Ligation of CD40 Activates Interleukin 1β-converting Enzyme (Caspase-1) Activity in Vascular Smooth Muscle and Endothelial Cells and Promotes Elaboration of Active Interleukin 1β

(Received for publication, January 24, 1997, and in revised form, April 9, 1997)

Uwe Schönbeck‡§, François Mach‡§, Jean-Yves Bonnefoy¶, Harald Loppnow**, Hans-Dieter Flad‖, and Peter Libby‡ ‡‡

From the ‡Vascular Medicine and Atherosclerosis Unit, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, the ¶Geneva Biomedical Research Institute, 14 chemin des Aulx, 1228 Geneva, Switzerland, the #Department of Immunology and Cell Biology, Research Center Borstel, 23845 Borstel, Germany, and the **Martin-Luther-University, Ernst-Grube-Straße 40, 06097 Halle (Saale), Germany

Inflammation contributes to a variety of arterial diseases including atherosclerosis. Interleukin 1β (IL-1β) in its activated mature 17-kDa form may mediate aspects of vascular inflammation. As shown previously, human vascular wall cells, such as smooth muscle cells (SMC), express the IL-1β precursor upon stimulation and the IL-1β-converting enzyme (ICE) constitutively but do not produce mature IL-1β or express ICE activity. How SMC, the most numerous cell type in arteries, may release active IL-1β has therefore remained a perplexing problem. We report here that stimulation of human vascular SMC and endothelial cells (EC) through CD40 ligand, a mediator recently localized in human atheroma, induced elaboration of the IL-1β precursor as well as activation of cell-associated ICE. In addition to the constitutively expressed 45- and 30-kDa immunoreactive ICE proteins, vascular cells incubated with recombinant human CD40 ligand (rCD40L) (but not IL-1 or TNF) showed an increase of a 20-kDa immunoreactive ICE protein by Western blot analysis. Furthermore, SMC and EC stimulated through rCD40L processed recombinant human IL-1β precursor (pIL-1β), generating a cleavage product of approximately 17 kDa. Appearance of both the 20-kDa immunoreactive ICE protein and pIL-1β processing activity required at least 6 h of stimulation with 0.3 or 1.0 μg/ml rCD40L, respectively, and was inhibited by pre-incubation of the ligand with an anti-CD40L antibody. Stimulation of vascular SMC and EC through rCD40L resulted in the release of biologically active IL-1β, indicating processing of the native IL-1β precursor induced by the ligand. These findings establish a novel mechanism of IL-1β activation in human vascular cells and, moreover, indicate a new pathway of ICE-activation, which could participate in inflammatory aspects of atherogenesis and other disease states.

Interleukin 1 (IL-1) figures importantly in many physiological and pathological processes, notably inflammatory diseases including atherosclerosis. Two distinct genes give rise to the two IL-1 isoforms denoted IL-1α and IL-1β that bind to common receptors. Interleukin 1α, often membrane-associated, can act by contact with neighboring target cells (1). Interleukin 1β, when secreted in its mature form, can act at a distance in a paracrine manner. Acquisition of biological activity for IL-1β (but not IL-1α) requires processing into the mature, 17-kDa protein (2–5). Upon stimulation, monocytes produce a cell-associated 33-kDa precursor form of IL-1β (2, 3). Maturation of the IL-1β precursor into the active 17-kDa form results from cleavage at the Asp116-Ala117 site by a cysteine protease denoted IL-1β-converting enzyme (ICE) (6–10). ICE in turn is synthesized as a precursor molecule of 45 kDa, which is thought to be autocatalytically cleaved to form an active homodimeric enzyme of 20- and 10-kDa subunits (p20/p10)11(11, 12). ICE was the prototype of a group of cysteine proteases, now called the caspase family (13). In addition to ICE (caspase-1), this protease family includes pro-apoptotic enzymes, such as human ICH-1 (caspase-2) or CPP32 (caspase-3). Each of these homologous enzymes share the active site cysteine and aspartate cleavage clefts. Studies of the enzymatic specificity of ICE demonstrated highly selective proteolytic activity, i.e. requiring aspartic acid in the P1-position (9, 14). Interleukin 1β and the apoptotic mediator CPP32 are among the substrates of ICE (15, 16). Although ICE can autoactivate (17), the initial mechanisms of activation and regulation of ICE-processing remain unknown.

Most studies investigating expression of ICE or IL-1β activation have focused on monocytes or monocyte-derived cell lines. However, normal arteries contain few if any mononuclear phagocytes. IL-1 derived from vascular smooth muscle (SMC) and endothelial cells (EC) may initiate local immune and inflammatory responses and induce expression of adhesion molecules (18–20) and chemoattractant cytokines, e.g. IL-8 or IL-1 itself (21–25), that can then recruit the "professional" phagocytes. In particular, inflammatory components of atherogenesis may involve IL-1 (26, 27). Although human atherosclerotic plaques contain both IL-1β and ICE (28, 29), the mechanisms that activate either the cytokine or the enzyme remain undefined.

* This work was supported in part by Grant HL-43364 of the NHLBI, National Institutes of Health (to P. L.), a grant from the Fonds National Suisse pour la Recherche Scientifique (to F. M.), and Grant Scho 614/1-1 of the Deutsche Forschungsgemeinschaft (to U. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Contributed equally to this work.

‡ To whom correspondence should be addressed: Vascular Medicine and Atherosclerosis Unit, Cardiovascular Division, Dept. of Medicine, Brigham and Women’s Hospital, Harvard Medical School, 221 Longwood Ave., LMRC 307, Boston, MA 02115. Tel.: 617-732-6628; Fax: 617-732-6661; E-mail: plibby@bustoff.bwh.harvard.edu.
Recent work has demonstrated co-expression of CD40 and its ligand CD40L in human atherosclerotic plaques, indicating a possible role for this receptor-ligand pair in vascular pathology (30). CD40L, originally described as a 33-kDa activation-induced transiently expressed CD4+ T cell surface molecule (31–34), is also expressed on macrophages, endothelial cells, and smooth muscle cells (30). Previous studies of the interactions between CD40L and its receptor CD40 concentrated on the role of these leukocyte-surface proteins in T cell-dependent B cell differentiation and activation (35). CD40 ligation regulates a variety of activities, including B cell growth, differentiation, and death (35, 36), cytokine production by monocytes (37), and expression of leukocyte adhesion molecules on EC (38–40). Recent reports from several groups linked CD40/CD40L interaction to the mechanisms of apoptosis (41–43), a process in which ICE and other caspases play major roles, as reviewed elsewhere (44). We therefore tested the hypothesis that CD40L modulates the expression and/or activity of ICE and thus of IL-1β in cells of the vascular vessel wall, particularly smooth muscle and endothelial cells.

We demonstrate here that recombinant human CD40L (rCD40L) induces de novo synthesis of the IL-1β precursor and coordinates expression of a 20-kDa immunoreactive ICE protein with the expression of biological ICE-activity in human vascular smooth muscle and endothelial cells. Moreover, supernatants of the rCD40L-stimulated cultures, but not supernatants from cells exposed to a variety of other mediators, contained biological IL-1β activity.

**EXPERIMENTAL PROCEDURES**

Cell Isolation and Culture—Human vascular SMC were isolated from saphenous veins by explant outgrowth (45) and cultured in Dulbecco’s modified Eagle’s medium supplemented with 1% -glutamine, 1% penicillin/streptomycin (BioWhittaker, Walkersville, MD), and 10% FCS (Atlanta Biologicals, Norcross, GA). Human vascular EC were isolated from saphenous veins by collagenase treatment (1 mg/ml; Worthington Biochemicals, Freehold, NJ) and cultured in dishes coated with fibronectin (1.5 µg/cm²; Upstate Biotechnology Incorporated, Lake Placid, NY) as described elsewhere (46). Cells were maintained in medium 199 (M199; BioWhittaker), supplemented with 1% penicillin/streptomycin, 5% FCS, 100 µg/ml heparin (Sigma), and 50 µg/ml ECGF (endothelial cell growth factor; Pel-Freez Biological, Rogers, AR). Both cell types were subcultured following trypsinization (0.5% trypsin (Worthington Biochemicals), 0.2% EDTA (EM Science, Gibbstown, NJ)) in 75-cm² culture flasks (Becton Dickinson, Franklin Lakes, NJ) and used throughout passages 2 to 4. Culture media and FCS contained less than 15% t-aminohexanoic acid as determined by the amoebocyte assay-analysis (QLC-1000; BioWhittaker). Human vascular SMC and EC were characterized by immunostaining with an anti-smooth muscle cell a-actin (Dako, Carpinteria, CA) and anti-vWF antibody (von Willebrand factor; Dako), respectively. In some experiments, the cells were cultured for 24 h prior to the experiment in medium lacking FCS. Vascular EC were cultured in M199 supplemented with 0.1% human serum albumin (Immuno-US-Incorporated, San Diego, CA).

For detection of IL-1β activity, the mouse thymocyte cell line D10.G4.1 (kindly provided by Dr. Andrew Lichtman, Brigham and Women’s Hospital, Boston, MA) and human dermal fibroblasts were isolated and cultured as described previously (48).

**Metabolic Labeling and Immunoprecipitation—**Human vascular SMC or EC cultured in 75-cm² flasks were washed twice and incubated for 24 h prior to the experiment in the absence of serum. The medium was replaced by medium lacking methionine and cysteine but supplemented with rCD40L (49) in the presence of 60 µCi/ml [35S]protein labeling mix (NEN Life Science Products). All experiments were performed in the presence of the endotoxin inhibitor polymyxin B (1 µg/ml; Sigma). After 24 h at 37 °C, cells were harvested by scraping in immunoprecipitation buffer (50 mM Tris-HCl, 0.1% SDS, 0.1% sodium deoxycholate, 1% Nonidet P-40, 150 mM NaCl, 5 mM EDTA, 20 µg/ml soybean trypsin inhibitor, 0.1% NaN₃ phenylmethylsulfonyl fluoride, 0.2 units/ml aprotonin, 0.025% sodium azide). Cell-extracts were centrifuged (30 min, 4 °C, 10,000 × g) and supernatants were precleared with non-immune rabbit serum (18 h, 4 °C; Vector, Burlingame, CA). After centrifugation (10 min, 4 °C, 10,000 × g), proteins of the supernatants were immunoprecipitated (2 h, 4 °C) with the IL-1β-specific polyclonal rabbit antibody (Upstate Biotechnology Inc.). After addition of protein A-agarose (1.5 h, 4 °C; Life Technologies, Inc.), precipitates were centrifuged (2 min, 4 °C, 300 × g), and the pellet was resuspended in 50 µl of SDS-PAGE sample buffer (0.2 µTrits (pH 6.8), 5% glycerol, 0.1% SDS, 3% β-mercaptoethanol, 0.1 mg/ml bromphenol blue, final concentrations). After heating for 10 min at 95 °C, the samples were separated by SDS-PAGE, transferred on polyvinylidene difluoride membranes (Millipore, Bedford, MA), and exposed to autoradiography film (NEN Life Science Products).

Western Blotting—Cell extracts, equalized in total protein, were separated by standard SDS-PAGE under reducing conditions, and transferred to polyvinylidene difluoride membranes using a semi-dry blotting apparatus (3.0 mA/cm², 30 min; Bio-Rad, Hercules, CA). Blots were blocked (2 h.), and dilution of first and second antibody was made in 5% defatted dry milk, PBS, 0.1% Tween 20. After 1 h of incubation with the respective primary antibody (1:1,000 polyclonal rabbit anti-IL-1β (Upstate Biotechnology Inc.), 1:200 polyclonal goat anti-IL-1β-converting enzyme (M19, Santa Cruz Biotechnology, Santa Cruz, CA)) blots were washed four times (15 min in PBS, 0.1% Tween 20) and the secondary peroxidase-conjugated goat anti-rabbit antibody (1:10,000; Jackson ImmunoResearch, West Grove, PA) was added for another hour. Finally, after 4 times washing (20 min, PBS, 0.1% Tween 20), immunoblots were developed using the Western blot chemiluminescnece system (NEN Life Science Products) or the chromogenic system adding diaminobenzidine (50 µg/ml; Sigma) in substrate buffer (17 m citric acid, 65 m H2PO4, 0.1% H2O2, 0.01% (w/v) Thimerosal) to the blots. Independently produced antibodies directed against IL-1β (rabbit polyclonal anti-IL-1β (Santa Cruz) and the mouse monoclonal antibody F13b (50)) as well as ICE (rabbit polyclonal α-ICE (p51) antibody (51)) yielded similar results in the experiments performed, indicating that the reagents employed specifically recognize the intended proteins.

**Processing Assay—**Cultured human vascular SMC and EC as well as monocytes were harvested by scraping in processing buffer (10 mM HEPES, 1 mM dithiothreitol, 10% glycerol; final concentrations; Sigma). After 3 freeze-thaw cycles, 30 µl of cell extract (containing equal amounts of total protein as determined for control cultures) were resolved at 37 °C with 50 µg of recombinant human IL-1β precursor (pIL-1β; Cis, Pine Brook, NJ). All assays were performed in a final volume of 50 µl. The processing was stopped by adding 10 µl SDS-PAGE (5 ×) sample buffer and heating the samples (10 min, 95 °C). Finally, the samples were separated by SDS-PAGE and were analyzed by immunoblotting as described above. Specificity of the processing was analyzed by pre-incubation (10 min, 37 °C) of cell extracts with 100 µg ICE-inhibitor (An-Tyr-Val-Ala-Asp-H (aldehyde); Pepitide Institute, Osaka, Japan) (8) prior to addition of the precursor.

**Measurement of IL-1β Activity—**Human vascular SMC or EC were incubated for 24 h with the respective stimuli (None, rCD40L, or IL-1β) in the absence or presence of the anti-CD40L antibody (5 µg/ml). The culture supernatants were added in the absence or presence of the neutralizing IL-1β antibody (1 µg/ml; Endogen, Cambridge, MA) to (i) the murine thymocyte cell line D10.G4.1 or (ii) subconfluent fibroblast cultures (MRC-5 cells; 2 × 10⁶ cells/m). The IL-1 assay was performed as described previously (48, 52). Briefly, after 72 h of stimulation, cells were pulsed for the final 24 h with tritiated thymidine ([3H]thymidine, 5 µCi/well, NEN Life Science Products) in 96-well plates and harvested, and [3H]thymidine incorporation (disintegrations per minute per culture × S.D.) was determined. The mean of triplicate cultures was determined. Alternatively, fibroblasts were fixed with paraformaldehyde (2%), stained with crystal violet (10% in methanol), and lysed by incubation with 100 µl of SDS (1%), and finally, absorbancy was measured at 550 nm.

19570
Regulation of ICE Activity in Vascular Cells by CD40L

RESULTS AND DISCUSSION

Stimulation of Human Vascular Cells with Recombinant Human CD40L Induces de Novo Synthesis of the 33-kDa IL-1β Precursor—Human vascular SMC and EC express CD40 protein and respond to its ligand CD40L (30, 38–40). Stimulation of vascular cells with rCD40L induced concentration-dependent de novo synthesis of the 33-kDa IL-1β precursor, as shown for human vascular SMC by metabolic labeling and immunoprecipitation (Fig. 1). Induction of the protein required at least 1 µg/ml rCD40L. The precipitated IL-1β protein migrated at approximately 33 kDa as expected for the precursor form (53–55). Smaller forms of IL-1β, i.e. the biologically active mature form with a molecular mass of 17 kDa, were neither detected in cell extracts nor culture supernatants. Similar results were obtained with human vascular EC (data not shown).

Recombinant Human CD40L Increases Expression of a 20-kDa Immunoreactive ICE Protein—Human vascular SMC and EC produce the IL-1β precursor (1, 21, 56) but do not release mature forms of IL-1β upon stimulation with IL-1α, IL-1β, TNF, endotoxin etc. (51). We therefore further analyzed the effect of rCD40L on the expression and/or activation of the ICE, the enzyme responsible for production of biologically active, mature IL-1β (6–10). We first investigated whether or not stimulation of vascular cells affected the expression of ICE proteins. In monocytes or monocyctic cell lines, this enzyme exists as a 45-kDa zymogen, an intermediate form of 30 kDa, and active subunits of 20 (p20) and 10 kDa (p10) (6, 17). An antibody raised against the p20 subunit detects a 45-, 30-, and 20-kDa band in vascular SMC and EC (51). Neither regulation of these immunoreactive ICE proteins nor biologically active ICE forms have been previously found in vascular cells. However, stimulation of vascular cells through rCD40L increased the expression of the 20-kDa immunoreactive ICE protein, as illustrated here for SMC (Fig. 2). This increase did not occur in cells cultured in the presence of serum, an unphysiologic condition for SMC (47). Thus, the following experiments were performed using vascular cells cultured in the absence of serum. Howard et al. (57) showed that activation of ICE requires co-expression with IL-1β. We therefore further explored the influence of recombinant human mature IL-1β (rIL-1β) or rIL-1β/rCD40L co-stimulation on ICE expression. However, rIL-1β either alone or in combination with rCD40L did not alter the expression of the 20-kDa immunoreactive ICE protein (Fig. 2). Appearance of the 20-kDa immunoreactive ICE protein depended on the rCD40L concentration (Fig. 3A). Furthermore, the increase of the 20-kDa immunoreactive ICE protein depended on the time of stimulation with rCD40L, first detected after 2 h (Fig. 3B). The early detection of the ICE protein could be due to processing rather than de novo synthesis of the constitutively expressed ICE precursor. The increasing strength of the 20-kDa band, compared with the weak zymogen band, may be due to additional de novo synthesis and subsequent processing of the ICE precursor, which is highly autocatalytic. In addition to the p20 subunit, active ICE contains the p10 subunit (12). We did not detect the p10 subunit in these analyses because the anti-p20 antibody used does not recognize p10.

Induction of IL-1β Converting Enzyme Activity in Vascular Cells by Recombinant Human CD40L—To analyze whether the increase of the 20-kDa immunoreactive ICE protein in
Western blotting with an IL-1β-antibody. Aliquots (30 μl) of these cell extracts (20,000 cells/μl) were incubated for 10 min at 37°C with pIL-1β (50 ng/μl; 33 kDa) and analyzed by Western blotting with an IL-1β-specific antibody. As a control, monocytic cell extracts (MØ) were also applied. Time- and concentration-dependence of the induction of ICE-activity was investigated using cell extracts from SMC cultures stimulated for 24 h with the indicated concentrations of rCD40L (B) or with 5 μg rCD40L/ml for the indicated times (C). Cell extracts were analyzed for processing of exogenous pIL-1β as described above. Specificity of the processing was analyzed by pre-incubating cell extracts from cultures stimulated for 24 h with 10 μg/ml rCD40L with an ICE-inhibitor, before addition of the recombinant IL-1β precursor. As a standard, recombinant mature human IL-1β (rIL-1β, 20 ng/ml; 17 kDa) was applied on the right lanes.

rCD40L-stimulated vascular cells correlates with the induction of biologically active ICE, we performed processing assays in which cell extracts were incubated with recombinant human IL-1β precursor (pIL-1β). We monitored processing of exogenous pIL-1β into smaller fragments of the cytokine by Western blot analysis. Extracts of vascular cells stimulated with rCD40L, but not extracts from unstimulated or IL-1β-stimulated cultures, processed pIL-1β to an IL-1β immunoreactive form of approximately 17 kDa (Fig. 4A). The cleavage product obtained in the processing assay using lysates of rCD40L-stimulated vascular cells comigrated with the band observed with monocytic cell extracts. Monocytic cell extracts, used here as a positive control, express biologically active ICE (8), which cleaves the IL-1β precursor into the mature 17-kDa form. Recombinant human CD40L, coordinate the induction of IL-1β processing activity and the increase of the 20-kDa immunoreactive ICE protein. Detection of IL-1β processing activity required extracts of SMC or EC stimulated with at least 1 μg/ml rCD40L (Fig. 4B) for 2–6 h (Fig. 4C), a concentration-and time-dependence described above for the induced increase of the 20-kDa immunoreactive ICE protein. A selective ICE-inhibitor (8) inhibited the processing of pIL-1β (Fig. 4, B and C). Extracts of SMC or EC cultures stimulated with rCD40L (5 μg/ml) processed the precursor within 1–2 min (Fig. 5A) and required material derived from at least 10,000 cells/μl (Fig. 5B). Compared with the original source of ICE, the monocyte, extracts of human vascular SMC and EC contained approximately 30-fold less ICE activity. Specific ICE activity was obtained by using monocyte extracts of 0.3–1.6 mg/ml total protein, whereas SMC or EC extracts of 10–50 mg/ml total protein were required. However, SMC comprise the most numerous cell type in arteries, which normally contains few if any monocytes. Therefore, activation of ICE by CD40 ligation on human vascular SMC may be crucial to initiate the inflammatory response in the normal vessel wall. Thus SMC, rather than monocytes, may be the biologically relevant cell type for local IL-1β precursor processing at sites of initiation of vascular inflammatory responses.

**Human Vascular SMC and EC Release Biologically Active IL-1β after Stimulation through rCD40L—As demonstrated above, rCD40L-induced IL-1β precursor expression in vascular cells and also activated ICE. However, we did not detect native, mature immunoreactive IL-1β in extracts or supernatants of SMC and EC cultures stimulated with rCD40L in radioimmunoprecipitation (Fig. 1) or Western blot experiments (data not shown). These findings may result from a concentration of mature IL-1β produced by SMC or EC below the detection limit of these techniques.** Thus, we employed bioassays to detect IL-1β activity in the supernatants of rCD40L-stimulated vascular cells. Supernatants of rCD40L-stimulated SMC markedly enhanced the IL-1-dependent proliferation of the mouse thymocyte cell line D10.G4.1 (Fig. 6). The induction of the prolif-
CD40L, as well as IL-1, is involved in atherogenesis, as cells in atheroma express CD40, its ligand. This pathway may have particular relevance for IL-1-induced sclerotic diseases. Moreover, inhibition of CD40L/CD40 signaling may represent a therapeutic strategy for inflammatory and host defense processes involving blood vessels. Ligation may prove important in initiating and sustaining inflammation in the vessel wall. We employed (proliferation of D10.G4.1 cells or human dermal fibroblasts) yielded similar results (data not shown). The proliferation of D10.G4.1 cells or human dermal fibroblasts depended on the concentration and required ≥1 μg/ml rCD40L (Fig. 6B). Thus, the concentration dependence of the induction of biological IL-1β activity in the supernatant correlated with the concentration required for both, the induction of ICE activity and the expression of the IL-1β precursor. Both of the conventional IL-1 bioassays employed (proliferation of D10.G4.1 cells or human dermal fibroblasts) yielded similar results (data not shown).

The presented data indicate that CD40/CD40L interaction regulates IL-1β activity in the vessel wall by both induction of IL-1β precursor expression and activation of the IL-1β-converting enzyme. This pathway may have particular relevance for atherogenesis, as cells in atheroma express CD40, its ligand CD40L, as well as IL-1β, a cytokine implicated in regulation of many aspects of vascular pathology. As other stimuli tested previously (e.g., IL-1α, IL-1β, TNFα, IL-8, and endotoxin) do not cause release of active IL-1β from vascular wall cells, CD40 ligation may prove important in initiating and sustaining inflammatory and host defense processes involving blood vessels. Moreover, inhibition of CD40L/CD40 signaling may represent a novel therapeutic target in arterial inflammation and athero-sclerotic diseases.

Acknowledgments—We thank Maria Muszynski, Curran Murphy, and Elissa Simon-Morrissey (Brigham & Women’s Hospital) for skillful assistance.

REFERENCES

1. Lopovop, H., and Libby, P. (1992) Exp. Cell Res. 196, 283–290
2. Lonnemann, G., Endres, S., van der meer, J. W., Cannon, J. G., Koch, K. M., Lonnemann, G., Endres, S., van der Meer, J. W., Cannon, J. G., Koch, K. M., van der Meer, J. W., and Diwan, V. M. (1995) Cell 81, 801–808
3. MOSLEY, B., Dower, S. K., Gillis, S., and Cosman, D. (1987) J. Biol. Chem. 262, 7081–7086
4. Black, R. A., Kronheim, S. R., Cantrell, M., Chambers, S. P., Aldape, R. A., Raybuck, S. A., and Lonnemann, G. (1989) Nature 338, 702–707
5. Howard, A. D., Kostura, M. J., Thompken, N. A. D., Ding, C. K., Jinjaco, G., Weidner, J., Saley, J., Pogue, K. A., Chaplin, D. P., Dunford, R. A., Schmidt, J. A., and Tocci, M. J. (1991) J. Immunol. 147, 2864–2869
6. Creer, D. P., Koziolczy, K. J., Mosley, B., Nelson, N., Van Ness, K., Greenstreet, T. A., March, C. R., Kronheim, S. R., Druck, T., Cunnell, A. L., Huebner, E., and Black, R. A. (1992) Science 256, 97–99
7. Walker, N. P. C., Talanian, R. V., Brady, K. D., Dang, L. C., Bump, N. J., Ferenz, C. R., Franklin, S. G., Guyard, T., Hackett, M. C., Hammill, D. L., Bung, L., Huglin, M., Houy, M., Wankovich, J. A., McGuiness, L., Orlowicz, E., Paskin, M., Pratt, C. A., Reif, P., Sunnannaa, M., Welch, J. P., Xiong, L., Moller, A., Tracey, D. E., and Kamen, R. (1994) Cell 78, 379–388
8. Wilson, K. P., Black, J.-A. F., Thomson, J. A., Kim, E. E., Griffith, J. P., Navia, M. A., Muroek, M. A., Chambers, S. P., Aldape, R. A., Raybuck, S. A., and Lonnemann, G. (1994) Nature 370, 270–275
9. Alnemri, E. S., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wang, W. W., and Yuan, J. (1999) Cell 97, 171
10. Sleath, P. R., Hendrickson, R. C., Kronheim, S. R., March, C. J., and Black, R. A. (1990) J. Biol. Chem. 265, 14526–14528
11. Tewari, M., Quan, T. L., O'Rourke, K., Desnoyers, S., Zeng, Z., Beider, D. R., Porier, G. G., Salvesen, G. S., and Dix, V. M. (1994) Cell 84, 801–808
12. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Labezken, Y. A., Munday, N. A., Rauj, S. M., Smulson, M. E., Yamin, T.-Y., Yu, V. L., and Miller, D. K. (1995) Nature 376, 37–43
13. Yamin, T.-Y., Ayala, J. M., and Miller, D. K. (1996) J. Biol. Chem. 271, 13273–13282
14. Dustin, M. L., Rothlein, R., Ruan, B. A., Dinarello, C. A., and Springer, T. A. (1989) J. Exp. Med. 170, 78, 1091–1101
15. Lederman, S., Yellin, M. J., Krichevsky, A., Belko, J., Lee, J. J., and Chess, L. (1992) J. Exp. Med. 175, 1081–1091
16. Noelle, J. R., Yoffe, T. M., Shepherd, D. M., Stamenkovic, I., LEDbetter, J. A., and Aruffo, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6556–6554
17. Armitage, R. J., Fanslow, W. C., Strickshine, L., Sato, T. A., Clifford, K. N., Macduff, B. M., Anderson, D. M., Gimpel, S. D., Davis-Smith, T., Maliszewski, C. R., Clark, E. A., Smith, C. A., Grabstein, K. H., Cosman, D., and Spriggs, M. K. (1992) Nature 357, 80–82
18. Graf, D., Konrathauer, U., Mages, H. W., Senger, G., and Kroczek, R. A. (1992) Eur. J. Immunol. 22, 3191–3194
19. Foy, T. M., Aruffo, A., Bajorek, J., Buhlmann, J. E., and Noelle, R. J. (1996) Annu. Rev. Immunol. 14, 591–617
Regulation of ICE Activity in Vascular Cells by CD40L

36. Gauchat, J.-F., Henchoz, S., Mazzei, G., Aubry, J.-P., Brunner, T., Blasey, H., Life, P., Talabot, D., Flores-Romo, L., Thompson, J., Kishi, K., Butterfield, J., Dahinden, C., and Bonnefoy, J.-Y. (1993) Nature 365, 340–343
37. Alderson, M. R., Armitage, R. J., Tough, T. W., Stockshine, L., Fanslow, W. C., and Springs, M. K. (1993) J. Exp. Med. 178, 669–674
38. Alderson, M. R., Armitage, R. J., Tough, T. W., Strockbine, L., Fanslow, W. C., and Springs, M. K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4342–4346
39. Hollenbaugh, D., Mischel-Petty, N., Edwards, C. P., Simons, J. C., Denfeld, R. W., Kiener, P. A., and Aruffo, A. (1995) J. Exp. Med. 182, 33–40
40. Yellin, M. J., Brett, J., Baum, D., Matsushima, A., Szablocs, M., Stern, D., and Chess, L. (1995) J. Exp. Med. 182, 1857–1864
41. Holder, M. J., Wang, H., Milner, A. E., Casamayor, M., Armitage, R., Spriggs, M. K., Fanslow, W. C., MacLennan, I. C. M., Gregory, C. D., and Gordon, J. (1993) Eur. J. Immunol. 23, 2368–2371
42. Merino, R., Grillot, D. A. M., Muthukumar, S., Fanslow, W. C., Bondada, S., and Nunez, G. (1995) J. Immunol. 155, 3830–3838
43. Ruggiero, G., Caceres, E. M., Voordouw, A., Noteboom, E., Graf, D., Kroczek, R. A., and Spits, H. (1996) J. Immunol. 156, 3737–3746
44. Henkart, P. A. (1996) Immunity 4, 195–201
45. Ross, R., and Kariya, B. (1980) in Handbook of Physiology, The Cardiovascular System (Ilohr, D. F., Somlyo, A. P., and Sparks, H. Y., eds) pp. 66–91, American Physiological Society, Bethesda, MD
46. Gibb, M. A., Jr., Cotran, R. S., and Folkman, J. (1974) J. Cell. Biol. 60, 673–684
47. Libby, P., and O’Brien, K. (1983) J. Cell. Physiol. 115, 217–223
48. Loppnow, H., Flad, H.-D., Durrbaum, I., Musehold, J., Petting, R., Ulmer, A. J., Herzberg, H., and Brandt, E. (1989) Immunobiology 179, 283–291
49. Mazzei, G. J., Edgerton, M. D., Losberger, C., Lecoanet-Henchoz, S., Grauer, P., Durandy, A., Gauchat, J.-F., Bernard, A., Allet, B., and Bonnefoy, J.-Y. (1995) J. Biol. Chem. 270, 7025–7028
50. Herzberg, H., Blum, B., Rosspeck, W., Frenzel, B., Brandt, E., Ulmer, A. J., and Flad, H.-D. (1989) Scand. J. Immunol. 30, 549–562
51. Schönbeck, U., Herzberg, M., Petersen, A., Wohlenberg, C., Gerdes, J., Flad, H.-D., and Loppnow, H. (1997) J. Exp. Med. 185, 1287–1294
52. Orencole, S. F., and Dinarello, C. A. (1990) Cytokines I, 14–22
53. Auron, P. E., Webb, A. C., Rosewater, L. L., Mucci, S. F., Alexander, R., Wolff, S. M., and Dinarello, C. A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7907–7911
54. Clark, B. D., Collins, K. L., Gandy, M. S., Webb, A. C., and Auron, P. E. (1986) Nucleic Acids Res. 14, 7897–7900, Correction (1987) Nucleic Acids Res. 15, 568
55. March, C. J., Mosley, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Gillis, S., Henneey, C. S., Kranheim, S. H., Grabstein, K., Conlon, P. J., Hopp, T. P., and Cosman, D. (1985) Nature 315, 641–647
56. Libby, P., Ordovas, J. M., Auger, K. R., Robbins, A. H., Birinyi, L. K., and Dinarello, C. A. (1986) Am. J. Pathol. 124, 179–185
57. Howard, A. D., Oksana, C. P., Griffin, P. R., Peterson, E. P., Lenny, A. B., Ding, G. J.-F., Pickup, D. J., Thornberry, N. A., Schmidt, J. A., and Toce, M. J. (1995) J. Immunol. 154, 2321–2332