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Immunology

Expression of Ia-like antigens by human vascular endothelial cells is inducible *in vitro*: Demonstration by monoclonal antibody binding and immunoprecipitation

(phytohemagglutinin/HLA-DR antigens/HLA-A, -B antigens)

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ABSTRACT **The expression of Ia-like antigens by cultured human endothelial cells has been investigated by means of monoclonal antibody binding to intact cells and by immunoprecipitation of radioiodinated membrane proteins. Primary growing and confluent cultures of human umbilical vein endothelium express little, if any, detectable Ia-like antigens under standard culture conditions. However, treatment of primary cultures with the lectin phytohemagglutinin induces the expression of Ia-like antigens. This action of the lectin uniformly affects all the endothelial cells in a culture, does not depend on cell division, and is associated with a cell shape change. The data presented in this report provide unequivocal serological and biochemical demonstration of Ia-like antigens on human vascular endothelial cells. The fact that the expression of Ia-like antigens by endothelium can be induced may have important implications for organ transplantation and for regulation of the immunological response.**

Human class II histocompatibility antigens or Ia-like molecules (e.g. HLA-DR, DC/MB/MT/LB, or SB antigens) are essential for effective communication among cells participating in the immunological response (1). In humans and rodents, class II antigens are expressed most abundantly by B lymphocytes, activated T lymphocytes and antigen-presenting cells, such as cells of the monocyte/macrophage line, Langerhans cells of the skin, and dendritic cells of lymphoid organs (2, 3). Thus, Ia-like antigens have been traditionally associated with immunologically active cells. Recent morphological studies, using immunofluorescence microscopy in frozen tissue sections (4–7) or antibody binding assays of dispersed tissue cells (8), suggest that human endothelium in some vascular beds also appears to express Ia-like antigens. In rat tissues, some workers report the absence of Ia antigens on endothelium (9, 10), although a recent report indicates that certain subclasses of Ia antigens may be expressed (11). With such morphological methods, however, Ia-like antigenic positivity could be caused by passive adsorption of antigen from the blood either onto the endothelial surface or into the underlying basement membrane.

Serological and biochemical studies of isolated human vascular endothelial cells have the potential to clarify whether this tissue does express Ia-like antigens. Cultured human umbilical vein endothelial (HUVE) cells (12) show some susceptibility to cytoxicity by anti-HLA-DR xenosera or allosera, suggesting that they express HLA-DR antigens or other Ia-like molecules (13–15). However, in these studies, the level of cytotoxicity was limited and the anti-human serological reagents available may have contained reactivities toward antigens other than Ia-like molecules. Furthermore, a recent binding study using monoclonal antibodies has suggested that endothelial cells may lack Ia-like antigens (16).

The expression of HLA-DR or other Ia-like antigens by vascular endothelium would have at least two important biological implications. First, the presence of Ia-like antigen-bearing endothelial cells within an organ graft could significantly enhance its immunogenicity and hence its likelihood of transplant rejection (17). In the mixed lymphocyte reaction, a model of transplant rejection, Ia-like antigens on the stimulator lymphocyte population serve as the major provokers of allogeneic responder lymphocyte proliferation (18). Interestingly, cultures of HUVE cells also induce allogeneic lymphocytes to proliferate (19) and a role for endothelial Ia-like molecules has been suggested in this phenomenon (20). Second, Ia-like antigen-bearing endothelial cells might function as immune accessory cells. Indeed, it has been shown that endothelial cell cultures may replace cells of the monocyte/macrophage line in supporting both phytohemagglutinin (PHA)-induced proliferation of T lymphocytes (21) and antigen-induced proliferation of lymphocytes in a secondary *in vitro* immunological response (22–24), and evidence has been obtained to support a functional role for endothelial Ia-like molecules in antigen presentation. In most antigen-presentation experiments, however, minor contamination by known Ia-like antigen-positive immune accessory cells (e.g., monocytes or dendritic cells) was not ruled out. Still, the suggestion that endothelium can act as an immune accessory tissue has raised new possibilities for regulation of the immunological response.

For these reasons, we felt it was important to establish unambiguously whether vascular endothelium expresses Ia-like molecules. We have used binding assays with several recently available mouse monoclonal antibodies reactive with human Ia antigens and immunoprecipitation of radioiodine-labeled proteins with a strong xenoserum. We report here that primary cultures of HUVE cells express few, if any, Ia-like molecules under standard culture conditions. However, exposure of these cultures to PHA induces a uniform expression of Ia-like molecules by endothelial cells. This inducible expression of Ia-like molecules by vascular endothelium has important implications for organ transplantation and for regulation of the immune response.

MATERIALS AND METHODS

HUVE cells were harvested by collagenase treatment of two to six normal-term umbilical cord segments and pooled in medium 199/20% (vol/vol) heat-inactivated fetal bovine serum supplemented with penicillin at 125 units/ml, streptomycin at 125 μg/ml, and 2 mM L-glutamine (medium A; all components

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Abbreviations: HUVE cells, human umbilical vein endothelial cells; PHA, phytohemagglutinin; P_i/NaCl, phosphate-buffered saline.

from M. A. Bioproducts, Walkersville, MD) as described (11). Replicate primary cultures were plated (day 0) in either Costar 96-well microtiter plates or Costar 24-well plates (M. A. Bioproducts). The cultures were washed (day 1) to remove unattached blood and endothelial cells and refed with medium A containing PHA (from *Phaseolus vulgaris*, type IV; leucoagglutinin from Sigma) at 0–8 μg/ml. Cultures normally reached confluence (*ca.* 1×10^5 cells per cm^2) by day 3 and were assayed in different experiments on days 1–5. In some experiments, PHA was added after confluence was reached (day 3) rather than on day 1.

Antibody-binding experiments (25) were carried out at ice temperature using *ca.* 1.5×10^4 endothelial cells in confluent monolayers in Costar 96-well microtiter plates. Each well was washed twice with Dulbecco's phosphate-buffered saline (P_i/NaCl) containing 1% bovine serum albumin (fraction V; Sigma). Substitution of heat-inactivated 20% (vol/vol) human serum in Dulbecco's P_i/NaCl instead of 1% albumin to block nonspecific binding of mouse immunoglobulin to cellular F_c receptors had no effect on antibody binding to HUVE cells. Each mouse monoclonal antibody (diluted 1:100 from an ascitic fluid) was added in 100 μl of Dulbecco's P_i/NaCl/albumin and incubated for 1 hr; unbound specific antibody was removed by washing three times with the same saline solution. ^{125}I-Labeled sheep anti-mouse immunoglobulin F(ab')$_2$ fragment (New England Nuclear, diluted 1:20 or 1:10) or affinity-purified ^{125}I-labeled rabbit anti-mouse immunoglobulin (a gift of Abul Abbas, Brigham and Women's Hospital) was added in 50 μl of Dulbecco's P_i/NaCl/albumin and incubated for 1 hr. Both the specific mouse monoclonal antibodies and the radiochemical-detecting antibodies were added in excess of cellular binding capacity (i.e., above saturation) as determined by serial dilutions. Unbound iodinated reagent was removed by washing four times with Dulbecco's P_i/NaCl/albumin. The radioactivity bound to cells was transferred to an assay tube by eluting each microtiter well twice with 200 μl of 10% $NaDodSO_4$ (Bio-Rad) and assayed by using a Beckman model Gamma 8000. The following specific mouse monoclonal reagents were used—BBM.1 (IgG2b), directed against human β_2-microglobulin (26); W6/32 (IgG2a), directed against an HLA-A, -B monomorphic heavy chain determinant (27) (both gifts of Peter Parham, Stanford University); DA2 (IgG1) and Genox 3.53 (IgG1) (28) (from Frances Brodsky and Peter Parham, Stanford University), LB3.1 (IgG2b) (a gift of Peter Knudsen, Harvard University, characterization unpublished), I2 (IgG2a) and I-LR1 (IgG2b) (29) (gifts of Lee Nadler, Sidney Farber Cancer Institute), anti-Ia (IgG2b) (New England Nuclear), anti-DR clone 1 (IgG1) (Bethesda Research Laboratories), and clone 19/9 (IgG2b) (Pel-Freez), all directed against monomorphic or supratypic determinants of human Ia-like molecules; and K1210 (IgG1), K1616 (IgG1), and MPC11 (IgG2b) (gifts of Donna Mendrick and Abul Abbas, Brigham and Women's Hospital)—as nonbinding mouse monoclonal antibodies (controls for nonspecific binding). The JY human lymphoblastoid cell line (a gift of Jack Strominger, Harvard University) was used as a positive Ia-like antigen-bearing control.

Immunocytochemical localization of antibody binding was carried out by the immunoperoxidase avidin–biotin complex technique (30). Confluent HUVE monolayers, grown on 1.2-cm-diameter glass coverslips (Bellco Glass), were lightly fixed with 0.1% glutaraldehyde (Polysciences) in Dulbecco's P_i/NaCl for 10–15 min on ice and the excess aldehyde was quenched with 0.05 M Tris·HCl (pH 7.5) on ice for 30 min. Monoclonal antibodies were added as above except that the binding and washing steps were carried out at room temperature. This procedure did not alter monoclonal antibody binding as assessed with ^{125}I-labeled sheep anti-mouse immunoglobulin F(ab')$_2$ fragment. Biotin-conjugated horse anti-mouse immunoglobulin and succinylated avidin–biotin-conjugated horseradish peroxidase complex (Vector, Burlingame, CA) were bound in second and third steps, respectively. Horseradish peroxidase binding was localized by the diaminobenzidine (Sigma) reaction followed by light microscopy.

For immune precipitation, 1×10^6 lymphoblastoid or nonenzymatically suspended HUVE cells (by 5 mM EDTA in P_i/NaCl/albumin) were washed three times with Dulbecco's P_i/NaCl and radioiodinated by using Iodo-bead catalyst (Pierce) in 500 μl of the P_i/NaCl containing 250 μCi of $Na^{125}I$ (1 Ci = 3.7×10^{10} becquerels; New England Nuclear) for 15 min at room temperature. The reaction was stopped by transferring the cell suspension to a clean tube without the Iodo-bead. The cells were immediately washed three times with Dulbecco's P_i/NaCl/albumin/10 mM KI. The final cell pellet was extracted with 0.5 ml of 2% Nonidet P-40 (Sigma) in 25 mM NaCl/25 mM Tris·HCl, pH 7.4/0.1 mM phenylmethylsulfonyl fluoride (Sigma) for 30 min at ice temperature and the detergent extract was clarified by centrifugation. Nonspecifically precipitating material was removed by sequential incubation with normal rabbit serum and heat-killed formalin-fixed *Staphylococcal* A bacteria and centrifugation. Specific precipitations were carried out by a modification (31) of the method of Kessler (32) from the residual supernatant and using normal rabbit serum as a negative control, rabbit anti-human β_2-microglobulin (33) (a reagent that reacts with all HLA-A, -B antigens) as a positive control, and rabbit anti-human p23,30, (34) (a reagent that reacts with all known human Ia-like molecules, gift of Jack Strominger, Deborah Shackelford, and James Kaufman, Harvard University) to assess the presence of human Ia-like antigens. The immunoprecipitated products were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (all reagents from Bio-Rad) on a 25-cm 7–15% linear acrylamide gradient using the buffer system of Laemmli (35). The gel was impregnated with EN^{3}HANCE (New England Nuclear), dried, and analyzed by fluorography on Kodak X-Omat film.

RESULTS

The binding of mouse monoclonal antibodies reactive against human Ia-like antigens was used to measure expression of Ia-like molecules by HUVE cells in confluent primary cultures. Binding of anti-Ia-like reagents to endothelial monolayers was consistently less than 10% of that of the anti-HLA-A, -B reagents and, in more than 40 separate primary cultures, this level was not distinguishable from that of the nonbinding monoclonal reagents. A representative experiment is shown in Table 1 ("control"). To rule out the possibility that this negative result was caused by lack of access to relevant portions of the cell surface, we examined binding of monoclonal antibodies to monodisperse cell suspensions of endothelial cells. In two separate experiments, the binding of anti-Ia monoclonal reagents was less than 10% of that of anti-HLA-A, -B reagents. In contrast, the same anti-Ia-like monoclonal antibodies bound to the human lymphoblastoid cell line JY at a level approaching that for the anti-HLA-A, -B reagents. Thus, primary cultures of HUVE cells at confluence express little, if any, Ia-like antigens.

Although primary cultures of HUVE cells under our standard culture conditions did not appear to express significant amounts of Ia-like antigens, it was possible that, under certain biological conditions, Ia-like antigens could be induced. For example, Ia antigens on mouse macrophages can be induced *in vitro* by lectin-generated lymphokines (36–38). Furthermore, lectin-generated human lymphokines have been shown to inhibit bovine

Table 1. Binding of monoclonal antibodies to confluent human endothelial cell monolayers

Monoclonal antibody	Antibody binding, cpm per well		
	Control	PHA treated	
		Day 1	Day 3
W6/32 (HLA-A, -B)	20,630 ± 458	33,533 ± 18,178	49,831 ± 5,732
LB3.1 (human Ia)	1,494 ± 2,138	24,843 ± 897	18,161 ± 364
I2 (human Ia)	47 ± 230	14,303 ± 1,637	12,401 ± 1,858

Primary HUVE cells were replicate plated in Costar 96 microtiter wells (day 0). PHA (4 μg/ml) was added on day 1 to subconfluent (dividing) cultures or on day 3 to confluent (nondividing) cultures or not at all (control). On day 5, monoclonal antibody binding was assayed in duplicate on confluent monolayers (*ca.* 1.5 × 10^4 cells per well) using ^{125}I-labeled sheep anti-mouse immunoglobulin F(ab')$_2$. Results are mean ± SEM. Data are corrected for nonspecific monoclonal antibody binding (*ca.* 1,800 cpm per well) and are representative of more than 20 such experiments; no effect of PHA treatment on nonspecific binding of first or second antibodies was noted.

endothelial cell migration (39), indicating that certain lymphocyte products can affect this cell type. HUVE cells grown in the presence of medium conditioned by PHA-treated human peripheral blood mononuclear cells showed marked enhancement of binding by mouse monoclonal antibodies reactive with human Ia-like antigens. Surprisingly, medium containing PHA, incubated in the absence of mononuclear cells, also caused an increase in Ia-like antigen expression whereas control medium without PHA did not. Thus, PHA alone appeared to be inducing the expression of Ia-like antigens by HUVE cells in culture. In our standard protocol, PHA was added to cultures in fresh medium A on day 1 (so as to avoid effects on plating efficiency). By day 3, PHA-treated HUVE cells clearly express Ia-like antigens. The level of binding of the monoclonal antibodies reactive with human Ia-like antigens increases as the dose of PHA increases (Fig. 1). The effect of PHA on the expression of Ia-like antigens was nearly maximal at a PHA dose of 4 μg/ml, and this dose was used routinely in subsequent experiments.

The relationship of endothelial growth state to the expression of Ia-like antigens and their induction by PHA was examined. In the absence of PHA, subconfluent actively dividing HUVE cultures do not bind monoclonal antibodies reactive with Ia-like antigens (Table 2). In our usual protocol, PHA was added to subconfluent cultures (day 1) and an increase in Ia-like antigen expression was noted 48 hr later. By day 3, in both PHA-treated and untreated cultures, stable confluent monolayers had formed in which there was little evidence of cell division. When PHA was added to confluent nondividing monolayers (day 3) instead of to dividing cultures (day 1), the expression of Ia-like antigens was nevertheless fully induced by day 5 (Table 1). Thus, cell division appears neither necessary (Table 1) nor sufficient (Table 2) for the expression of Ia-like antigens.

Several additional observations regarding the effects of PHA on the endothelial cultures were made. First, the level of binding of monoclonal antibodies reactive with HLA-A, -B antigens also increased with PHA treatment to nearly double the initial value (Table 1). Second, PHA induced a change in cell shape, causing the cells to assume a more spindle-shaped morphology. This change was observable by 24 hr but was most apparent at confluence, when the untreated cells assumed a polygonal morphology. Third, PHA was not cytotoxic, as judged by absence of cell retraction and detachment and by preservation of trypan blue dye exclusion, at all doses used. Finally, there was no obvious mitogenic effect of PHA on HUVE cells, consistent with previous observations (18).

Although monoclonal antibody binding is a method of great specificity, we wished to confirm our results by a different method. Immunoprecipitation of radiochemically labeled endothelial cell surface proteins was used to establish that PHA does indeed induce expression of an Ia-like molecule. Rabbit anti-human p23,30 specifically precipitated a two-chain Ia-like complex from both radioiodinated JY human lymphoblastoid cells (Fig. 2, lane 1) and PHA-treated HUVE cells (Fig. 2, lane 3). This complex is not detected by precipitation from a cell extract of untreated endothelial cells containing equal amounts

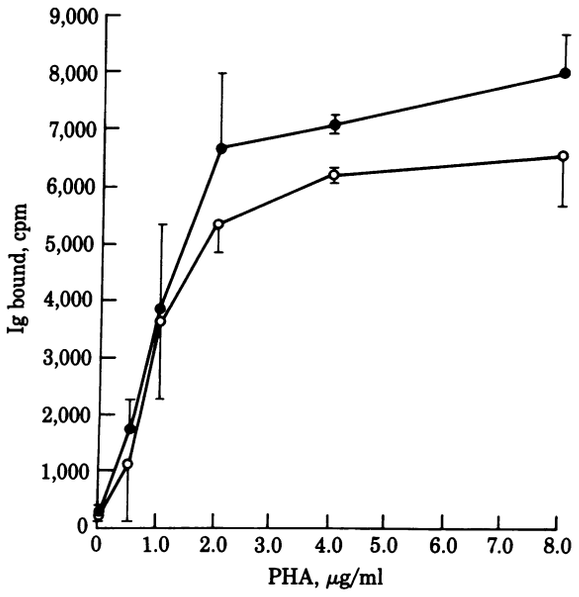

FIG. 1. Dose–response curve of Ia-like-antigen induction by PHA treatment of HUVE cells. Binding of two mouse monoclonal antibodies reactive with Ia-like antigens [LB3.1 (●) and I2 (○)] to HUVE cells, assayed with ^{125}I-labeled sheep anti-mouse Ig, was determined in triplicate and corrected for nonspecific binding of a nonreactive mouse monoclonal antibody (*ca.* 1,500 cpm per well).

Table 2. Binding of monoclonal antibodies to actively dividing human endothelial cells

Monoclonal antibody	Antibody binding, cpm per well
W6/32 (HLA-A, -B)	8,587 ± 1,296
BBM.1 (HLA-A, -B)	11,461 ± 3,320
LB3.1 (human Ia)	369 ± 133
I2 (human Ia)	292 ± 142
NEN anti-Ia (human Ia)	(−10 ± 164)

Monoclonal antibody binding to replicate plated subconfluent (actively dividing) cultures (day 1) was assayed in Costar 24 wells using ^{125}I-labeled rabbit anti-mouse immunoglobulin. Each well contained *ca.* 5 × 10^4 cells. Data represent means of triplicate determinations (except duplicate for I2) ± SD and are corrected for nonspecific monoclonal antibody binding (*ca.* 1,500 cpm per well).

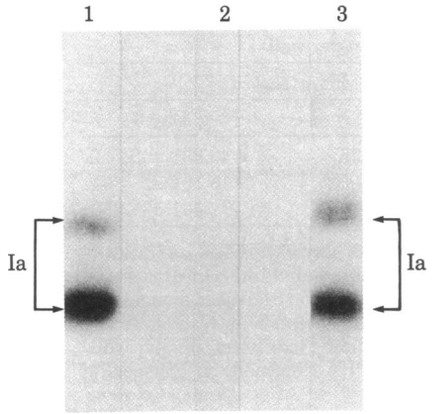

FIG. 2. Fluorograph of proteins immunoprecipitated by rabbit anti-human p23,30 serum from JY lymphoblastoid cells (lane 1) and from HUVE cells grown in the absence (lane 2) and presence (lane 3) of PHA. Equal amounts of radioiodine were incorporated into 2×10^6 cells in each group, and immunoprecipitated protein was subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis on a 7–15% acrylamide gradient gel. The portions of the lanes shown extend (approximately) from M_r 50,000 (top) to M_r 20,000 (bottom). Lane 1 was exposed for 1 day whereas lanes 2 and 3 were exposed for 14 days, reflecting the significantly lower percentage of surface protein composed of Ia-like antigens on HUVE cells compared with a human lymphoblastoid B-cell line.

of trichloracetic acid-precipitable cell surface radioactivity (Fig. 2, lane 2). Similar precipitation results were obtained using LB3.1, one of the anti-Ia-like mouse monoclonal reagents (data not shown). These observations confirm biochemically that PHA induces the expression of Ia-like antigens by endothelial cells. It is noteworthy that, even after induction of Ia-like-antigen expression by PHA, endothelial cells express far less of these antigens per unit of cell surface-labeled protein than the JY human lymphoblastoid cell line (Fig. 2).

It was important to determine whether PHA induced Ia-like-antigen expression by all (or most) endothelial cells or whether a small percentage of endothelial cells or a contaminating non-endothelial subpopulation (e.g., monocytes or dendritic cells) could account for the increase of these molecules. At the light-microscope level, immunoperoxidase staining of PHA-treated cultures showed a uniform pattern of binding of both LB3.1 and I2 (reactive with Ia-like antigens). This staining was greater than background staining with K1210 (negative binding control) but less than that with W6/32 or BBM.1 (positive anti-HLA-A, -B controls). In contrast, no specific staining of untreated cells with LB3.1 or I2 was noted. These morphological data are consistent with the level of antibody binding measured in our radioimmunobinding assay. Most importantly, it was clear that there was no subpopulation of intensely Ia-like antigen-positive-staining cells that could account for the net increased binding to the treated cultures.

DISCUSSION

To establish unequivocally the presence of Ia-like antigens on human vascular endothelial cells, we have examined primary cultures of human umbilical vein endothelium by two methods: (*i*) monoclonal antibody binding and (*ii*) immunoprecipitation of radiolabeled proteins with xenosera. Our data indicate that subconfluent (growing) and confluent (quiescent) primary cultures of human umbilical vein endothelial cells under our standard culture conditions do not express readily detectable Ia-like antigens. The difference between our data and those of previous workers (13–15) may be explained by differences in methodological sensitivity, (i.e., cytotoxicity assays may detect fewer molecules than monoclonal antibody binding or immunoprecipitation) or it may be that our reagents are more specific. In either case, the number of Ia-like molecules per cell, if any, must be small. Most excitingly, our data show that the expression of Ia-like antigens in endothelial cultures can be induced by treatment with PHA. Direct visualization of monoclonal antibody binding by immunocytochemistry showed a uniform increase of Ia-like-antigen expressions by all of the endothelial cells. No intensely staining subpopulation was detected. This generalized expression of Ia-like antigens has since been confirmed by indirect immunofluorescence analysis with a fluorescence flow cytometer.

The mechanism of action of PHA on endothelium is not yet established. It is possible that PHA acts on some subpopulation to produce a factor that in turn acts on the remaining cells in the culture. The most likely candidate for an intermediary cell type is a T lymphocyte since a lectin-generated lymphokine from rat T lymphocytes has been shown to induce expression of Ia antigens by mouse macrophages (36–38) and the doses of PHA used in our experiments are stimulatory for human T lymphocytes. Two preliminary observations have suggested that T lymphocytes may in fact mediate the action of PHA on endothelial cells. First, the lectin concanavalin A, another T lymphocyte mitogen, also appears to induce the expression of endothelial cell Ia-like antigens at concentrations that stimulate T lymphocytes. Second, treatment of primary endothelial cultures with an anti-human T-cell monoclonal antibody and complement before the addition of PHA appears to abrogate both the induction of Ia-like antigens and the increased expression of HLA-A, -B antigens without altering endothelial cell viability. Interestingly, Nunez and Stastny* have reported an effect of lymphocyte products on endothelial cell HLA-A, -B antigen expression and suggest a possible effect on Ia-like antigen expression. Additional experiments to establish a role for T lymphocytes in the expression of endothelial cell Ia-like antigens are now in progress.

The central point of this paper is that the expression of Ia-like antigens by endothelium is inducible. Lipsky and Kettman (16) have reported that the human vascular endothelial cells used in their experiments to support PHA stimulation of T lymphocytes were Ia-like antigen negative. Our results suggest that these cells may have become Ia-like antigen positive during the course of their experiment. This prediction may be functionally important since recent observations have suggested that Ia-antigen-positive human accessory cells are necessary for the support of lectin-induced proliferation of T lymphocytes (40).

The monoclonal antibodies reactive with Ia-like antigens used most extensively in these studies (LB3.1, I2, and New England Nuclear anti-Ia) are monomorphic (i.e., they react with all cells) but only recognize products of the HLA-DR locus. It is possible that other Ia-like antigens could be expressed. However, Genox 3.53 and I-LR1, which recognize some Ia-like products of other genetic loci (DC-1 and SB, respectively; see refs. 41 and 42), also do not bind to cells grown under our standard conditions. Furthermore, the xenoserum used in this study to immunoprecipitate radiolabeled cell surface proteins appears to recognize all Ia-like antigens yet failed to detect these molecules in untreated cells. In a preliminary experiment, PHA-treated endothelial cells pooled from several donors bind both Genox 3.53 and I-LR1, suggesting that multiple Ia-like antigens are coincidently induced.

* Nunez, G. & Stastny, P. Association of Histocompatibility Testers, San Francisco, May 1982, p. A23 (abstr.).

We have also observed that PHA treatment of endothelial cultures resulted in significant increases in HLA-A, -B antigens (class I antigens) per cell, suggesting that there is a PHA-dependent increase in several products encoded by the major histocompatibility complex. A similar pattern of coincident increase of class I and class II histocompatible antigen expression has been observed in human monocytes treated with lectin-generated lymphokines.* In contrast, monoclonal reagents reactive with six other endothelial cell surface antigens not expressed on lymphocytes did not show an increase in binding. Thus, PHA treatment of HUVE cells appears to act selectively and does not lead to a generalized increase of all cell surface proteins. The possible interrelationships of the induction of Ia-like antigens, the increase in HLA-A, -B antigens, and the observed concomitant change in cell shape require further study.

The observation that the expression of Ia-like antigens is inducible on vascular endothelium has two important implications. First, it suggests that the contribution of vascular endothelium to the immunogenicity of organ grafts may be variable and thus potentially subject to pharmacologic manipulation. Second, the immunological accessory functions of endothelium, suggested by the *in vitro* experiments of other workers, (21–24), also may be variable and provide a mechanism of physiological immune regulation. Investigation of whether cell-derived factors such as monokines or lymphokines can modulate endothelial histocompatibility antigen expression and the role that this might play in lymphocyte–endothelial cell communication will provide further insights into these questions.

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