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*J Immunol* 1999; 162:1648-1655;

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Vascular Endothelial Cell Expression of ICAM-1 and VCAM-1 at the Onset of Eliciting Contact Hypersensitivity in Mice: Evidence for a Dominant Role of TNF-α

Julie F. McHale, Olivier A. Harari, Diane Marshall, and Dorian O. Haskard

We have studied vascular endothelial activation and increased expression of ICAM-1 and VCAM-1 at the onset of the elicitation phase of oxazolone contact hypersensitivity in mice. By measuring the local uptake of i.v. administered radiolabeled anti-ICAM-1 and anti-VCAM-1 mAb, we found that endothelial ICAM-1 and VCAM-1 was increased by 4 h after challenge, 2 h later than the first peak of ear swelling and 125I-labeled human serum albumen uptake. Increased expression of endothelial ICAM-1 and VCAM-1 was significantly greater in sensitized animals than in naive animals. Anti-TNF-α antiserum significantly inhibited both the increase in ear thickness (p < 0.01), and the up-regulation of ICAM-1 and VCAM-1 expression (p < 0.01 for both) at 4 h. In contrast, the combination of anti-IL-1α and IL-1β had only a small inhibitory effect on ICAM-1 expression (p < 0.05) and no significant effect on increased ear thickness or on VCAM-1 expression. A mixture of anti-TNF-α, anti-IL-1α, and IL-1β was no more inhibitory for endothelial ICAM-1 and VCAM-1 expression than anti-TNF-α alone. ICAM-1 and VCAM-1 expression at 4 h was unaffected by a combination of mAb against α4 and β2 integrins, whereas expression at 24 h was significantly inhibited (p < 0.05), suggesting that the release of TNF-α and other cytokines involved in the initiation of the response may not require leukocyte traffic or other leukocyte functions involving these integrins. We conclude that the early up-regulation of endothelial ICAM-1 and VCAM-1 during the elicitation of contact hypersensitivity is primarily due to the immune-dependent local release of TNF-α. The Journal of Immunology, 1999, 162: 1648–1655.

The contact hypersensitivity (CS) response in mice is a model of clinical allergic contact dermatitis and also a widely employed model for investigating mechanisms of T lymphocyte-mediated inflammation (reviewed in Ref. 1). About 2 h after topical application of Ag to the ear of sensitized mice, there is marked edema due to mast cell degranulation and release of vasoactive mediators such as histamine and serotonin (2, 3). This is followed by leukocyte migration into the tissues, and a second larger peak of ear swelling occurring about 24 h, after which inflammation spontaneously subsides (3). There is evidence that local release of cytokines, including TNF-α, IL-1, and IFN-γ, is critical for the optimal generation of the CS reaction (4, 5).

Leukocyte adhesion to endothelium is the first step in their emigration into the tissues; therefore, it is of fundamental importance to the generation of inflammatory responses. Recently, there has been a major increase in our understanding of the mechanisms involved in leukocyte-endothelial cell interactions, with leukocytes undergoing a series of adhesion and activation events consisting of rolling, firm adhesion, and transmigration into the tissues (6, 7). ICAM-1 and VCAM-1 are members of the Ig superfamily and are expressed on endothelial cells in response to cytokines such as TNF and IL-1 that leads to the up-regulation or de novo induction of a variety of chemotactants and surface adhesion molecules including ICAM-1 and VCAM-1 (19). In view of the limitations of standard immunohistochemistry for quantifying endothelial cell Ag expression, we have previously developed in porcine models of inflammation techniques for quantifying endothelial cell luminal adhesion molecule expression in vivo by measuring the uptake of radiolabeled Abs, administered i.v. (20–23). A similar approach has since been used to study endothelial cell adhesion molecule expression in the mouse (24–27). In the present study, we have adapted the radiolabeled Ab targeting approach to
investigate the kinetics of expression of ICAM-1 and VCAM-1 in relation to edema during the early phase of the response to Ag challenge in CS and to explore the requirement for TNF-α and IL-1.

Materials and Methods

Animals

Female BALB/c mice were purchased from Harlan (Bicester, Oxon, U.K.) and were maintained according to U.K. Home Office regulations. Mice were studied at 6–10 wk of age.

Abs cytokines and cell lines

Recombinant murine (m) IL-1α and IL-1β were purchased from Peprotec (London, U.K.). Abs to polyclonal anti-mouse IL-1α and IL-1β and a gift were from Dr. Roberto Solari (Glaxo-Wellcome Laboratories, Stevenage, U.K.). rmTNF-α and sheep polyclonal anti-mouse TNF-α antiserum were a gift from Dr. Tony Meagher (National Institute for Biological Standards and Control, Potter’s Bar, U.K.). Control sheep serum was purchased from Dakopatts (Glostrup, Denmark).

The hybridoma lines for the rat anti-mouse mAb YN-1/1.7.4 (IgG2a, anti-ICAM-1), M/7.2.7 (IgG1, anti-VCAM-1), M18/2 (IgG2a, anti-β2 integrin (CD18)), and PS/2 (IgG2b, anti-α4 integrin (CD49d)) were obtained from the American Type Culture Collection (Manassas, VA). Irrelevant control mAbs (rat IgG1) and (rat IgG2a) were raised against DNP and were a gift from Dr. David Gray (Imperial College School of Medicine, Hammersmith Hospital, London, U.K.). Hybridomas were cultured in heat-inactivated 10% FCS (Life Technologies, Paisley, Scotland) in RPMI 1640 (Life Technologies) in 5% CO2 at 37°C. Abs were purified from tissue culture supernatants by protein G-affinity chromatography (Pharmacia, Uppsala, Sweden).

The murine endothelial cell line bEND-3 was kindly supplied by Dr. M. Robinson (Celltech Ltd, Slough, U.K.) and grown in DMEM with 10% FCS, 50 μg/ml penicillin (Life Technologies), 50 μg/ml streptomycin (Life Technologies), and 2 mM l-glutamine (ICN Biomedicals, Costa Mesa, CA) in 5% CO2 at 37°C. The monocyte-derived cell line J774 was obtained from Dakopatts (Glostrup, Denmark). Hybridomas were cultured in heat-inactivated 10% FCS (Life Technologies, Paisley, Scotland) in RPMI 1640 (Life Technologies) in 5% CO2 at 37°C. Abs were purified from tissue culture supernatants by protein G-affinity chromatography (Pharmacia, Uppsala, Sweden).

Contact sensitivity

Animals were sensitized 5 days before the experiment by the application of 1% 4-ethoxyethyl-2-phenyl-2-oxazolin-5-one (oxazolone) (Sigma, St. Louis, MO) in acetone/olive oil (4:1) (50 μl) onto the shaved flank. The CS response was subsequently elicited by applying 1% oxazolone in acetone/olive oil (10 μl) to the right ear, while the left ear was treated with acetone/olive oil alone. Ear thickness was measured using an engineer’s micrometer (RS232, Cedar, Sheffield, U.K.), and expressed as the absolute increase (Δ) in mm over baseline.

Ab radiolabeling

All radioisotopes were purchased from Amersham International (Amer sham, Little Chalfont, U.K.). Control anti-DNP mAb and human serum albumin (HSA) were labeled with125I using the Iodogen method (28). Anti-VCAM-1 was labeled with111In using a diethylenetriaminepentaacetic acid (DTPA)-coupling technique (29). Anti-ICAM-1 was labeled with99mTc using the reduction method (30). After each Ab labeling, the percentage of the total isotope bound to Ab was determined using instant thin layer chromatography (Gelman Sciences, Ann Arbor, MI). Typically greater than 95% of isotope was bound to Ab.

Targeting of radiolabeled mAb and HSA

Expression of ICAM-1 and VCAM-1 in vivo was assessed using i.v. administered radiolabeled mAb. Animals received an i.v. injection via the tail vein of a mixture (20 μg each) of99mTc-labeled anti-ICAM Ab (YN1),111In-labeled anti-VCAM Ab (M/7.2.7), and125I-labeled control IgG1 or IgG2a anti-DNP Ab 5 min before the end of each experiment. The IgG1 or IgG2a anti-DNP Abs gave identical results and were used interchangeably as the negative control, in view of the anti-VCAM-1 (IgG1) and anti-ICAM-1 (IgG2a) being of different isotypes. For each animal, syringes were weighed before and after injection to allow accurate determination of the injected dose (ID). Five minutes after injection, each animal was killed with an i.p. overdose of pentobarbitone sodium (Rhone Mereux, Harlow, Essex, U.K.). The thorax was then opened, and 20 ml PBS containing 10 μmol of heparin (Leo Laboratories, Prince Risborough, U.K.) perfused into the left ventricle via a 23-gauge needle. The perfusate was aspirated from an incision in the right atrium. At the end of each experiment the distal 9 mm of each ear was removed and weighed; the radioactivity was counted in an automated gamma-counter. A standard aliquot of the injected solution was also counted to allow calculation of ID. After corrections for background, spill over between isotopes and decay, the Ab uptake for each sample was expressed as percent of ID/100 mg of tissue. Localization of i.v. injected125I-HSA was assessed in the same way as uptake of radiolabeled mAb except that the counts were not corrected for weight of tissue.

Adhesion assay

The adhesion of J774 cells to bEND-3 cells was assayed using calcein-AM-labeled J774 cells. bEND-3 cells were plated at confluence in 96-well tissue culture plates and stimulated with 10 ng/ml rmTNF-α for 8 h. The J774 cells were harvested from flasks and activated by incubating with 1 ng/ml of 48-phorbol-12,13-dibutyrate (Sigma) for 30 min. They were then centrifuged at 1200 rpm, and resuspended in 3 μM calcein-AM (Sigma) in medium for 30 min at 37°C. After washing, calcein-labeled J774 cells were added to the bEND-3 cells at a density of 106 cells/well. After a 30 min incubation, the nonadherent J774 cells were removed by aspiration and washing, and fluorescent activity that remained on the plate was read in a Cytofluor plate reader (NEC Technologies, Boxborough, MA). A standard curve of J774 cell number was performed by preparing serial dilutions of the stock suspension and reading 100 μl samples on the same plate. The adhesion of J774 cells to bEND-3 cells in the presence of blocking Abs was expressed as a percentage, calculated from the formula: (fluorescence intensity in the presence of blocking Ab ÷ fluorescence intensity in the absence of Ab) × 100.

Immunohistochemistry

To localize the uptake in the ear of i.v. injected mAbs, ears were snap frozen, sectioned, and stained using a biodiylated rabbit anti-rat secondary Ab and streptavidin-biotin horse radish peroxidase, as previously described (23).

Cytokine-induced skin lesions

Cutaneous inflammatory lesions were induced by intradermal (i.d.) injections of rmTNF-α, rmIL-1α, or rmIL-1β, each given at 5 ng in 50 μl. Animals were anesthetized by intramuscular injection of a mixture of xylazine 1 mg/kg (Bayer, Wuppertal, Germany) and ketamine 0.5 mg/kg (Parke-Davis, Earligh, U.K.). The flanks were shaved and one i.d. injection was given on each side. At the end of the experiment, the dorsal skin was removed and 0.7 cm diameter discs were collected using a biopsy punch, including the entire injection site. Radioactivity localized in skin discs was then counted in a gamma-counter.

Statistics

Because the sample sizes in the experiments performed in this project were usually less than 10, a normal distribution was not assumed; therefore, comparisons were made using the Mann-Whitney U test.

Results

Differentially radiolabeled mAb were injected i.v. into BALB/c mice to measure the expression of ICAM-1 and VCAM-1 on the luminal surface of endothelial cells during the evolution of the CS response elicited by the application of 1% oxazolone to one ear of the sensitized mice. In each mouse, the uptake of mAb was expressed as a sensitized ear by the percentage of ID/100 mg that localized in the challenged ear by the percentage of ID/100 mg that localized in the uninflamed-control ear. As shown in Fig. 1, the LR of the125I-labeled irrelevant control mAb did not significantly deviate from 1 at any of the time points studied, indicating that any increase in uptake of anti-ICAM-1 and anti-VCAM-1 mAb was not due to nonspecific exudation of Ig. In contrast, both99mTc-labeled anti-ICAM-1 mAb and111In-labeled anti-VCAM-1 mAb showed significantly increased localization in the inflamed ear at 4 h (mean ± SD, LR 1.59 ± 0.13 and 1.55 ± 0.09 for anti-ICAM-1 and anti-VCAM-1 respectively; both p < 0.05 compared with baseline) and 8 h (mean ± SD LR 1.50 ± 0.1 and 1.55 ± 0.12 for anti-ICAM-1 and
anti-VCAM-1 respectively, both \( p < 0.05 \) compared with baseline) after challenge, suggesting increased tissue expression of both adhesion molecules at these early time points (Fig. 1A). By conducting further similar experiments in which uptake of radiolabeled mAb was measured at later time points after oxazolone challenge, we found evidence for a maintained increase in ICAM-1 and VCAM-1 expression up to 48 h, after which time expression declined (Fig. 1B).

To provide additional evidence that the increased uptake of the anti-ICAM-1 and anti-VCAM-1 mAb was due to the specific binding of mAb to adhesion molecule within the ear, we performed an experiment in which the radiolabeled mAb were injected in the presence of an excess of unlabeled mAb. This established that a 10-fold excess of unlabeled anti-ICAM-1 mAb could totally block the increased uptake into ears of the radiolabeled anti-ICAM-1 but not the uptake of radiolabeled anti-VCAM-1 mAb, while the converse was true for a 10-fold excess of unlabeled anti-VCAM-1 (Fig. 2). Similarly in the same experiment an excess of unlabeled nonbinding control Ab did not affect the increased uptake of either the radiolabeled anti-ICAM-1 or the radiolabeled anti-VCAM-1 (Fig. 2). To directly demonstrate that the specific uptake of mAb was due to binding by endothelial cells within the inflamed ears, we injected unlabeled anti-ICAM-1, anti-VCAM-1 mAb, or anti-DNP control mAb i.v. and detected mAb localization in unchallenged and oxazolone-challenged ears by immunohistochrometry, using biotinylated rabbit anti-rat Ig for detection. As shown in Fig. 3, in mice injected with anti-ICAM-1 or anti-VCAM-1 mAb, there was no immunohistochemical evidence of specific binding of rat Ig to cells other than endothelial cells, either in the intravascular or extravascular compartment. No endothelium-associated rat Ig was detected on examination of the oxazolone-challenged ear of a mouse injected with irrelevant control anti-DNP mAb, whereas rat Ig was clearly detectable, bound to endothelium in the ears of mice injected with anti-ICAM-1 or anti-VCAM-1 mAb. In unchallenged ears, there was no endothelial uptake of irrelevant control anti-DNP mAb or anti-VCAM-1 mAb, but there was a low level uptake of anti-ICAM-1, consistent with a degree of constitutive ICAM-1 expression.

We next performed parallel experiments looking at the time course of altered ear thickness and the uptake of 125I-labeled albumin, to relate the endothelial expression of ICAM-1 and VCAM-1 to existing measures of the CS response. A significant increase in ear thickness was seen 2 h after 1% oxazolone challenge (Fig. 4A). This fell slightly by 4 h (\( p < 0.05 \) compared with 2 h), increased again by 8 h (\( p < 0.05 \) compared with 4 h), and reached a plateau at 24–48 h. A similar time course was found for the uptake of 125I-labeled albumin (Fig. 4B), with an initial increase in uptake detectable at 2 h, a fall at 4 h (\( p < 0.05 \) compared with 2 h), followed by a secondary rise at 8 h (\( p < 0.05 \) compared with 4 h). Therefore, these data indicate that during the initial phase of the CS response the increased endothelial expression of ICAM-1 and VCAM-1 is preceded in time by an increase in ear thickness and in endothelial permeability to albumin, and that the subsequent kinetics of these changes are distinct from those of increased endothelial cell expression of ICAM-1 and VCAM-1.

Because agents such as oxazolone may have a direct proinflammatory effect unrelated to antigenicity, we compared the localization of the anti-ICAM-1 and anti-VCAM-1 Abs in the oxazolone-challenged ears of mice that had either been previously sensitized or had undergone sham-sensitization with vehicle alone. As shown in Fig. 5B, although at 4 h there were slight increases in anti-ICAM-1 and anti-VCAM-1 uptake relative to control Ab in sham-sensitized mice, these increases were not statistically significant. Furthermore, in sham-sensitized mice there was no increase in uptake of ICAM-1 or VCAM-1 detectable at 24 h. Thus, as with the increase in ear thickness (Fig. 5A), the endothelial expression of both ICAM-1 and VCAM-1 is largely immune-dependent at both 4 and 24 h after oxazolone challenge.
In a preliminary experiment, we established that direct injection of IL-1α, IL-1β, or TNF-α into mouse skin led to increased uptake of both anti-VCAM-1 and anti-ICAM-1 mAb 4 h after injection. Furthermore, the increased uptake of anti-VCAM-1 and anti-ICAM-1 Abs into skin in response to local cytokine injection could be completely inhibited by i.p. injection of the appropriate anti-TNF-α, IL-1α, or IL-1β antiserum but not by an irrelevant control antiserum (Fig. 6). Next, we explored the possible roles of IL-1α, IL-1β, and TNF-α in the early increase in endothelial ICAM-1 and VCAM-1 expression seen at 4 h after oxazolone challenge. As shown in Fig. 7, anti-TNF-α antiserum significantly inhibited both the increase in ear thickness (p < 0.01) and the up-regulation of ICAM-1 and VCAM-1 expression (p < 0.01 for both). The combination of anti-IL-1α and IL-1β antiserum had a small inhibitory effect on ICAM-1 expression (p < 0.05) but was significantly less inhibitory than anti-TNF-α (p < 0.05). Anti-IL-1α and IL-1β had no significant inhibitory effect on increased ear thickness or on VCAM-1 expression. Finally, a mixture of anti-TNF-α, anti-IL-1α, and IL-1β antiserum was no more inhibitory than anti-TNF-α alone for expression of ICAM-1 or VCAM-1, but had a small but significantly (p < 0.05) greater inhibitory effect than anti-TNF-α alone on ear swelling.

In an attempt to clarify the involvement of leukocytes in the initial release of TNF-α and perhaps other cytokines responsible for the up-regulation of ICAM-1 and VCAM-1 expression, an experiment was conducted using a mixture of inhibitory Abs to leukocyte integrins. In a preliminary experiment the combination of anti-β2 (mAb M18/2) and anti-α4 (mAb PS/2) integrins totally abrogated the adhesion in a static adhesion assay of the monocytic cell line J774 to a TNF-α-stimulated monolayer of the murine endothelial cell line bEND-3 (data not shown). Mice that had received an i.p. injection of this combination of mAb against β2 and α4 integrins 1 h before challenging with oxazolone showed a similar increase in ICAM-1 and VCAM-1 expression (Fig. 8B) and in ear thickness (Fig. 8A) at 4 h after challenge as mice that were
pretreated with control mAb. In contrast, at 24 h after challenge the combination of anti-β2 and anti-α4 integrins led to inhibition of ICAM-1 and VCAM-1 expression by 55 and 58%, respectively (p, 0.05 for each compared with mice treated with control mAb), accompanied by a significant inhibition of the increase in ear thickness (p, 0.05 compared with mice treated with control mAb). It should be noted that plasma concentrations of anti-β2 and anti-α4 integrin mAb were adequate to block leukocyte-endothelial cell adhesion at 4 h, because plasma taken 1, 2, and 4 h after i.p. injection fully inhibited adhesion of J774 to TNF-α-stimulated bEND-3 cells when tested ex vivo (data not shown). These data show that the increase in endothelial ICAM-1 and VCAM-1 expression by 4 h after cytokine stimulation by i.v. injection of 99mTc-labeled anti-ICAM-1 mAb, 111In-labeled anti-VCAM-1 mAb, and 125I-labeled control mAb injected i.v. 5 min before the end of the experiment. Data points are mean ± SD of groups of six mice. *, p < 0.01 compared with sham-sensitized mice.

**FIGURE 5.** Expression of ICAM-1 and VCAM-1 after challenge of sensitized and sham-sensitized mice. Mice were challenged with oxazolone after sensitization 5 days previously with oxazolone or with vehicle alone. A, Increase (Δ) in ear thickness at the time of Ab injection. B, Endothelial expression of ICAM-1 and VCAM-1 at 4 and 24 h after challenge as measured by uptake of 99mTc-labeled anti-ICAM-1 mAb, 111In-labeled anti-VCAM-1 mAb, and 125I-labeled control mAb injected i.v. 5 min before the end of the experiment. Data points are mean ± SD of groups of six mice. *, p < 0.01 compared with sham-sensitized mice.

**FIGURE 6.** Effect of recombinant cytokines on expression of ICAM-1 and VCAM-1 in mouse skin. Animals received i.p. injections of either 200 μl of control serum or 50 μl, 100 μl, or 200 μl of antiserum to (A) TNF-α, (B) IL-1α, and (C) IL-1β. Cutaneous inflammation was induced 24 h later by i.d. injection of (A) TNF-α, (B) IL-1α, and (C) IL-1β (each 5 ng) or by PBS alone. Endothelial expression of ICAM-1 and VCAM-1 was measured 4 h after cytokine stimulation by i.v. injection of 99mTc-labeled anti-ICAM-1 mAb, 111In-labeled anti-VCAM-1 mAb, and 125I-labeled control mAb 5 min before the end of the experiment. The LR shown are mAb uptake in cytokine-stimulated skin divided by mAb uptake in unstimulated skin. Values are the mean ± SD of two skin spots in three animals for each group. *, p < 0.05 compared with animals treated with control serum.

**Discussion**

Although there is now a considerable literature indicating the importance of endothelial cell activation and expression of adhesion molecules for leukocyte-endothelial cell interactions (7, 31), there is still relatively little detailed understanding of the precise dynamics of adhesion molecule expression during the course of inflammatory responses in vivo. In this study, we have adapted techniques that we have previously developed in the pig (20–23) to measure endothelial expression of ICAM-1 and VCAM-1 in the mouse CS response to oxazolone and to test the involvement of cytokines in their expression during the early phase of the response.

This study is the first example of the use of this technique to study the mechanisms underlying endothelial adhesion molecule expression in a localized immune-mediated inflammatory lesion in the mouse. By simultaneously injecting differentially radiolabeled anti-ICAM-1, anti-VCAM-1, and control mAb into individual animals, we were able to internally control the experiments and obtain data with far fewer animals than would be required if separate animals were injected with each of the three Abs labeled with the same isotype. We do not think that differences between the isotopes or radiolabeling procedures used for each Ab are likely to have influenced the results significantly because 1) the data obtained in the delayed-type hypersensitivity model are expressed as a LR between the two ears of the uptake of individual Abs and 2) a 10-fold excess of unlabeled Abs fully and specifically inhibited the increased uptake of both anti-ICAM-1 and anti-VCAM-1 mAb. Furthermore, it is unlikely that the uptake of anti-ICAM-1 and
anti-VCAM-1 mAb could be attributed to nonspecific protein exudation because 1) the injected mAb were found by immunohistochemistry to localize specifically to endothelium, 2) increased uptake of anti-ICAM-1 and anti-VCAM-1 mAb was first detectable at 4 h after challenge, lagging in time behind increased ear thickness and uptake of 125I-HSA, both of which showed biphasic increases, with peaks at 2 and 24 h but with intermediate troughs at 4 h. It is notable that the biphasic kinetics of the increase in ear thickness were very similar to those previously reported in this model (2).

We believe that the uptake of anti-ICAM-1 and anti-VCAM-1 mAb in the inflamed ears is a direct reflection of expression of the corresponding Ags on the luminal surface of endothelial cells, and that binding of Abs to circulating leukocytes contributed insignificantly to the results. Thus not only did the counting of injected radioactivity associated with peripheral blood leukocytes show only minimal binding of anti-ICAM-1 mAb and no detectable binding of anti-VCAM-1 mAb (data not shown), but the in vivo experimental protocol involved the removal of circulating blood by perfusion before the end of each experiment. The sensitivity of the radiolabeled mAb technique for quantifying changes in endothelial surface molecule expression makes it a very powerful tool for studying surface Ag expression during endothelial activation in vivo, particularly for molecules such as ICAM-1 that are constitutively expressed and therefore relatively difficult to quantify using immunohistochemistry.

We have recently reported that anti-TNF-α mAb inhibited the immune-mediated endothelial expression of VCAM-1, both during the early and late phases of the DTH reaction to purified-protein derivative in the pig (23). However, further studies, exploring the relative importance of TNF and IL-1 for the induction of endothelial cell adhesion molecules in this pig model, have been hampered by the lack of sufficient quantities of appropriate neutralizing Abs. By adapting the approach to mice, we have been able to compare the inhibitory effects on the CS response of neutralizing antisera to TNF-α and IL-1α/β. Although anti-TNF-α was able to inhibit both the increase in ear thickness and the up-regulation of ICAM-1 and VCAM-1 at 4 h after challenge, anti-IL-1 antisera had only a small inhibitory effect on ICAM-1 expression and did not affect VCAM-1 expression or ear thickness. Therefore, these observations extend observations showing impaired ear swelling at
24 h during the CS response in mice treated with anti-TNF-α blocking mAb (4) and indicate that TNF-α is a significantly more important cytokine than IL-1α or IL-1β for the early generation of CS in this model. However, it should be noted that the local injection of IL-1R antagonist has been shown to suppress ear swelling measured at 24 and 48 h during the CS response to dinitrofluorobenzene (5) and it is possible that IL-1 may perform an important role in amplifying or prolonging the inflammatory reaction after the initial effects of TNF-α. Such a pre-eminence of TNF-α in the cytokine cascade has been proposed in the context of rheumatoid synovitis (32).

It is well established that β2 and α4 integrins are critical for the optimal development of the CS response (11–16), in large part probably due to their roles in mediating leukocyte interactions with endothelium, although these integrins are likely also to be involved in leukocyte migration and activation within subendothelial tissue. We observed that a combination of anti-β2 integrin and anti-α4 integrin mAb was able to significantly inhibit endothelial expression of ICAM-1 and VCAM-1 at 24 h but had no effect on the early expression at 4 h after Ag challenge. These data are consistent with the maintenance of ICAM-1 and VCAM-1 expression, as measured at 24 h, being dependent upon the on-going actions of mediators released after leukocyte emigration and activation within the tissue. On the other hand, the grounds that the combination of anti-β2 and anti-α4 integrins could be expected to result in widespread inhibition of leukocyte function, the failure of the combination of anti-β2 integrin and anti-α4 integrin mAb to inhibit the increase in endothelial ICAM-1 and VCAM-1 expression at 4 h after challenge suggests that the initial release of TNF-α may not require leukocyte traffic or other leukocyte functions involving these integrins.

The mechanism for the early release of TNF-α remains unclear but could well involve release from keratinocytes or mast cells, both of which are known to express TNF-α capable of stimulating endothelial adhesion molecule expression (33–38). In fact, the 2-h lag that was observed between the early peak in ear swelling and the initial endothelial expression of ICAM-1 and VCAM-1 is consistent with the simultaneous release by mast cells of TNF-α and rapidly acting vasoactive mediators (2, 3), because expression of ICAM-1 and VCAM-1 by endothelial cells is delayed due to the requirement for new protein synthesis (39, 40). Whether the mast cell is the source of the TNF-α responsible for ICAM-1 and VCAM-1 expression at the onset of CS may be determined by experiments using mast cell-deficient mice (3, 41).

Because the early increase in ICAM-1 and VCAM-1 expression was dependent upon previous sensitization, the early release of TNF-α and other mediators would appear to be at least in part related to cognate recognition of Ag within the ear. However, it remains possible that nonspecific proinflammatory actions of oxazolone could contribute to mediator release and/or endothelial activation in this model (42). For example, it is possible that oxazolone might have a direct action on endothelium, because the up-regulation of adhesion molecules has been shown to be induced on endothelial cells by incubation with haptens in vitro (43). Because we saw no significant up-regulation of endothelial ICAM-1 or VCAM-1 expression in sham-sensitized oxazolone-challenged mice, 1% oxazolone is not likely to be sufficient in itself for the up-regulation of endothelial ICAM-1 or VCAM-1 expression in our model but conceivably may serve to enhance the effects on endothelium of TNF-α and/or other mediators.

In conclusion, by using radiolabeled mAb targeting in vivo, we have analyzed the endothelial expression of ICAM-1 and VCAM-1 at the onset of the CS inflammatory response. We have shown that initial up-regulation of endothelial ICAM-1 and VCAM-1 expression follows the first peak of ear swelling by 2 h and is an immune-mediated phenomenon requiring the local release of TNF-α. The further application of this technique will allow us to relate in detail the expression of ICAM-1 and VCAM-1 to that of P- and E-selectins (44–47) and other endothelial cell determinants critical for leukocyte recruitment, and will enable the acquisition of a detailed understanding of how endothelial activation and expression of surface molecules relates to the timing and nature of this important T cell-mediated inflammatory response.

Acknowledgments

We thank Drs. David Gray, Tony Meagher, and Roberto Solari for their generous gifts of recombinant cytokines and Abs.

References


