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Immunology

CD40 on human endothelial cells: Inducibility by cytokines and functional regulation of adhesion molecule expression

(E-selectin/intercellular adhesion molecule 1/vascular cell adhesion molecule 1/T lymphocyte/cell-mediated immunity)

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ABSTRACT **Cultured human umbilical vein endothelial cells (EC) constitutively express a low level of CD40 antigen as detected by monoclonal antibody binding and fluorescence flow cytometric quantitation. The level of expression on EC is increased about 3-fold following 24 h treatment with optimal concentrations of tumor necrosis factor, interleukin 1, interferon β, or interferon γ; both interferons show greater than additive induction of CD40 when combined with tumor necrosis factor or interleukin 1. Expression of CD40 increases within 8 h of cytokine treatment and continues to increase through 72 h. A trimeric form of recombinant murine CD40 ligand acts on human EC to increase expression of leukocyte adhesion molecules, including E-selectin, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1. CD40 may be detected immunocytochemically on human microvascular EC in normal skin. We conclude that endothelial CD40 may play a role as a signaling receptor in the development of T-cell-mediated inflammatory reactions.**

CD40 is a type I cell surface protein of M_r 50,000 expressed principally by B cells. It has also been found on dendritic cells, follicular dendritic cells, thymic epithelial cells, and monocytes (1–4). Cloning of CD40 has revealed it to be a member of the nerve growth factor receptor (NGFR)/tumor necrosis factor receptor (TNFR) family of proteins (5, 6). Stimulation of B cells through CD40 induces B7 costimulator expression (7), interleukin 6 (IL-6) synthesis (8), proliferation (1, 9), and isotype switching/IgE production (10, 11). Crosslinking of CD40 on monocytes induces tumoricidal activity, and in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF), IL-3, or interferon γ (IFN-γ), engagement of CD40 augments tumor necrosis factor (TNF) and IL-6 production (4). In thymic epithelial cells, CD40 signaling results in GM-CSF production (3). In mice that have lost CD40 through homologous recombination, immunoglobulin class switching for T-dependent antigens is defective, although responses to T-independent antigens are normal (12). Such CD40 "knockout" mice do not form germinal centers in their lymphoid organs, further implicating CD40 in B-cell development and maturation. A ligand for CD40 has been cloned and identified as a $CD4^+$ T-cell activation antigen (11, 13, 14). CD40 ligand is a type II membrane protein with homology to TNF, and the functional form is presumed to be a homotrimer (15). Mutation of this molecule is the defect responsible for X-linked hyper-IgM syndrome in humans (16–20). Defects similar to those in CD40 knockout mice have been noted in mice that have lost CD40 ligand through homologous recombination (12, 21).

Endothelial cells (ECs), like B cells, dendritic cells, and monocytes, interact with T cells in the development of immune responses. ECs express costimulatory molecules and, when induced by IFN-γ to express class II major histocompatibility complex molecules, can induce proliferation of allogeneic $CD4^+$ T cells (22–27). EC also constitutively and inducibly express adhesion molecules that serve to recruit circulating leukocytes to local sites of antigenic challenge (28). In light of the numerous immunologic functions served by ECs and the emerging role of CD40 in immune responses, we have examined CD40 expression on ECs, both *in vitro* and *in vivo*, and also analyzed the functional effect of CD40 ligand on ECs.

MATERIALS AND METHODS

Cytokines and CD40 Ligand Trimer. Recombinant human TNF (5.3×10^6 units/mg), IFN-γ (2.5×10^7 units/mg), IFN-β (5×10^7 units/mg), and IL-1α (5×10^7 units/mg) were a gift of Biogen. Recombinant human IL-4 (5×10^6 units/mg) and IL-6 (1.68×10^8 units/mg) were obtained from Genzyme. Recombinant human GM-CSF (1.5×10^7 units/mg) was obtained from R & D Systems. Trimeric recombinant murine CD40 ligand was formed from monomers tagged with a leucine zipper tail; such preparations of murine CD40 ligand trimer have been shown to be biologically active on human cells, delivering stimulatory signals to B cells (11, 15). All cytokines used in this study are free of detectable endotoxin by the Limulus assay.

Monoclonal Antibodies (mAbs). Murine mAbs, tested as blocking agents or used as specific antibody for fluorescence flow-cytometric analysis of surface molecules, were partly purified from ascites fluid or serum by ammonium sulfate precipitation or Protein G columns (GIBCO/BRL). Antibodies used in this study include M2 and M3 (anti-CD40; both IgG1; ref. 4), E1/6 [anti-vascular cell adhesion molecule 1 (VCAM-1)/CD106; IgG1; gift from M. Bevilacqua, Amgen; ref. 29], TS2/9 [anti-CD58/lymphocyte function-associated antigen 3 (LFA-3); IgG1; gift from T. Springer, Center for Blood Research, Boston], K16/16 (nonbinding IgG1; gift from Donna Mendrick, Brigham and Women's Hospital, Boston); and H4/18 (anti-CD62E/E-selectin; IgG1; ref. 30). Fluorescein isothiocyanate (FITC)-conjugated mAb anti-VCAM-1/CD106 (clone G11B1; IgG1), anti-E-selectin/CD62E (clone 1.2B6; IgG1), and isotype control FITC-conjugated IgG1 were obtained from Antigenix America, New York. FITC-conjugated mAb anti-intercellular adhesion molecule 1 (ICAM-1)/CD54 (clone B-C14; IgG1) and anti-HLA-class I-ABC (clone B-H9) were obtained from Biosource International, Camarillo, CA.

Abbreviations: EC, endothelial cell; mAb, monoclonal antibody; IL-*n*, interleukin *n*; IFN-β, interferon β; IFN-γ, interferon γ; TNF, tumor necrosis factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; FITC, fluorescein isothiocyanate; LFA-3, lymphocyte function-associated antigen 3; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; UEA-1, *Ulex europaeus* agglutinin 1.

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Cell Isolation and Culture. Human ECs were isolated from umbilical veins and cultured as previously described on gelatin-coated, tissue-culture plastic (Falcon) (31, 32). In the experiments reported, ECs at passage level 2–4 were incubated with cytokines and other reagents as indicated in either Medium 199 (GIBCO) supplemented with 20% (vol/vol) fetal bovine serum (FBS) (GIBCO), 50 μg of endothelial cell growth factor per ml (Collaborative Biomedical Products, Bedford, MA), 100 μg of heparin per ml (Sigma), 2.5 mM glutamine (GIBCO), 100 units of penicillin per ml, and 100 μg of streptomycin per ml (GIBCO) or RPMI 1640 (GIBCO) supplemented with 10% (vol/vol) FBS, 2.5 mM glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin (per ml). No consistent differences in the ECs were noted between these two conditions, and they are described interchangeably. These serially passaged EC cultures stain uniformly for von Willebrand factor expression, lack any bone marrow-derived contaminants detectable by antibody staining (26), and are free of detectable T cells by functional assays—e.g., adhesion molecule expression or cytokine release in response to phytohemagglutinin.

Flow Cytometry. To recover cells for flow cytometry, cells were washed twice with Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS) and incubated for 30 min with PBS/5 mM EDTA at 37°C. Cells were recovered from the plates and washed once with PBS/1% bovine serum albumin (BSA) before being incubated with either unconjugated specific first antibody diluted in PBS/5% goat serum or FITC-conjugated specific antibody for 30 min at 4°C, according to the recommendations provided by the supplier. For indirect immunofluorescence labeling, cells were then washed two times with PBS/1% BSA and incubated with a secondary FITC-conjugated $F(ab')_2$ goat anti-mouse IgG (heavy & light chain; Boehringer Mannheim) at a 1:100 dilution in PBS/5% goat serum for 30 min at 4°C. After indirect or direct immunofluorescence labeling, cells were washed one time in PBS/1% BSA and two times in PBS and then fixed with 2% paraformaldehyde before being analyzed. Cells were analyzed by using a Becton Dickinson FACSort running LYSIS II software. Corrected mean fluorescence values were calculated as follows: for each treatment the mean fluorescence value for the isotype-matched control antibody was subtracted from the mean fluorescence value for the specific antibody.

Immunohistochemistry. Skin biopsies were obtained from healthy human adult volunteer donors in accordance with protocols approved by the Yale University Human Investigations Committee and immediately snap frozen in OCT. Cryostat sections (4 μm) were cut and stained as described (33) by using the double staining protocol involving biotinylated *Ulex europaeus* agglutinin 1 (UEA-1, Vector Laboratories) as a panendothelial cell marker (red) and mAb M2 to detect CD40 (blue).

RESULTS

CD40 Expression on Cultured Human EC. We used indirect immunofluorescence flow-cytometric analysis to examine ECs for expression of CD40. As shown in Fig. 1, cultured human ECs constitutively express low levels of CD40 (estimated at 5–10 × 10^3 copies per cell), and this expression is maintained at similar levels over several passages (data not shown). Similar staining was observed with each of two different anti-CD40 mAbs (data not shown). To determine if CD40 expression was upregulated by various cytokines, we incubated our EC cultures with concentrations of individual recombinant cytokines known to produce optimal effects on endothelial expression of adhesion or MHC molecules. TNF and IFN-γ were found to be the most effective at enhancing CD40 expression, producing about a 3-fold increase in CD40 expression after 24 h (Fig. 1). IFN-β and IL-1α also increased CD40 expression, although somewhat less effectively than TNF or IFN-γ. IL-4 was not effective and in some cases appeared to decrease constitutive levels of CD40. IL-6 and GM-CSF had no effect on CD40 expression (data not shown). We next examined the concentration dependence of CD40 induction by TNF and IFN-γ and compared this to induction of VCAM-1 in the same cultures. As shown in Fig. 2, induction of both VCAM-1 and CD40 increased over the same range of cytokine concentrations. As expected, TNF was consistently more effective at inducing VCAM-1 expression than was IFN-γ. The half-maximal dose of TNF for induction of CD40 and VCAM-1 expression was 1.3 and 1.2 units/ml, respectively, while the half-maximal dose of IFN-γ was 17 units/ml for CD40 expression and 20 units/ml for VCAM-1 expression.

During inflammatory reactions, cells are usually exposed to multiple cytokines. We therefore examined the effect of combinations of cytokines on CD40 upregulation (Table 1). Interestingly, several combinations produced greater than additive effects on increasing CD40 expression. Specifically, both IFN-γ and IFN-β each produced greater than additive upregulation of CD40 when combined with either TNF or IL-1α. Combinations of IFN-γ and IFN-β or TNF and IL-1α were not synergistic. Again, IL-4 had no positive effect on CD40 expression in combination with any other cytokine. In

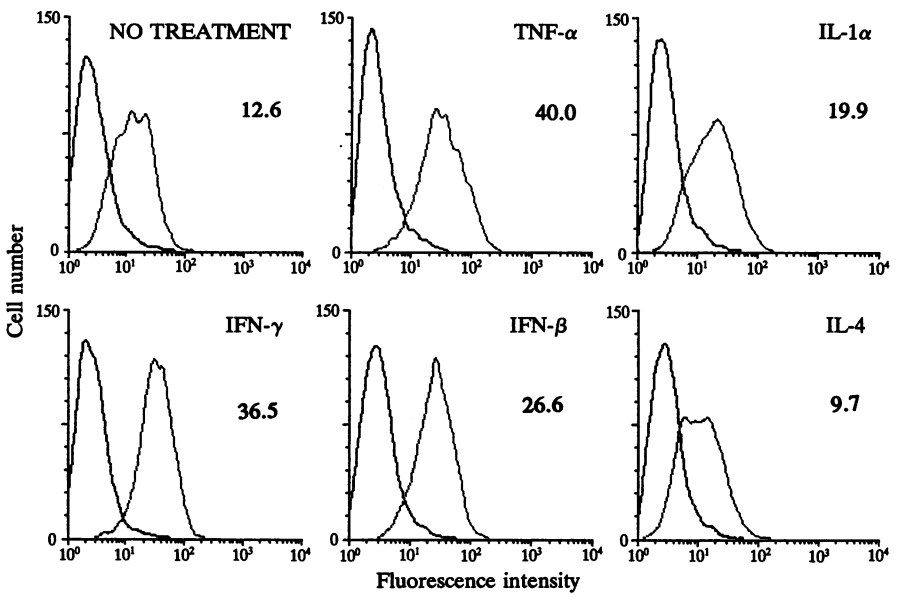

FIG. 1. Expression of CD40 on untreated and cytokine-treated ECs. All treatments were for 24 h. The following concentrations of cytokines were used: TNF, 100 units/ml; IL-1α, 100 units/ml; IFN-γ, 1000 units/ml; IFN-β, 1000 units/ml; and IL-4, 1000 units/ml. Each panel is a histogram representing cell number (*y* axis) vs. fluorescence intensity (*x* axis) for 5000 cells. CD40 is detected with mAb M2 and in each panel is compared to binding of irrelevant nonbinding mAb K16/16; both antibodies are quantified by indirect immunofluorescence flow cytometry as described in *Materials and Methods.* Numbers in the panels represent corrected mean fluorescence intensities. Data shown are from one of three experiments with similar results.

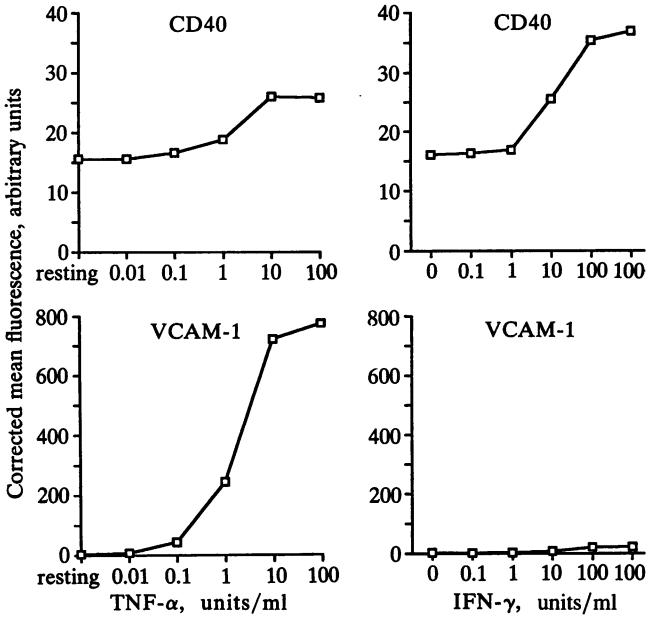

FIG. 2. Effect of TNF or IFN-γ concentration on upregulation of CD40 or VCAM-1 expression. All treatments were for 24 h. Data are the corrected mean fluorescence intensities determined by indirect fluorescence flow cytometry as described in the legend to Fig. 1. Data shown are from one of three experiments with similar results.

fact, when added with IFN-γ or especially with IFN-β, IL-4 reduced the level of induction by these cytokines by 39% and 80%, respectively.

A time course of CD40 induction on ECs is shown in Fig. 3. In this experiment, basal CD40 expression was elevated by a combination of TNF and IFN-γ as early as 8 h after cytokine exposure and continued to increase through the course of the experiment (72 h). VCAM-1 was induced more rapidly than was CD40, being detectable by 4 h, peaking at 24 h, and declining thereafter.

CD40 Function on Cultured Human EC. We next addressed the question of whether CD40 expressed on ECs is functional as a receptor by utilizing a trimeric form of the murine CD40 ligand (CD40LT). We incubated ECs with various concentrations of this molecule (0.1–10 μg/ml) and then examined the cells by indirect immunofluorescence flow cytometry for expression of EC activation markers. As shown in Fig. 4, stimulation through CD40 by CD40LT induced VCAM-1 (CD106) and E-selectin (CD62E) expression, while LFA-3 (CD58) expression was unaffected. ICAM-1 (CD54) expression was

Table 1. Effects of different combinations of cytokines on CD40 expression on ECs

	Cotreatment				
Treatment	None	TNF-α	IL-1α	IFN-γ	IFN-β
None	12.6				
TNF-α	40.0				
IL-1α	19.9	37.3			
IFN-γ	36.5	93.4*	57.8*		
IFN-β	26.6	81.3*	41.2*	36.2	
IL-4	9.7	39.8	18.8	27.2	15.4

ECs were incubated with one or two of the indicated cytokines for 24 h before CD40 expression was measured by flow cytometry. The concentrations of cytokines used were as follows: TNF-α, 100 units/ml; IL-1α, 100 units/ml; IFN-γ, 1000 units/ml; IFN-β, 1000 units/ml; and IL-4, 1000 units/ml. The values shown are corrected mean fluorescence.

*These combinations of cytokines have a greater than additive effect on CD40 expression of ECs. The data shown are representative of three similar experiments.

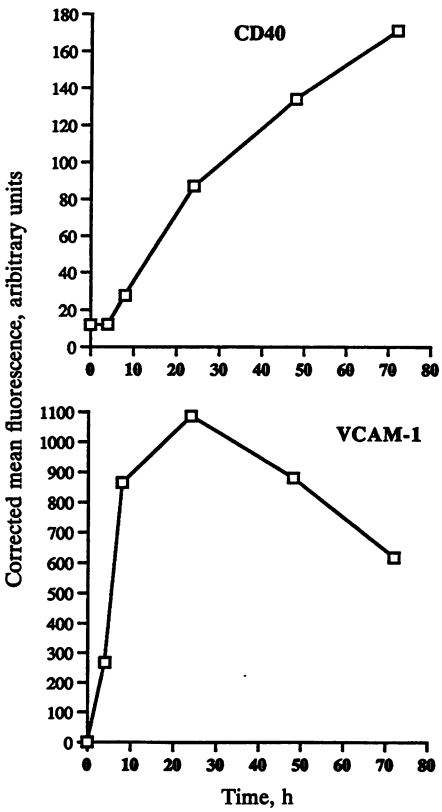

FIG. 3. Effect of time of cytokine treatment on expression of CD40 or VCAM-1. All treatments used 100 units of TNF and 1000 units of IFN-γ per ml. Data are the corrected mean fluorescence intensities determined by indirect fluorescence flow cytometry as described in the legend to Fig. 1. Data shown are from one of two experiments with similar results.

also increased by this treatment (data not shown). To confirm that the effect of the CD40LT was mediated through CD40, we added an inhibitory anti-CD40 antibody (M2) to the cultures and examined adhesion molecule expression by direct immunofluorescence flow cytometry. Preincubation of ECs with mAb M2 effectively blocked the ability of CD40LT to upregulate expression of E-selectin, VCAM-1, or ICAM-1 by 77%, 91%, and 90%, respectively, compared with cells pretreated with a control IgG (data not shown). In contrast, pretreatment of a replicate culture with mAb M2 did not block TNF induction of E-selectin, VCAM-1, or ICAM-1 (data not shown).

CD40 Expression on EC *in Vivo*. To support the relevance of our *in vitro* findings, we used immunohistochemistry to analyze normal human skin for cellular patterns of expression of CD40. As shown in Fig. 5, microvascular ECs, identified by positive staining with UEA-1 lectin (red), stain positively for CD40 (blue). The staining intensity of anti-CD40 antibodies varies among ECs in different microvessels in the same specimens, and in general, venular and capillary ECs appeared to stain more strongly than arteriolar ECs. We also note that keratinocytes appear to express low levels of CD40; keratinocyte staining can be distinguished from more intense staining localized to a dendritic population of epidermal cells, presumably Langerhans cells. Little staining is noted on other vascular cell populations—e.g., smooth muscle cells and pericytes—or on skin fibroblasts.

DISCUSSION

CD40 was originally described as a B-cell antigen but has more recently been shown to be expressed on thymic epithelial cells, dendritic cells, and monocytes (1–4). Our report now extends

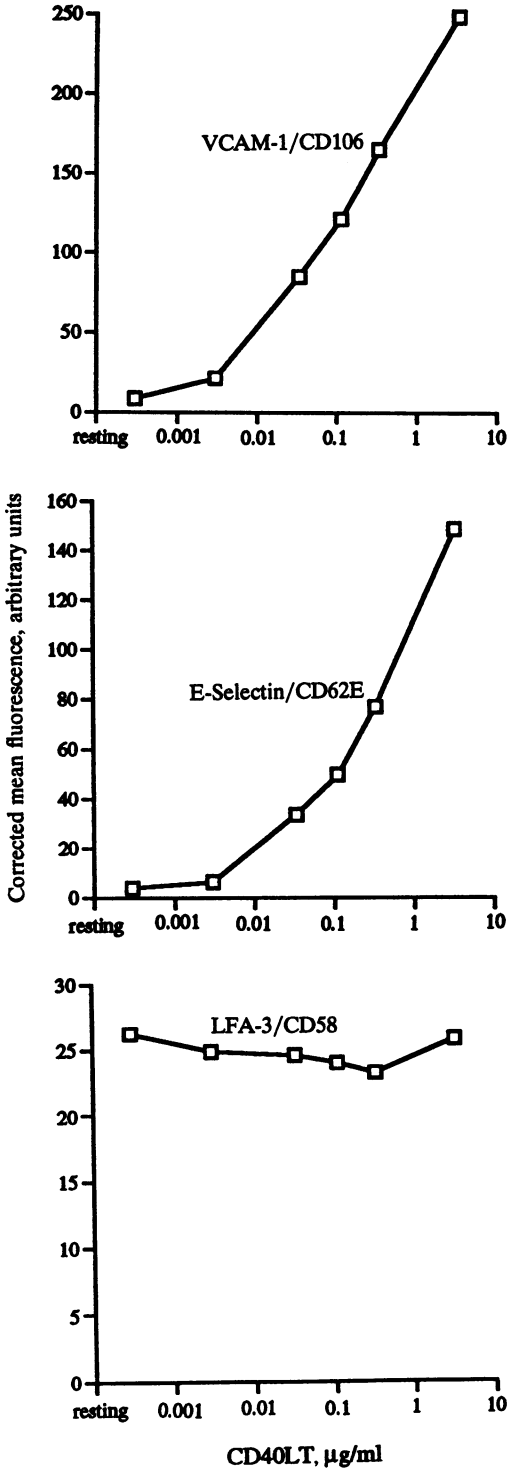

FIG. 4. Effect of recombinant murine CD40 ligand trimer (CD40LT) concentration on endothelial expression of VCAM-1, E-selectin, and LFA-3. All treatments were for 24 h. Data are the corrected mean fluorescence intensities determined by indirect fluorescence flow cytometry as described in the legend to Fig. 1. Data shown are from one of three experiments with similar results.

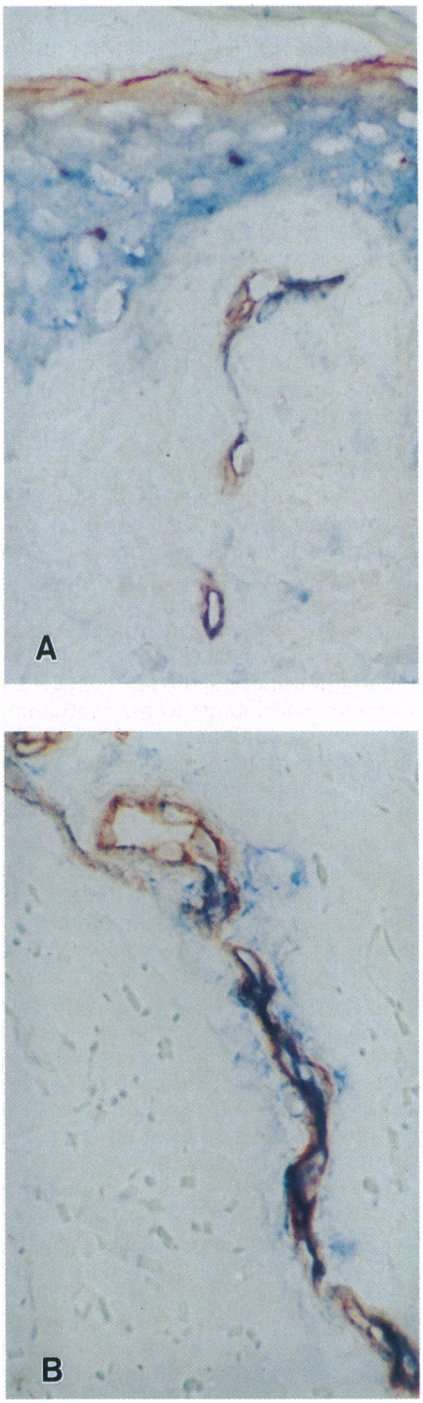

FIG. 5. Expression of CD40 on ECs and other cells in normal human skin. Sections are double stained with UEA-I (red) to identify all ECs and with anti-CD40 mAb M2 (blue) to identify CD40-expression cells. At low power (*A*) microvessels in the dermis intensely express CD40. CD40 also appears to be expressed at lower levels by keratinocytes (light blue) and more intensely by an intraepidermal dendritic cell population, probably Langerhans cells. Dermal fibroblasts are not stained. UEA-I staining is also observed on cornified cells in the epidermis; the specificity of this reaction is unclear. At high power (*B*), the microvascular ECs can be clearly seen to be doubly stained, but the staining intensity varies between the two microvessels shown. Data shown are from one of three experiments with similar results.

this list to include human vascular ECs. Cultured ECs constitutively express CD40, and this expression can be increased by cytokines. We also find endothelial expression of CD40 *in vivo* on normal human skin. A previous report failed to find significant CD40 staining of ECs in the thymus (3), and our finding of CD40-positive ECs in skin may reflect tissue-specific expression of this molecule. Moreover, CD40 expression on keratinocytes and dendritic-like cells in the epidermis indicates a more ubiquitous distribution of the CD40 molecule than originally thought. More thorough analysis of a variety of human tissues will need to be performed to evaluate the full range of CD40 expression *in vivo*.

Signaling through CD40 appears to have distinct effects on different cell types. In B cells, the effects on proliferation, isotype switching, and germinal center formation are well known (1, 9, 10, 34, 35). In monocytes, tumoricidal activity is induced and cytokine production is stimulated (4). Thymic epithelial cells have previously been shown to express GM-CSF after treatment with IFN-γ or IL-1α or after cross-linking CD40-bound mAbs (3). We found that endothelial CD40 can mediate signals that lead to increases in adhesion molecule expression. We are as yet uncertain what other functions in ECs may be activated through CD40, but we did not see any induction in ECs of either IL-6 or GM-CSF production (data not shown). These negative observations distinguish the actions of CD40 ligand on ECs from those of TNF. This difference is not surprising because although CD40 shares structural features with TNF receptors, these are confined to the extracellular, ligand-binding domains rather than to the intracellular regions thought to be relevant for signaling.

The ability of CD40 to deliver signals that increase adhesion molecule expression is consistent with a role for this molecule in the development of immune inflammation. $CD4^+$ T cells activated via their antigen receptor are rapidly induced to express the CD40 ligand. Preliminary observations suggest that ECs may increase this effect even further. Activated $CD4^+$ T cells may then interact with CD40-expressing ECs to induce leukocyte adhesion molecules and with monocytes to induce cytokine production, contributing to local inflammation. Activated T cells themselves produce cytokines, such as IFN-γ and TNF, which can further increase endothelial CD40 expression, thus potentially magnifying the response. However, it is important to note that the same T cell-derived cytokines, namely IFN-γ and TNF, can directly induce EC adhesion molecules. The CD40 signal may be redundant or may provide a unique profile of endothelial activation that selectively favors one kind of inflammatory reaction over another.

The expression of CD40 on ECs raises the theoretical possibility that this molecule could be used to deliver a signal to CD40 ligand-expressing T cells. Several experiments, however, have failed to detect any change in T-cell responsiveness when endothelial CD40 is blocked by antibody binding (unpublished observations). The unidirectional nature of the signal mediated by CD40 and its ligand is consistent with the general pattern observed in studies of other members of the TNF/TNF receptor family of ligand pairs.

To date, defects in CD40 ligand in humans (X-linked hyper-IgM) and "knockout" of CD40 or CD40 ligand in mice have primarily been reported to result in defects in humoral responses—i.e., in B-cell maturation, isotype switching, and germinal center formation (12, 16–21, 35, 36). However, hyper-IgM syndrome patients are susceptible to infection by *Pneumocystis carinii*, a hallmark of compromised cell-mediated immunity. A role for CD40 in cell-mediated immunity is further suggested by the molecular effects of inhibitory mAbs to this receptor in murine graft-vs.-host disease (37, 38). It will be interesting to learn if altered EC responses in these patients contribute to this lesion.

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1. Clark, E. A. & Ledbetter, J. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4494–4498.
2. Freudenthal, P. S. & Steinman, R. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7698–7702.
3. Galy, A. H. & Spits, H. (1992) *J. Immunol.* **149,** 775–782.
4. Alderson, M. R., Armitage, R. J., Tough, T. W., Strockbine, L., Fanslow, W. C. & Spriggs, M. K. (1993) *J. Exp. Med.* **178,** 669–674.
5. Stamenkovic, I., Clark, E. A. & Seed, B. (1989) *EMBO J.* **8,** 1403–1410.
6. Torres, R. M. & Clark, E. A. (1992) *J. Immunol.* **148,** 620–626.
7. Ranheim, E. A. & Kipps, T. J. (1993) *J. Exp. Med.* **177,** 925–935.
8. Clark, E. A. & Shu, G. (1990) *J. Immunol.* **145,** 1400–1406.
9. Banchereau, J., de Paoli, P., Valle, A., Garcia, E. & Rousset, F. (1991) *Science* **251,** 70–72.
10. Jabara, H. H., Fu, S. M., Geha, R. S. & Vercelli, D. (1990) *J. Exp. Med.* **172,** 1861–1864.
11. Spriggs, M. K., Armitage, R. J., Strockbine, L., Clifford, K. N., Macduff, B. M., Sato, T. A., Maliszewski, C. R. & Fanslow, W. C. (1992) *J. Exp. Med.* **176,** 1543–1550.
12. Kawabe, T., Naka, T., Yoshida, K., Tanaka, T., Fujiwara, H., Suematsu, S., Yoshida, N., Kishimoto, T. & Kikutani, H. (1994) *Immunity* **1,** 167–178.
13. Armitage, R. J., Fanslow, W. C., Strockbine, L., Sato, T. A., Clifford, K. N., Macduff, B. M., Anderson, D. M., Gimpel, S. D., Davis, S. T. & Maliszewski, C. R. (1992) *Nature (London)* **357,** 80–82.
14. Hollenbaugh, D., Grosmaire, L. S., Kullas, C. D., Chalupny, N. J., Braesch, A. S., Noelle, R. J., Stamenkovic, I., Ledbetter, J. A. & Aruffo, A. (1992) *EMBO J.* **11,** 4313–4321.
15. Fanslow, W. C., Srinivasan, S., Paxton, R., Gibson, M. G., Spriggs, M. K. & Armitage, R. J. (1994) *Sem. Immunol.* **6,** 267–278.
16. Allen, R. C., Armitage, R. J., Conley, M. E., Rosenblatt, H., Jenkins, N. A., Copeland, N. G., Bedell, M. A., Edelhoff, S., Disteche, C. M. & Simoneaux, D. K. (1993) *Science* **259,** 990–993.
17. Aruffo, A., Farrington, M., Hollenbaugh, D., Li, X., Milatovich, A., Nonoyama, S., Bajorath, J., Grosmaire, L. S., Stenkamp, R. & Neubauer, M. (1993) *Cell* **72,** 291–300.
18. DiSanto, J. P., Bonnefoy, J. Y., Gauchat, J. F., Fischer, A. & de Saint, B. G. (1993) *Nature (London)* **361,** 541–543.
19. Fuleihan, R., Ramesh, N., Loh, R., Jabara, H., Rosen, R. S., Chatila, T., Fu, S. M., Stamenkovic, I. & Geha, R. S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2170–2173.
20. Korthauer, U., Graf, D., Mages, H. W., Briere, F., Padayachee, M., Malcolm, S., Ugazio, A. G., Notarangelo, L. D., Levinsky, R. J. & Kroczek, R. A. (1993) *Nature (London)* **361,** 539–541.
21. Xu, J., Foy, T. M., Laman, J. D., Elliott, E. A., Dunn, J. J., Waldschmidt, T. J., Elsemore, J., Noelle, R. J. & Flavell, R. A. (1994) *Immunity* **1,** 423–431.
22. Pober, J. S., Collins, T., Gimbrone, M. A., Cotran, R. S., Gitlin, J. D., Fiers, W., Clayberger, C., Krensky, A. M., Burakoff, S. J. & Reiss, C. S. (1983) *Nature (London)* **305,** 726–729.
23. Pober, J. S., Gimbrone, M. A., Jr., Cotran, R. S., Reiss, C. S., Burakoff, S. J., Fiers, W. & Ault, K. A. (1983) *J. Exp. Med.* **157,** 1339–1353.
24. Hughes, C. C. W., Savage, C. O. S. & Pober, J. S. (1990) *J. Exp. Med.* **171,** 1453–1467.
25. Savage, C. O. S., Hughes, C. C. W., Pepinsky, R. B., Wallner, B. P., Freedman, A. S. & Pober, J. S. (1991) *Cell. Immunol.* **137,** 150–163.
26. Savage, C. O. S., Hughes, C. C. W., McIntyre, B. W., Picard, J. K. & Pober, J. S. (1993) *Transplantation* **56,** 128–134.
27. Adams, P. W., Lee, H. S., Waldman, W. J., Sedmak, D. D., Morgan, C. J., Ward, J. S. & Orosz, C. G. (1992) *J. Immunol.* **148,** 3753–3760.
28. Pober, J. S. & Cotran, R. S. (1990) *Transplantation* **50,** 537–544.
29. Rice, G. E. & Bevilacqua, M. P. (1989) *Science* **246,** 1303–1306.
30. Pober, J. S., Bevilacqua, M. P., Mendrick, D. L., Lapierre, L. A., Fiers, W. & Gimbrone, M. A., Jr. (1986) *J. Immunol.* **136,** 1680–1687.
31. Gimbrone, M. A., Jr. (1976) *Prog. Hemostasis Thromb.* **3,** 1–28.
32. Thornton, S. C., Mueller, S. N. & Levine, E. M. (1983) *Science* **222,** 623–625.
33. Petzelbauer, P., Bender, J. R., Wilson, J. & Pober, J. S. (1993) *J. Immunol.* **151,** 5062–5072.
34. Foy, T. M., Laman, J. D., Ledbetter, J. A., Aruffo, A., Claassen, E. & Noelle, R. J. (1994) *J. Exp. Med.* **180,** 157–163.
35. Korpelainen, E. I., Gamble, J. R., Smith, W. B., Goodall, G. J., Qiyu, S., Woodcock, J. M., Dottore, M., Vadas, M. A. & Lopez, A. F. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11137–11141.
36. Renshaw, B. R., Fanslow, W. C., Armitage, R. J., Campbell, K. A., Liggitt, D., Wright, B., Davison, B. L. & Maliszewski, C. R. (1994) *J. Exp. Med.* **180,** 1889–1900.
37. Durie, F. H., Aruffo, A., Ledbetter, J., Crassi, K. M., Green, W. R., Fast, L. D. & Noelle, R. J. (1994) *J. Clin. Invest.* **94,** 1333–1338.
38. Durie, F. H., Foy, T. M., Masters, S. R., Laman, J. D. & Noelle, R. J. (1994) *Immunol. Today* **15,** 406–411.