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Differential Up-Regulation of Circulating Soluble and Endothelial Cell Intercellular Adhesion Molecule-1 in Mice

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Although circulating levels of soluble intercellular adhesion molecule-1 (sICAM-1) are frequently used as an indicator of the severity of different immune, inflammatory, or neoplastic diseases, little is known about the factors that govern plasma sICAM-1 concentration and its relationship to the membranous form of ICAM-1 (mICAM-1) expressed on vascular endothelial cells. Plasma sICAM-1 concentration (measured by enzyme-linked immunosorbent assay) and mICAM-1 expression (measured using the dual radiolabeled monoclonal antibody technique) in different vascular beds (eg, lung, small intestine, and spleen) were monitored in wild-type (C57BL) and ICAM-1-deficient mice, before and after administration of tumor necrosis factor (TNF)-α. In wild-type mice, TNF-α elicited time-dependent increases in lung and intestine mICAM-1 (plateau achieved at 12 hours), with a corresponding increase in plasma sICAM-1 (peaked at 5 hours and then declined). The initial increases in mICAM-1 and pulmonary leukocyte sequestration (measured as lung myeloperoxidase activity) induced by TNF-α preceded any detectable elevation in sICAM-1. In ICAM-1-deficient mice, plasma sICAM-1 was reduced by ~70%, with >95% reductions of mICAM-1 in lung and intestine, and >75% reduction in splenic accumulation of anti-ICAM-1 antibody. Although TNF-α doubled plasma sICAM-1 in ICAM-1-deficient mice, mICAM-1 was unaffected in all tissues. Either splenectomy or pretreatment with cycloheximide resulted in an attenuated TNF-induced increase in sICAM-1, without affecting mICAM-1 expression. These findings indicate that plasma sICAM-1 concentration does not accurately reflect the level of ICAM-1 expression on endothelial cells in different vascular beds. (Am J Pathol 1997, 151:205–214)

Intercellular adhesion molecule (ICAM)-1 is a cell surface glycoprotein of the immunoglobulin superfamily that has been shown to mediate various cell-cell interactions, including leukocyte adhesion to vascular endothelial cells[1–4] as well as tumor progression and metastasis.[5,6] This adhesion molecule is constitutively expressed on the surface of endothelial cells, and its expression can be increased by exposure to endotoxin or inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β.[7–11] Immunohistochemical and flow cytometric analyses have revealed that a variety of human diseases, including allograft rejection,[12] autoimmune disorders,[13] asthma,[14] inflammatory bowel diseases,[15] atherosclerosis,[16] and malignant tumors,[17] are associated with an increased expression of ICAM-1 on vascular endothelial cells and/or circulating immune cells. These findings in different patient populations, coupled to animal studies demonstrating abrogation of disease progression or severity after administration of ICAM-1 blocking antibodies,[18] support the view that cell-membrane-associated ICAM-1 (mICAM-1) plays an important role in the pathogenesis of several disease processes.

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A soluble isoform of ICAM-1 (sICAM-1) has been described.[19,20] sICAM-1 appears to contain most of the extracellular portion of mICAM-1, and it is readily detected in normal serum by enzyme-linked immunosorbent assay (ELISA).[20] Soluble isoforms of endothelial cell adhesion molecules, including sICAM-1, sP-selectin, and sE-selectin, are generally thought to represent shed fragments of their membrane-bound counterparts.[21] Inasmuch as activated endothelial cells in inflamed tissue are believed to shed mICAM-1, plasma sICAM-1 concentration is considered to reflect the expression of mICAM-1 on endothelial cells.[22] Consequently, the plasma sICAM-1 level has been widely used in the clinical setting to monitor the inflammatory disease activity and the level of endothelial cell activation.[21] Elevated serum sICAM-1 levels have been reported in various immune, inflammatory, ischemic, and neoplastic disorders.[14,15,21,22] Although sICAM-1 measurements in human plasma are being reported with increasing frequency, there is little experimental evidence from animal studies that supports its use as an index of ICAM-1 expression on endothelial cells. This consideration seems appropriate in view of a recent report that proposes the production and secretion of multiple spliced variants of ICAM-1 by murine endothelial cells.[23]

Until recently, quantification of endothelial cell adhesion molecule expression has not been possible in intact animals. Estimates of endothelial cell surface expression of ICAM-1, VCAM-1, P-selectin, and E-selectin in different vascular beds can now be obtained using differentially radiolabeled binding and nonbinding monoclonal antibodies (MAbs).[9–11,24–26] The dual radiolabeled MAb technique allows for the detection of constitutive and induced expression of adhesion receptors on vascular endothelium with a precision not previously attained using histochemical procedures. This technique has been successfully applied to mice, rats, and pigs, where it yields information on adhesion molecule expression in multiple organ systems.

The overall objective of the present study was to define the relationship between ICAM-1 expression on endothelial cells of different vascular beds and the concentration of sICAM-1 in mouse plasma. The dual radiolabeled MAb technique was used to measure mICAM-1 expression in different vascular beds (lung, small intestine, and spleen) while sICAM-1 concentration in plasma was quantified by ELISA. Experiments were performed in wild-type and ICAM-1-deficient mice, and an effort was made to address issues related to the role of the spleen in producing sICAM-1, the contributions of *de novo* synthesis and cell surface shedding to plasma sICAM-1 levels, and the dependence of cytokine-induced pulmonary leukosequestration on lung mICAM-1 and sICAM-1.

Materials and Methods

Animals

Male C57BL/6J mice (n = 118) and ICAM-1-deficient mice (n = 28)[27] weighing 20 to 25 g were obtained from Harlan Laboratories (Frederick, MD) and Pharmacia-UpJohn (Kalamazoo, MI). The studies were approved by and performed in accordance with the guidelines of the Louisiana State University Medical Center Animal Care and Use Committee.

Splenectomy

In some mice, a splenectomy was performed to assess the contribution of the spleen to sICAM-1 levels. For mice undergoing this surgical procedure, anesthesia was induced using ketamine hydrochloride (Ketaset; 150 mg/kg intramuscularly (i.m.)) and xylazine (Rompun; 10 mg/kg i.m.). After laparotomy from a left subcostal incision, the splenic artery and vein were ligated and then the spleen was removed. Sham mice underwent a left subcostal incision, and the spleen was exteriorized and then replaced into the peritoneal cavity. The incision was closed with sterile sutures and the mice were allowed to recover for 7 days before the experiment.

Quantification of ICAM-1 Expression

Monoclonal Antibodies

The MAbs used for the *in vivo* characterization of ICAM-1 were YN-1, a rat immunoglobulin G_{2b} (IgG_{2b}) directed against mouse ICAM-1,[28] and P-23, a murine IgG_1 directed against human P-selectin,[29] which does not bind to any mouse antigen. MAb YN-1 was produced at Bayer Corp. (West Haven, CT), whereas P-23 was generated at Pharmacia-UpJohn (Kalamazoo, MI).

Radioiodination of Monoclonal Antibodies

The binding MAb directed against ICAM-1 (YN-1) was labeled with ^{125}I (DuPont NEN, Boston, MA), whereas the nonbinding MAb (P-23) was labeled with ^{131}I. Radioiodination of the MAbs was performed by the iodogen method.[30] Briefly, 250 μg of protein was incubated with 250 μCi of $Na^{125}I$ and

125 μg of iodogen at 4°C for 12 minutes. After radioiodination, the radiolabeled MAbs were separated from free ^{125}I by gel filtration on a Sephadex PD-10 column (Pharmacia LKB, Uppsala, Sweden). The column was equilibrated with phosphate buffer containing 1% bovine serum albumin and was eluted with the same buffer. Two fractions of 2.5 ml each were collected, the second of which contained the labeled antibody. Absence of free ^{125}I or ^{131}I was ensured by extensive dialysis of the protein-containing fraction. Less than 1% of the activity of the protein fraction was recovered from the dialysis fluid. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis showed normal heavy and light chain moieties of expected molecular weight. Labeled MAbs were stored in 500-μl aliquots at 4°C and used within 3 weeks after the labeling procedure. The specific activity of labeled MAbs was 0.5 μCi/μg.

Animal Procedures

Anesthesia was induced using ketamine hydrochloride (150 mg/kg i.m.) and xylazine (10 mg/kg i.m.). The right carotid artery and the left jugular vein were cannulated with polyethylene tubing. To measure ICAM-1 expression, a mixture of ^{125}I-labeled anti-ICAM-1 MAb (YN-1; 6 μg), a dose of unlabeled anti-ICAM-1 MAb (54 μg), and an amount of ^{131}I-labeled P-23 necessary to ensure a total ^{131}I injected activity of 400,000 to 600,000 cpm, was administered through the jugular vein cannula (total volume, 200 μl) after collecting blood samples for the sICAM-1 assay. Pilot studies using a 6-μg dose of ^{125}I-labeled YN-1, in conjunction with 24 to 84 μg of cold YN-1, showed that the combination of 6 μg of ^{125}I-labeled YN-1 and 54 μg of cold YN-1 provided optimal activity to accurately assess ICAM-1 expression in the lung under constitutive and stimulated conditions.

Blood samples were obtained through the carotid artery catheter at 5 minutes after injection of the MAb mixture. Thereafter, the animals were heparinized (1000 U/kg sodium heparin) and rapidly exsanguinated by vascular perfusion with sodium bicarbonate buffer via the jugular vein and simultaneous blood withdrawal via the carotid artery. The inferior vena cava was then severed at the thoracic level and the carotid artery was perfused with sodium bicarbonate buffer. After completion of the exchange transfusion, organs were harvested and weighed.

Calculation of ICAM-1 Expression

^{125}I (binding MAb) and ^{131}I (nonbinding MAb) activities in different organs and in 100-μl aliquots of cell-free plasma were counted in a 14800 Wizard 3 gamma counter (Wallac, Turku, Finland), with automatic correction for background activity and spillover. The injected activity in each experiment was calculated by counting a 4-μl sample of the mixture containing the radiolabeled MAbs. The radioactivities remaining in the tube used to mix the MAbs, the syringe used to inject the mixture, and jugular vein catheter were subtracted from the total calculated injected activity. The accumulated activity of each MAb in an organ was expressed as the percentage of the injected dose (%ID) per gram of tissue. ICAM-1 expression was calculated by subtracting the nonspecific tissue accumulation of MAb from the accumulation of binding MAb as follows: ICAM-1 expression = (%ID/g for ^{125}I) − (%ID/g for ^{131}I) × (%ID for ^{125}I in plasma)/(%ID for ^{131}I in plasma). This formula corrects the tissue accumulation of nonbinding MAb for the relative plasma levels of both binding and nonbinding MAbs to estimate the nonspecific tissue accumulation of MAb.[11] This value, expressed as %ID/g, was converted to micrograms of MAb per gram of tissue by multiplying the above value by the total injected binding MAb (micrograms), divided by 100.

Soluble ICAM-1 Determinations

Plasma samples from C57BL/6J and ICAM-1-deficient mice were assayed using a commercially available ELISA kit for mouse sICAM-1 (Endogen, Cambridge, MA) and expressed as micrograms of sICAM-1 per milliliter of plasma. All plasma samples for sICAM-1 measurements were obtained before administration of MAbs for mICAM-1 determinations or before harvesting the tissues for measuring myeloperoxidase (MPO) activity.

Tissue Myeloperoxidase Activity

After exsanguination of mice, tissue samples were excised, rinsed with ice-cold saline, blotted dry, and frozen at −70°C until thawing for determination of MPO activity using methods previously described.[31] Briefly, the tissues were homogenized in 20 mmol/L phosphate buffer (pH 7.4) and centrifuged at 6000 × *g* for 20 minutes at 4°C. The pellet was homogenized and sonicated with an equivalent volume of 50 mmol/L acetic acid (pH 6.0) containing 0.5% (w/v) hexadecyltrimethylammonium hydroxide. Peroxidase activity was deter-

mined by measuring the H_2O_2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine and expressed as units per gram of tissue.

Experimental Protocols

To assess the relationships among plasma sICAM-1 concentration, mICAM-1 expression on vascular endothelial cells, and leukocyte recruitment into tissues, the following general protocol was employed. Constitutive expression of mICAM-1 was determined by injecting the mixture of radiolabeled (^{125}I-labeled YN-1 and ^{131}I-labeled P-23) and unlabeled (YN-1) MAbs into untreated mice, with the tissues harvested as described above. The magnitude of TNF-α-induced ICAM-1 expression in different tissues was determined at various times (2, 5, 9, 12, and 24 hours) after intraperitoneal (i.p.) injection of a single dose (25 μg/kg) of recombinant murine TNF-α (1.3×10^5 U/μg; R&D Systems, Minneapolis, MN). This dose was recently shown to induce maximal ICAM-1 expression in different vascular beds of the mouse.[24] Tissue MPO activity was measured in different organs of C57BL/6J (wild-type) mice at various times (0, 2, 5, 9, 12, and 24 hours) after injection of TNF-α (25 μg/kg). In ICAM-1-deficient mice, MPO was measured both under baseline conditions and at 2 hours after treatment with TNF-α (25 μg/kg).

We have previously demonstrated that ICAM-1-deficient mice maintain significant plasma levels of sICAM-1 despite profound reductions in mICAM-1 in all organs studied except the spleen, suggesting that the spleen is a major source of circulating sICAM-1.[24] To assess the contribution of the spleen to sICAM-1 levels, mICAM-1 expression as well as sICAM-1 levels were measured in sham and splenectomized, wild-type mice. sICAM-1 levels were measured in some ICAM-1-deficient mice that underwent the surgical procedures. The mice that underwent surgical procedures were allowed to recover for 7 days before the experiment. Plasma sICAM-1 levels and mICAM-1 expression were determined in the above experiments, both under baseline conditions and 5 hours after treatment with TNF-α (25 μg/kg).

In another series of experiments, several animals were pretreated with cycloheximide, a protein synthesis inhibitor, at 1 hour before TNF-α injection, using a dose of 14 mg/kg (i.p.).[32] To assess the role of proteolytic cleavage in generating sICAM-1, animals were pretreated (10 minutes before TNF-α injection) with different doses of iodoacetamide (1, 2, 4, and 8 mg/kg i.p.), a protease inhibitor that was previously shown to specifically inhibit interferon-γ induced sICAM-1 production by keratinocytes in culture.[33]

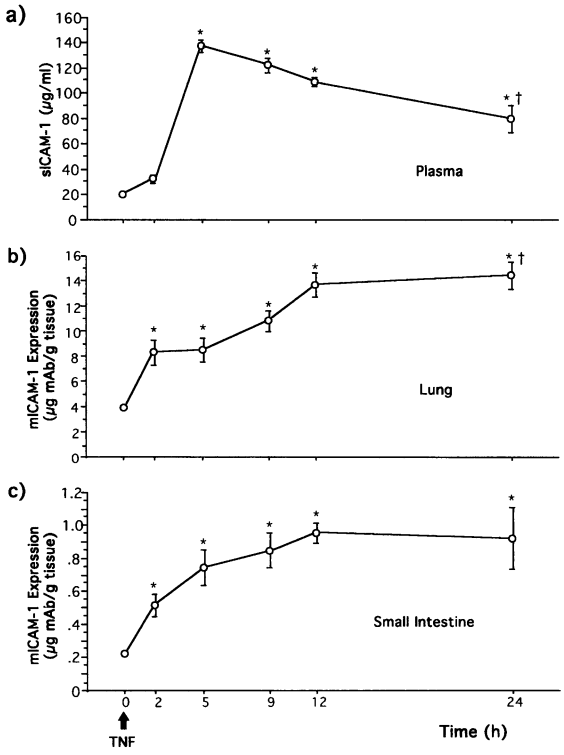

Figure 1. *Time course of changes in soluble* (a) *and membranous ICAM-1 (lung* (b) *and small intestine* (c) *after administration of recombinant TNF-α (25 μg/kg) in wild-type mice. Mean values ± SE are shown.* *P < *0.05* versus *basal (0 hour) value;* †P < *0.05* versus *5-hour value. n ≥ 4 mice per time point.*

Statistics

The data were analyzed using an analysis of variance with the Scheffé's (*post hoc*) test. Either a paired or unpaired Student's *t*-test was used when only two groups were compared. All values are presented as means ± SE. Statistical significance was set at $P < 0.05$.

Results

Time Course of Changes in Soluble and Membranous ICAM-1 after Stimulation with TNF-α (Figure 1)

There was no significant increase in plasma sICAM-1 (Figure 1a) until 2 hours after TNF-α administration; sICAM-1 levels increased after 2 hours, reaching a peak at 5 hours, followed by a gradual decline over the subsequent 20-hour period. Membranous ICAM-1 in the pulmonary (Figure 1b) and intestinal (Figure 1c) vascular beds was significantly elevated

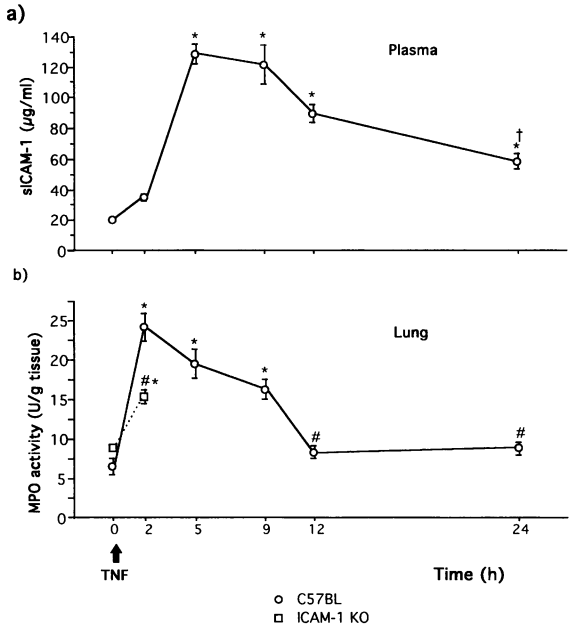

Figure 2. *Time course of changes in sICAM-1* (**a**) *and MPO activity* ($n \geq 6$ *mice per time point*) *in the lung* (**b**) *after administration of recombinant TNF-α* (*25 μg/kg*) *to wild-type mice.* □, *measurements made in ICAM-1-deficient mice* ($n = 5$ *mice per time point*). *Mean values ± SE are shown.* *P < 0.05 versus *constitutive (0 hour) value.* †P < 0.05 versus *5-hour value.* #P < 0.05 versus *2-hour value in wild-type mice.*

even at 2 hours after TNF-α administration, increasing to a plateau at 12 hours, where it remained for the remainder of the experiment. With the exception of the spleen, similar patterns of mICAM-1 expression were elicited in other organs such as heart, skeletal muscle, pancreas, mesentery, and other regions of the gastrointestinal tract (discussed below).

Temporal Relationship between TNF-α-Induced Changes in Tissue MPO Activity (Leukocyte Sequestration) and sICAM-1 (Figure 2)

The kinetics of TNF-induced changes in sICAM-1 observed in this series of experiments were very similar to that noted in Figure 1, that is, sICAM reached a peak level at 5 hours and then gradually declined. In the same animals, TNF-α elicited an abrupt increase in MPO activity in the lung, which reached a peak at 2 hours and then rapidly declined to baseline values at 12 hours. MPO activity in the lung of ICAM-1-deficient mice was significantly increased 2 hours after TNF-α administration. However, the magnitude of the increase in lung MPO of ICAM-1-deficient mice was significantly lower than that observed in the lungs of wild-type (C57BL/6J) mice but still significantly higher than the baseline

Table 1. *Plasma sICAM-1 Concentration and Tissue YN-1 Accumulation in the Lung, Small Intestine, and Spleen of Wild-Type and ICAM-1 Deficient Mice, under Baseline Condition and after Stimulation with Recombinant TNFα (25 μg/kg for 5 Hours)*

	sICAM-1 (μg/ml)		YN-1 (μg MAb/g tissue)					
			Lung		Small bowel		Spleen	
	Basal	TNF	Basal	TNF	Basal	TNF	Basal	TNF
C57BL/6J (n = 6 or 7)	20.26 ± 1.08	137.57 ± 4.99*	3.913 ± 0.097	8.459 ± 0.941*	0.220 ± 0.011	0.744 ± 0.107*	3.475 ± 0.187	3.888 ± 0.210
ICAM-1-deficient (n = 4)	6.68 ± 0.65†	12.43 ± 1.47†	0.024 ± 0.006†	0.052 ± 0.011†	0.10 ± 0.002†	0.013 ± 0.002†	0.818 ± 0.085†	0.683 ± 0.061†
% ICAM-1-deficient/C57BL/6J	33.0	9.0	0.6	0.6	4.5	1.7	23.5	17.6

* $P < 0.05$ *versus* basal.
† $P < 0.05$ *versus* C57BL/6J.

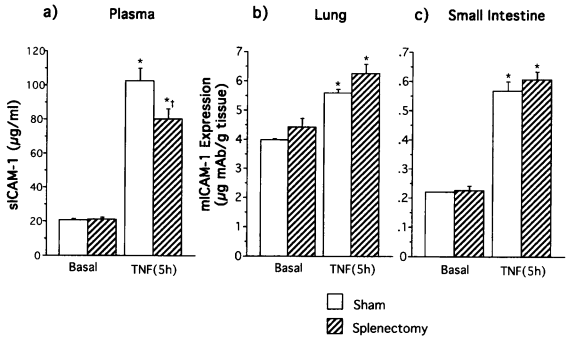

Figure 3. *Effects of splenectomy on plasma sICAM-1 concentration* (a) *and mICAM-1 expression in the lung* (b) *and the small intestine* (c). *Mean values ± SE are shown. Constitutive (basal), sham: n = 6; splenectomy: n = 6; recombinant TNF-α (25 μg/kg, 5h)-induced, sham: n = 8; splenectomy: n = 8.* *$P < 0.05$ versus *constitutive (basal) value.* †$P < 0.05$ versus *sham value.*

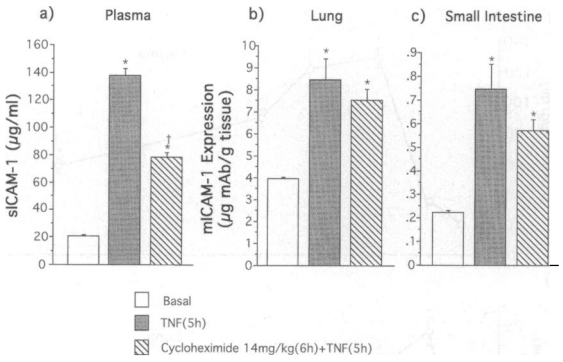

Figure 4. *Effects of cycloheximide on circulating sICAM-1 production* (a) *and mICAM-1 expression in the lung* (b) *and the small intestine* (c). *Mean values ± SE are shown. Constitutive (basal): n = 6; TNF-α (25 μg/kg, 5h)-induced: n = 7; pretreated with cycloheximide (14 mg/kg) before TNF-α (25 μg/kg, 5h) injection: n = 5.* *$P < 0.05$ versus *constitutive (basal).* †$P < 0.05$ versus *TNF-α-induced.*

value. TNF-α administration did not elicit a significant increase in tissue MPO activity in the other organs studied.

Plasma sICAM-1 and mICAM-1 Expression in ICAM-1-Deficient Mice (Table 1)

Although plasma levels of sICAM-1 as well as mICAM-1 in the lung and small intestine of ICAM-1-deficient mice were greatly reduced relative to wild-type mice, sICAM-1 levels remained at 33% of the wild-type level and splenic mICAM-1 was approximately 25% of the value detected in wild-type mice under baseline conditions. These substantial levels of sICAM-1 and splenic mICAM-1 occurred in the face of a profound reduction in mICAM-1 in lung, small intestine, and other organs (data not shown). This pattern continued to exist after administration of TNF-α to wild-type and ICAM-deficient mice (Table 1).

Effects of Splenectomy on sICAM-1 Production (Figure 3)

Although basal plasma sICAM-1 levels were unaffected by splenectomy, removal of the spleen did significantly attenuate (by 28%) the increased plasma sICAM-1 normally elicited by TNF-α in wild-type mice. Splenectomy did not affect either constitutive or induced expression of mICAM-1 in the pulmonary and intestinal vascular beds. In ICAM-1-deficient mice (two to three per group), splenectomy resulted in a 35% reduction in the TNF-α-induced increase in sICAM-1, whereas basal plasma sICAM-1 concentration was unaffected by splenectomy in the mutant mice (data not shown).

Effects of Cycloheximide on Plasma sICAM-1 and mICAM-1 (Figure 4)

Pretreatment of wild-type mice with cycloheximide significantly attenuated the TNF-α-induced increase in plasma sICAM-1 (Figure 4a). On the other hand, TNF-α-induced mICAM-1 expression either in the lung (Figure 4b) or small intestine (Figure 4c) was not significantly affected.

Effects of Iodoacetamide on Plasma sICAM-1 and mICAM-1 (Figure 5)

Pretreatment with iodoacetamide significantly attenuated the TNF-α-induced increase in plasma sICAM-1 (Figure 5a) without affecting the corresponding expression of mICAM-1 in either the lung (Figure 5b) or small intestine (Figure 5c). Increasing doses

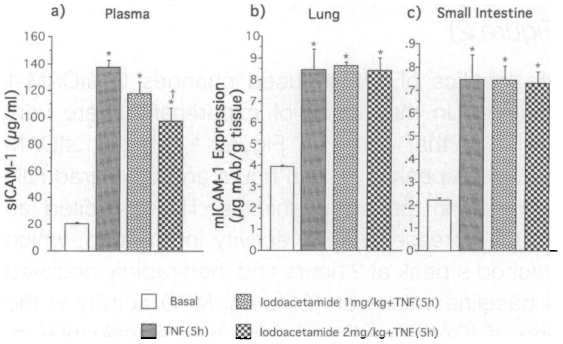

Figure 5. *Effects of iodoacetamide on plasma sICAM-1 concentration* (a) *and mICAM-1 expression in the lung* (b) *and the small intestine* (c). *Mean values ± SE are shown. Constitutive (basal): n = 6; TNF-α (25 μg/kg, 5h)-induced: n = 7; pretreated with iodoacetamide (1 mg/kg) before TNF-α (25 μg/kg, 5h) injection: n = 5; pretreated with iodoacetamide (2 mg/kg) before TNF-α (25 μg/kg, 5h) injection: n = 5.* *$P < 0.05$ versus *constitutive (basal).* †$P < 0.05$ versus *TNF-α-induced.*

of iodoacetamide (4 and 8 mg/kg) did not elicit further reductions in sICAM-1 (data not shown).

Discussion

The widespread application of sICAM-1 measurements in plasma as an index of mICAM expression on endothelial cells and of inflammatory disease activity is based on the assumption that the plasma level of sICAM-1 correlates with the density of mICAM-1 expression on vascular endothelium. The overall objective of the present study was to test this assumption by analyzing the relationship between circulating levels of sICAM-1 and the expression of mICAM-1 on endothelial cells in different vascular beds of the mouse, both under baseline conditions and after cytokine challenge. Such an analysis was only recently made possible by the development of a technique that allows for *in vivo* quantification of endothelial cell adhesion molecule (ECAM) expression using differentially radiolabeled, ECAM-specific MAbs.[9] The recent development of an ELISA for detection of sICAM-1 in mouse plasma also allowed for an assessment of sICAM-1 changes in experimental models of inflammation.[34] Hence, In the present study, the dual radiolabeled MAb and ELISA techniques were used to simultaneously quantify mICAM-1 and sICAM-1, respectively.

Although studies performed on cultured human endothelial cells have revealed a close correlation between the production of sICAM-1 and the expression of mICAM-1 on monolayers activated by either TNF-α, IL-1, or endotoxin,[35] comparable efforts to establish such a correlation has met with limited success in studies conducted *in vivo*. For example, it was reported that there is no correlation between mICAM-1 density, assessed by immunohistochemistry on mucosal biopsies, and plasma sICAM-1 in patients with inflammatory bowel disease, despite an apparent positive correlation between disease activity and sICAM-1 levels.[15] Jaeschke and co-workers[34] have recently shown that endotoxin elicits significant increases in both plasma sICAM-1 and hepatic mICAM-1, assessed by an immunohistochemical staining score. However, the rise in sICAM-1 (initially occurring at 3 hours) significantly preceded the increase in mICAM-1. Although these studies raise some question about the nature of the relationship between sICAM-1 and mICAM-1 *in vivo*, interpretation of these findings is rendered difficult due to uncertainties about the quantitative resolution of immunohistochemical techniques for detection of mICAM-1.[9] Our study provides objective quantitative data that lends support to the view that sICAM-1 does not accurately reflect the density of mICAM-1 (and presumably the state of endothelial cell activation) in the vasculature. We found that the kinetics of TNF-α-induced increases in sICAM-1 appeared to be dissociated from the changes in mICAM-1 noted in different vascular beds. For example, at 24 hours after TNF stimulation, mICAM-1 levels remain elevated above the level noted at 5 hours; however, sICAM-1 levels were clearly falling at the later time point. Furthermore, we were unable to demonstrate a clear correlation between sICAM-1 and mICAM-1 under TNF-α-stimulated conditions in ICAM-1-deficient mice (Table 1), after splenectomy (Figure 3) or after pretreatment with cycloheximide (Figure 4). These observations clearly indicate that sICAM-1 is not a reliable predictor of mICAM-1 expression on endothelial cells.

The cellular processes that account for the generation of plasma sICAM-1 remain undefined. Two mechanisms are frequently invoked to explain the existence of sICAM-1 in plasma: 1) differential splicing of ICAM-1 mRNA[36] and 2) proteolytic cleavage of the membrane-bound form of ICAM-1.[33] The former mechanism is supported by studies that demonstrate a distinct mRNA encoding sICAM-1 in different human cell lines[36] and the existence, in wild-type and ICAM-1-deficient mice, of three different alternatively spliced isoforms of ICAM-1 that are missing the complete domains of the adhesion molecule.[23] Our study provides some data that tend to support this mechanism. First, the dissociated kinetics of appearance of plasma sICAM and endothelial cell mICAM-1 could be explained by temporal differences in the synthesis (and membrane translocation) of mICAM-1 and the synthesis (and secretion) of a spliced variant of ICAM-1 (sICAM-1). Second, our finding that TNF-stimulated sICAM-1 levels are more profoundly influenced by cycloheximide treatment than mICAM-1 expression in different vascular beds would suggest that sICAM-1 generation might not be linked to the synthesis of mICAM-1. Third, our measurements of sICAM-1 and mICAM-1 in wild-type and ICAM-1-deficient mice indicate that, despite the absence of mICAM-1 in most organs, the plasma level of sICAM-1 in ICAM-1 deficient mice remains between 25 and 30% of the wild-type value. As significant shedding of mICAM-1 appears unlikely in the ICAM-1-deficient mice, the latter observation would suggest that the spliced variants of ICAM-1 could account for one-fourth to one-third of the sICAM-1 detected in plasma of unstimulated wild-type mice.

The general assumption that sICAM-1 is derived from the proteolytic cleavage of mICAM-1 is largely

based on the observation that sICAM-1 contains most of the structure (and some function) of the extracellular portion of mICAM-1[20] and that sICAM-1 production appears to be correlated with mICAM-1 expression on cultured human endothelial cells.[35] Support for a shedding component to sICAM-1 production is provided by a recent report that demonstrated an attenuated release of sICAM-1 from cultured human keratinocytes pretreated with the protease inhibitor iodoacetamide.[33] Based on this observation, we examined whether iodoacetamide affects TNF-α-induced increases in mICAM-1 and plasma sICAM-1 in wild-type mice. We observed a partial (30%) attenuation of the cytokine-induced increase in sICAM-1 (Figure 5) in iodoacetamide-treated animals, although this effect was not accompanied by an increased mICAM-1 in any vascular bed as might be expected if iodoacetamide were interfering with mICAM-1 shedding. Such a reciprocal relationship between surface expression of a shed adhesion molecule and its soluble (shed) form has been demonstrated for L-selectin.[37] Hence, the responses to iodoacetamide cannot be clearly interpreted at the present time, and the possibility that shedding of mICAM-1 accounts for some of the increment in plasma sICAM-1 noted in cytokine challenged animals cannot be discounted.

Irrespective of whether sICAM-1 results from shedding of mICAM-1, differential splicing of ICAM-1 mRNA, or both processes, the responses observed in splenectomized mice would suggest that this organ contributes significantly to plasma sICAM-1. Our results indicate that splenectomy reduces the TNF-induced increase in plasma sICAM-1 levels by approximately 25% in both wild-type and ICAM-1-deficient mice. This contribution of the spleen to circulating sICAM-1 is supported by our demonstration of the existence of substantial mICAM-1 in the spleen of ICAM-1-deficient mice (Table 1), which is consistent with a recent report showing that the spleen of ICAM-1-deficient mice immunostains intensely for the alternatively spliced isoforms of ICAM-1 described above.[23] Our observation that the spleen is a major source of sICAM-1 raises the possibility that the accumulation of anti-ICAM-1 MAb (YN1) in this organ may not accurately reflect mICAM-1 expression on splenic vascular endothelium. It is possible that the MAb YN-1 binds to sICAM-1 that is trapped in the spleen, either as free sICAM-1 or as an immune complex (YN-1 bound to sICAM-1) engulfed by the reticuloendothelial system.[10]

There is some evidence in the literature that invokes an anti-inflammatory role for soluble forms of endothelial cell adhesion molecules. This anti-inflammatory action of soluble adhesion molecules is generally attributed to competition, with their membrane-bound counterparts, for relevant counter-receptors expressed on activated leukocytes.[38–40] sICAM, for example, has been shown to dose-dependently inhibit the adhesion of lymphocytes to cerebral endothelial cells.[39,40] Although our study does not directly address the anti-inflammatory role of sICAM, the time-dependent, TNF-induced changes in plasma sICAM-1 relative to lung MPO may bear on this issue. We observed that the TNF-induced pulmonary leukosequestration, which is blunted in ICAM-1-deficient mice, rapidly dissipates at a time when sICAM-1 is rapidly rising to achieve peak levels in the plasma. As these changes are occurring at a time when lung mICAM-1 is rising, it is tempting to speculate that the elevated sICAM-1 may be one of the contributors to the rapid demargination of leukocytes from the lung that is observed within 3 to 5 hours after TNF administration.

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