

Increased Expression of Endothelial Cell Adhesion Molecules Due to Mediator Release from Human Foreskin Mast Cells Stimulated by Autoantibodies in Chronic Urticaria Sera

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Histamine-releasing antibodies that act against the epitope of the α chain of Fc ϵ RI (anti-Fc ϵ RI α antibody) that may affect pathogenesis in serum of patients with chronic urticaria. We assessed the capability of anti-Fc ϵ RI α antibody in sera from patients with chronic urticaria to release histamine and cytokines, and to induce the expression of endothelial cell adhesion molecules. We also assessed the release of inflammatory mediators from cultured foreskin mast cells, and expression of endothelial cell adhesion molecules on human dermal microvascular endothelial cells. Cells were pretreated with mast cell-conditioned media: culture media of mast cells treated with sera from chronic urticaria patients containing anti-Fc ϵ RI α antibody. Histamine release from human foreskin mast cells challenged with sera, increased after both 20 min and 16 h intervals. Leukotriene D₄ release also increased at both 20 min and 16 h. Tumor necrosis factor- α increased significantly in foreskin mast cell culture challenged with

sera of chronic urticaria patients. After the stimulation of human dermal microvascular endothelial cells with the conditioned media, the expression of intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin increased significantly. Treatment of the conditioned media with anti-tumor necrosis factor- α monoclonal antibody partially inhibited the expression of intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin. The data suggest that sera from patients with chronic urticaria containing anti-Fc ϵ RI α antibody release mediators and tumor necrosis factor- α by activating human foreskin mast cells. This release can play a pathogenic role in chronic urticaria by activating endothelial cells, in part due to the actions of tumor necrosis factor- α from mast cells. *Key words: anti-Fc ϵ RI α antibody/cell adhesion molecules/chronic urticaria/endothelial cells/mast cells. J Invest Dermatol 118:658–663, 2002*

Urticaria is a common complaint that affects as much as 2% of the population. It is often transient but can be chronic, and there is no identifiable exogenous allergen in most cases of chronic urticaria (CU; Champion, 1990). Intradermal injection of autologous serum elicits an immediate weal and flare response and mast cell degranulation in the majority of patients with severe CU (Grattan *et al*, 1986; 1990). Hide *et al* (1993) reported data that implicate an autoimmune mechanism (anti-IgE antibody) as the precipitating event in CU and Gruber *et al* (1988) noted that IgM anti-IgE antibodies are found in cold urticaria, which suggests a pathogenetic role for IgG.

More recent studies have suggested that there are autoantibodies in the serum of patients with CU that mediate histamine release from skin mast cells *in vitro* and *in vivo* (Grattan *et al*, 1991; Hide *et al*, 1993; Fiebiger *et al*, 1995; Niimi *et al*, 1996; Zweiman *et al*, 1996). These results suggest that anti-Fc ϵ RI α antibody is relevant to the pathogenesis of severe CU (Sabroe and Greaves, 1997; Tong *et al*, 1997; Ferrer *et al*, 1998, 1999). Grattan *et al* (1991) first described histamine releasing autoantibodies in chronic idiopathic urticaria. They suggested that histamine releasing autoantibodies are important in the pathogenesis of CU by stimulating or facilitating degranulation of basophils and cutaneous mast cells through cross-linking cell surface IgE receptors. Zweiman *et al* (1996) reported that serum histamine releasing activity from patients with CU appears in an IgG-containing fraction of the serum, which may contain IgE in some cases. Tong *et al* (1997) noted that a large fraction of patients with CU have antibodies directed to functional Fc ϵ RI α and suggested that CU may be autoimmune in origin. It has also been reported that histamine release from mast cells in CU is complement dependent (Ferrer *et al*, 1999).

Therefore, mast cells are considered to be the primary effector cells in CU by releasing a variety of inflammatory mediators, such as leukotriene, tryptase, prostaglandins, histamine, interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)- α (Walsh *et al*,

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Abbreviations: CU, chronic urticaria; HDMEC, human dermal microvascular endothelial cells; VCAM-1, vascular cell adhesion molecules 1.

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1991; Bradding *et al*, 1992; Moller *et al*, 1993). Mast cells are frequently found in close proximity to blood vessels. Release of mast cell mediators can cause inflammation, as well as accumulation and activation of other cells, including endothelial cells and leukocytes.

The principal histologic finding in most cases of CU is dermal edema. There is a variable cellular infiltrate around vessels, in which neutrophils and eosinophils predominate in the early lesion; in the advanced lesion lymphocytes usually predominate and are accompanied by neutrophils and eosinophils (Jones *et al*, 1983; Charlesworth *et al*, 1989; Frew and Kay, 1990; Thiers, 1996; Sabroe *et al*, 1999). The migration of leukocytes from the blood into the sites of tissue inflammation is an essential process; inflammation with the adhesion of leukocytes to cell adhesion molecules, such as the intercellular cell adhesion molecules-1 (ICAM-1), vascular cell adhesion molecules-1 (VCAM-1), and E-selectin, which are regulated by various cytokines (Jones *et al*, 1983; Osborn *et al*, 1989; Stoolman, 1989; Springer, 1990; Shimizu *et al*, 1991).

In this study, we purified human foreskin mast cells and assessed the capability of anti-FcεRIα antibody in sera from patients with CU to promote histamine and cytokine secretion, from the mast cells. Purified human foreskin mast cells were challenged and cultured with sera that showed positivity to an anti-FcεRIα antibody detection test. Furthermore, we examined the effects of sera of CU patients containing anti-FcεRIα antibody and mast cell-conditioned media pretreated with anti-FcεRIα antibody, on the expression of endothelial cell adhesion molecules by enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Subjects Sera were obtained from 21 normal, healthy volunteers (11 men and 10 women; mean age = 35; range = 20–52) and from 41 patients with CU (24 men and 17 women; mean age = 33; range = 22–49). Weals in CU patients occurred at least twice a week, disappeared within 24 h and lasted for more than 2 mo. Patients with clinical evidence of urticarial vasculitis or physical urticaria, including cholinergic urticaria, dermatographism, and delayed pressure urticaria were excluded from the study. The diagnosis for delayed pressure urticaria can be made from carefully examining the patient's medical history and confirmed according to the method described by Barlow *et al* (1993). Anti-histamine treatment was stopped at least 48 h and systemic corticosteroids or immunosuppressive drugs at least 1 wk before serum collection. Sera were collected and stored at –70°C until used.

Production of human recombinant soluble FcεRIα The gene segment encoding the extracellular portion of human FcεRIα was provided by Dr M.H. Jouvin (Harvard Medical School, Boston, MA). In order to generate the soluble form of human FcεRIα, the gene encoding the extracellular portion of human FcεRIα was subcloned into pMT/V5-His vector (pMT/V5-His-hFcεRIα) under control of metallothionein promoter (Invitrogen, San Diego, CA). The sequence of the plasmid was confirmed by automatic sequencing. A *Drosophila* Schneider cell line stably expressing soluble human FcεRIα was generated by transfection with pMT/V5-His-hFcεRIα together with pCoHYGRO containing the *Escherichia coli* hygromycin B-phosphotransferase gene under control of the *Drosophila copia* promoter (Invitrogen). Transfection was performed according to standard CaPO₄ protocols (Di Nocera and David, 1983), and transfected cells were selected with hygromycin (300 μg per ml) for 6 wk. Expression was induced in cells by addition of CaPO₄ to the culture medium at a final concentration of 500 μM for 36 h before use. The soluble FcεRIα was obtained from the supernatants of stable transfected insect cells.

ELISA for anti-FcεRIα antibody Sera were screened for the presence of anti-FcεRIα antibody using ELISA. A volume of 50 μl of 2 μg soluble FcεRIα per ml, diluted with carbonate buffer (pH 9.6), was plated in 96-well flat-bottom plates and incubated overnight at 4°C. A total of 100 μl of sera diluted 1 : 200 in Hank's balanced salt solution with divalent cations (Irvine Scientific, Santa Ana, CA) and 1% bovine serum albumin (Sigma, St Louis, MO) were added to each well and the plates were incubated at 37°C for 1 h. After washing, 100 μl of peroxidase-conjugated goat anti-human IgG (Sigma) or IgM (Sigma) diluted 1:1000 by Hank's balanced salt solution with divalent cations and

5% newborn calf serum (GibcoBRL, Gaithersburg, MD), was added to each well and plates were incubated for 1 h. The plates were washed again, and the binding of antibody was quantified colorimetrically by the addition of tetramethylbenzidine (1 mg per ml; Sigma). One milliliter of a 100 mg per ml stock solution (tetramethylbenzidine in acetone) was added to 100 ml of distilled water. Ten microliters of 30% H₂O₂ was added immediately prior to use. The chromogenic reaction was stopped with 25 μl 8 M H₂SO₄ and the plates were read spectrophotometrically at 450 nm on an ELISA reader (Dynatech Laboratories, Alexandria, VA). All were performed in triplicate.

Isolation of human newborn foreskin mast cells Samples of human foreskin obtained from infants (1–5 d) were placed in RPMI-1640 medium immediately after surgical excision. Using a chopper, the skin was finely diced into 3 mm fragments and subsequently washed with Tyrod's buffer (pH 7.4) containing 137 nM NaCl, 0.36 nM NaH₂PO₄, 2.6 nM KCl, 1.0 nM CaCl₂, 1.5 nM MgCl₂, 119 nM NaHCO₃, 5.5 nM glucose, and 1.0 g gelatin per l (TGCM buffer). The method used for the digestion of skin fragments was modified from that described by Benyon *et al* (1986) in which chopped foreskin was added to an enzyme buffer containing collagenase (0.25 mg per ml, type I), hyaluronidase (0.08 mg per ml, type I), and DNase (0.16 mg per ml). The tissue was incubated in enzyme buffer (1 g tissue per 10 ml) for 20, 20, and 30 min, respectively. The dispersed mast cells were harvested by filtration through nylon mesh and washed with erythrocyte-lysing buffer (0.1 mol per l NH₄Cl, 0.001 mol per l KH₂CO₃, and 2 × 10⁻⁶ mol per l ethylenediamine tetraacetic acid) and TGCM buffer. The dispersed mast cells were separated using a rough Percoll gradient (density 1.0410), quantified, and assessed for purity by alcian and trypan blue staining. The purity range of partially purified mast cells was 25–60%.

Human dermal microvascular endothelial cells (HDMEC) culture HDMEC were isolated from foreskins by trypsinization and Percoll gradient centrifugation as described previously (Lee *et al*, 1992; Swerlick *et al*, 1992a, 1992b). Cells were cultured in endothelial basal media (Clonetics, San Diego, CA) with 5 ng epidermal growth factor per ml (Clonetics), 1 μg hydrocortisone acetate per ml (Sigma), 5 × 10⁻⁵ M dibutyryl cyclic adenosine monophosphate (Sigma), 100 U per ml penicillin, 100 μg streptomycin per ml, 250 μg amphotericin B per ml (Sigma), and 30% human serum (Irvine). The resulting cell culture was free of contaminating fibroblasts as assessed by morphologic and immunologic criteria. Experiments were conducted with HDMEC at passages 2–6.

Mediator release and cytokine production from human foreskin mast cells The partially purified skin mast cells (2 × 10⁵ cells) in TGCM (1 ml) designated for mediator measurement were incubated with the sera of CU patients (100 μl) at 37°C for 20 min, or 16 h, in a CO₂ incubator. Mast cells for cytokine production were incubated in a CO₂ incubator for 20 min, or 16 h, in both the presence and absence of HDMEC. Reactions were terminated in an ice bath and samples were centrifuged at 4°C. The supernatants were assayed for the presence of histamine, leukotrienes, and cytokines. Because they decompose readily in air, 0.1% gelatin (final concentration) was added to those supernatants designated for measurement of leukotrienes.

Histamine assay Histamine was analyzed using the automated fluorometric method with a dialyzer (Astoria Analyzer Series 300, Astoria-Pacific International, Clackamas, ON) as described by Siraganian (1974). The limit of detection of the assay was approximately 1–5 ng of histamine per ml, and the amount of histamine released was expressed as a percentage change of the total histamine present in the unstimulated cells.

Leukotriene determination by radioimmunoassay The leukotriene content in each of the cell supernatants was determined by radioimmunoassay as described previously (Aharony *et al*, 1983). The leukotriene antibody was diluted in buffered saline (5 mM MES, HEPES adjusted to pH 7.4 with 1 M NaOH) containing 0.1% gelatin. Each assay tube contained 100 μl of sample supernatant, antibody (50 μl of a 1:1000 dilution), and 50 μl of [³H]leukotriene D₄ (LTD₄; 2500–3000 cpm) in buffered saline. Incubations were for 2 h at 4°C, and the reaction was terminated by addition of 0.5 ml dextran-coated charcoal (200 mg charcoal and 20 mg dextran mixed with 100 ml buffered saline). Five minutes after incubation the mixture was centrifuged at 800 × g and 4°C. A total of 0.4 ml of the supernatant was added to Aquasol (NEN Life Science Products, Boston, MA) and counted using a liquid scintillation spectrometer (Packard, model 3225). Standard curves were constructed in the presence of antigen using LTD₄. The detection

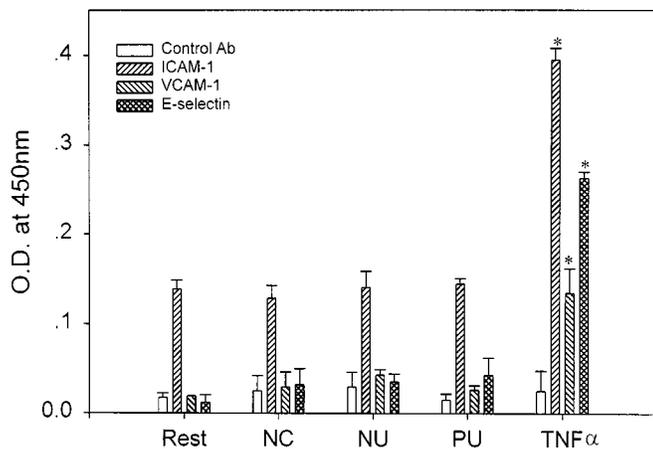


Figure 1. Regulation of the expression of endothelial adhesion molecules by pretreatment with the sera of patients with CU. After 16 h of incubation with normal control sera (NC), anti-FcεRIα antibody-negative sera of CU patients (NU), anti-FcεRIα antibody-positive sera of CU patients (PU), and TNF-α, the expression of adhesion molecules was assessed by ELISA. Results are presented as mean ± SD. All data points were performed in triplicate. Results shown are representative of those found in more than 10 separate experiments and with nine different sera.

limit of the assay was 0.045 pmol. LTD₄ release was expressed as pmol per 2×10^6 cells.

ELISA for detection of adhesion molecule expression HDMEC were plated in 96-well flat-bottomed microtiter plates and allowed to grow to confluence over 24 h at a concentration of 4×10^4 cells per well. HDMEC were incubated with sera from anti-FcεRIα antibody-negative controls, anti-FcεRIα antibody-negative CU patients, or anti-FcεRIα antibody-positive CU patients. A total of 100 μl of anti-ICAM-1 antibody (84H10, Immunotech Inc., Westbrook, ME), anti-VCAM-1 antibody (51-10C9, Pharmingen, San Diego, CA), or anti-E-selectin antibody (1.2B6, Immunotech) was added to each well, and the plates were then incubated at 37°C for 1 h. After washing, 100 μl of peroxidase-conjugated goat anti-mouse IgG (Sigma), diluted 1:500, was added to each well and the plates were incubated for an additional 1 h. The plates were washed again, and the binding of antibody was quantified colorimetrically by the addition of tetramethylbenzidine (1 mg per ml; Sigma). One millimeter of a 100 mg per ml stock solution (tetramethylbenzidine in acetone) was added to 100 ml of distilled water. Ten microliters of 30% H₂O₂ was added immediately prior to use. The chromogenic reaction was stopped with 25 μl 8 M H₂SO₄ and the plates were read spectrophotometrically at 450 nm on an ELISA reader. Controls included incubation with an irrelevant isotype-matched mouse monoclonal antibody and omission of the primary antibody (blanks). The results are expressed as optical density after subtraction of blank values.

Statistical analysis Mean values and SEM, were calculated for all experiments. Data were analyzed using the Wilcoxon signed rank test or repeated measures of analysis of variance. *p*-values of less than 0.05 were considered significant.

RESULTS

Detection of anti-FcεRIα antibody, expression of adhesion molecules on HDMEC, and isolation of human foreskin mast cells When values were higher than the mean optical density of the normal controls plus three times the standard deviation, the optical density was considered reactive. Fourteen (34%) of 41 sera samples from CU patients had reactivity to anti-FcεRIα antibody.

The expression of adhesion molecules on HDMEC after stimulation with the anti-FcεRIα antibody-positive sera of CU patients is shown in **Fig 1**. The expression of ICAM-1, VCAM-1, and E-selectin on HDMEC were not altered by pretreatment with the anti-FcεRIα antibody-positive sera of patients with CU at any

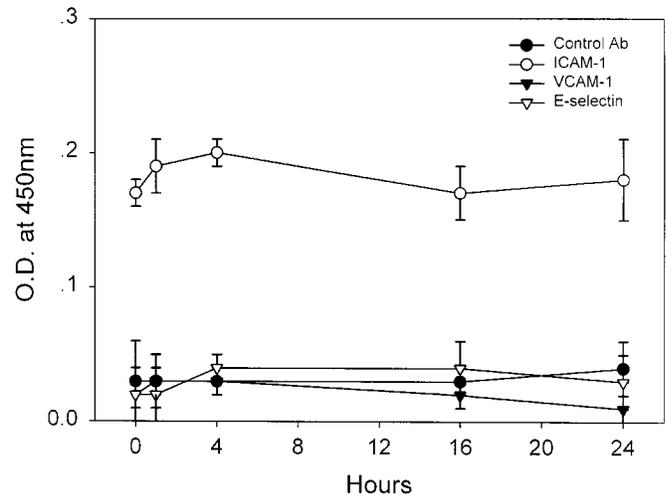


Figure 2. Time course of the induction of the expression of endothelial adhesion molecules in response to anti-FcεRIα antibody-positive sera of CU patients. Following pretreatment with the anti-FcεRIα antibody-positive sera of CU patients, the expression of endothelial adhesion molecules was assessed by ELISA. After 1, 4, 16, and 24 h of incubation. Results are presented as mean ± SD. Results are presented as mean ± SD. All data points were performed in triplicate. Results shown are representative of three separate experiments and with two different sera.

time during incubation (**Fig 2**). The culture of HDMEC with unstimulated control, normal healthy control sera, and anti-FcεRIα antibody-negative sera also failed to express ICAM-1, VCAM-1, and E-selectin; however, incubation with TNF-α used as positive control, increased or induced the expression of ICAM-1, VCAM-1, and E-selectin significantly (**Fig 1**).

The number of human foreskin mast cells obtained by enzyme digestion and rough Percoll gradient was 2×10^5 per g of tissue. Purity ranged from 25% to 60% (**Table I**).

Effects of CU sera on mediator release, and production of cytokines, in foreskin mast cells Spontaneous release of mast cells (2×10^5) after 20 min, and 16 h incubation periods at 37°C was $10.0 \pm 1.05\%$ and $13.5 \pm 0.90\%$, respectively (**Table I**). Human foreskin mast cells challenged by sera (100 μl) containing anti-FcεRIα antibody for periods of either 20 min or 16 h increased histamine secretion markedly ($25.3 \pm 1.72\%$, $45.7 \pm 2.68\%$) compared with the spontaneous rates of release (**Table I**). Human foreskin mast cells challenged by 100 μl, 200 μl, or 300 μl of anti-FcεRIα antibody-positive sera for 20 min also showed LTD₄ release (1.09 ± 0.07 pmol per 10^6 cells, 1.30 ± 0.03 pmol per 10^6 cells, and 1.08 ± 0.02 pmol per 10^6 cells, respectively), rather than spontaneous release (0.16 ± 0.06 pmol per 10^6 cells). LTD₄ release for the 16 h treatment increased from 0.22 ± 0.06 to 2.09 ± 0.19 pmol per 10^6 cells (**Table I**). The amount of LTD₄ released was very low compared with guinea pig lung mast cells activated with the ovalbumin/anti-ovalbumin antibody reaction (87.5 ± 5.79 pmol per 10^6 cells). The amount of LTD₄ released by the high concentration (300 μl) in CU patients' sera was less than released by the low concentration (100 μl) of CU patients' sera. Human foreskin mast cells incubated with the sera of CU patients for 16 h increased TNF-α production (376.7 ± 67.45 pg per 2×10^5 cells), but did not increase IL-13, compared with spontaneous release (203.6 ± 24.93 pg per 2×10^5 cells; **Table I**). TNF-α and IL-13 produced from foreskin mast cells 20 min after incubation were not detected by ELISA (data not shown).

Human foreskin mast cells challenged with the sera (100 μl) from anti-FcεRIα-negative CU patients or normal sera for 20 min or 16 h slightly increased histamine release, LTD₄, and TNF-α

Table I. Effects of sera from CU patients on the mediator release and production of cytokines in mast cells isolated from human newborn foreskins^a

Incubation time	Histamine release (%)		LTD ₄ (pmol per 2 × 10 ⁶ cells)		TNFα (pg per 2 × 10 ⁵ cells)		IL-13 (pg per 2 × 10 ⁵ cells)
	20 min	16 h	20 min	16 h	20 min	16 h	20 min and 16 h
Spontaneous	10.0 ± 1.05	13.5 ± 0.90	0.16 ± 0.06	0.22 ± 0.06	ND	203.6 ± 24.93	ND
(+)Sera (100 μl) ^b	25.3 ± 1.72**	45.7 ± 2.68**	1.09 ± 0.07**	2.09 ± 0.19**	ND	376.7 ± 67.45**	ND
EC + SM	9.6 ± 1.39	11.7 ± 1.84	NE	0.25 ± 0.08	ND	202.8 ± 29.80	ND
EC + SM + Sera	26.5 ± 2.06**	39.3 ± 3.79**	1.49 ± 0.20**	1.98 ± 0.74**	ND	385.9 ± 70.29**	ND
(-)Sera (100 μl) ^c	13.8 ± 2.74	16.0 ± 2.38	NE	0.44 ± 0.17	ND	244.4 ± 29.14	ND
Normal sera (100 μl)	13.9 ± 1.76	15.6 ± 2.35	NE	0.21 ± 0.09	ND	231.5 ± 22.00	ND

^aMast cells (2 × 10⁵ cells) from newborn foreskins were isolated using various enzymes (0.25 mg collagenase per ml, 0.08 mg hyaluronidase per ml, and 0.16 mg DNase per ml) using the Percoll gradient technique, and challenged with sera from CU patients for 20 min or 16 h. The values of EC alone did not detect in all kinds of experiments.

^bSera from anti-FcεRIα-positive CU patients.

^cSera from anti-FcεRIα-negative CU patients. ND, no detection; EC, endothelial cells; SM, skin mast cells; NE, no experiment. Each value except TNF-α (n = 5), all EC + SM, all (-) sera, and all normal sera (n = 4) represents the mean ± SEM (n = 6).

**p < 0.001 compared with spontaneous release.

production in mast cells, but this increase was not statistically significant, compared with the spontaneous rates of release (Table I); however, one sample of anti-FcεRIα antibody-negative sera (100 μl; n = 4) remarkably increased the production of TNF-α (329.8 pg per 2 × 10⁵ cells). This value was similar to anti-FcεRIα antibody-positive sera.

Effects of CU sera on mediator release and the production of cytokines in foreskin mast cells cocultured with HDMEC When foreskin mast cells were cocultured with HDMEC and stimulated with the anti-FcεRIα antibody-positive sera of CU patients for 16 h, the release of histamine (39.3 ± 3.79%), and production of LTD₄ (1.98 ± 0.74 pmol per 10⁶ cells) and TNF-α (385.9 ± 70.29 pg per 2 × 10⁵ cells) did not increase significantly, compared with mast cells stimulated only by patients' sera (45.7 ± 2.68%, 2.09 ± 0.19 pmol per 10⁶ cells, 376.7 ± 67.45 pg per 2 × 10⁵ cells, respectively) (Table I). Histamine release and LTD₄ production were not increased in the mast cells cocultured with HDMEC stimulated with anti-FcεRIα-positive sera of CU patients for 20 min compared with mast cells stimulated only by patients' sera. The lack of production of IL-13 from the mast cells challenged with the sera of CU patients may be due to the small number of mast cells used.

The mast cells cocultured with HDMEC stimulated with the anti-FcεRIα-negative sera or normal sera did not significantly increase histamine release, LTD₄, and TNF-α production (data not shown).

Effects of cultured supernatants from anti-FcεRIα antibody-positive sera-treated mast cells on the expression of adhesion molecules Direct transfer of culture supernatants from anti-FcεRIα antibody-positive sera-treated mast cells (conditioned media) to HDMEC enhanced the expression of ICAM-1, VCAM-1, and E-selectin after 4 h (Fig 3), but culture supernatants from normal human sera or anti-FcεRIα antibody-negative sera did not affect expression (data not shown). After 16 h of stimulation with media conditioned to HDMEC, enhanced expression of ICAM-1 and VCAM-1 persisted, whereas E-selectin expression returned to basal levels (Fig 4).

Effects of blocking antibodies to TNF-α on the expression of adhesion molecules by conditioned media The potential contribution of mast cell-generated TNF-α on the expression of adhesion molecules was examined with blocking antibodies. HDMEC were treated for 4–16 h with conditioned media in the presence or absence of antibody, and the expression of adhesion molecules was then measured. As shown in Fig 5, antibodies to TNF-α inhibited enhanced ICAM-1, VCAM-1, and E-selectin expression. This pattern of inhibition was reproduced in three

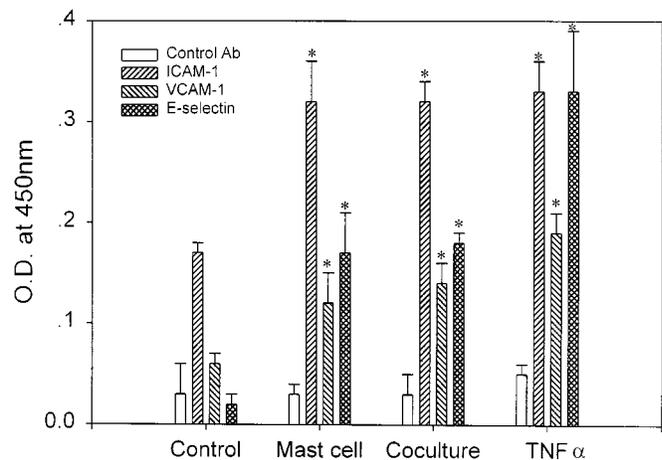


Figure 3. Effect of conditioned medium from anti-FcεRIα antibody-positive sera-treated mast cells on the induction of the expression of endothelial adhesion molecules. HDMEC were treated with culture supernatant from culture media only (control), anti-FcεRIα antibody-positive sera-treated mast cells (mast cell), culture supernatant from anti-FcεRIα antibody-positive sera-treated mast cells-endothelial cell coculture (coculture), and TNF-α for 4 h. The expression of endothelial adhesion molecules was assessed by ELISA. Results are presented as mean ± SD. All data points were performed in triplicate. Results shown are representative of six separate experiments and with five different sera. *p < 0.05 vs untreated HDMEC.

additional experiments. To know whether the anti-human IgG antibody in some of the sera classified as having a FcεRI antibody might induce the expression of endothelial cell adhesion molecules directly and to determine whether coculture supernatants may have synergistic effects for the expression of cell adhesion molecules, we performed a coculture supernatant study. After the stimulation of HDMEC with mixed culture supernatants of mast cells and HDMEC stimulated with the anti-FcεRIα antibody-positive sera of CU patients, ICAM-1 and VCAM-1 expression were enhanced at 4 h and 16 h. E-selectin expression was induced at 4 h, but at not at 16 h; however, this expression on HDMEC did not increase significantly after pretreating with the mixed culture supernatants of mast cells and endothelial cells treated with anti-FcεRIα antibody-positive sera of CU patients, compared with pretreatment of conditioned media of mast cells treated with those sera.

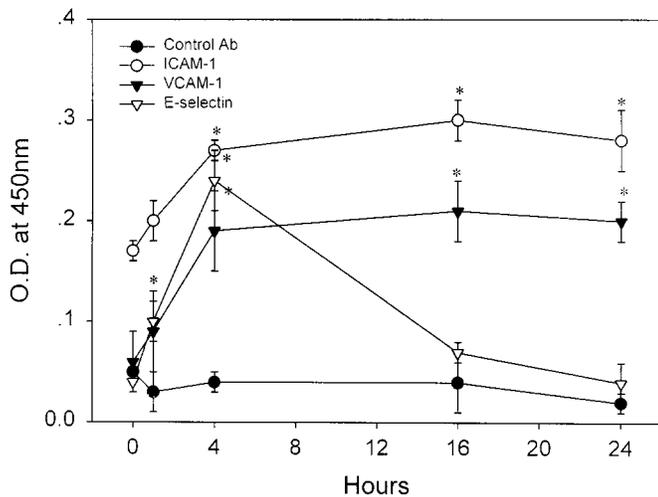


Figure 4. Time course of the induction of the expression of endothelial adhesion molecules in response to culture supernatants from anti-Fc ϵ RI α antibody-positive sera-treated mast cells. Following the pretreatment of culture supernatants from anti-Fc ϵ RI α antibody-positive sera-treated mast cells, the expression of endothelial adhesion molecules was assessed by ELISA after 1, 4, 16, and 24 h of incubation. Results are presented as mean \pm SD. All data points were performed in triplicate. Results shown are representative of three separate experiments and two different sera. * $p < 0.05$ vs untreated HDMEC.

DISCUSSION

The purity (25–60%) of human foreskin mast cells obtained using the rough Percoll gradient method was higher than has been reported for dispersed skin mast cells (8–25%; Benyon *et al*, 1986; **Table I**); we observed by microscopy that the morphology of skin mast cells had not changed (data not shown). The yield obtained was similar to that of other workers (2×10^5 cells per g tissue), and therefore, the Percoll gradient method would seem to be satisfactory for the purification of skin mast cells. The amount of histamine released was higher than reported by other laboratories (Benyon *et al*, 1986; Zweiman *et al*, 1996), and similar to that of another report (Grattan *et al*, 1991). Although the amount of LTD $_4$ released from skin mast cells activated with CU patients' sera was very low, this release has not been reported previously.

It is well known that mast cells prepackage TNF- α in granules, and store it for release (Walsh *et al*, 1991). TNF- α was detected in mast cells stimulated with the sera of CU patients for 16 h. This result suggests that TNF- α may be released immediately after synthesis in preference to the release of preformed TNF- α .

Pretreatment of HDMEC *in vitro* with conditioned media led to an increase in the expression of ICAM-1, VCAM-1, and E-selectin, whereas this effect was not found with culture supernatants of anti-Fc ϵ RI α antibody-negative CU serum-treated mast cells, CU serum with anti-Fc ϵ RI α antibody only, or normal human serum. These results suggest that this effect could be due to a soluble mediator released by anti-Fc ϵ RI α antibody-activated mast cells.

The expression of ICAM-1 and VCAM-1 on HDMEC can be increased by IL-1 α or TNF- α . The onset of increase occurs at 4–6 h after induction and reaches a maximum level at 16–24 h. In this study, the expression of ICAM-1 and VCAM-1 started to increase at 1 h and reached a peak at 16 h and their presence was evident at 24 h (Pober and Cotran, 1990; Swerlick *et al*, 1992a). In this study, the induction of E-selectin reached a peak at 4 h and then disappeared quickly. Thus stimulation of HDMEC by conditioned media causes an increase in the expression of endothelial cell adhesion molecules, similar to stimulation with IL-1 α or TNF- α . By using blocking antibodies and attempting to measure the TNF- α levels in

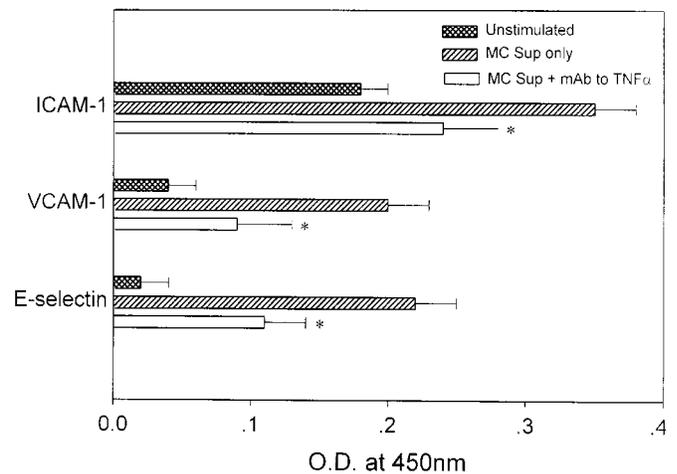


Figure 5. Effect of anti-TNF- α monoclonal antibody on the expression of endothelial adhesion molecules after pretreatment with culture supernatants from anti-Fc ϵ RI α antibody-positive sera-treated mast cells. HDMEC were treated at 16 h for ICAM-1 and VCAM-1 expression or at 4 h for E-selectin expression with media only (unstimulated) and culture supernatants (MC Sup), in the presence or absence of blocking antibodies to TNF- α . The expression of endothelial adhesion molecules was assessed by ELISA. Results are presented as mean \pm SD. All data points were performed in triplicate. Results shown are representative of three separate experiments and two different sera. * $p < 0.05$ vs MC Sup.

conditioned media, we were able to show that the action of anti-Fc ϵ RI α antibody could at least in part, be attributed to the production of TNF- α from mast cells and endothelial activation.

The expression of endothelial cell adhesion molecules (ICAM-1, VCAM-1, E-selectin) are induced by bacterial endotoxin or various cytokines (e.g., IL-1, IL-4, TNF- α , IL-13, histamine, IFN- γ). Thus, cell adhesion molecule expression can be induced by certain factors such as IL-1, IL-4, or IL-13 from mast cells, mononuclear cells, T lymphocytes, or the endothelial cell itself. In addition, previous studies have shown that some circulating antibodies can activate endothelial cells directly. Anti-endothelial cell antibody can play a pathogenic role in scleroderma by activating endothelial cells, in part due to the autocrine or paracrine actions of IL-1 (Carvalho *et al*, 1996). Also, anti-endothelial cell antibody can induce the expression of ICAM-1 on HDMEC in Behçet's disease (Lee *et al*, 1999). We suspect that the mediators (histamine, LTD $_4$, TNF- α , etc.) released from mast cells challenged with sera of CU patients stimulate the endothelial cells during coculture, and then endothelial cells may produce some cytokines. Therefore, we designed a coculture supernatant study to examine whether the anti-human IgG antibody in some of the sera classified as having a Fc ϵ RI antibody might induce the expression of endothelial cell adhesion molecules directly and to determine whether coculture supernatants may have synergistic effects for the expression of cell adhesion molecules. Our results showing no synergistic effects suggests that anti-Fc ϵ RI α antibody did not affect the activation of endothelial cells directly and there are no additive autocrine effects of endothelial cell cytokines produced by mast cell activation.

Although the exact pathogenesis of CU is still unknown, it seems that anti-Fc ϵ RI α antibody plays a part in CU by activating mast cells, TNF- α production, and endothelial cells and enhancing adhesion molecule expression. Activation of endothelial cells will facilitate leukocyte traffic and thus initiate inflammation.

Circulating functional autoantibodies to the high-affinity IgE receptor (Fc ϵ RI) as well as to IgE have been found in approximately one-third of patients with chronic idiopathic urticaria and anti-human IgG autoantibody might have picked up IgE/anti-IgE

complexes (as well as anti-Fc ϵ RI antibody) bound to the soluble α -subunit on the ELISA. In this study, we used sera that had not been preincubated with anti-IgE antibody or had not been treated with heat. Thus, some of the sera classified as having anti-Fc ϵ RI antibody might have had anti-IgE antibody. The possibility of histamine-releasing activity by some sera containing anti-IgE antibody cannot be excluded at the present time. Additional studies using a purified anti-Fc ϵ RI antibody will be needed.

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