Elevated Soluble Intercellular Adhesion Molecule-1 Levels in Obesity: Relationship to Insulin Resistance and Tumor Necrosis Factor-α System Activity

Marek Strączkowski, Piotr Lewczuk, Stella Dzienis-Strączkowska, Irina Kowalska, Agnieszka Stępień, and Ida Kinalska

Intercellular adhesion molecule-1 (ICAM-1) is 1 of the possible factors linking obesity and diabetes with cardiovascular disease, however, the mechanism of the increase in ICAM-1 concentration in obesity remains unclear. Therefore, the aim of the present study was to assess plasma soluble ICAM-1 (sICAM-1) levels in obese subjects with normal glucose tolerance and to evaluate whether those levels may be related to insulin resistance and tumor necrosis factor-α (TNFα) system activity. The study was performed in 8 lean and 15 obese subjects. Anthropometric and biochemical parameters were measured, and insulin sensitivity was evaluated using the euglycemic hyperinsulinemic clamp technique (insulin infusion, 50 mU $\times$ kg^{-1} $\times$ h^{-1}). Obese subjects were markedly more hyperinsulinemic and insulin resistant and had higher plasma soluble TNF receptor 2 (sTNFR2) and sICAM-1 levels. sICAM-1 was related positively to body mass index (BMI), waist-to-hip ratio (WHR), percent of body fat, glycated hemoglobin (HbA_{1c}), plasma insulin and triglycerides (TG), TNFα, and sTNFR2 and negatively to insulin sensitivity. Multiple regression analysis showed that only sTNFR2 and insulin sensitivity were independent predictors of sICAM-1 concentrations and were responsible for 66% of sICAM-1 variability. We conclude that an increase in plasma sICAM-1 concentration in obesity is related to TNFα system activation and insulin resistance.

OBESITY IS A MAJOR risk factor for the development of impaired glucose tolerance and type 2 diabetes mellitus.[1] Insulin resistance is considered the most important pathophysiological link between these disorders.[2] Insulin resistance is also believed to play a role in other pathological states associated with obesity, such as hypertension, cardiovascular disease, atherosclerosis, and dyslipidemia.[3] Therefore, much of the experimental work has focused on mechanisms leading from obesity and insulin resistance to atherosclerosis.

An early event in the pathogenesis of atherosclerosis involves adhesion of circulating leukocytes to the endothelium and their subsequent passage to the arterial intima.[4] This process is mediated by the adhesion molecules, which appear on the cell surface of the activated endothelium and are also present in circulation in the soluble forms.[5] One of the adhesion molecules is intercellular adhesion molecule-1 (ICAM-1), its soluble form (sICAM-1) may serve as a marker of its endothelial expression.[6] Increased sICAM-1 levels were found in ischemic heart disease and peripheral vascular disease, and it is suggested that sICAM-1 may be useful as an index of endothelial cell activation in atherosclerosis.[7] Plasma sICAM-1 concentrations are also related to the risk of future myocardial infarction in apparently healthy men.[8]

Increased plasma sICAM-1 levels were observed in obesity,[9] impaired glucose tolerance,[10] and type 2 diabetes mellitus,[11-13] and it is suggested that it may play a role in diabetic macrovascular complications.[13,14] The relationship between sICAM-1 and insulin resistance in healthy men was also found.[15] Alternatively, elevated sICAM-1 in insulin-resistant subjects may be the result of increased tumor necrosis factor-α (TNFα) activity. TNFα is a potent activator of ICAM-1 expression[16-18] and also induces insulin resistance by acting via an autocrine-paracrine mechanism in adipose tissue and skeletal muscle.[19] TNFα has 2 cell surface receptors, TNFR1 and TNFR2, which are also present in plasma in soluble forms (sTNFR1 and 2). sTNFR2 is increased in obesity[20] and related to insulin resistance.[21] Because sTNFR2 is a more stable protein than TNFα, it might serve as the best predictor of TNFα system activation in obesity.[20] The role of TNFα system activation in inducing increased sICAM-1 levels in obesity has not yet been determined.

Therefore, in the present study, we assess sICAM-1 levels in obese subjects with normal glucose tolerance, and we evaluate whether those levels may be related to insulin resistance and TNFα system activity.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the Medical Academy, Białystok. A total of 23 subjects were recruited for this study, 8 lean persons (body mass index [BMI] $<$ 27.8; 3 males and 5 females) and 15 obese (BMI $>$ 27.8; 5 males and 10 females) without ischemic heart disease, hypertension, peripheral vascular disease, infections, or any other serious medical problems. Before participating in the study, physical examination and resting electrocardiography were performed. All subjects underwent oral glucose tolerance test (OGTT), and they all had normal glucose tolerance according to World Health Organization (WHO) criteria. All subjects gave written informed consent before entering the study.

All analyses were performed after an overnight fast. The BMI was calculated as body weight $\times$ height^{-2} (kg/m^2). The waist-to hip ratio (WHR) was also estimated. The waist circumference was measured at the smallest circumference between the rib cage and the iliac crest, with the subject in the standing position. The hip circumference was measured at the widest circumference between the waist and the thighs. Percent of body fat was estimated by bioelectric impedance analysis using the Tanita TBF-511 Body Fat Analyzer (Tanita Corp, Tokyo,

From the Departments of Endocrinology and Pediatric Neurology, Medical Academy, Białystok, Poland; and the Max-Planck-Institute for Experimental Medicine, Goettingen, Germany.

Submitted February 21, 2001; accepted May 1, 2001.

Address reprint requests to Marek Strączkowski, MD, Department of Endocrinology, Medical Academy, M.C. Skłodowskiej 24a, 15-276 Białystok, Poland.

0026-0495/02/5101-0033$35.00/0
doi:10.1053/meta.2002.28095

Japan). On that basis, fat mass (FM) and fat-free mass (FFM) were assessed.

Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique as described by DeFronzo et al[22] and modified by Ponchner et al.[23] On the morning of the study, 2 venous catheters were inserted into antecubital veins, 1 for the infusion of insulin and glucose and the other in the contralateral hand for blood sampling; that hand was heated to approximately 60°C. Insulin (Actrapid HM, Novo Nordisk, Copenhagen, Denmark) was given as a primed-continuous intravenous infusion for 2 hours at 50 mU $\times$ kg^{-1} $\times$ h^{-1}, resulting in constant hyperinsulinemia of approximately 550 pmol/L. Arterialized blood glucose was obtained every 5 minutes, and 40% dextrose (2.22 mol/L) infusion was adjusted manually to maintain plasma glucose levels at 5.0 mmol/L. The glucose infusion rate approached stable values during the final 40 minutes of the study, and the rate of whole-body glucose uptake (M value) was calculated as the mean glucose infusion rate from 80 to 120 minutes, corrected for glucose space and normalized per kilogram of FFM (M_{ffm}).

Fasting blood samples were also taken from the antecubital vein before the beginning of the clamp for the determination of glycated hemoglobin (HbA_{1c}), plasma lipids, TNFα, sTNFR1, sTNFR2, and sICAM-1. For the determination of plasma TNF system and sICAM-1, samples were frozen at -70°C.

Plasma glucose was measured immediately by the enzymatic method using a glucose analyzer. Plasma insulin was measured in duplicate with the Medgenix EASIA test (BioSource Europe, Nivelles, Belgium). The minimum detectable concentration was 1.05 pg/L and the intra-assay and interassay coefficients of variation (CVs) were below 5.5% and 10%, respectively. In that method, human and animal proinsulins present no cross-reaction. HbA_{1c} was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany). Plasma cholesterol and triglycerides (TG) were assessed by the enzymatic methods (Cormay, Warsaw, Poland). Plasma FFA were measured by the colorimetric method.[24]

Plasma TNFα concentrations were measured by the Immunoassay Kit (BioSource International, Camarillo, CA) with the minimum detectable concentration 1.7 pg/mL and with the intra-assay and interassay CVs below 5.2% and 8.5%, respectively. Plasma sTNFR1 and sTNFR2 were determined with the EASIA kits (BioSource Europe). The minimum detectable concentration was 0.05 ng/mL for sTNFR1 and 0.1 ng/mL for sTNFR2. The intra-assay and interassay CVs for both receptors were below 6.5% and 9%, respectively. sTNFR1 EASIA does not cross-react with sTNFR2, and TNFα does not interfere with the assay.

sICAM-1 concentration in plasma was analyzed by a sandwich enzyme-linked immunosorbent assay (ELISA) method (R&D Systems Europe, Abingdon, UK). The detection limit of the method was 0.35 ng/mL. The intra-assay and interassay CVs were below 4.8% and 10.1%, respectively.

All of the statistics were performed with the STATISTICA 5.0 program (StatSoft, Krakow, Poland). To evaluate differences between groups, Mann-Whitney U test was used. To estimate the relationships between variables, simple and multiple regression analyses were performed. The level of significance was accepted at P less than .05.

RESULTS

Anthropometric and biochemical characteristics of the studied groups are given in Table 1. In the present study, obese subjects were markedly more insulin resistant ($P < .005$) and hyperinsulinemic ($P < .02$). They also had significantly greater TNFα system activity as measured by sTNFR2 levels ($P < .005$ in comparison to lean subjects). No significant differences in TNFα and sTNFR1 concentrations were observed between the studied groups.

Table 1. Anthropometric and Biochemical Characteristics of the Studied Groups

	Lean Subjects (n = 8)	Obese Subjects (n = 15)
Age (yr)	41.87 ± 16.56	42.86 ± 11.48
BMI (kg/m^2)	23.77 ± 2.29	34.43 ± 5.94*
WHR	0.817 ± 0.06	0.881 ± 0.07*
Percent of body fat	21.06 ± 5.89	40.00 ± 11.21*
FFM (kg)	55.03 ± 5.15	56.52 ± 9.62
FM (kg)	15.12 ± 5.65	44.61 ± 23.55*
Plasma glucose (mmol/L)	4.99 ± 0.82	5.44 ± 0.47
Plasma insulin (pmol/L)	81.88 ± 45.57	141.34 ± 63.83*
Plasma FFA (mmol/L)	576.25 ± 326.32	588.67 ± 265.65
Plasma cholesterol (mmol/L)	5.05 ± 1.19	5.87 ± 0.87
Plasma TG (mmol/L)	1.23 ± 0.77	1.71 ± 0.74
HbA_{1c} (%)	5.66 ± 0.31	6.01 ± 0.47
M_{ffm} ($\mu mol \times kg^{-1} \times min^{-1}$)	55.79 ± 17.42	28.82 ± 12.90*
TNFα (pg/mL)	4.91 ± 2.01	5.41 ± 2.76
sTNFR1 (ng/mL)	2.13 ± 0.41	2.27 ± 0.53
sTNFR2 (ng/mL)	3.53 ± 0.28	4.81 ± 1.06*
sICAM-1 (ng/mL)	160.12 ± 35.91	243.20 ± 70.66*

NOTE. Data are expressed as means ± SD.

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; FFM, fat-free mass; FM, fat mass; FFA, free fatty acids; TG, triglycerides; M_{ffm}, whole-body glucose uptake normalized for FFM; TNFα, tumor necrosis factor-α; sTNFR1, soluble TNFα receptor 1; sTNFR2, soluble TNFα receptor 2; sICAM-1, soluble intercellular adhesion molecule 1.

*$P < .05$.

Plasma sICAM-1 values were markedly higher in the obese group ($P < .005$). No difference in sICAM-1 concentrations between males and females was observed ($P = .77$). When obese subjects were analyzed separately, no significant difference in sICAM-1 levels was observed between subjects with visceral (WHR > 0.90) versus peripheral type of obesity ($P = .64$).

Correlations between sICAM-1 and other examined variables are shown in Table 2. sICAM-1 levels were related positively to BMI, WHR, percent of body fat, HbA_{1c}, plasma insulin and TG, TNFα, and sTNFR2 and negatively to insulin sensitivity (Fig 1). No significant correlation between sTNFR1 and sICAM-1 was found.

To evaluate factors responsible for the increase in sICAM-1 concentrations, multiple regression analysis was performed. sTNFR2 was chosen as the best predictor of TNFα activity because it was most strongly related to sICAM-1. Only M_{ffm} and sTNFR2 were independently determining sICAM-1 levels (Table 3). In a stepwise regression analysis, both M_{ffm} and sTNFR2 were responsible for 66% of sICAM-1 variability ($P < .00005$) with age and plasma FFA slightly improving correlation coefficient ($R^2 = .74$, $P < .00005$) and with other variables not entering the regression model.

DISCUSSION

Increased plasma levels of adhesion molecules, including sICAM-1, in obesity was reported previously.[9] In that report, the role of obesity in promoting endothelial activation was also demonstrated by the marked reductions of soluble adhesin

Table 2. Correlations Between Plasma sICAM-1 Concentrations and Other Clinical Parameters

	r	*P*
Age (yr)	.35	.11
BMI (kg/m^2)	.43	.04
WHR	.44	.03
Percent of body fat	.44	.03
FFM (kg)	−.11	.62
FM (kg)	.23	.28
Plasma glucose (mmol/L)	.25	.24
Plasma insulin (pmol/L)	.42	.04
Plasma FFA (mmol/L)	.27	.20
Plasma cholesterol (mmol/L)	.38	.07
Plasma TG (mmol/L)	.46	.03
HbA_{1c} (%)	.13	.55
M_{ffm} (μmol × kg^{-1} × min^{-1})	−.74	.0001
TNFα (pg/mL)	.52	.01
sTNFR1 (ng/mL)	.19	.38
sTNFR2 (ng/mL)	.76	.0001

Table 3. Multiple Regression Analysis Results With Plasma sICAM-1 Concentration as the Dependent Variable (R^2 = .81)

	Beta	SE	*P*
Age (yr)	0.07	0.25	.77
BMI (kg/m^2)	−0.18	0.44	.69
WHR	−0.19	0.29	.51
Percent of body fat	−0.06	0.48	.89
Plasma glucose (mmol/L)	0.17	0.31	.58
Plasma insulin (pmol/L)	−0.04	0.23	.86
Plasma FFA (mmol/L)	0.25	0.16	.13
Plasma cholesterol (mmol/L)	0.13	0.19	.50
Plasma TG (mmol/L)	−0.38	0.21	.10
M_{ffm} (μmol × kg^{-1} × min^{-1})	−0.49	0.21	.04
sTNFR2 (ng/mL)	0.83	0.29	.01

levels observed after weight loss due to caloric restriction.[9] Therefore, the investigators suggested that obesity by itself could overstimulate adhesin production. Our data show that the elevation of sICAM-1 in obesity is related to 2 factors, insulin resistance and increased TNFα system activity. Both parameters also change after weight loss.[25] Although those parameters are closely related to each other, as TNFα induces insulin resistance, the present study suggests that both variables act at least partly via different mechanisms. The role of insulin resistance in increasing soluble adhesion molecules levels was examined by Chen et al.[15] A significant relationship between insulin resistance and sICAM-1 concentration was found, and it was independent of all other variables.[15] It was suggested that insulin resistance may be an important factor responsible for sICAM-1 elevation, and that this may give a possible explanation for the previously observed increase in blood levels of soluble adhesion molecules in type 2 diabetes, dyslipidemia, and hypertension.[15] The present study also stresses the importance of insulin resistance in increasing sICAM-1 concentrations. The insulin levels itself, however, does not seem to influence independently sICAM-1, as the relationship between insulin and sICAM-1 was not significant after adjustment for other variables. In the study of Jilma et al,[26], no effect of hyperinsulinemia on sICAM-1 blood levels was reported.

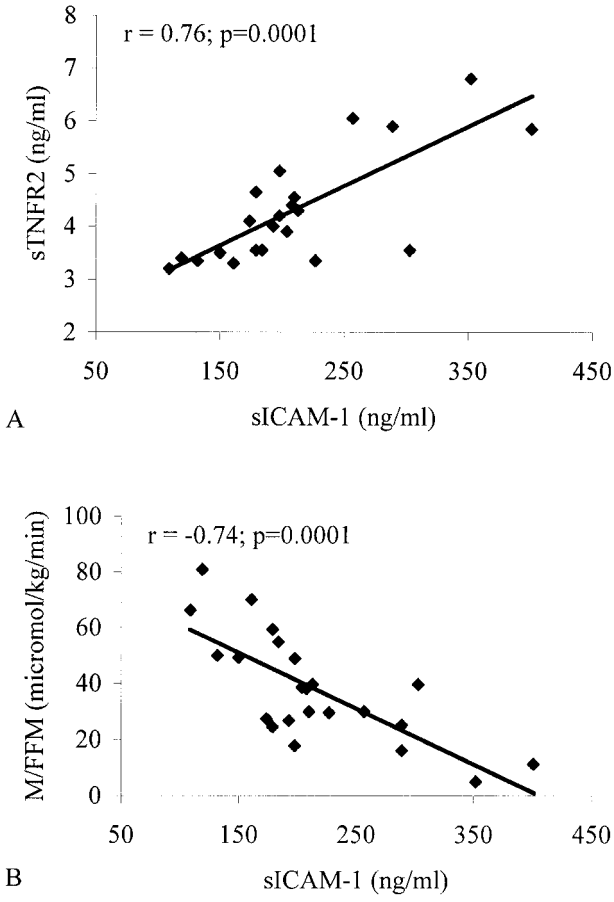

Fig 1. Correlations between sICAM1 and sTNFR2 (A) and sICAM-1 and M_{ffm} (B).

Another possible activator of sICAM-1 in obesity is TNFα. It can stimulate ICAM-1 expression on hematopoietic cells, fibroblasts, endothelial cells, and vascular smooth muscle cells.[27] The role of TNFα in stimulating ICAM-1 expression was observed in many pathologic states, for instance, in inflammatory diseases. However, no data are available about such an action in obesity. In fact, in obesity, TNFα acts rather in an autocrine-paracrine manner,[19] inducing insulin resistance in skeletal muscle and adipose tissue, and only a slight increase in circulating TNFα is observed.[19] Those values are much lower than those observed in inflammatory and other diseases and also lower that those required to mediate general systemic effects.[19] The increase in blood levels of TNFα in obesity is accompanied by the increase in sTNFR2.[20] Soluble forms of TNFα receptors originate from the cell-surface receptors,[20] and it is suggested that they act as a reservoir of TNFα by stabilizing its bioactivity.[28] Our study suggests that even small TNFα system activation observed in obesity is sufficient to elevate sICAM-1 concentration. Probably, TNFα-dependent increase in sTNFR2 might prolong circulating TNFα function. It was also reported that TNFα can induce ICAM-1 expression on cells that normally do not express this molecule,[29] so it is

possible that such an action takes place locally in adipose tissue or skeletal muscle of obese subjects. The limitation of the present study is that it does not reveal any cause-effect relationship. We can only hypothesize in the context of previously known relationships that increased sICAM-1 is the result of insulin resistance and TNFα system activation. There may be other, as yet undetermined, pathways that may also be involved.

Previous studies focused on plasma glucose levels as the possible cause of sICAM-1 increase. However, no significant effect of glucose on sICAM-1 in normoglycemic men was observed.[9] This is consistent with our results, because no marked relationship between glucose and sICAM-1 was found. In contrast, hyperglycemia might be an important factor inducing ICAM-1 expression in type 2 diabetic subjects.[12]

We conclude that in normoglycemic obese subjects an increase in sICAM-1 concentration is present, and this may be 1 of the mechanisms linking obesity with cardiovascular disease. Elevation in sICAM-1 levels in obesity is related to TNFα system activation and insulin resistance.

REFERENCES

1. Chan JM, Stampfer MJ, Ribb EB, et al: Obesity, fat distribution and weight gain as risk factors for clinical diabetes in man. Diabetes Care 17:961-969, 1994
2. Ferrannini E: Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: Problems and prospects. Endocr Rev 19:477-490, 1998
3. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988
4. Munro JM, Cotran RS: Biology of disease: The pathogenesis of atherosclerosis: Atherogenesis and inflammation. Lab Invest 58:249-261, 1988
5. Gearing AJH, Hemingway I, Pigott R, et al: Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, VCAM-1: Pathological significance. Ann N Y Acad Sci 667:324-331, 1992
6. Leeuwenberg JFM, Smeets EF, Neefjes JJ, et al: E-selectin and intercellular adhesion molecule-1 are released by human endothelial cells in vivo. Immunology 77:543-549, 1993
7. Blann AD, McCollum CN: Circulating endothelial cell/leukocyte adhesion molecules in atherosclerosis. Thromb Haemost 72:151-154, 1994
8. Ridker PM, Hennekens CH, Roitman-Johnson B, et al: Plasma concentrations of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 351: 88-92, 1998
9. Ferri C, Desideri G, Valenti M, et al: Early upregulation of endothelial adhesion molecules in obese hypertensive men. Hypertension 34:568-573, 1999
10. Ferri C, Desideri G, Baldoncini R, et al: Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. Diabetes 47:660-667, 1998
11. Steiner M, Reinhardt KM, Krammer B, et al: Increased levels of soluble adhesion molecules in type 2 (non-insulin dependent) diabetes mellitus are independent of glycemic control. Thromb Haemost 72: 979-984, 1994
12. Ceriello A, Falleti E, Bortolotti N, et al: Increased circulating intercellular adhesion molecule-1 levels in type 2 diabetic patients: The possible role of metabolic control and oxidative stress. Metabolism 45:498-501, 1996
13. Fasching P, Waldhausl W, Wagner OF: Elevated circulating adhesion molecules in NIDDM—Potential mediators of diabetic macroangiopathy. Diabetologia 39:1242-1244, 1996
14. Wagner OF, Jilma B: Putative role of adhesion molecules in metabolic disorders. Horm Metab Res 29:627-630, 1997
15. Chen NG, Holmes M, Reaven GM: Relationship between insulin resistance, soluble adhesion molecules, and mononuclear cell binding in healthy volunteers. J Clin Endocrinol Metab 84:3485-3489, 1999
16. Pigott R, Dillon LP, Hemingway IH, et al: Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys Res Commun 187:584-589, 1992
17. Boehme MW, Waldherr R, Kist A, et al: Kinetics of soluble TNF receptors and soluble adhesion molecules ICAM-1, E-selectin and VCAM-1 under systemic rhTNF therapy. Eur J Clin Invest 26:404-410, 1996
18. Hou J, Baichwal V, Cao Z: Regulatory elements and transciption factors controlling basal and cytokine induced expression of the gene encoding intercellular adhesion molecule 1. Proc Natl Acad Sci USA 91:11641-11645, 1994
19. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: A key component of the obesity-diabetes link. Diabetes 43:1271-1278, 1994
20. Hotamisligil GS, Arner P, Atkinson RL, et al: Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. Diabetes 46:451-455, 1997
21. Fernandez-Real JM, Broch M, Ricart W, et al: Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. Diabetes 47:1757-1762, 1998
22. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979
23. Ponchner M, Heine RJ, Pernet A, et al: A comparison of the artificial pancreas (glucose controlled insulin infusion system) and a manual technique for assessing insulin sensitivity during euglycemic clamping. Diabetologia 26:420-425, 1984
24. Duncombe WS: The colorimetric microdetermination of nonesterified fatty acids in plasma. Clin Chim Acta 9:122-135, 1964
25. Katsuki A, Sumida Y, Murashima S, et al: Serum levels of tumor necrosis factor-α are increased in obese patients with non-insulin dependent diabetes mellitus. J Clin Endocrinol Metab 83:859-862, 1998
26. Jilma B, Dallinger S, Hergovich N, et al: Effects of hyperinsulinemia on plasma levels of circulating adhesion molecules. J Clin Endocrinol Metab 85:1748-1751, 2000
27. Couffinhal T, Duplaa C, Labat L, et al: Tumor necrosis factor-α stimulates ICAM-1 expression in human vascular smooth muscle cells. Arterioscler Thromb 13:407-414, 1993
28. Aderka D, Engelmann H, Maor Y, et al: Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 175:323-329, 1992
29. Dustin ML, Singer KH, Tuck DT: Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon γ and is mediated by intercellular adhesion molecule 1 (ICAM-1). J Exp Med 167:1323-1340, 1988