Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 459 (2003) 269–273
www.elsevier.com/locate/ejphar

# Docosahexaenoic acid inhibits cytokine-induced expression of P-selectin and neutrophil adhesion to endothelial cells

Yusheng Wang, Qing Liu, Henrik Thorlacius*

*Department of Surgery, Malmö University Hospital, Lund University, 20502 Malmö, Sweden*

Received 13 June 2002; received in revised form 19 November 2002; accepted 26 November 2002

**Abstract**

Dietary polyunsaturated fatty acids, such as docosahexaenoic acid, may inhibit pathological processes involving endothelial cell activation. Herein, it was found that treatment of endothelial cells with docosahexaenoic acid dose dependently reduced neutrophil adhesion provoked by tumor necrosis factor-α (TNF-α). In fact, pretreatment with 100 μM of docosahexaenoic acid for 24 h decreased TNF-α-induced neutrophil adhesion by 50%. Moreover, this pretreatment with docosahexaenoic acid (100 μM, 24 h) down-regulated TNF-α-induced endothelial cell surface expression of P-selection by 75%. Importantly, immunoneutralization of P-selectin reduced neutrophil adhesion to TNF-α-activated endothelial cells by more than 50%, indicating a significant role of P-selectin in this model. On the other hand, CXC chemokines, i.e. macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemoattractant (KC), are also important regulators of neutrophil activation and adhesion. However, pretreatment with docosahexaenoic acid had no effect on TNF-α-provoked production of MIP-2 and KC in endothelial cells. Our study provide evidence that docosahexaenoic acid inhibits expression of P-selectin and subsequent adhesion of neutrophils to endothelial cells in response TNF-α, which may help explain the anti-inflammatory effects exerted by docosahexaenoic acid.


*Keywords:* Adhesion; Chemokine; Endothelium; Fatty acid; Neutrophil; TNF-α (tumor necrosis factor-α)

## 1. Introduction

Tumor necrosis factor-α (TNF-α) is a powerful regulator of leukocyte recruitment and has been suggested to be a potential target in several inflammatory diseases (Deventer, 1997; Ksontini et al., 1998). The extravasation process of leukocytes is a multistep process supported by a coordinated expression of adhesion molecules (Butcher, 1991). Initial leukocyte rolling is considered to be principally mediated by the selectin family of adhesion molecules (P-, E- and L-selectin) (Carlos and Harlan, 1994). On the other hand, firm adhesion of inflammatory cells has been shown to be mediated by integrins on leukocytes, which bind to members of the immunoglobulin gene superfamily, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells (Butcher, 1991; Carlos and Harlan, 1994). Moreover, activation and movement of subsets of leukocytes are regulated by specific families of chemokines (Zlotnik et al., 1999). The CXC chemokines, including macrophage inflammatory protein-2 (MIP-2) (Tekamp-Olson et al., 1990) and cytokine-induced neutrophil chemoattractant (KC) (Oquendo et al., 1989), are considered to be functional homologues of human GRO chemokines (Anisowicz et al., 1987; Richmond et al., 1988) and predominately activate neutrophils.

The composition of fatty acids in the diet influences the risk of inflammatory diseases (Simopoulos et al., 1994). For example, it has been reported that an increase in the intake of omega-3 polyunsaturated fatty acids, e.g. docosahexaenoic acid, may induce clinical improvement in inflammatory bowel disease, rheumatoid arthritis and atherosclerosis (Simopoulos et al., 1994) although the detailed anti-inflammatory mechanisms remain elusive. It is interesting to note that previous studies have reported that docosahexaenoic acid counteracts TNF-α-induced expression of E-selectin, ICAM-1 and VCAM-1 and subsequent adhesion of monocytes to activated endothelium (De Caterina et al., 1994, 1995, 2000; Weber et al., 1995), which may help explain the protective effect of omega-3 polyunsaturated fatty acids in cardiovascular diseases. In

* Corresponding author. Tel.: +46-40-331000; fax: +46-40-336207.
*E-mail address:* henrikthorlacius@hotmail.com (H. Thorlacius).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(02)02849-2

contrast to monocytes, neutrophil–endothelium interactions and tissue recruitment are critically dependent on P-selectin in response to TNF-α (Robinson et al., 1999; Mansson et al., 2000). Moreover, considering that neutrophils are important effector cells in causing tissue injury in several inflammatory diseases, it may be hypothesized that the protective effects of docosahexaenoic acid discussed above may, at least in part, be related to inhibitory effects on P-selectin and/or CXC chemokine expression and subsequent neutrophil adhesion to TNF-α-activated endothelium.

Based on these considerations, the aim of the present study was to examine the impact of docosahexaenoic acid on TNF-α-induced expression of P-selectin, CXC chemokines and neutrophil adhesion to endothelial cells.

## 2. Materials and methods

### 2.1. Cells

The polyoma transformed murine endothelioma cell line eEnd.2 was cultured in Dulbecco's minimal essential medium supplemented with 10% fetal calf serum, L-glutamine, penicillin, streptomycin and subcultured twice weekly as described previously (Williams et al., 1989). Neutrophils were freshly isolated from C57/Bl6 mice. The bone marrow was flushed aseptically out of the femurs and humeri bones with ice-cold phosphate-buffered saline (PBS) and then neutrophils were isolated by using Ficoll-Paque™ Research Grade (Amersham Pharmacia Biotech, Uppsala, Sweden). The purity of bone marrow neutrophils was higher than 70% as assessed by Turk stain in a haematocytometer. Neutrophils were resuspended in culture medium until use in the adhesion assay.

### 2.2. Adhesion assay

Endothelial cells were plated at a density of $2 \times 10^4$ cells per well in 96-well plates. When confluent, the cells were incubated with 1, 10 and 100 μM of docosahexaenoic acid 24 h prior to TNF-α challenge. The endothelial cells were stimulated with negative control medium or recombinant TNF-α (R&D Systems Europe, Abingdon, Oxon, UK) at a final concentration of 100 ng/ml 4 h before incubation with neutrophils. In separate experiments, a monoclonal antibody directed against P-selectin (RB40.34, Pharmingen, San Diego, CA, USA) at a final concentration of 1, 10 and 100 μg/ml was added 3.5 h after application of TNF-α (100 ng/ml) and 30 min before incubation with neutrophils in order to define the role of P-selectin in TNF-induced neutrophil adhesion. Next, $2 \times 10^4$ neutrophils were incubated for 20 min on the confluent endothelial cells at 37 °C. Subsequently, the wells were washed with PBS three times in order to remove nonadherent cells. Next, 50 μl 0.05 M phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide was added into each well followed by 150 μl of tetramethylbenzididne and incubated for 10 min at 25 °C. At the end, 100 μl of 0.5 M of $H_2SO_4$ was added in order to stop the oxidation reaction. The enzyme activity was determined spectrophotometrically as the myeloperoxidase-catalyzed change in absorbance occurring in the redox reaction of $H_2O_2$–tetramethylbenzididne (450 nm, 25 °C). A standard curve was constructed using defined quantities of neutrophils in the same plate.

### 2.3. Enzyme linked immunoadsorbant assay

Culture medium from TNF-α stimulated endothelial cells was collected and the level of immunoreactive murine MIP-2 and KC protein in cell culture supernatant was determined by use of a double-antibody specific Quantikine Enzyme linked immunoadsorbant assay (ELISA) kits (R&D Systems Europe). A standard curve was constructed using recombinant murine MIP-2 and KC (R&D Systems Europe). The minimum detectable concentration of MIP-2 and KC is less than 2 pg/ml in this assay.

### 2.4. Flow cytometry

Endothelial cells ($2 \times 10^5$) were cultured in 12-well plates with Dulbecco's minimal essential medium, 10% fetal calf serum in 37 °C for 24 h with or without 100 μM of docosahexaenoic acid. Then, TNF-α at the final concentration of 100 ng/ml was added for 4-h stimulation and subsequently the cells were isolated by trypsination. Isolated cells were centrifugated at $300 \times g$ for 5 min. Pelleted cells were washed with PBS once and then mixed with a fluorescein isothiocyanate-labeled antibody directed against P-selectin (1 μg, RB40.34, Pharmingen) or a fluorescein isothiocyanate-labeled control antibody (1 μg,

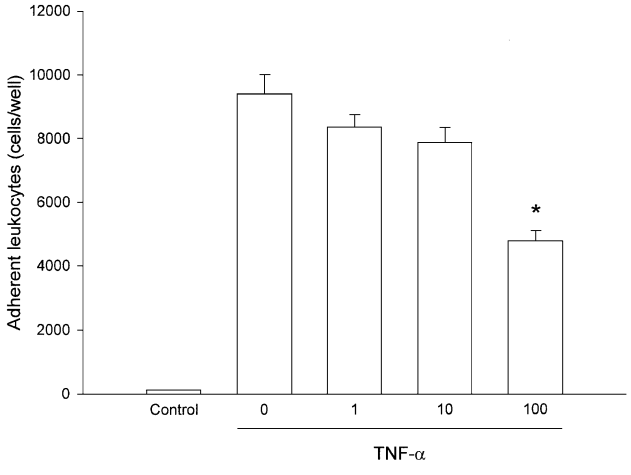


Fig. 1. Neutrophil adhesion to endothelial cells stimulated with 100 ng/ml of tumor necrosis factor-α (TNF-α) for 4 h. Nontreated cells served as negative control (control). Endothelial cells were pretreated with docosahexaenoic acid (0, 1, 10 and 100 μM) 24 h prior to TNF-α challenge. Data represents mean ± S.E.M. and asterisk indicate significant difference ($P < 0.05$ vs. TNF alone, $n = 8$).

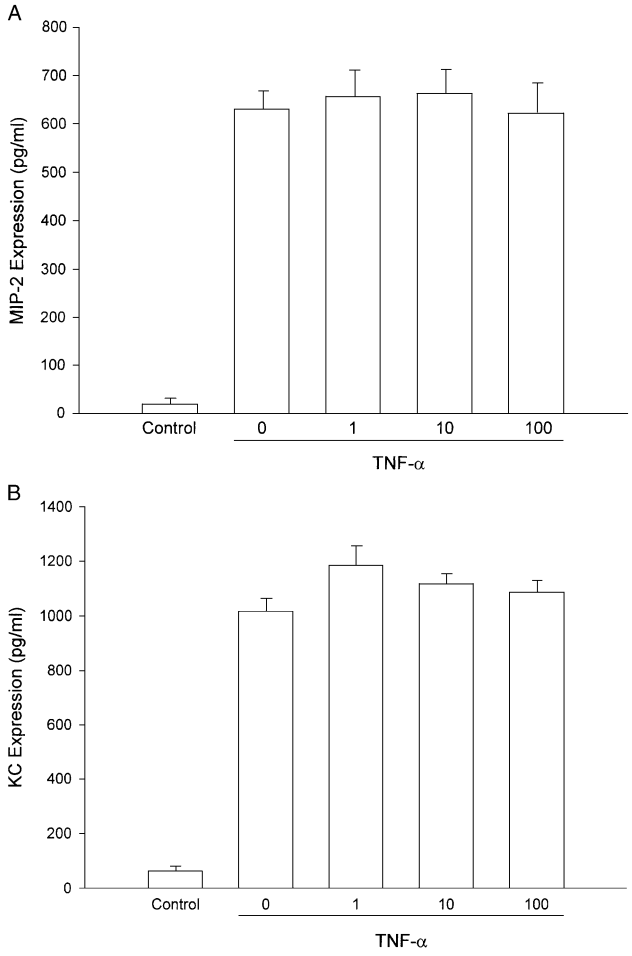


Fig. 2. Expression of (A) macrophage inflammatory protein-2 (MIP-2) and (B) KC. Endothelial cells were treated with 100 ng/ml of tumor necrosis factor-α (TNF-α) for 4 h. Nontreated cells served as negative control (control). Endothelial cells were pretreated with docosahexaenoic acid (0, 1, 10 and 100 μM) 24 h prior to TNF-α challenge. Data represents mean ± S.E.M. and $n = 8$.

R3-34, Pharmingen) for 20 min in the dark at 4 °C. Washed and resuspended cells were kept on ice in the dark until analysis in a flow cytometer (Coulter Epics XL-MCL Beckman Coulter) within 30 min.

### 2.5. Statistics

Statistical evaluations were performed using the Kruskal-Wallis one way analysis of variance on ranks for unpaired samples (Dunn's post hoc test was used). The results are presented as mean values ± S.E.M. and $n$ represents number of experiments. Differences were considered to be significant at $P < 0.05$.

## 3. Results

Myeloperoxidase, a marker of neutrophils, was determined after incubating isolated neutrophils with cultured endothelial cells. The numbers of neutrophils per well was based on myeloperoxidase calculations from a standard curve using defined numbers of neutrophils. In negative control experiments, the number of neutrophils was 121 ± 9 cells per well (Fig. 1). Administration of TNF-α for 4 h markedly increased neutrophil adhesion to 9424 ± 611 cells per well (Fig. 1, $P < 0.05$ vs. negative control). Notably, we found that pretreatment of endothelial cells with docosahexaenoic acid for 24 h dose dependently (0–100 μM) reduced neutrophil adhesion elicited by TNF-α (Fig. 1). In fact, 100 μM of docosahexaenoic acid decreased TNF-α-induced neutrophil adhesion by 50% (Fig. 1, $P < 0.05$ vs. TNF-α alone).

Specific ELISA was used to determine the levels of MIP-2 and KC protein in the cell culture supernatant. As shown in Fig. 2, TNF-α challenge markedly increased MIP-2 and

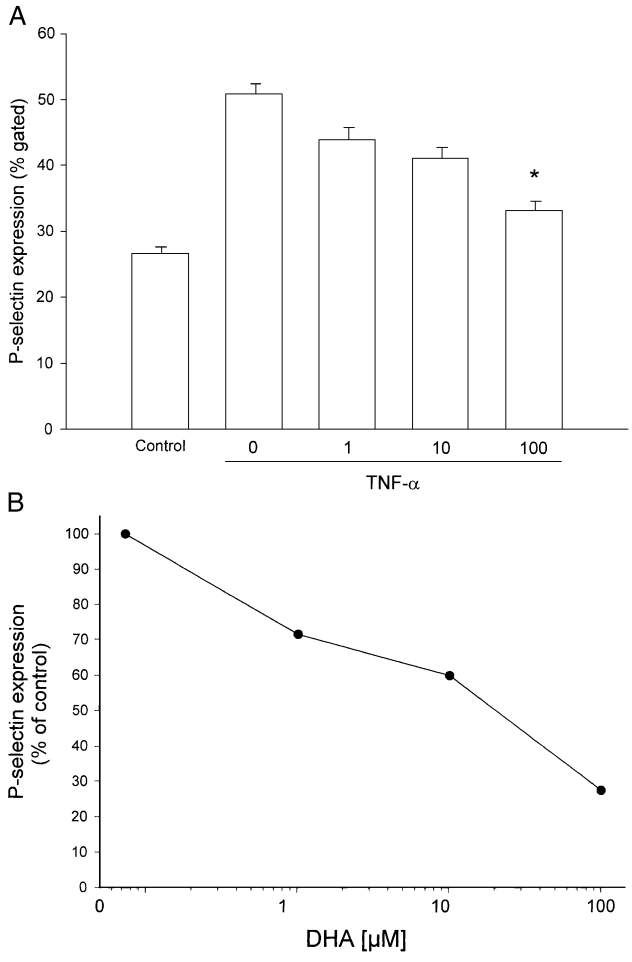


Fig. 3. Flow cytometric expression of P-selectin on the surface of endothelial cells (A) showing the percentage of endothelial cells expressing P-selectin. Endothelial cells were treated with 100 ng/ml of tumor necrosis factor-α (TNF-α) for 4 h. Nontreated cells served as negative control (control). Endothelial cells were pretreated with docosahexaenoic acid (0, 1, 10 and 100 μM) 24 h prior to TNF-α challenge. In (B), the susceptibility of TNF-α-induced P-selectin expression on endothelial cells to docosahexaenoic acid treatment. The effect of docosahexaenoic acid is dose-dependent with the $IC_{50}$ at 20 μM. Data represents mean ± S.E.M. and asterisk indicate significant difference ($P < 0.05$ vs. TNF alone, $n = 8$).

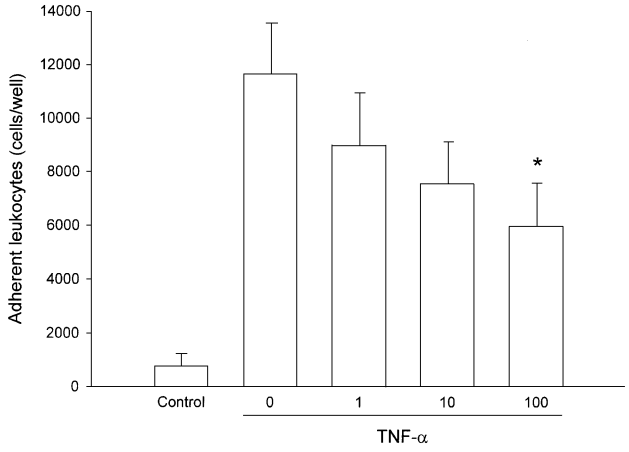


Fig. 4. Role of P-selectin in tumor necrosis factor-α (TNF-α)-induced neutrophil adhesion. A monoclonal antibody against P-selectin (0, 1, 10 and 100 μg/ml) was added to the endothelial cells 3.5 h after stimulation with 100 ng/ml of TNF-α and 30 min prior to incubation with neutrophils. Nontreated cells served as negative control (control). Data represents mean ± S.E.M. and asterisk indicate significant difference ($P<0.05$ vs. TNF alone, $n=8$).

KC expression in endothelial cells ($P<0.05$ vs. negative control). It was found that docosahexaenoic acid did not affect TNF-α-induced production of MIP-2 (Fig. 2A, $P>0.05$ vs. TNF-α alone) and KC (Fig. 2B, $P>0.05$ vs. TNF-α alone).

Next we wanted to delineate the role of P-selectin in the inhibitory effect of docosahexaenoic acid on TNF-α-induced adhesion of neutrophils. We found that TNF-α markedly increased endothelial cell surface expression of P-selectin (Fig. 3, $P<0.05$ vs. negative control), which is in line with a previous report (Weller et al., 1992). Interestingly, it was observed that pretreatment with 100 μM of docosahexaenoic acid for 24 h markedly decreased (75% reduction) TNF-α-induced expression of P-selectin on the surface of endothelial cells (Fig. 3A, $P<0.05$ vs. TNF-α alone). As shown in Fig. 3B, the $IC_{50}$ of docosahexaenoic acid on TNF-α-induced P-selectin expression on endothelial cells was found to be nearly 20 μM. Furthermore, we found that an anti-P-selectin antibody dose dependently reduced TNF-α-provoked adhesion of neutrophils (Fig. 4), demonstrating that P-selectin indeed plays an important role in this experimental system.

## 4. Discussion

Adhesion of leukocytes to activated endothelial cells constitutes an important component in inflammatory reactions and immune surveillance (Butcher, 1991; Carlos and Harlan, 1994). There is an accumulating body of evidence in the literature suggesting that polyunsaturated fatty acids exerts potent anti-inflammatory effects (De Caterina et al., 1994, 1995, 2000; Weber et al., 1995; Sperling et al., 1993). The present study demonstrates that docosahexaenoic acid has the capacity to inhibit TNF-α-induced adhesion of neutrophils. Moreover, the inhibitory effect of docosahexaenoic acid appears to be due to a down-regulation of TNF-α-induced expression of P-selectin, whereas TNF-α regulated expression of CXC chemokines is insensitive to docosahexaenoic acid treatment.

An accumulating body of evidence suggest that the content of dietary fatty acids may influence a number of important diseases, such as atherosclerosis and rheumatoid arthritis (Simopoulos et al., 1994). In particular, it has been reported that docosahexaenoic acid inhibits monocyte adhesion and reduces endothelial cell expression of VCAM-1 and ICAM-1 (De Caterina et al., 1994, 1995, 2000; Weber et al., 1995). Our present study extends on previous observations by demonstrating that docosahexaenoic acid also inhibits neutrophil adhesion to activated endothelial cells. In fact, our data shows for the first time that docosahexaenoic acid decreases TNF-α-induced expression of P-selectin, which is an important adhesion molecule regulating neutrophil extravasation into tissues in vivo (Robinson et al., 1999; Mansson et al., 2000). Notably, we found that the $IC_{50}$ of docosahexaenoic acid on TNF-α-induced expression of P-selectin on endothelial cells was nearly 20 μM. It is interesting to note that a previous study on TNF-α-induced VCAM-1 expression and monocyte adhesion on endothelial cells reported an $IC_{50}$ of docosahexaenoic acid around 10 μM (Weber et al., 1995). Considered together, it may be suggested that the anti-inflammatory effect of docosahexaenoic acid on adhesion molecule expression is around 10–20 μM. Indeed, we found that an anti-P-selectin antibody significantly reduced TNF-α-induced adhesion of neutrophils, indicating the important role of P-selectin in this experimental system. These findings suggest that docosahexaenoic acid has the capacity to negatively regulate cytokine-mediated interactions between neutrophils and activated endothelial cells. However, inhibition of P-selectin function did not completely block neutrophil adhesion, suggesting that also other adhesion molecules may be involved in this in vitro model of cell adhesion in response to TNF-α. In fact, a previous study has reported that both E-selectin and ICAM-1 may support TNF-α-mediated neutrophil adhesion to endothelial cells in vitro (Weller et al., 1992; Ishii et al., 1992; Lo et al., 1989). Such a role of P-selectin-independent pathways may help explain our finding that pretreatment with docosahexaenoic acid partially reduced neutrophil adhesion whereas P-selectin expression was almost completely inhibited in response to TNF-α challenge. In this context, it is important to note that our data do not exclude that docosahexaenoic acid may negatively regulate TNF-α-induced neutrophil adhesion via mechanisms other than inhibition of P-selectin expression.

Chemokines are important regulators of leukocyte navigation in tissues (Zlotnik et al., 1999). In particular, CXC chemokines (i.e. MIP-2 and KC) control tissue recruitment of neutrophils and we have previously shown that the function of MIP-2 and KC is a critical component in TNF-α-provoked

adhesion and extravasation of neutrophils (Liu et al., 2000; Schramm et al., 2000; Zhang et al., 2001). In fact, it has been reported that docosahexaenoic acid may reduce cytokine-induced expression of the CXC chemokine IL-8 although the observed decrease was small, i.e. less than 20% (De Caterina et al., 1995). Therefore, we next wanted to determine the potential effect of docosahexaenoic acid on the expression of MIP-2 and KC triggered by TNF-α. Herein, we found that docosahexaenoic acid had no effect on TNF-α-induced expression of MIP-2 and KC, suggesting that CXC chemokines do not constitute targets of docosahexaenoic acid and do not help to explain the anti-inflammatory effect of docosahexaenoic acid on TNF-α-induced adhesion of neutrophils.

Taken together, our novel data demonstrate that docosahexaenoic acid has the capacity to reduce neutrophil adhesion to cytokine-activated endothelial cells in a dose-dependent fashion. In addition, these results suggest that the anti-inflammatory effect of docosahexaenoic acid is related to a reduction in TNF-α-induced expression of P-selectin on endothelial cells, whereas induction of CXC chemokines is insensitive to docosahexaenoic acid. Thus, our study may help explain the observed beneficial effects of docosahexaenoic acid in numerous conditions characterized by pathological inflammation and tissue accumulation of neutrophils.

## Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (K2000-04P-13411-01A, K2002-73-X-14273-01A), Crafoordska stiftelsen (20010968), Harald och Greta Jaenssons stiftelse, Greta och Johan Kocks stiftelse, Fröken Agnes Nilsson stiftelse, Franke and Margareta Bergqvists stiftelse för främjande av cancerforskning, Nanna Swartz stiftelse, Ruth och Rickard Julins stiftelse, Einar och Inga Nilssons stiftelse, Svenska Läkaresällskapet (2001-907), Blanceflors stiftelse, Teggers stiftelse, Allmäna sjukhusets i Malmö stiftelse för bekämpande av cancer, MAS fonder.

## References

Anisowicz, A., Bardwell, L., Stenman, G., Sager, R., 1987. Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells. Proc. Natl. Acad. Sci. 84, 7188–7192.

Butcher, E.C., 1991. Leukocyte–endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 67, 1033–1036.

Carlos, T.M., Harlan, J.M., 1994. Leukocyte–endothelial adhesion molecules. Blood 84, 2068–2101.

De Caterina, R., Cybulsky, M.A., Clinton, S.K., Gimbrone Jr., M.A., Libby, P., 1994. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. Arterioscler. Thromb. 14, 1829–1836.

De Caterina, R., Cybulsky, M.A., Clinton, S.K., Gimbrone Jr., M.A., Libby, P., 1995. Omega-3 fatty acids and endothelial leukocyte adhesion molecules. Prostaglandins Leukot. Essent. Fat. Acids 52, 191–195.

De Caterina, R., Liao, J.K., Libby, P., 2000. Fatty acid modulation of endothelial activation. Am. J. Clin. Nutr. 71, 213S–223S.

Deventer, S.J.H., 1997. Tumor necrosis factor and Crohn's disease. Gut 40, 443–448.

Ishii, Y., Lo, S.K., Malik, A.B., 1992. Neutrophil adhesion to TNF alpha-activated endothelial cells potentiates leukotriene B4 production. J. Cell. Physiol. 153, 187–195.

Ksontini, R., Mackay, S.L.D., Moldawer, L.L., 1998. Revisiting the role of tumor necrosis factor α and the response to surgical injury and inflammation. Arch. Surg. 133, 558–567.

Liu, Q., Wang, Y., Thorlacius, H., 2000. Dexamethasone inhibits tumor necrosis factor-alpha-induced expression of macrophage inflammatory protein-2 and adhesion of neutrophils to endothelial cells. Biochem. Biophys. Res. Commun. 271, 364–367.

Lo, S.K., Van Seventer, G.A., Levin, S.M., Wright, S.D., 1989. Two leukocyte receptors (CD11a/CD18 and CD11b/CD18) mediate transient adhesion to endothelium by binding to different ligands. J. Immunol. 143, 3325–3329.

Mansson, P., Zhang, X.W., Jeppsson, B., Johnell, O., Thorlacius, H., 2000. Critical role of P-selectin-dependent rolling in tumor necrosis factor-α-induced leukocyte adhesion and extravascular recruitment in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 190–196.

Oquendo, P., Alberta, J., Wen, D.Z., Graycar, J.L., Derynck, R., Stiles, C.D., 1989. The platelet-derived growth factor-inducible KC gene encodes a secretory protein related to platelet alpha-granule proteins. J. Biol. Chem. 264, 4133–4137.

Richmond, A., Balentien, E., Thomas, H.G., Flaggs, G., Barton, D.E., Spiess, J., Bordoni, R., Derynck, R., 1988. Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to beta-thromboglobulin. EMBO J. 7, 2025–2033.

Robinson, S.D., Frenette, P.S., Rayburn, H., Cummiskey, M., Ullman-Cullere, M., Wagner, D.D., Hynes, R.O., 1999. Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. Proc. Natl. Acad. Sci. 96, 11452–11457.

Schramm, R., Liu, Q., Thorlacius, H., 2000. Expression and function of MIP-2 are reduced by dexamethasone treatment in vivo. Br. J. Pharmacol. 131, 328–334.

Simopoulos, A.P., Koletzko, B., Anderson, R.E., Hornstra, G., Mensink, R.P., Weksler, B.B., Harris, W.S., De Caterina, R., Muggli, R., Sprecher, H., 1994. The 1st Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL): fatty acids and lipids from cell biology to human disease. J. Lipid Res. 35, 169–173.

Sperling, R.I., Benincaso, A.I., Knoell, C.T., Larkin, J.K., Austen, K.F., Robinson, D.R., 1993. Dietary omega-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. J. Clin. Invest. 91, 651–660.

Tekamp-Olson, P., Gallegos, C., Bauer, D., McClain, J., Sherry, B., Fabre, M., van Deventer, S., Cerami, A., 1990. Cloning and characterization of cDNAs for murine macrophage inflammatory protein 2 and its human homologues. J. Exp. Med. 172, 911–919.

Weber, C., Erl, W., Pietsch, A., Danesch, U., Weber, P.C., 1995. Docosahexaenoic acid selectively attenuates induction of vascular cell adhesion molecule-1 and subsequent monocytic cell adhesion to human endothelial cells stimulated by tumor necrosis factor-alpha. Arterioscler. Thromb. Vasc. Biol. 15, 622–628.

Weller, A., Isenmann, S., Vestweber, D., 1992. Cloning of the mouse endothelial selectins: expression of both E- and P-selectin is inducible by tumor necrosis factor-α. J. Biol. Chem. 267, 15176–15183.

Williams, R.L., Risau, W., Zerwes, H.G., Drexler, H., Aguzzi, A., Wagner, E.F., 1989. Endothelioma cells expressing the polyoma middle T oncogene induce hemangiomas by host cell recruitment. Cell 57, 1053–1063.

Zhang, X.W., Wang, Y., Liu, Q., Thorlacius, H., 2001. Redundant function of macrophage inflammatory protein-2 and KC in tumor necrosis factor-alpha-induced extravasation of neutrophils in vivo. Eur. J. Pharmacol. 427, 277–283.

Zlotnik, A., Morales, J., Hedrick, J.A., 1999. Recent advances in chemokines and chemokine receptors. Crit. Rev. Immunol. 19, 1–47.