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Monocytes Stimulate Expression of the Bcl-2 Family Member, A1, in Endothelial Cells and Confer Protection Against Apoptosis

Karen E. Noble1, R. Gitendra Wickremasinghe, Chris DeCornet, Panayiotis Panayiotidis, and Kwee L. Yong2

We have investigated the molecular mechanisms underlying the ability of peripheral blood monocytes to block apoptosis induction in endothelial cells. Monocytes stimulated the expression of the bcl-2 homologue A1 in serum-starved endothelial cells after 6 h of coincubation, with elevated A1 levels persisting for up to 21 h. IL-1 and TNF also stimulated A1 expression at 6 h, but A1 transcript levels fell by 21 h. Direct cellular contact with monocytes was required for stimulation of A1 mRNA in endothelial cells. Stimulation of endothelial cell A1 mRNA by monocytes was not inhibited by anti-β2 integrin Abs, but anti-platelet endothelial cell adhesion molecule-1 (PECAM-1) mAb reduced A1 transcript levels at 21 h. Studies employing either TNF on its own, or anti-TNF in endothelium/monocyte cocultures showed that TNF plays a role in the early (6-h) stimulation of A1, but is less important for the sustained elevation of A1 levels at 21 h. Serum-starved endothelial cells demonstrated increased survival and decreased apoptosis after coculture with monocytes. IL-10 reduced A1 mRNA expression in, as well as survival of, endothelial cells that were cocultured with monocytes. In comparison with A1, Bcl-2 was expressed at low levels and was up-regulated by monocytes only at 21 h, while neither Bax nor Bcl-xL levels were altered by monocytes. The interaction of monocytes with endothelium during the course of an inflammatory reaction may provide survival signals to endothelial cells. The Journal of Immunology, 1999, 162: 1376–1383.

Homoeostasis of the vascular endothelium is maintained by the balance between cell proliferation and cell death. Apoptosis of vascular endothelial cells (EC)3 accompanies the vascular restructuring that occurs throughout growth and development as well as in tissue repair and remodeling. On the other hand, EC damage and apoptosis may also contribute to disease pathology, such as in the endothelial leakage syndrome and veno-occlusive disease that complicate bone marrow transplantation, perhaps as a consequence of radiation (1). Critically situated at the boundary between blood and tissues, the endothelium is a focus for inflammatory and immune processes. EC receive signals from humoral factors, inflammatory mediators, and physical forces from both circulation and tissues. These signals not only alter EC function and behavior, in many cases inducing proinflammatory and prothrombotic properties, but may also influence the survival and integrity of vascular endothelium. LPS (2), TNF-α (3), and other cytokines, for example, induce EC death (4), while other growth factors such as fibroblast growth factor and vascular endothelial growth factor delay apoptosis (5).

Direct cell:cell contact can also influence cellular responses, and adherent leukocytes recruited to an area of inflammation may alter EC function and survival. Monocytes are found consistently at sites of inflammation. The recruitment of these cells from peripheral blood is considered to involve sequential adhesive events at the vascular interface (6). There is now increasing evidence that such adhesive events are not only the basis for cell migration, but can also lead to cellular activation, thus playing a role in the regulation of inflammatory responses (7, 8). Monocytes interacting with unstimulated endothelium are induced to express tissue factor, mediated in part by engagement of ICAM-1 (9). Monocytes also induce expression of E-selectin on EC via a cell:cell contact-dependent pathway in which β2 integrins play a role (10). In a similar manner, monocytes may also influence the integrity and survival of EC.

The balance between cell survival and apoptosis is dependent upon the relative expression of specific genes whose products interact to determine the final outcome of apoptotic signals. Proteins belonging to the bcl-2 family appear to play a central and prominent role in maintaining cell viability (11). bcl-2 is an intracellular protein that blocks apoptosis and prolongs survival (12). EC express bcl-2 at low levels, but also the bcl-2 homologues A1 (13) and bcl-xL (8), which promote cell survival, as well as Bax, which causes accelerated apoptosis. While bcl-2 is up-regulated by growth factors (5), A1 appears to be regulated by inflammatory cytokines (13).

In this study, we have examined the ability of monocytes to influence expression of A1 in EC and investigated whether coculture with monocytes can rescue EC from apoptosis. We have also characterized the cellular interactions responsible for these effects.

Materials and Methods

Cell isolation and culture

Endothelial cells. EC were isolated from human umbilical veins by collagenase treatment and established in culture in Iscove’s modified Dulbecco’s medium (Sigma, Poole, U.K.), 20% FCS (Seralab, Loughborough, London, United Kingdom

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2 Address correspondence and reprint requests to Dr. Kwee L. Yong, Department of Haematology, University College London, 98 Chenes Mews, London WC1E 6HX, U.K.
3 Abbreviations used in this paper: EC, endothelial cell(s); PECAM, platelet endothelial cell adhesion molecule; PI, propidium iodide.

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Table I. Primers used for amplification of A1 and for construction of mimic template

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>A1 forward</td>
<td>GAC TAT CTT CAG TGC GTC CTA CAG</td>
</tr>
<tr>
<td>A1 reverse</td>
<td>GGG CAA TTT GCT GTC GTA GAA G</td>
</tr>
<tr>
<td>Luciferase forward</td>
<td>TCG TG TAT GCA CAA A AA ACT CTC</td>
</tr>
<tr>
<td>Luciferase reverse</td>
<td>TAC CTT ACG CAG CCA GTT CTA TG</td>
</tr>
</tbody>
</table>

U.K.), 20 U/ml heparin, and 50 μg/ml EC growth supplement (Sigma) on fibronectin-coated tissue culture flasks.

Leukocytes. Monocytes were isolated from peripheral blood by starch sedimentation and centrifugation over Nycroprep (S.G.1.068), followed by platelet depletion by centrifugation over autologous plasma, as detailed elsewhere (10). Neutrophils were purified from venous blood using double density centrifugation (Histopaque 1119 and 1077; Sigma), and washed twice in HBSS with 5 mM glucose and 2% FCS. Neutrophils of by this method were >95% pure, as assessed by morphology, and >99% viable by trypan blue exclusion. To obtain lymphocytes, mononuclear cells were depleted of monocytes by incubating with anti-CD14-coated Dynabeads for 10 min at room temperature. The resulting lymphocyte suspensions contained less than 1.5% monocytes, as determined by double staining for CD3 and CD14 and analysis by flow cytometry.

All reagents used for cell isolation were screened routinely for endotoxin contamination using the E-Toxase Limulus amoebocyte lysate assay (Sigma), and contained less than 0.03 endotoxin U/ml.

Serum starvation of EC

Confluent EC in 25-cm² flasks or six-well plates were washed with HBSS and then serum starved using 0.1% FCS and a supplement of insulin/transferrin/sodium selenite (RPMI/0.1% FCS/TIS) for 5 h. EC cultures were then coincubated with monocytes, lymphocytes, or neutrophils resuspended in RPMI/0.1% FCS/TIS at 1 × 10⁶ cells/flask (1:1 cell ratio), IL-1 (20 U/ml), TNE (100 U/ml), or medium as control for up to 21 h. In some experiments, monocytes were separated from EC by millipore filters (0.45 μm, Transwell; Costar, Cambridge, MA) to prevent direct cell:cell contact. Functional inhibition experiments were performed by preincubating monocytes with mAbs against CD11b (M1/70, Boehringer Mannheim, East Sussex, U.K.); 7E3, a gift of Dr. Barry Coller, Stony Brook, NY); CD18 (60.3, gift of Dr. John Harlan, University of Washington, Seattle), VLA-4 (Becton Dickinson, Cowley, U.K.), L-selectin (DREG-56, gift of Dr. Rothlein, Boehringer Ingelheim, CT), or EC with anti-platelet endothelial cell adhesion molecule-1 (PECAM-1) (1:3, gift of Dr. P. Newman, Blood Research Institute, Milwaukee, WI) for 5 min at room temperature before use in cocultures. All mAbs were used at 20 μg/ml. In all experiments, an isotype-matched mAb was used as a negative control. In experiments designed to investigate the role of TNF and IL-1 in EC/monocyte interactions, an isotype-matched mAb was used as a negative control. In exper-

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RNA extraction and competitive RT-PCR

Monocytes were removed from EC by incubation with 2 mM EDTA, at 37°C for 3 min, and EC were harvested from the flasks using trypsin/EDTA. The purity of the resulting EC preparation was >99%, as assessed by staining with anti-CD14 mAb for contaminating monocytes using the alkaline phosphatase anti-alkaline phosphatase method. Total RNA was isolated as described (14). One microgram of RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Life Technologies). Each reaction contained a fixed amount of the appropriate PCR mimic as an internal standard. The primers used for amplification of A1 cDNA are detailed in Table I. The reaction was conducted for 34 cycles using an annealing temperature of 56°C, yielding a 286-nucleotide product from A1 CDNA and a 470-nucleotide product from the A1 mimic. PCR products were fractionated by agarose gel electrophoresis and photographed under UV illumination. Band intensities were quantified by laser densitometric scanning (Molecular Dynamics, Sunnyvale, CA; Personal Densitometer) and normalized with respect to the intensity of the mimic band obtained in each amplification. The results were expressed as a ratio of the A1 band intensity relative to the intensity of the actin band obtained by amplification of the same cDNA (A1/A1 mimic divided by actin/actin mimic). Data relating to bcl-2, bcl-x₀, or bax band intensities were treated in an analogous fashion.

Determination of cell survival

Adherent cells were removed with the use of trypsin/EDTA. Nonadherent and adherent cells were pooled and centrifuged at 1000 rpm for 5 min. Cell viability was assessed using trypan blue exclusion. Cytosin preparations were stained with May-Grunwald-Giemsa. Apoptotic cells were identified by the presence of nuclear condensation and fragmentation (17). All slides were assessed by two observers in a blinded fashion. The remaining cells were used in a FACs-based assay employing dual staining with annexin V-FITC/propidium iodide (PI, Apoptosis Detection Kit; R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. Samples were analyzed on a flow cytometer (FACScan; Becton Dickinson).

Statistics

Results are expressed as mean ± SEM. Data were compared using Student’s t test, and a p value of <0.05 was considered significant.

Results

Monocytes stimulate A1 expression in EC

EC were cultured to confluence and rinsed, and the culture medium was replaced with serum-free medium, as detailed above. After 5 h, monocytes (1 × 10⁶/flask), IL-1 (20 U/ml), or medium alone was added to each flask. Following removal of monocytes, EC were harvested and analyzed for A1 and actin mRNA expression by RT-PCR. EC grown in complete culture medium (EC − 5 h) expressed A1 mRNA, which was decreased after 5 h of serum starvation (EC + 0 h, p < 0.01, Fig. 1A). EC cocultured with monocytes for an additional 6 h (EC + MO + 6 h) contained more A1 mRNA (A1:actin ratio 0.33 ± 0.03) than did EC cultured alone for the same period of time (EC + 6 h, A1:actin ratio 0.03 ± 0.01, p < 0.01, n = 5). Peripheral blood monocytes do not express A1 mRNA under these conditions (Fig. 1A), and thus could not have contaminated RT-PCR of EC. In addition, EC harvested for RT-PCR and survival analysis contained less than 1% contaminating monocytes, as determined by staining with anti-CD14.

The ability of monocytes to augment A1 expression was tested more rigorously by varying the input of selected cDNA samples over 100-fold range while maintaining constant levels of mimic templates in PCR reactions (Fig. 1B). When cDNA from serum-starved EC cocultured with monocytes (EC + MO + 6 h) was tested, the relative intensity of the A1 amplification product was at least equivalent to or exceeded that of the actin product when 0.1 or 1 μl of cDNA was used. When cDNA from control EC (11-h serum starvation in the absence of monocytes, EC + 6 h) was tested, the relative intensities of A1 PCR products were substantially lower than that of actin products at all input levels (Fig. 1B). The A1 band intensity became equivalent to the A1 mimic band intensity at CDNA inputs between 0.1 and 1 μl in the EC + MO + 6 h sample. In contrast, A1 bands did not achieve equivalence with the mimic bands when the EC + 6 h sample was tested, even at cDNA input levels of 1 μl. A similar titration using cDNA from EC after 5-h serum starvation, i.e., before the addition of monocytes (EC 0 h), additionally confirmed that A1 mRNA expression was very low in these cells (data not shown).
Stimulation with IL-1 (EC + IL-1) also increased A1 expression in serum-starved EC (Fig. 1, A and C). Expression of A1 in response to IL-1 was short-lived, in that it declined by more than one-half at 21 h (A1:actin ratio 0.07 ± 0.06 at 21 h, cf 0.23 ± 0.05 at 6 h, p < 0.01, n = 4, Fig. 1C). In contrast, the stimulation of A1 expression by monocytes was sustained (Fig. 1C), and levels at 21 h were not significantly lower than those at 6 h.

Neither neutrophils nor lymphocytes had any significant effect on the expression of A1 in EC under these conditions (Fig. 2). Monocyte suspensions contained a variable (5–15%) number of contaminating lymphocytes. Despite repeated attempts to deplete CD3+ cells, we never achieved less than a 5% level of contaminating lymphocytes. Therefore, we cannot discount the possible contribution of a small number of lymphocytes to the A1 response seen in our EC/monocyte cocultures.

**Monocytes increase survival of EC**

In one series of experiments, the degree of apoptosis in EC cultures was assessed by morphologic analysis of cytospin preparations. Serum starvation increased the percentage of apoptotic EC, from 6 ± 1.76% to 28.7 ± 5.8% at 21 h (p < 0.01, n = 4). When serum-starved EC were cultured in the presence of monocytes, however, there was a significant decrease in apoptosis, to 18 ± 3.2% (p < 0.01, n = 4, Fig. 3A).

These results obtained by morphologic assessment of apoptosis were confirmed by FACS analysis using annexin V-FITC/PI staining of EC. Apoptotic cells stain positive for annexin V, while necrotic cells take up both PI and annexin V. Thus, live cells are found in the dual negative (lower left) quadrant. Fig. 3B shows FACS histograms from one representative experiment. In four experiments, the percentage of live EC in serum-starved cultures was

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FIGURE 1. Monocytes stimulate A1 mRNA in EC. A, Confluent EC were subjected to serum starvation for 5 h before the addition of monocytes or IL-1 (at 0 h), and were then incubated for an additional 6 or 21 h. RT-PCR for A1 mRNA was performed as described. Times are given relative to the addition of monocytes/IL-1 to serum-starved cultures of EC. MO, monocytes; EC −5 h, EC grown in complete medium; EC −2 h, EC after 3-h serum starvation; EC + MO, EC cocultured with monocytes; EC + IL-1, EC stimulated with IL-1 (20 U/ml). Bands from one representative experiment are shown, A1:actin ratios are displayed as a histogram at the bottom of the figure. Open triangles, mimic bands; closed triangles, specific PCR product. The position of m.w. markers is indicated. B, Comparison of A1 mRNA and actin mRNA levels at varying dilutions of input cDNA. cDNA (0.01–1 μl) from EC after 11 h of serum starvation (EC + 6 h), or after 5-h serum starvation, followed by 6-h incubation with monocytes (EC + MO + 6 h), were used in competitive PCR assays for actin or A1 sequences. A1 mimic templates were used at a 100-fold lower concentration than in A. C, Monocytes stimulate EC expression of A1 mRNA with different kinetics compared with IL-1. Data from four experiments are given as the mean ± SE of the A1:actin ratios.
62.7 ± 4.8%, which was significantly lower than control EC grown in complete medium (87.8 ± 3.7%, p < 0.01, n = 4). Coculture with monocytes increased the proportion of live EC in serum-starved cultures to 75.8 ± 5.3 (p < 0.05, n = 4, Fig. 3C). Apoptotic cells eventually take up PI because of loss of integrity of the cell membrane, and thus appear in the annexin-positive, PI-positive upper right quadrant. Hence, the percentage of apoptotic EC determined by this method is considerably lower than that obtained by morphologic analysis.

**Monocyte stimulation of A1 expression requires cell:cell contact**

To determine whether contact between monocytes and EC was needed to stimulate A1 expression, additional experiments were conducted in which monocytes were cultured on a 0.45-μm filter above EC. When contact between monocytes and EC was prevented in this way (EC + MO filter, Fig. 4A), EC displayed much lower A1 mRNA levels when compared with EC that had been cocultured in contact with monocytes (EC + MO, Fig. 4A). In two separate experiments that were analyzed at 6 h, the levels of A1 mRNA were reduced by 100 and 57% in EC separated from monocytes, compared with parallel cultures in which EC were in contact with monocytes. When A1 expression was determined at 21 h, there was 50.2 ± 7.5% reduction in A1 mRNA levels in EC + MO on filters, compared with contact cocultures (p < 0.01, n = 4, Fig. 4B). This suggests that contact between monocytes and EC is required to achieve optimal induction of A1 expression in HUVEC.

One possible explanation for these findings is that adherent monocytes are necessary to generate a signal in EC that leads to increased A1 expression. Alternatively, monocyte adherence may result in the release of a soluble factor that stimulates A1 expression. We attempted to distinguish between these two possibilities by plating EC on a 0.45-μm filter above an EC/monocyte coculture. EC cocultured with monocytes (EC + MO +21 h) showed greatly augmented A1 levels, while EC that had been cultured on the filter above the coculture (EC above EC + MO +21 h) displayed much lower A1 levels, which were only slightly higher than control EC (EC +21 h, Fig. 4C). EC cultured on filters showed increased A1 expression when stimulated with IL-1 (A1:actin ratio

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**FIGURE 3.** Monocytes increase survival of serum-starved EC. EC cultures were set up essentially as described, and cells were harvested at 21 h for survival analysis. A, Cytospin preparations were assessed for morphologic evidence of apoptosis. Mean ± SE of percentage apoptotic cells in three separate experiments. B, Dual staining with annexin V-FITC/PI was used to determine the percentages of live, apoptotic, and necrotic cells. FACS histograms from one representative experiment are shown. The percentages of live EC are as follows: control nonserum-starved EC, 90.2%; serum-starved EC, 61.5%; and serum-starved EC cocultured with monocytes, 76.5%. C, Results of three separate experiments are shown, and SEs have been omitted for clarity, but did not exceed 5% of the mean values shown. S/S, serum-starved.
0.11 at 21 h), confirming that culture on filters per se did not alter the ability of the cells to respond to agonists. We conclude therefore that monocytes in contact with EC induce A1 expression at least in part by contact-dependent mechanisms, which may involve the ligation of cell adhesion receptors.

Role of adhesion molecules in monocyte stimulation of A1 mRNA expression in EC

We therefore investigated whether the $\beta_2$ integrin/ICAM-1 adhesion pathway was important for the increase in A1 expression by EC induced after coculture with monocytes. FACS analysis of serum-starved EC confirmed that these cells expressed low/intermediate levels of ICAM-1, but undetectable levels of VCAM-1 and E-selectin (data not shown), a pattern similar to that in noncytokine-stimulated EC. Monocyte adherence to endothelium is mediated partly by $\beta_2$ integrins, and our initial studies confirmed that mAbs directed at either the $\alpha_5$-chain (CD11b) or the $\beta_2$-chain (CD18) were effective in blocking at least 50% of adhesion of monocytes to serum-starved EC (data not shown). When these same mAbs were included in EC/monocyte cocultures, however, there was no alteration in A1 mRNA, compared with control cocultures incubated with isotype-matched IgG (Fig. 5A). In contrast, anti-CD31 (PECAM-1) reduced A1 expression in EC/monocyte cocultures by 50 ± 6% at 21 h (Fig. 5B). Anti-CD31 had no effect on basal levels of A1 mRNA in EC cultures (data not shown).

Role of TNF in the induction of A1 expression

TNF is produced by monocytes and has been reported to cause up-regulation of A1 gene expression in EC (13). To determine a possible contribution of TNF to the induction of A1 in EC following coculture with monocytes, we included anti-TNF mAb at the start of coculture. Fig. 6 shows that the inclusion of anti-TNF mAb to EC/monocyte cocultures produced an almost complete inhibition of the A1 response at 6 h. At 21 h of coculture, however, anti-TNF mAb only achieved a modest reduction of 32.2 ± 15%. Interestingly, the inclusion of anti-TNF mAb completely abolished any increase in A1 expression when monocytes were separated from EC by filters (data not shown), suggesting that monocyte-derived TNF may be responsible for the soluble component of the A1 response in EC/monocyte cocultures. In contrast, anti-IL-1 mAb did not affect A1 mRNA expression either in EC/monocyte cocultures or when monocytes were cultured on filters (data not shown).

The addition of exogenous TNF to serum-starved EC led to an increase in A1 expression at 6 h, which reached 86 ± 14% of that induced by monocytes in parallel cultures. At 21 h, however, the level of A1 mRNA expression in TNF-stimulated EC was only 23 ± 12% of that in EC cocultured with monocytes. These results confirm that TNF contributes significantly to the early increase in A1 levels in EC/monocyte cocultures, but is less important for the sustained increase in A1 at 21 h. These results are consistent with the lesser inhibitory action of anti-TNF mAb at 21 h (see above).

Effect of IL-10 on monocyte-induced A1 expression in EC

Stimulation of TNF synthesis in monocytes is followed by the induction of IL-10, which acts in an autocrine fashion to inhibit further TNF production (18), thus limiting the inflammatory response. When IL-10 was added to EC/monocyte cocultures, no induction of A1 expression was seen at 6 h (Fig. 7A). Hence, IL-10 blocked the ability of monocytes to increase A1 expression in EC after 6 h of coculture. The inhibitory effect of IL-10 was less evident when A1 expression was analyzed at 21 h (Fig. 7A). This
provides further support for a role for TNF in the initial induction of A1 expression by monocytes, while suggesting that non-TNF-dependent pathways mediate the sustained levels of A1 at later time points.

We then asked whether the inhibitory effect of IL-10 on A1 expression by EC in cocultures was reflected in decreased survival of EC. When IL-10 was present in EC/monocyte cocultures, the percentage of apoptotic cells was increased compared with control cocultures in the absence of IL-10 (Fig. 7B). Thus, IL-10 inhibited A1 induction and also decreased survival of EC cocultured with monocytes.

Effect of monocytes on expression of other apoptosis-related genes in EC

*bax* mRNA was constitutively expressed in EC, and levels were not altered by serum starvation, coculture with monocytes, or IL-1 stimulation, except for a small increase after serum starvation at +6 h. It is unlikely, however, that changes in *bax* levels are responsible for the protective effect of monocytes on survival of serum-starved EC seen at 21 h because *bax* expression at this time was unchanged under all conditions (Fig. 8A). *bcl*-x*<sub>L</sub> mRNA was also expressed by unmanipulated EC, but mRNA levels were not altered by serum starvation, nor by subsequent coculture with monocytes or by stimulation with IL-1 (Fig. 8B). *bcl*-2 mRNA was expressed at low levels in EC and appeared to be up-regulated in response to coculture with monocytes, but with delayed kinetics compared with A1 (Fig. 8C).

Discussion

We have shown that monocytes induce expression of the antiapoptotic gene, A1, and at the same time confer protection against apoptosis in serum-starved EC. The induction of A1 in EC/monocyte cocultures was mediated by direct cell:cell contact as well as by TNF. IL-1 stimulation of EC also resulted in an increase in A1 mRNA. This was, however, short-lived and returned to near basal levels by 21 h. In contrast, A1 mRNA levels remained elevated for up to 21 h in EC cocultured with monocytes. IL-1 failed to protect serum-starved EC from apoptosis, probably as a result of the transient nature of A1 induction by this cytokine. It is possible that phagocytosis of apoptotic EC by monocytes may falsely increase the proportion of live EC in EC/monocyte cocultures. We did not observe this phenomenon, however, when we examined monocytes removed at the end of the coculture period. Furthermore, significant effects on A1 expression were seen even when monocytes were separated from EC by 0.45-μm pore filters. We were unable to extend our work to include analysis of A1 protein, due to the lack of a suitable Ab for use in Western blotting.

Our experiments suggest that actual cell:cell contact is necessary to generate a signal in EC that leads to the induction of A1, perhaps by the ligation of surface adhesion receptors. We were, however, unable to inhibit the induction of A1 by using mAbs against the CD11b/CD18 complex, despite the fact that these Abs are effective at inhibiting adhesion of monocytes to EC under both normal and serum-starved conditions. PECAM-1 (CD31) is a junctional adhesion molecule that mediates transendothelial migration of leukocytes (19). The reduction in A1 stimulation by anti-PECAM-1 mAbs in EC/monocyte cocultures raises the interesting
possibility that monocyte transmigration may be important in stimulating the increase in A1 mRNA in EC. It may be that the engagement of PECAM-1 on transmigrating leukocytes can lead to cell activation (20), which in turn releases signals to the EC to influence cell behavior and survival. Interestingly, the induction of E-selectin gene expression in EC/monocyte cocultures is also inhibited by anti-PECAM-1 mAbs (unpublished observations). It is not yet known whether direct engagement of PECAM-1 on EC results in cell activation.

At least part of the ability of monocytes to induce A1 expression by EC is mediated by TNF, an observation consistent with a previous report that TNF induces A1 mRNA in EC (13). Our results suggest that TNF plays a major role in the early A1 response of EC, but a lesser role at 21 h. Both soluble and membrane-bound TNF may be important; evidence for a role for soluble TNF is provided by the finding that anti-TNF mAb abolished A1 induction when EC were separated from monocytes by 0.45-μm filters (data not shown). Monocyte activation leads initially to the synthesis of inflammatory cytokines such as TNF and IL-1, which then act to stimulate the production of IL-10 (21), an immunomodulatory cytokine with an antiinflammatory role. One action of IL-10 is to prevent further secretion of inflammatory mediators by monocytes (22). Thus, the inhibitory action of IL-10 on A1 expression in EC/monocyte cocultures may be the result of suppression of TNF synthesis by monocytes.

Although we and others observed that TNF increased A1 expression in EC (13), prolonged exposure to TNF under some circumstances may in fact induce apoptosis in EC (3). Indeed, TNF induces apoptosis in many cell types, and many of the intracellular death signals downstream from the TNF receptor have now been identified (23). EC, however, are not killed by TNF alone unless coincubated with a protein or mRNA synthesis inhibitor (24).
antiapoptotic effect of TNF is mediated by the activation of NF-κB, which, by inducing TNF receptor-associated factors 1 and 2, and the inhibitors of apoptosis proteins, blocks the activation of the caspase cascade that leads to death (25). The action of TNF in inducing A1 expression in EC may represent another antiapoptotic mechanism. Retroviral-mediated transfer of A1 cDNA to human microvascular EC protects against death induced either by TNF in the presence of actinomycin D or by ceramide (24). Therefore, the increased survival of EC when cultured in the presence of monocytes may be the result of augmented A1 expression.

A1 contains BH1 and BH2 domains that mediate heterodimerization with the proapoptotic protein bax (26). Thus, A1 may function, like bcl-2 and bcl-xL, by inhibiting bax-induced cytochrome c release from mitochondria, and the subsequent activation of caspase-3. Monocytes had little effect on bax expression at 21 h, when the protective effect on EC was most marked, suggesting it is unlikely that alterations in bax levels influence EC survival in EC/monocyte cocultures. bcl-xL has been shown to protect against ceramide-induced apoptosis in EC. bcl-xL is constitutively expressed by EC, but levels were not significantly altered by coincubation with monocytes. bcl-2 mRNA levels in EC increased in response to coculture with monocytes, but with slower kinetics when compared with A1 mRNA. We cannot, therefore, exclude a possible role for bcl-2 protein in the protection of EC from apoptosis.

Lindner et al. have reported that mononuclear cells preactivated with either LPS or irradiation caused apoptosis of cultured EC (24). The observation that EC receive survival signals in the context of platelet-activating factor, which would in turn stimulate the proapoptotic protein bax (26). Thus, A1 may function, like bcl-2 and bcl-xL, by inhibiting bax-induced cytochrome c release from mitochondria, and the subsequent activation of caspase-3. Monocytes had little effect on bax expression at 21 h, when the protective effect on EC was most marked, suggesting it is unlikely that alterations in bax levels influence EC survival in EC/monocyte cocultures. bcl-xL has been shown to protect against ceramide-induced apoptosis in EC. bcl-xL is constitutively expressed by EC, but levels were not significantly altered by coincubation with monocytes. bcl-2 mRNA levels in EC increased in response to coculture with monocytes, but with slower kinetics when compared with A1 mRNA. We cannot, therefore, exclude a possible role for bcl-2 protein in the protection of EC from apoptosis.

The observation that EC receive survival signals in the context of an inflammatory response is important. Previous work has shown that the inflammatory cytokines, TNF and IL-1, are able to induce A1 expression (13), while growth factors and mitogens do not. This points to a role for A1 in protecting EC against death and damage in inflammation. In this study, we have demonstrated that monocytes also increase A1 expression, but, in contrast to the effect seen with cytokines, monocyte-induced A1 expression is sustained for a longer period of time. Vascular endothelium plays a pivotal role in the regulation of inflammatory responses; thus, the maintenance of EC integrity and survival is crucial to the effective development as well as the successful resolution of inflammation. Loss of integrity of EC in uncontrolled inflammation, for example, leads to the capillary leak syndrome, with resulting tissue edema, and the development of the adult respiratory distress syndrome. The elucidation of specific survival signals in the context of inflammation may open up avenues of intervention in the management of conditions in which uncontrolled inflammation leads to endothelial damage and death.

References