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Visualization of Cell Adhesion Molecules on Cardiovascular Cells by Confocal Microscopy

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Cell adhesion molecules (CAM) are important glycoproteins which mediate cell-cell interactions. The expression of these molecules are modulated by a variety of mediators engaged in cell activation.

Common methods applied for the detection of CAM are cell ELISA, FACS or radioactive binding studies. All these methods summarize the expression on a great number of cells and do not visualize CAM on single cells. The quantitative detection of CAM expression on cultured cells by conventional epifluorescence microscopy is difficult because of the rapid photobleaching of the fluorescent dye. We established a method to visualize CAM on single cells using confocal microscopy.

MATERIALS AND METHODS

Human coronary and pulmonary smooth muscle cells were cultured on 3well glass-slides (DMEM-medium with 15% FCS). At cell-confluency, medium was changed to 1% FCS for 48 hours in order to synchronize the cell cycle. Cells were fixed with ice-cold methanol (10 min) and incubated with monoclonal antibodies against I(nter) C(ellular) A(dhesion) M(olecule)-1 and V(ascular) C(ell) A(dhesion) M(olecule)-1 (Dianova, 1:50, 60 min). The antigen-antibody-complexes were detected by a second antibody coupled with Cy-3 (Dianova, 1:50, 60 min). Immunofluorescence measurements were performed with the confocal laser scan microscope Odyssey XL (Noran instruments) using the inverted microscope Diaphot 200 (Nikon). Specimen were studied with the 60x oil-imersion objective (NA 1,4). Probes were excited with the 515 nm line of a 10 mW multiline Argon ion laser and Cy-3 emission was detected at wavelengths >550 nm after passage through a 10 μm slit aperture. Cells were localized at a framerate of 30/sec and pictures of individual cells were taken by averaging 120 frames. CAM expression was determined by two different ways: a) mean fluorescense intensity (from 3 to 255 arbitrary units) of the pixels forming single cells and b) the sum of the pixel intensities per cell area. We analyzed 10 cells per well and determined the mean value for each individual cell preparation.

RESULTS

Under basal conditions there was a low expression of CAM on vascular smooth muscule cells. The mean fluorescence intensity of basal expression amounted to 7 ± 2 (ICAM-1) and 6 ± 1 (VCAM-1) (n=3 coronary cell preparations). Control experiments with an indirect cell ELISA also showed very similar basal expression values of both ICAM-1 and VCAM-1.

CAM are regulated by cytokines on endothelial cells as well as smooth musle cells (Carlos and Harlan 1994, Couffinhal et al. 1993). Using the ELISA technique we observed a marked stimulation of CAM expression with TNFα (10 ng/ml; 15 h) on a confluent cell monolayer (4-fold increase in optical density units). This stimulating effect

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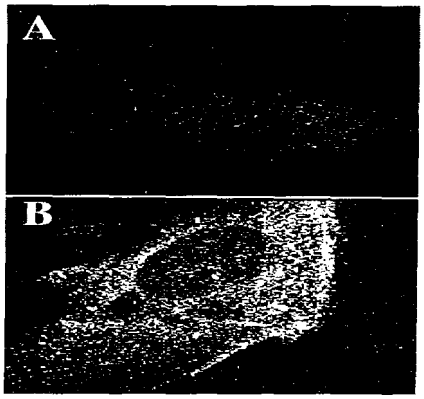

Fig. 1. ICAM-1 expression on single pulmonary SMC.

ICAM-1 expression on single human pulmonary SMC aunder conditions (IA) and mulet with TNF α 10 ng/ml

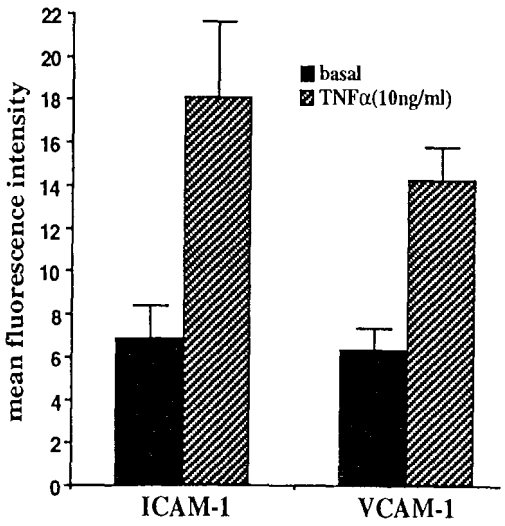

Fig. 2. Effects of TNFα on CAM expression of coronary SMC.

ICAM-1 and VCAM-1 expression (mean fluorescense intensity) on human coronary SMC under basal conditions (■)and after stimulation with TNFα (10 ng/ml, ■) for 15 hours (n=4-5).

of TNFα was detectable on single cells by confocal microscopy. The mean fluorescence intensity amounted to 18 ± 4 (ICAM-1) and 14 ± 4 (VCAM-1) (n=3) (Figs. 1 and 2)

The stimulation of CAM expression induced by TNFα is based on de novo synthesis of the glycoproteins. Measurements with the ELISA technique showed that the stimulation could be abolished by preincubation of the cells for 3 hours

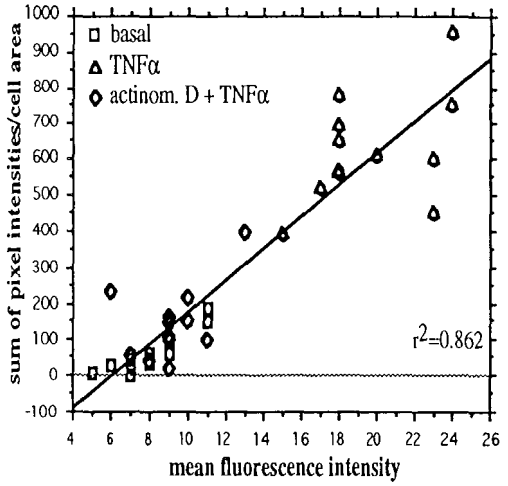

Fig. 3. ICAM-1 expression: correlation between mean fluorescence and the sum of fluorescence intensities per cell area.

ICAM-1 expression on human coronary SMC. Results from 34 individual cells under basal conditions (□), stimulated with TNFα (10 ng/ml, 15 h, Δ) and stimulated with TNF after preincubation with actinomycin D(1 μg/ml, 3h. ◊)are shown.

with the transcription-inhibitor actinomycin D (1 μg/ml) or cycloheximid (10 μg/ml), an inhibitor of protein biosynthesis. These inhibitory effects were also detectable on single cells by confocal microscopy. Fig. 3 demonstrates CAM expression on pulmonary smooth muscle cells under basal conditions, after TNFα-stimulation with and without actinomycin D preincubation. There is a good correlation between the two different detection methods we used to visualize CAM on single cells.

These data demonstrate that CAM expression could be visualized on singe cells by confocal microscopy. The method is sensitive to detect low levels of basal expression and the effects of exogenously added substances could be demonstrated onsingle cell level. This method will provide a new technique to study the modulation of CAM expression on different cell types.

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