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IFCC Professional Scientific Exchange Programme Report: Effects of cytokines on the expression of adhesion molecules on human umbilical vein endothelial cells (HUVECs)

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Introduction

The localisation of leukocytes plays a pivotal role in the different phases of an inflammatory process. Adhesion molecules are of eminent importance for the migration of leukocytes to sites of inflammation enabling the firm adhesion and diapedesis of leukocytes by interactions between integrins on leukocytes and adhesion molecules on the surface of endothelial cells.

Further investigations have proven that a wide range of proteins is summed up in what is now known as the family of cell adhesion molecules, comprising four major groups, i.e. selectins, mucin-like adhesion molecules (selectin ligands), integrins and members of the immunoglobuline superfamily. In acute and chronic inflammation the initial step lies in the recruitment of leukocytes. Thereby circulating leukocytes bind loosely to stimulated or injured endothelium via interactions between various selectins (L-, P-, E- selectin) and their ligands. Consequently, these leukocytes start to roll along the endothelium, a situation characterised by the repeated forming and breaking of loose bonds between adhesion molecules on the surface of each cell type. This process is accompanied by an increased secretion of cytokines by the endothelial cells leading to the activation of leukocytes, which is response strengthen their integrin adhesiveness (e.g. LFA-1, VLA-4). Subsequently, the rolling leukocytes become immobilised by the formation of firm interactions between leukocytes and the endothelium supported by ICAM-1, ICAM-2, VCAM-1 and MadCAM-1. Finally, the fixed leukocytes start to migrate across the endothelium while interacting with PECAM-1, ICAM-1 and VCAM-1. Simultaneously, a wide variety of cytokines is released into circulation as a response of the cellular immune system to the infection (Figure 1).

It has been reported that cytokines have regulatory effects on cell adhesion molecules expression. However, not all cytokines and combination of cytokines released during inflammatory and immunological processes into the blood have been investigated thoroughly so far. In the present study we investigate the influence of cytokines which target endothelial cells, i.e. IL-1 beta, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-a, IFN-g and the combination of IL-2, IL-4, IL-6, IL-8 and IL-10 with IL-1 beta, TNF-alpha or IFN-gamme on the expression of adhesion molecules. Furthermore, we determined the effects of a mixture of all evaluated cytokines under in vitro conditions.

Material and methods

Human umbilical vein endothelial cells were isolated and cultured using a modified standard procedure. Briefly, fresh human umbilical veins were washed with PBS (without Ca2+ and Mg2+), filled with pre-warmed 0.05% collagenase solution and incubated at 37°C for 6 minutes. Thereafter the veins were perfused with medium 199 containing 20% foetal calf serum (pH 7.4). Cells were collected from the perfusate by centrifugation at 900 rpm for 5 minutes and seeded into culture flasks (75 cm2) pre-coated with human fibronectin. Endothelial cells were cultured in complete medium containing Medium 199, 20% FCS, 10000 U/mL penicillin, 10000 µg/mL streptomycin, 100 mg/L low molecular weight heparin, 3 mmol/L L-glutamine and 30 mg/L endothelial cell growth supplement (ECGS). The confluent primary monolayers were washed with PBS, coated with fresh culture medium and harvested by gentle scraping. The cell suspension was transferred into each well of four 6-well culture

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plates pre-coated with human fibronectin and cultured in a humidified incubator set at 37.4°C and 5% CO2. Only cells from these first subcultures were used for the experiments described below.

Appropriate stimulant was added in 1 mL of freshly prepared medium leaving out the additives heparin and ECGS and endothelial cells were stimulated for 16 h at 37.4 oC and 5 % CO2. The final concentration of all cytokines used in the experiments were as follows: IL-1 beta (10 ng/mL), IL-2 (20 ng/mL), IL-4 (20 ng/mL), IL-6 (20 ng/mL), IL-8 (20 ng/mL), IL-10 (20 ng/mL), TNF-alpha (20 ng/mL) and IFN-gamma (100 ng/mL).

After the incubation period cells were detached by trypsinisation and the adhesion molecule surface expression on unstimulated and stimulated cells was measured by means of flow-cytometric analysis using FITC- and PE-labelled monoclonal mouse derived antibodies. Results are given as percentage changes in cell surface molecule expression versus respective control values. Results are expressed as mean ± confidence interval (P = 0.95). Data were analysed using ANOVA analysis.

Results

The effects of in vitro incubation of HUVEC monolayers with cytokines and cytokine-mixtures on adhesion molecule expression are demonstrated in Table I.

Conclusion

Experiments described herein have illustrated the effects of a number of pro- and anti-inflammatory cytokines on the expression of adhesion molecules on the surface of cultured human umbilical vein endothelial cells. This in vitro study has demonstrated the up-regulating influence of pro-inflammatory cytokines on certain adhesion molecules being crucial for leukocytes to transmigrate to the sites of inflammation during acute graft rejection. The application of a number of cytokine combinations especially those including the anti-inflammatory cytokines IL-4, IL-10 or IFN-gamma led to the down-regulation of adhesion molecule expression. These observations might be related to their properties in preventing hyper-reactions of the cellular immune system. Our in vitro model demonstrated evidence for the stimulatory and modulatory action of certain cytokines and therefore render it necessary to further investigate the mechanisms of modulation and their intracellular pathways.

In the second part of study, in vitro model dealt with the effects of two specific cytokine combinations on the expression of surface molecules on human umbilical vein endothelial cells. It has been demonstrated that combination of cytokines led to significant increase in E-selectin, VCAM-1 and ICAM-1 expression and to the induction of P-selectin. We have also underlined that TNF-alpha – within both combinations – predominantly affected adhesion molecule expression. Furthermore, we

Stimulation with Cytokines	P-selectin CD 62P	E-selectin CD 62E	VCAM-1 CD 106	ICAM-1 CD 54	PECAM-1 CD 31	L-selectin ligand CD 34
IL-1 beta	97 ± 5	361 ± 52*	256 ± 23*	294 ± 39*	84 ± 5	53 ± 6**
IL-2	99 ± 5	99 ± 5	117 ± 8	114 ± 9	95 ± 7	87 ± 5
IL-4	96 ± 6	61 ± 7*	226 ± 25*	98 ± 5	84 ± 3	46 ± 3**
IL-6	98 ± 6	112 ± 7	101 ± 6	109 ± 8	95 ± 7	98 ± 8
IL-8	93 ± 6	95 ± 4	102 ± 4	99 ± 7	99 ± 5	107 ± 9
IL-10	107 ± 7	108 ± 5	117 ± 6	113 ± 8	110 ± 8	103 ± 9
TNF-alpha	119 ± 17	2238 ± 187**	889 ± 53**	531 ± 49*	78 ± 5	58 ± 11**
IFN-gamma	97 ± 7	111 ± 7	210 ± 34*	193 ± 22*	84 ± 5	53 ± 9**
ALL Cytokines	243 ± 29*	585 ± 95*	677 ± 37*	231 ± 18*	45 ± 3*	57 ± 11**

Table I
Effects of different cytokines on the cell surface expression of adhesion molecules on cultured HUVECs. Results are expressed as percentage changes in mean fluorescence intensity versus respective control value (= 100% MFI) and are expressed as mean ± confidence intervala; *p<0.05, **p<0.001.

a ... confidence interval for n = 20; t(P,f) = 2.09; P = 0.95

found that endothelial cells show different behaviour concerning surface molecule expression under multiple or single cytokine stimulation. Finally, our data emphasise the importance of endothelial cells during inflammatory processes expressing adhesion molecules, which are indispensable for leukocyte transmigration.

Abbreviations used:

Abbreviations used: : HUVEC - human umbilical vein endothelial cells LFA-1 - ligand for beta2 integrins, CD11a/CD18 VLA-4-verylate antigen-4,CD49d/CD29 ICAM-1 - intercellular cell adhesion molecule-1 ICAM-2 - intercellular cell adhesion molecule-2 VCAM-1- vascular cell adhesion molecule-1 PECAM-1- platelet-endothelial cell adhesion molecule-1 IL-1b - interleukin b IL-2 - interleukin 2 IL-4 - interleukin 4 IL-6 - interleukin 6 IL-8 - interleukin 8 IL-10 - interleukin 10 TNF-a-tumor necrosis factor-a I IFN-g-interferon-g FCS-fetal calf serum

References

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