Antibody Mediated Ligation of Platelet/Endothelial Cell Adhesion Molecule-1 (PECAM-1) on Neutrophils Enhances Adhesion to Cultured Human Dermal Microvascular Endothelia…

Kuwabara, Hiroko
Tanaka, Sumiko
Sakamoto, Haruhiko
Oryu, Makoto
Uda, Hirotsugu

(Citation)
The Kobe journal of the medical sciences,42(4):233-241

(Issue Date)
1996-08

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0000963

Kobe J. Med. Sci. 42, 233/241, August 1996

ANTIBODY MEDIATED LIGATION OF PLATELET / ENDOTHELIAL CELL ADHESION MOLECULE-1 (PECAM-1) ON NEUTROPHILS ENHANCES ADHESION TO CULTURED HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS

Hiroko KUWABARA, Sumiko TANAKA, Haruhiko SAKAMOTO, Makoto ORYU, and Hirotsugu UDA

Second Division, Department of Pathology, Kagawa Medical University, 1750-1, Miki-Cho, Kita-Gun, Kagawa, 761-07, Japan

INDEXING WORDS

platelet / endothelial cell adhesion molecule-1(PECAM-1); neutrophil; DMvE cells

SYNOPSIS

We investigated the effects on leukocyte adhesion to cultured human dermal microvascular endothelial (DMvE) cell by antibody mediated ligation of platelet / endothelial cell adhesion molecule-1 (PECAM-1) on neutrophils. Preincubation of neutrophils with anti-PECAM-1 antibody markedly enhanced their adhesion to DMvE cells, while the adhesion-increasing action was cancelled out by anti-CR3 or anti-LFA-1β(CD18) antibody. These findings suggest that ligation of PECAM-1 on neutrophils increases their adhesion to cultured DMvE cells depending on the β2 integrin family, and PECAM-1 may have a crucial role in dermal inflammation.

Received for publication : August 31, 1996

Authors' names in Japanese : 桑原宏子, 田中澄子, 阪本晴彦 尾立磨琴, 宇多弘次

INTRODUCTION

Expression of certain adhesion molecules is essential for leukocyte movement in inflammatory lesions. PECAM-1 (platelet / endothelial cell adhesion molecule-1), one of recently characterized cell adhesion molecules, belongs to the immunoglobulin gene superfamily and is identical to the hec 7 and CD 31 antigens. It exists on the surface of platelets, monocytes, neutrophils, some T cell subsets, and the intercellular junctions of cultured endothelial cells.[13)15)]

Antibody mediated ligation of adhesion molecules is believed to deliver the signal for engagement of the natural ligand, and recently, Berman and Muller[5)] reported that the ligation of PECAM-1, using anti-PECAM-1 monoclonal antibody, on monocytes and neutrophils increases the binding capacity of leukocyte CR3 (CD11b / CD18, also known as Mac-1, a member of the β2 integrin family), and that anti-PECAM-1 treated monocytes increase the adhesion to human cultured umbilical vein endothelial cells. The β2 integrin family includes LFA-1(CD11a / CD18) and p150,95 (CD11c / CD18) as well as CR3, and these have a common β subunit.[16)] The enhancement of cell adhesion to the endothelium by the β2 integrin family is well known[3)] and the family also plays an important role in general leukocyte functions such as phagocytosis and chemotaxis.[7)] In fact, inherited deficiency of β2 integrin compromises the phagocytic and migratory capacities of circulating granulocytes and monocytes, leading to life-threatening bacterial infections.[1)2)]

In the dermatological fields, neutrophils play an important role in the process of acute inflammation or disease. For the emigration of neutrophils from the blood into the dermis, tight adhesion of neutrophils to the dermal endothelium is the prerequisite step, and cytogenetically, cultured human endothelial cells differ significantly between those derived from fetal umbilical cords and those derived from vessels in various other sites.[4)14)] In this experiment, we examined whether the ligation of PECAM-1 on neutrophils, using monoclonal anti-PECAM-1 antibody, enhances the adhesion to dermal microvascular endothelial (DMvE) cells in culture and whether the enhanced action depends on the β2 integrin family. This is the first report on anti-PECAM-1 treated neutrophil adhesion assay using cultured DMvE cells.

MATERIALS AND METHODS

Separation of neutrophils

Heparinized peripheral blood from a healthy adult was doubly diluted with PBS and centrifuged on a Monopoly Resolving Medium (Dainippon Pharmaceutical, Osaka, Japan) at

60g for 30minutes at 4°C. The centrifugation was continued at 180g for another 30 minutes at 4°C, and the leukocytes layer obtained were rinsed in PBS three times. The concentration of neutrophils was adjusted in 5 % bovine serum albumin (BSA) in RPMI-1640 (Nipro, Osaka, Japan) (BSA-RPMI).

Antibodies

Monoclonal anti-PECAM-1 (R&D Systems, Abingdon, UK, 1:1000), anti-CR3 (CD-11b / CD18) and anti-LFA-1β (CD18) (Dakopatts, Denmark, 1:100 and 1:200, respectively) antibodies were used. The anti-LFA-1β (CD18) antibody inhibits lymphocyte and granulocyte functions which are dependent on CD18.[10]

Adhesion of neutrophils to human dermal microvascular endothelial (DMvE) cells

DMvE cells from neonatal donors (Cell system, Kirkland, WA) were cultured into a monolayer on the collagen gel of a slide glass before the experiment. Conditions were 37°C, 5% CO_2 and chamber size 1.1 cm x 0.7 cm (Heraeus Biotechnology, Germany). 150 μl neutrophils of 1.5 x 10^6 / well in BSA-RPMI were preincubated with or without monoclonal antibodies at 37°C for 5 minutes and then added to the DMvE cells. These materials were incubated under the conditions of 37°C, 5% CO_2 for 15 minutes, rinsed well in BSA-RPMI, fixed in 1 % glutalaldehyde for 2 hours, and stained with hematoxylin-eosin for microscopic observation. The number of neutrophils in contact with DMvE cells was counted. The neutrophils migrating through the endothelial monolayer were determined using the following points. When the neutrophils adhering to the upper surface of the monolayer came into focus by microscope, those beneath the endothelial cells were out of focus. Moreover, neutrophils migrating through the monolayer became quite flattened between the monolayer and the substratum.[17] Each experiment was performed in triplicate.

Transmission electronmicroscopical observations

The adherence of anti-PECAM-1 treated neutrophils to DMvE cells was electronmicroscopically observed. The fixed material in 2.5% glutalaldehyde was post-fixed in 1% osmium tetroxide for 1 hour, dehydrated through a graded ethanol series, embedded by inverting a Beem Capsule full of Epok 812 over each chamber section, and polymerized. After removing a block containing tissue sections by brief heating, ultrathin sections were cut, stained with uranyl acetate and lead citrate, and observed with a Hitachi- H600 electron microscope.

Statistical significance

Adhering neutrophils to 50 DMvE cells were counted in each experiment. Statistical significance was evaluated by Student's *t* test.

RESULTS

Fig.1. shows the mean number of neutrophils adhering to one DMvE cell. The number of adhered anti-PECAM-1 treated neutrophil to DMvE cells was markedly greater than that treated by PBS ($P<0.01$)(Fig.1). The anti-PECAM-1 treated neutrophils were bound to the upper surface of the DMvE cells or adjoining periphery, but were very rarely present on the under surface. Electronmicroscopically, the cultured cells had eccentrically arranged nuclei with one or two distinct nucleoli, and the cytoplasm contained a large number of mitochondria and vacuoles. In these respects, they resembled the DMvE cells (8). Anti-PECAM-1 treated neutrophils adhered to the surface of endothelium extending their pseudopodia (Fig.2). All of these light and elecron-microscopical findings were similar to those for the anti-PECAM-1 treated leukocytes adhering to the apical surface of the umbilical vein endothelium (11). Approximately 80% of the adhesion could be blocked by anti-CR3 or anti-LFA-1β(CD18) (Fig.1&3).

DISCUSSION

Inflammatory cell accumulation into the dermis from the blood vessel involves rolling, tight adhesion and transendothelial migration. Each step is mediated by a distinct family of cell adhesion molecules, and tight adhesion to the endothelium is prerequisite. PECAM-1 has a distinct role in the transendothelial migration phase of leukocytes.[11] In fact, concentrated PECAM-1 is present throughout the endothelial junction and anti-PECAM-1 antibody blocks transendothelial migration of both monocytes and neutrophils. Interactions of PECAM-1 both on leukocytes and the endothelium are essential for the transendothelial migration. On the other hand, PECAM-1 is not directly related to the adhesion events on the endothelium, since anti-PECAM-1 antibody does not block adhesion to the endothelium.[11] In our experiment, anti-PECAM-1 treated neutrophils enhanced the adherence above or adjoining the DMvE cells by extending their pseudopodia. This morphological change of neutrophils was the same as that of leukocytes adhering to the apical surface of umbilical vein endothelium.[11] The enhancement of adherence was cancelled out by anti-CR3 or anti-LFA-1β(CD18) antibody, suggesting that PECAM-1 mediated adherence to the DMvE cells depends on the β2 integrin. Berman *et al.* [5]

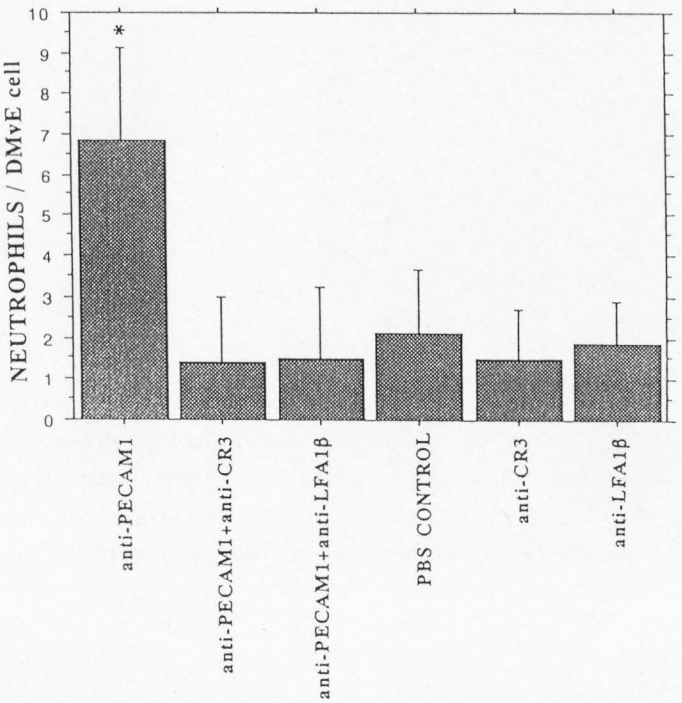

Figure 1. The mean number of neutrophils adhering to one DMvE cell. Bar shows SD. Anti-PECAM-1 treated neutrophils markedly enhance the adherence to DMvE cells (*P<0.01 is significant difference compaired with PBS control), and this enhancement is cancelled out by anti-CR3 or anti-LFA-1β(CD18) (significant differences compaired with only anti-PECAM-1-treated neutrophils).

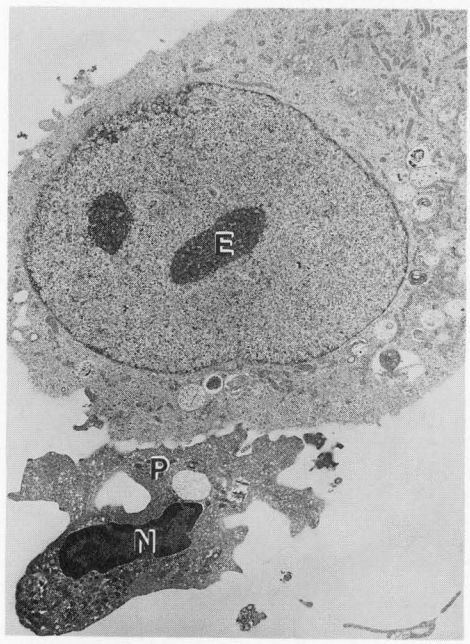

Figure 2. Electronmicroscopy shows that anti-PECAM-1 treated neutrophils adhere to the endothelium by extending a pseudopod (N: nucleus of neutrophil, P: pseudopod, E; DMvE cell) (x3,000).

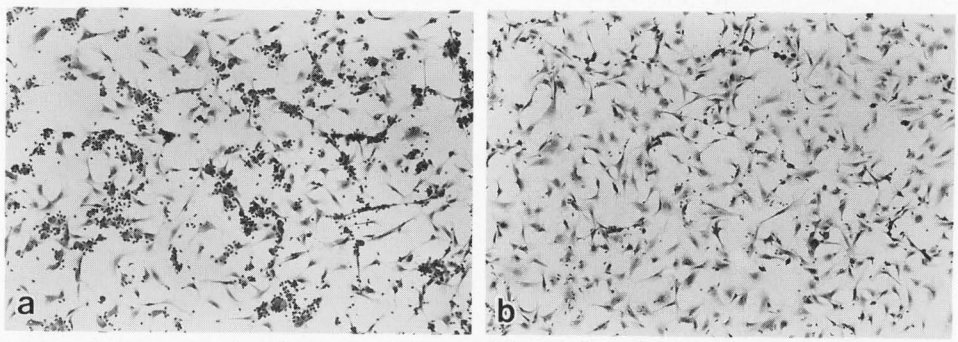

Figure 3. (a) Many anti-PECAM-1 treated neutrophils are adhered to the DMvE cells. (b) The enhanced adherence is blocked by anti-CR3 antibody.

reported that anti PECAM-1 treated neutrophils increase the CR3 activity on neutrophils by rosetting assay of erythrocytes. CR3 is a member of the β2 integrin family (CD18) with LFA-1 and p150,95, and they cooperate for adherence to the endothelial cells through a counter-receptor for ICAM-1 on the endothelium.[6)9)] This neutrophil-endothelium adhesion mediated by β2 integrin is a tight adhesion. We conclude here that the ligation of PECAM-1 on neutrophils to enhance its adhesion to the apical surface of dermal microvascular endothelium depends on the β2 integrin.

Neutrophils play an important role in the process of acute inflammation or disease, secreting cytokines such as TNF-α[18)] and enzymes such as proteinases,[12)] and phagocyting microorganisms. The β2 integrin enhances the phagocytic activity, as well as the adherence to the endothelium[7)]. Ligation of PECAM-1 on neutrophils may play an important role for phagocytosis in the dermal matrix, as well as for adherence to the endothelium of dermal microvessels. In the process of dermatological inflammation or disease, PECAM-1 seems to play a critical role in the accumulation and phagocytic activity of neutrophils.

REFERENCES

1.Anderson, D.C., Schmalstieg, F.C., Arnaout, M.A., Kohl, S., Tosi, M.F., Dana, N., Buffone, G.J., Hughes, B.J., Brinkley, B.R., Dickey, W.D., Abramson, J.S., Springer, T., Boxer, L.A., Hollers, J.M.,and Smith, C.W.: J. Clin. Invest. 1984. 74. 536/551. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (GP138): common relationship to diminished cell adherence.

2.Arnaout, M.A., Pitt ,J., Cohen, H.J., Melamed, J., Rosen, F.S., and Colten, H.R.: N. Eng. J. Med. 1982. 306. 693/699. Deficiency of a granulocyte-membrane glycoprotein (gp150) in a boy with recurrent bacterial infections.

3.Arnaout, M.A.: Blood 1990.75.1037/1050.Structure and function of the leukocyte adhesion molecules CD11/ CD18.

4.Belloni, P.N. and Nicolson, G.L.: J. Cell Physiol. 1988. 136. 398/410. Differential expression of cell surface glycoproteins on various organ-derived microvascular endothelia and endothelial cell cultures.

5.Berman, M.E., and Muller, W. A.: J. Immunol. 1995. 154. 299/307. Ligation of platelet / endothelial cell adhesion molecule 1 (PECAM-1 / CD31) on monocytes and neutrophils increases binding capacity of leukocyte CR3 (CD11b / CD18).

6.Diamond, M.S., Staunton, D.E., de Fougerolles, A.R., Stacker, S.A., Garcia-Aguilar, J., Hibbs, M.L., and Springer, T.A.: J. Cell Biol. 1990. 111. 3129/3139. ICAM-1(CD54): a

counter receptor for Mac-1 (CD11b / CD18).

7.Fallman, M., Andersson, R., and Andersson, T.: J. Immunol.1993;151: 330/338. Signaling properties of CR3 (CD11b / CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles.

8.Imcke, E., Ruszczak, Z.B., Mayer-da Silva, A., Detmar, M., and Orfanos, C.E.: Arch Dermatol Res. 1991. 283. 149/157. Cultivation of human dermal microvascular endothelial cells in vitro: immunocytochemical and ultrastructural characterization and effect of treatment with three synthetic retinoids.

9.Larson, R.S., Corbi, A.L., Berman, L., and Springer, T.: J. Cell Biol.1989. 108.703/712. Primary structure of the leukocyte function-associated molecule-1 α subunit: an integrin with an embedded domain defining a protein superfamily.

10.McMichael, A.J., *et al.*, eds. Oxford University Press, 1987. Leukocyte typing III. White cell differentiation antigens.

11.Muller, W.A., Weigl, S.A., Deng, X, and Phillips, D.M.: J. Exp.Med. 1993. 178. 449/460. PECAM-1 is required for transendothelial migration of leukocytes.

12.Naito, K., Morioka, S., and Ogawa, H.: J. Invest. Dermatol. 1982. 79. 303/306. The pathogenic mechanisms of blister formation in bullous pemphigoid.

13.Newman, P.J., Berndt, M.C., Gorski, J., White II, G.C., Lyman, S., Paddock, C., and Muller, W.A.: Science 1990. 247. 1219/1222. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily.

14.Nichols, W.W., Buynak, E.B., Bradt, C., Hill, R., Aronson, M., Jarrel, B.E., Muller, S.N., and Levine, E.M.: J.Cell Physiol. 1987. 132. 453/462. Cytogenic evaluation of human endothelial cultures.

15.Ohto, H., Maeda, H., Shibata, Y., Chen, R.F., Ozaki, Y., Higashihara, M., Takeuchi, A., and Tohyama, H.: Blood 1985. 66. 873/881. A novel leukocyte differentiation antigen: two monoclonal antibodies TM2 and TM3 define a 120-kd molecule present on neutrophils , monocytes, platelets, and activated lymphoblasts.

16.Sanchez-Madrid, F., Nagy, J.A., Robbins, E., Simon, P., and Springer, T.A.: J. Exp.Med. 1983.158.1785/1803. A human leukocyte differentiation antigen family with distinct α-subunits and a common β subunit: The lymphocyte function-associated antigen (LFA-1) , the C3bi complement receptor (OKM1/ Mac-1), and the p150,95 molecule.

17.Smith, C.W., Marlin, S.D., Rothlein, R., Toman, C., and Anderson, D.C.: J. Clin.Invest. 1989. 83.2008/2017. Cooperative interactions of LFA-1 and Mac-1 woth intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro.

18. Yamazaki, M., Ikenami, M., and Sugiyama, T.: Br. J. Cancer 1989. 59. 353/355. Cytotoxin from polymorphonuclear leukocytes and inflammatory ascitic fluids.