Intercellular adhesion molecule 1 on monocytes mediates adhesion as well as trans-endothelial migration and can be downregulated using antisense oligonucleotides

Abstract The intercellular adhesion molecule 1 (ICAM-1) on endothelial cells is involved in the recruitment of leukocytes to inflammatory sites. In contrast to ICAM-1 expression on endothelial cells, little is known about its function in leukocytes in inflammation. Using ICAM-1-directed anti-sense oligodeoxynucleotides (ODNs), we examined the role of ICAM-1 expression on monocytes and lymphocytes for adhesion and trans-endothelial migration. As determined by flow cytometry, a downregulation of the ICAM-1 expression of 50% was observed on peripheral blood mononuclear cells (PBMCs) after their transfecion with anti-sense ODNs using cationic lipids. The decrease in the level of ICAM-1 expression in PBMCs was associated with a 36% inhibition of adhesion to interleukin-1β-stimulated endothelial cells and a 40% reduction of trans-endothelial migration. Gating on particular subsets of the PBMC, the down-regulation of ICAM-1 and the functional effects could be ascribed to monocytes, while no significant inhibition was found for lymphocytes. This could be explained by differences in cellular ODN uptake. Since the ligands of ICAM-1 are not expressed on endothelial cells, the results suggest a homotypic interaction among monocytes. In conclusion, in addition to ICAM-1 expression on endothelial cells, ICAM-1 expression on monocytes mediates adhesion and trans-endothelial migration. This might be relevant for the clinical use of ICAM-1-directed anti-sense ODNs for the treatment of inflammatory diseases, because monocytes appear to be suitable target cells in which to achieve anti-inflammatory effects.

Key words Anti-sense oligonucleotides · Inflammation · Intercellular adhesion molecule 1 · Monocytes · Trans-endothelial migration

Introduction

Inflammation is a multi-step process resulting from infection or tissue injury [18]. The immigration of leukocytes into sites of inflammation is initiated by tethering to activated endothelial cells and rolling along the vessel surface [3, 26]. As a result, the leukocytes may adhere to the endothelial cells and migrate into the inflamed tissue [8, 42]. Intercellular adhesion molecule-1 (ICAM-1, CD54), a member of the immunoglobulin superfamily, plays a central role in the recruitment of leukocytes to inflammatory sites. ICAM-1 is a 90- to 110-kDa trans-membrane glycoprotein expressed at low levels on vascular endothelial cells [12]. There is an increase of expression in response to pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α or interferon-γ [37], as it is found on cells in inflamed tissues of patients with inflammatory diseases [15]. Functional studies using monoclonal antibodies (mAbs) imply that the interaction between cytokine-induced ICAM-1 on endothelial cells and its ligands [the β2 integrins lymphocyte function-associated antigen-1 (LFA-1) and Mac-1] on leukocytes results in strong adhesion of leukocytes to endothelium and mediates their transmigiration [23, 28, 32, 36, 39, 40]. ICAM-1 is also constitutively expressed at low levels on lymphocytes, monocytes, and neutrophils and is upregulated during inflammation [7, 12, 13, 22]. In contrast to ICAM-1 expression on endothelial cells, little is known about the role of its expression on leukocytes.

In this study, we examined the role of ICAM-1 expression on peripheral blood mononuclear cells...
(PBMCs) for adhesion to endothelial cells and for trans-endothelial migration using anti-sense oligodeoxynucleotides (ODNs). ICAM-1-directed anti-sense ODNs were chosen, because specific activity was shown in cell cultures and in vivo [5, 6, 9, 43]. Furthermore, unlike mAbs, the use of anti-sense ODNs is not associated with intrinsic activities or steric hindrance. We could show that the downregulation of ICAM-1 expression on monocytes resulted in a significant reduction of adhesion and trans-endothelial migration when the ODN-treated cells were placed on an endothelial cell layer. Therefore, the data indicate a functional role of ICAM-1 during inflammation, both in endothelial cells and in monocytes.

**Materials and methods**

**Cells**

Human leukocytes were obtained (after informed consent) from volunteers by vein puncture. PBMCs were separated by Ficoll density-gradient centrifugation, as previously described [11]. For transfection, adhesion, and transmigration experiments, cells were cultured in RPMI 1640 medium (Boehringer Ingelheim Bioproducts, Heidelberg, Germany) supplemented with 10% heat-inactivated fetal calf serum (FCS; Boehringer Ingelheim Bioproducts), 100 IU/ml penicillin (Life Technologies, Karlsruhe, Germany), 100 μg/ml streptomycin (Life Technologies), and 2 mM L-glutamine (Life Technologies).

The endothelial cell line ECV304 (ATCC, Rockville, Md.), derived from spontaneously transformed human umbilical-vein endothelial cells (HUVECs) [45], was cultured in medium 199 (Life Technologies) supplemented with 10% FCS, L-glutamine (2 mM/l), penicillin (100 IU/ml), and streptomycin (100 μg/ml) and passed twice each week after trypsinization. HUVECs were obtained from PromoCell (Heidelberg, Germany) and were cultured, according to the manufacturer’s instructions, in endothelial cell-growth medium containing 2% FCS, 0.1 mg/ml epidermal growth factor, 1 μg/ml hydrocortisone, 1 ng/ml basic fibroblast growth factor, 0.4% bovine hypothalamic extract with heparin, 50 ng/ml amphotericin B, and 50 μg/ml gentamicin. The medium and the supplements were obtained from PromoCell (Heidelberg, Germany). For stimulation of endothelial cells, 200 U/ml IL-1β (PromoCell, Heidelberg, Germany) was added to the medium 16 h before analysis of ICAM-1 expression and before adhesion and transmigration assays.

**ODN synthesis and transfection**

The phosphorothiate ODNs used in this study were the ICAM-1-directed anti-sense ODN ISIS1570 (5’-TGGAGGGAATAAGCGCC-3’) [9] and the scrambled control ODNs scISI51570 (5’-CGGAGGATAAAGCGGTG-3’) and scISI52302 (5’-ACTGCTACCGTGCCAGCC-3’). They were synthesized using standard phosphorodiester chemistry by Interactiva (Ulm, Germany) and were purified by reverse-phase high-performance liquid chromatography and lyophilized after synthesis by the manufacturer. Before use, the ODNs were resolved in hydroxyethylpiperazine ethanol sulfonic acid (HEPES) buffer (20 mM, pH 7.4).

Transfection of PBMCs was performed using the cationic lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine/dioleoylphosphatidyl ethanolamine (Lipofectin, Life Technologies) was added to the cells, followed by the addition of 500 μl Opti-MEM containing the ODNs (final concentration 0.5 μM). The cells were incubated for 4 h at 37°C. Subsequently, the transfection medium was replaced by medium 199 containing 10% FCS. After incubation for 4 h at 37°C, the medium was substituted again with medium 199 containing 10% FCS and 200 U/ml IL-1β for stimulation of ICAM-1 expression.

FACS analyses and functional assays were performed 16 h after stimulation with IL-1β. For FACS analysis, endothelial cells were removed from the culture plate by trypsinization for 5 min with 1 ml trypsin/ethylene diamine tetraacetic acid (EDTA) at 37°C. We could show that this short-time trypsinization did not influence the surface expression of ICAM-1 significantly in comparison with cells harvested by a cell scraper.

**Immunofluorescence staining and flow cytometry**

After washing with phosphate-buffered saline (PBS), 1 × 10⁶ cells were incubated with the fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mAbs in 500 ml PBS containing 1% bovine serum albumin at 4°C for 30 min. The following mAbs were used: CD3-FITC (clone SK7), CD11a-FITC (clone G-25.2), CD14-FITC (clone M1/69), CD15-FITC (clone M6A), CD19-FITC (clone S1251), CD45-FITC (clone HP2/1), and ICAM-1/CD54-PE (clone LB-2). Isotype-identical mAbs served as controls (IgG1 and IgG2b FITC/PE-conjugated). All mAbs were supplied by Becton-Dickinson (Heidelberg, Germany) except the CD49d-FITC antibody, which was obtained from Immunotech (Marseille, France). Following antibody staining, cells were washed and suspended in PBS buffer. The cells were analyzed using a Becton Dickinson FACScan (Heidelberg, Germany) with a 2-W argon ion laser. Fluorescence was measured using 530-nm, 15-nm-width (FITC) and 575-nm, 36-nm-width (PE) band-pass filters. Data were analyzed using Becton Dickinson Cell Quest software after gating on viable cells. For dual-color immunofluorescence analysis, a FL-1/FL-2 dot plot was used. The mean fluorescence intensity was calculated by the software and expressed in arbitrary units. For specific analyses of ICAM-1 expression on monocytes or lymphocytes after the transfection of ODNs, the two different cell types were identified by gating according to sideward-scattering and forward-scattering characteristics.

**Adhesion assay**

The adhesion of leukocytes to endothelial cells was examined as previously described [1, 33]. Briefly, 2 × 10⁶ PBMCs in 1 ml medium 199 were added to a confluent monolayer of ECV304 cells or HUVECs growing in a six-well culture plate and stimulated with 200 U/ml IL-1β 16 h before the assay. Cells were co-incubated for 90 min at 37°C. Subsequently, non-adherent cells were removed by gentle washing of the endothelial cell layer with 3 ml PBS on a shaker (IKA-VIBRAX-VXR, Janke and Kunkel, Staufen, Germany) for 1 min three times. The endothelial cell layer with the adherent leukocytes was trypsinated for