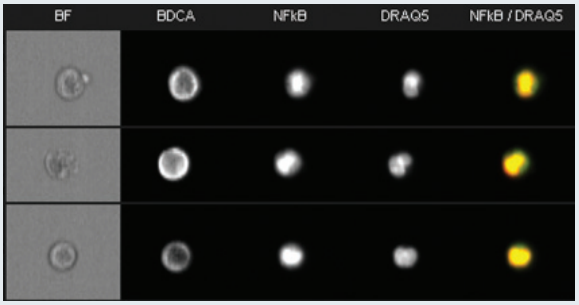
Figure 4.

Image similarity-based measurement of NF-κB nuclear localization

To measure nuclear translocation of NF-κB, we measured the "Similarity" of the NF-κB and DRAQ5 image pair for each pDC in the file (see the histograms below). The more nuclear the NF-κB is, the more similar the image pair will appear and the larger the similarity value will be. The R6 gate identifies the region of positive correlation for the score, and the percentage of pDC that fall within R6 is indicated in the upper right corner of the plots. Representative images of cells that fall within and to the left of R6 are shown.







ImageStream^X Specifications

Excitation Sources

Laser (nm)	Example Dyes
405	DAPI, Pacific Blue™
488	FITC, PE, ECD, PE-Cy5
560	Alexa Fluor® 546, Cy3
592	Texas Red®, Alexa Fluor® 594
658	Cy5, Alexa Fluor® 647, APC, APC-Cy-7

Imaging Performance

Magnification	20X	40X	60X
Numeric Aperture	0.5	0.75	0.9
Field of View (μm)	120 x 512	60 x 256	40 x 170
Imaging Rate (cells/sec)	2,000	1,000	600

Instrument Capabilities

Images per Cell	Up to 12
Imaging Modes	Brightfield, SSC, and fluorescent
Sample Throughput	1 sample/min nominal
Automated Processes	Startup, shutdown, and self-calibration

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