ALLOGENEIC INDUCTION OF THE HUMAN T CELL-INSTRUCTED MONOCYTE PROCOAGULANT RESPONSE IS RAPID AND IS ELICITED BY HLA-DR*

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The allogeneic mixed lymphocyte culture (MLC)[1] system has been widely used as an in vitro model for analysis of lymphocyte recognition mechanisms and the coupled responses. After recognition of allogeneic B cells and monocytes, lymphocytes undergo several phenotypic and functional changes, i.e., blast transformation and proliferation as well as differentiation to allospecific cytolytic and memory cells (reviewed in reference 1). The cellular locus of these well-recognized consequences of the recognition of allogeneic differences in molecules of the major histocompatibility complex is the T lymphocyte. The effector functions of the cells of the mononuclear phagocyte series, the monocytes and macrophages induced by the allogeneic response, have been less well delineated. Newman et al. (2) reported the induction of plasminogen activator in monocytes by cell-free MLC supernatants, and several conflicting results have been published on the generation of molecules able to initiate the coagulation protease cascade, e.g., procoagulant activity (PCA) of the tissue factor type in human allogeneic MLC (3–5). The first topic of controversy has been the inducibility of PCA per se in the allogeneic MLC: whereas Rickles et al. (3) were unable to observe PCA generation, Rothberger et al. (4) and van Ginkel et al. (5) demonstrated such responses.

The generation of procoagulant molecules by allogeneic stimulation is important to basic immunobiology because it is a poorly characterized effector mechanism of the lymphoid system and because microvascular thrombosis and perivascular and interstitial fibrin deposits are tissue lesions commonly encountered during rejection of human kidney and skin allografts (6, 7). We here explore the ability of human peripheral blood mononuclear cells (PBM) to recognize allogeneic differences and respond by induction of the monocyte PCA pathway. Our results demonstrate a relatively rapid procoagulant response that is temporally dissociated from T cell proliferation. We present data to suggest that this is a T cell-instructed activation pathway for induction of monocyte PCA response

[1] *Abbreviations used in this paper:* LPS, lipopolysaccharide; MLC, mixed lymphocyte culture; PCA, procoagulant activity; PBM, peripheral blood mononuclear cells; TdR, thymidine.

initiated by HLA-DR antigens and that it appears to be discordant with respect to proliferative allogeneic responses.

Materials and Methods

Cell Isolations. Venous blood was drawn from healthy volunteers into 5 U/ml (final) sodium heparin. PBM were isolated as previously described (10) on Ficoll-Hypaque density gradients followed by three washes in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) containing 2 mM L-Glutamine, 25 mM Hepes, and 50 μg/ml gentamycin. PBM were >94% viable by trypan blue exclusion and contained <2% polymorphonuclear leukocytes.

Monocytes were isolated on the basis of their receptor-mediated attachment to fibronectin on gelatin-coated surfaces (8, 9) as described in detail elsewhere (10). The monocytes used in reconstitution experiments were ≥97% nonspecific esterase positive (11) and >90% excluded trypan blue.

Nylon wool columns (12) were used to isolate T cells from nonadherent lymphocytes recovered from gelatin-fibronectin surfaces. When 0.5 g of nylon wool (Fenwal Laboratories, Deerfield, IL) was packed into 12-ml disposable plastic syringes and used to fractionate 3–6 × 10^7 cells, ≥94% of the nylon wool effluent cells reacted with OKT 11 (Ortho Diagnostic Systems Inc., Westwood MA), a monoclonal antibody against human E rosette-forming T lymphocytes (13). In some experiments, gelatin-fibronectin nonadherent cells were depleted of monocytes by Sephadex G-10 gel filtration (14) in which 8 ml of swollen gel was packed into 12-ml disposable plastic syringes over a glass wool layer; then 25–50 × 10^6 cells were passed through such columns. The effluent cells were ≥98% nonspecific esterase negative.

Mixed Lymphocyte Culture. MLC for analysis in the PCA pathway were carried out in 12 × 75-mm polypropylene tissue culture tubes (Falcon Labware, Oxnard, CA). Primary two-way allogeneic MLC were established in duplicate between 10^6 PBM from pairs of individual donors in 1.0 ml of either Leibovitz's L-15 medium supplemented with 2 mM L-glutamine, 50 μg gentamycin, and 10% heat-inactivated fetal calf serum (Irvine Scientific) or RPMI 1640 medium containing the same supplements plus 25 mM Hepes. Control cultures consisted of 10^6 PBM from single donors in 0.5 ml of the culture medium. In some MLC, cells from an established lymphoma B cell line, Daudi, were used to stimulate PBM. 10^6 Daudi cells were cultured with 10^6 PBM in 1.0 ml of RPMI 1640 medium after treatment of the Daudi cells with mitomycin C at 100 μg/ml for 25 min at 37°C, followed by three washes in RPMI 1640. The MLC were incubated for varying times in a humidified atmosphere of 5% CO_2, 95% air.

To assess the proliferative response, the MLC were performed in 96-well flat-bottomed tissue culture plates (Corning Glass Works, Corning, NY). 2 × 10^5 PBM from each individual of pairs of donors were cultured in triplicate in 0.2 ml of L-15 medium supplemented as described above. The cultures were harvested daily during a 7-d period, or after other time intervals as given in Results. The cells were cultured for 18 h in the presence of 1 μCi/well of [^{3}H]thymidine ([^{3}H]TdR) before harvesting on glass fiber filters with an automated harvester. The filters were washed, dried, placed in scintillant, and counted in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, CA).

Assay for PCA. The PCA content of the cells was assayed by their ability to accelerate the spontaneous clotting time of pooled, citrated platelet-poor, normal human plasma in a one-stage clotting assay (15). To analyze total PCA content, the cells were washed once after termination of the MLC, resuspended in serum-free RPMI 1640, and subjected to homogenization by three cycles of freezing-thawing and two cycles of sonication. Cell lysates from single-donor control cultures were tested for PCA either separately or after combining the lysates, without any difference in the final results. PCA expression by intact viable cells was determined on washed and resuspended cells without homogenization. In the coagulation assay 100 μl of cell suspension or homogenate and 100 μl of 25 mM $CaCl_2$ were added to 100 μl of normal human plasma. The clotting time, from addition of $CaCl_2$ to formation of a visible clot, was measured in glass tubes with constant rocking at 37°C.

The times were converted to milliunits of PCA by reference to a standard curve derived from rabbit brain thromboplastin standard (Difco Laboratories, Inc. Detroit, MI) at 37.5 mg dry weight/ml, which was assigned a value of 10^5 mU/ml. Serial dilutions were used to produce a log-log plot. For further comparison, a 10^3 mU PCA/ml sample corresponds to a clotting time of ~50 s.

In some clotting assays, human plasmas deficient in clotting factors VIII, IX, X (George King Bio-Medical, Inc., Overland Park, KS), or in factor VII (from Dr. Daryl Fair, Research Institute of Scripps Clinic), were used as substrates instead of normal human plasma. Functional cleavage of prothrombin was assayed as described earlier (16) with a mixture of purified prothrombin (Dr. Daryl Fair) and purified fibrinogen (Dr. Ed Plow, Research Institute of Scripps Clinic) as substrate.

Monoclonal Antibodies. The mouse monoclonal antibodies SG 157 (a generous gift from Dr. Sanna Goyert and Dr. Jack Silver, Michigan State University, East Lansing) and OKIaI (Ortho Diagnostic Systems, Inc.) to common determinants of HLA-DR have been described previously (17, 18). The antibodies were added to the MLC at the initiation of cultures. The mouse monoclonal antibody Leu 2a against human suppressor/cytotoxic T cells (19) was purchased from Becton, Dickinson & Co., Sunnyvale, CA.

Endotoxin Contamination. All chemical reagents, monoclonal antibodies, tissue culture media, and fetal calf sera used were tested for endotoxin contamination by the limulus amoebocyte lysate assay (E-toxate; Sigma Chemical Co., St. Louis, MO) and were negative at the level of 0.1 ng/ml.

Results

Induction of PCA in MLC. A total of 31 different cellular combinations in two-way MLC were established from 22 individuals and were analyzed for induction of monocyte PCA. In MLC harvested after 48 h and assayed for total cellular PCA, all allogeneic pairs developed a response in which the cellular PCA was at least two times higher than that of the autologous controls. In Table I the distribution of the response levels for the 31 MLC combinations are given as relative degrees of stimulation (stimulation index). Five of the six donor combinations tested for PCA expression by intact viable cells demonstrated at least a twofold PCA response relative to control.

Table II gives the mean PCA in MLC and control autologous cultures from 15 donor pairs after 48 h incubation in RPMI 1640 medium. In MLC, a PCA response approximately five times higher ($\bar{x} = 5.3 \pm 2.1$, range 2.1–13.2) than that in controls was observed. For comparison, after a 4-h incubation with 10 μg/ml of bacterial lipopolysaccharide (LPS), a model stimulus for monocyte PCA, PBM responded with a PCA of 736 ± 168 mU/10^6 cells (mean $\pm$ SD of six experiments). In these experiments, the relative degree of stimulation was 16.3 ± 4.1.

TABLE I

Induction of Monocyte PCA in Two-Way MLC: Rate of Stimulation in 31 Allogeneic Combinations

	Stimulation index[‡]			
	1–3	3–6	6–9	9–12
Number of allogeneic combinations	9	11	8	3

* MLC in L-15 or RPMI 1640 medium were assayed at 48 h for total cellular PCA.
‡ Stimulation index = [(PCA Donor A + Donor B)/(PCA Donor A + PCA Donor B)].

TABLE II

Induction of Monocyte Procoagulant Actitivy in Two-Way Allogeneic MLC

Cell culture*	Viable expression[‡]		Total cell content[§]	
	PCA (mU/10⁶ cells)	Stimulation index	PCA (mU/10⁶ cells)	Stimulation index[∥]
Autologous	10 ± 3	—	32 ± 11	—
Allogeneic	30 ± 15	3.8 ± 2.3	174 ± 113	5.3 ± 2.1

* 48-h culture of 10⁶ PBM from each donor in complete RPMI 1640 medium.
‡ Mean ± SD from 6 experiments.
§ Mean ± SD from 15 experiments.
∥ Stimulation index calculated as in legend to Table I.
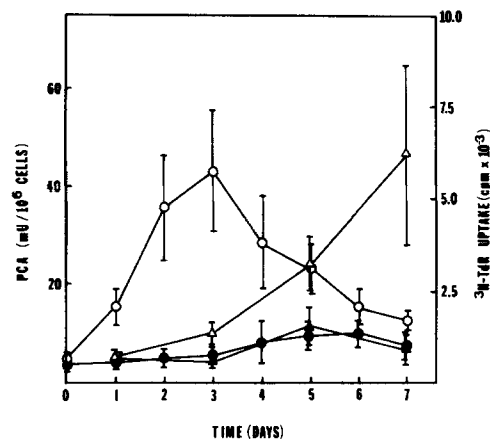

FIGURE 1.   Temporal dissociation of monocyte PCA response (○) (autologous control culture [●]) and T cell proliferation (△) (autologous control culture [▲]) in two-way allogeneic MLC. To assess PCA induction, MLC were established between 10⁶ PBM from 10 pairs of donors in 1.0 ml of L-15 medium and, after incubation, the cells assayed for total cellular PCA. For T cell proliferative response, 2 × 10⁵ PBM from each individual of the same pairs of donors were cultured in 0.2 ml of L-15 medium. T cell response was assessed by [³H]TdR incorporation.

Time Course of PCA Induction.   To assess the temporal profile of PCA induction, MLC pairs were harvested daily and assayed for total cellular PCA as well as PCA expression by intact cells. The PCA responses differed somewhat depending on the cell culture conditions used. In Leibovitz's L-15 medium, maximal PCA generation occurred on day 3 (Fig. 1), showing an almost ninefold increase of activity from the initiation of the culture. In RPMI 1640 medium, the induction of PCA was greater than in Leibovitz's L-15 medium (Fig. 2, *bottom*). After 4 d of culture the PCA reached a maximum 20-fold greater than the initial basal activity on day zero. When the PCA generated in MLC is compared with that in control autologous culture, a relatively sharp peak of maximal activity is seen on day 2 (Fig. 2, *top*). The viable PCA was maximally expressed on day 6 by reference to the stimulation index or on day 7 by reference to net PCA level.

Lack of Concordance Between PCA Generation and [³H]TdR Uptake.   It is readily apparent from data illustrated in Fig. 1 that in two-way allogeneic MLC the PCA response and the T cell proliferative response as measured by [³H]TdR uptake
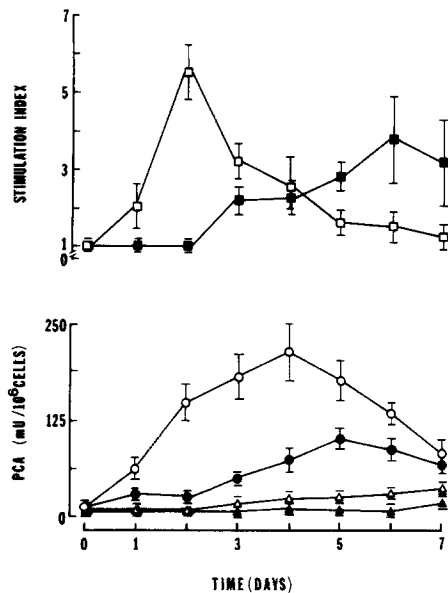
FIGURE 2.   Temporal profile of PCA induction in two-way allogeneic MLC. (*Bottom*) After incubation in RPMI 1640 medium, the cultures were assayed for total cellular (allogeneic [O], control [●]) (nine donor combinations) or cell surface-expressed PCA (allogeneic [△], control [▲]) (six donor combinations). Results are given as net PCA/10^6 cells. (*Top*) Responses for total cellular PCA (□) and viable cell PCA expression (■) are given as stimulation indices, calculated as: [(PCA donor A + donor B)/(PCA donor A + PCA donor B)].

TABLE III

Allogeneic Induction of Cellular PCA Using Daudi Lymphoblastoid B Cells

Culture*	PCA‡ (mU/10^6 PBM)	Stimulation index
Allogeneic (PBM + Daudi)	285 ± 158	9.8 ± 4.3
Control (PBM, Daudi)	30 ± 11	—

* Cultivation for 24 h in complete medium of 10^6 PBM, mixed with 10^6 mitomycin C-treated Daudi in 1 ml for allogeneic culture, then assayed for PCA. For control cultures, 10^6 PBM were separately cultured in 0.5 ml medium, and 10^6 mitomycin C-treated Daudi cells were separately cultured in 0.5 ml medium. Each was assayed separately for PCA and the values were summed.
‡ Mean ± SD of experiments (six cell donors).

were temporally dissociated. In a series of experiments consisting of 12 allogeneic MLC, linear regression analysis (detailed data not included) with a correlation coefficient (r) of 0.068 indicated a lack of concordance between the PCA responses on day 3 and the [^{3}H]TdR uptake on day 7. The analysis was performed using both net PCA generated and stimulation indices.

Induction of PCA by Allogeneic Lymphoblastoid B Cells.   The stimulatory capacity of Daudi cells was analyzed in one-way MLC with allogeneic PBM as responding cells. When the MLC were established at a Daudi/PBM ratio of 1:1 and incubated for 24 h, the PBM gave a 10-fold PCA response relative to autologous control culture (Table III). The time course of PCA generation for 10^6 PBM stimulated by 10^6 Daudi cells is characterized in Fig. 3. By 6 h a readily measurable increase
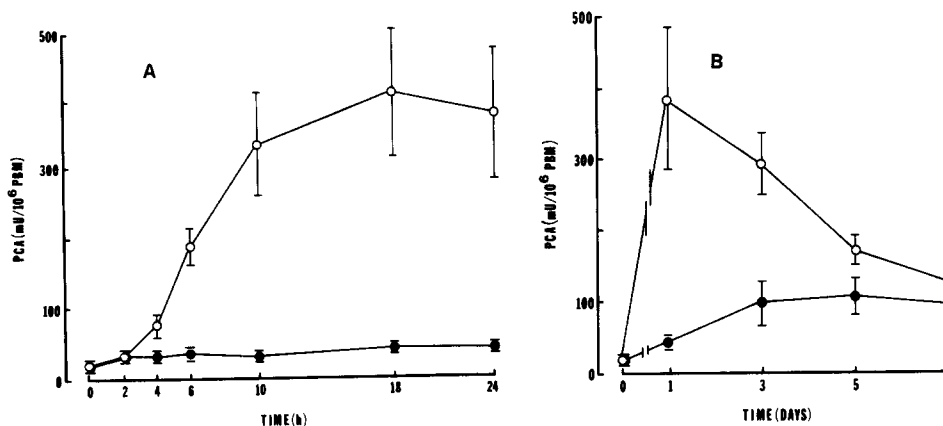
FIGURE 3. Time course of PCA induction in one-way allogeneic MLC. Cultures were established between 10^6 PBM and 10^6 mitomycin C-treated Daudi cells in 1.0 ml of RPMI 1640. Total cellular PCA (mean ± SD of three experiments) for MLC (O) and control autologous cultures (●) were determined.
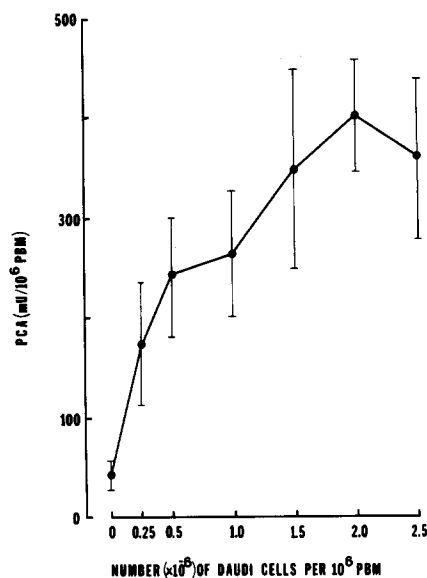

FIGURE 4. Stimulatory capacity of Daudi lymphoblastoid B cells in PCA generation using one-way MLC. Increasing numbers of mitomycin C-treated Daudi cells were added to 10^6 allogeneic PBM, the cell mixtures incubated for 24 h and assayed for total cellular PCA. The maximal stimulation by 2×10^6 Daudi corresponds to a Daudi/monocyte ratio of approximately 8:1.

of PCA from the basal activity was observed. Full PCA of ~ 400 mU/10^6 PBM was reached in 18 h. The activity remained relatively high through day 3, followed by a decline to control levels on day 7. A dose-response study consisting of four experiments is illustrated in Fig. 4. Increasing amounts of Daudi cells were cultured for 24 h with a constant number of PBM, and the PCA determined on cell lysates. An input of 2×10^6 Daudi cells stimulated the highest PCA response in 10^6 PBM, corresponding to a Daudi/monocyte ratio of approxi-

mately 8:1.

Cellular Locus of PCA Induced in Allogeneic MLC. To determine the cell type responsible for PCA generation, allogeneic MLC (PBM) were fractionated after 48 h culture into fibronectin-adherent monocytes and monocyte-depleted (Sephadex G-10 filtration) nonadherent lymphocyte populations. These fractions were then assayed for total cellular PCA. Adherent monocytes contained 179 ± 29 mU PCA/10^6 cells compared with 8.5 ± 1.5 mU PCA/10^6 cells for the lymphocyte-enriched fraction. In further experiments, MLC were established between Daudi cells and T cell-reconstituted monocytes (Daudi/monocyte/T cell ratio, 2:1:4) and after 24 h coculture, the cells were fractionated into adherent monocytes and monocyte-depleted nonadherent cells. The PCA response in the former was 1,140 ± 209 mU/10^6 cells and 44 ± 2 mU/10^6 cells in the latter. The vast majority of the PCA generated in MLC is thus produced by monocytes, consistent with the previous direct cytologic proof of the monocyte origin of PCA for bacterial lipopolysaccharide- and immune complex-stimulated PBM (15, 20, 21), with only negligible levels in lymphocytes or Daudi lymphoblastoid B cells.

Inhibition of the Allogeneically Induced PCA by Monoclonal Anti-HLA-DR Antibodies. To evaluate the role of HLA-DR (human Ia-like) antigens in the process of monocyte stimulation and consequent expression of procoagulant molecules, mouse monoclonal antibodies SG 157 and OKIa1 to common determinants of human HLA-DR were added to two-way MLC at the initiation of cultures. Both antibodies were able to inhibit 100% of the MLC-generated PCA response using 10^6 PBM from two individual donors (Fig. 5). The dose-response curve exhibited a narrow plateau of complete inhibition followed at higher antibody concentrations by lower degree of inhibition (Fig. 5). The latter phenomenon is probably explainable by trace contamination with bacterial endotoxin in the monoclonal antibodies. Both antibodies gave a weakly positive reaction in limulus assay for
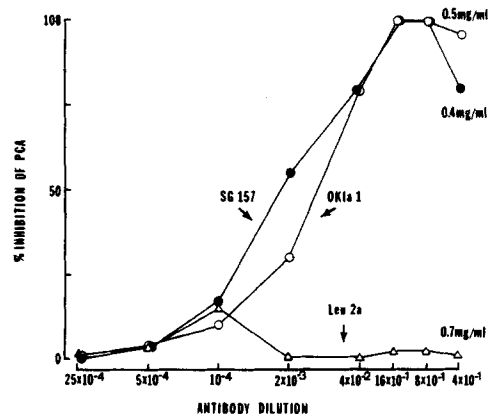
FIGURE 5. Effect of hybridoma monoclonal-derived antibodies to HLA-DR (OK Ia1 and SG-157) as well as control Leu-2 anti-T cell antibody on PCA generation in two-way allogeneic MLC. MLC were established between 10^6 PBM from pairs of donors and the antibodies added at the initiation of the 48-h cultures, after which the cells were assayed for total PCA. For comparison of the three antibodies, the total protein contents in the ascites fluids are given at the highest concentrations used.

bacterial endotoxin at the highest concentrations used in the blocking experiments. The use of an irrelevant control monoclonal antibody, Leu 2a, that was negative for bacterial endotoxin at all concentrations, had no effect on the PCA response (Fig. 5).

T Cell Dependence of the Allogeneic Induction of Monocyte Procoagulant Response.   Cellular requirements for the allogeneic procoagluant response of monocytes were assessed in one-way MLC between Daudi cells and unfractionated PBM, either purified monocytes (>97% nonspecific esterase positive) or monocytes reconstituted with nylon wool effluent T cells. After 24 h the cultures were assayed for PCA generation. Allogeneic stimulation of purified monocytes resulted in a mimimal increase of PCA that was marginally above the control level (Fig. 6). A response comparable to that in unfractionated PBM was restored by reconstitution of 0.5×10^6 monocytes with 2×10^6 syngeneic T cells before starting the MLC.

Characterization of the Procoagulant Activity Induced in Allogeneic MLC.   To examine functional characteristics of the procoagulant effector molecule produced by the monocyte and expressed on its surface, one-stage clotting assays were performed using plasma substrates selectively deficient in single known coagulation proteins. Lysates of alloactivated cells initiated full coagulation activity in plasmas deficient in factors VIII and IX of the intrinsic pathway (Table IV). The cells were unable to fully accelerate the coagulation rate of plasmas deficient in factors VII or X. Reconstitution with purified factor VII or X restored the procoagulant effect of the cells, indicating a requirement for these coagulation proteins.

The MLC homogenates did not induce clotting in a mixture of purified prothrombin and fibrinogen, ruling out the participation of prothrombinase-like activity described in certain other T cell-dependent pathways of monocyte procoagulant induction (16, 20, 22). These results with MLC-induced PCA are characteristic of tissue factor expression by monocytes and were comparable to the initiation of coagulation using a rabbit brain thromboplastin preparation as
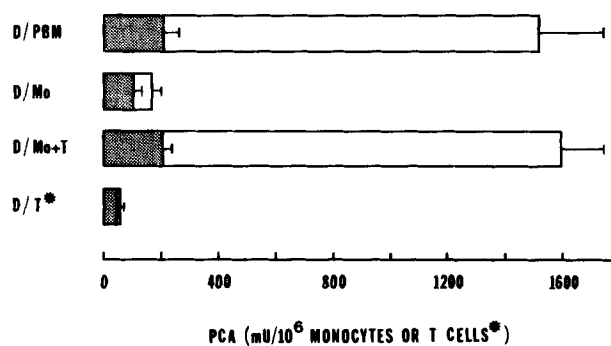

PCA ($mU/10^6$ MONOCYTES OR T CELLS[*])

FIGURE 6.   T cell requirement for optimal PCA response in one-way allogeneic MLC. 10^6 Daudi cells were used to stimulate 10^6 unfractionated PBM (D/PBM) and 0.5×10^6 purified monocytes either alone (D/Mo) or after reconstitution with 2×10^6 T cells (D/Mo + T). Similarly, 2×10^6 T cells were stimulated with 10^6 Daudi cells (D/T). The cultures were incubated for 24 h and assayed for total cellular PCA. Results given are the mean ± SD from six experiments. Shaded portions of the bars are controls obtained by summation of PCA values from participants of MLC cultured separately.

TABLE IV

Requirements of Allogeneically Induced Monocyte PCA for Known Coagulation Proteins

Test sample	PCA assay in various substrate plasmas (clotting time, seconds)				
	NHP*	X deficient	IX deficient	VIII defi-cient	VII defi-cient
Allogeneic culture lysate	54	337	62	55	158
Medium	203	>450	>450	>450	217
Thromboplastin	37	429	40	36	164

* Normal human plasma.

a source of tissue factor (Table IV).

Discussion

Monocyte PCA represents an inducible effector mechanism that has its molecular basis in the expression of cell membrane-associated tissue factor (23) or a recently described cellular serine protease that directly cleaves prothrombin to functional thrombin (16, 22). The synthesis and cell surface expression by monocytes and macrophages of effector molecules able to initiate the coagulation pathways can be induced by a variety of stimuli. These include bacterial LPS (24, 25), antigen-antibody complexes (21, 26), soluble protein antigens in presensitized individuals (27), and mouse hepatitis virus in responsive mouse strains (28).

The present study demonstrates that alloantigens are relatively potent inducers of monocyte PCA in MLC. 71% of 31 donor combinations analyzed in two-way cultures exhibited at least threefold responses relative to controls. When cells from a lymphoblastoid B cell line, Daudi, were used as a source of allogeneic stimulus, the mean relative degree of response was twice that for two-way MLC using PBM from pairs of individual donors. Daudi cells, which express only negligible amounts of basal or inducible PCA, are useful as a model stimulus to investigate the allogeneic PCA response.

The temporal characteristics of the induction of monocyte tissue factor, the procoagulant effector molecule implicated in the allogeneic response, are unique relative to previously described lymphoid procoagulant responses. In contrast to the 4-h maxima observed for immune complexes (21), endotoxin (15), and murine hepatitis virus (18), the two-way allogeneic response required 2–3 d. This induction is, however, rapid for allogeneic responses, as demonstrated by comparison with T cell proliferation in the same MLC. Daudi-stimulated responses were even more prompt, reaching maxima within 18 h.

Few studies of the allogeneic induction of lymphoid PCA have been described. Rickles et al. (3), performing MLC with cells from four donors and assays for PCA after 24, 48, and 72 h, were unable to observe the PCA response. Rothberger et al. (4) subsequently observed enhancement of tissue factor type cellular PCA in allogeneic cultures. Maximal PCA relative to control was observed after 7 d of culture. In these experiments both the MLC and the control autologous cultures produced significant increments of PCA as early as day one,

a result attributed to factors in serum-supplemented tissue culture media. Such an activation, also consistent with endotoxin contamination, which is relatively ubiquitous in commercially available sera and media, would have obscured early increases of PCA due to allogeneic stimulation. Van Ginkel et al. (5) confirmed the induction of tissue factor expression in MLC harvested on day seven but give no information on the temporal course of induction of PCA.

Induction of monocyte PCA response only after 7 d for full activity is remarkably slow and sharply contrasts with other known monocyte PCA-induction pathways, which usually result in maximal activity within 4–6 h (20, 29, 30). This suggests that the cellular pathway and metabolic requirements may differ significantly from those so far described. The apparent discrepancies between the present study and prior results in which either no response was observed or it occurred only relatively late may in part be influenced by differences in methodology. The lower basal or control levels of PCA in our studies clearly permit identification of significant stimulation earlier during the response. Monocyte activation by alloantigens and resultant procoagulant expression also may be dependent on experimental conditions similar to differences described for the T cell proliferative response in MLC (31). The temporal dissociatioh and the lack of quantitative correlation between allogeneic induction of monocyte PCA and T cell proliferation are not surprising in view of the different responding cells. There may also be differences in the allogeneic molecules that are recognized, the required cellular interactions, and the signals and their mode of action.

The PCA response resulting from allogeneic stimulation was blocked by the addition of monoclonal antibodies to HLA-DR antigens. Similarly, the use of polyclonal alloantisera (32) or xenoantisera (33, 34) or monoclonal xenoantisera (35–37) has resulted in various degrees of inhibition of T cell proliferation in human allogeneic MLC. These results have been interpreted to indicate the blocking of stimulatory epitopes on DR (Ia-like) molecules on the stimulator cells, i.e., B cells and monocytes. The same locus of blocking by monoclonal anti-DR antibodies is also possible in our experiments. The relative ease of complete blocking would suggest a major role for DR, and as such, provides the first evidence for specificity in the allogeneic PCA response. Antigens encoded by other locuses are either not stimulatory, or the resulting stimulation is below the limit of detection in our system. Another possible target of blocking with anti-DR could be the T cell collaboration with the effector monocyte, shown in the present report to exist for the MLC-induced procoagulant response. In the mouse, the T cells and monocytes required for full PCA response after stimulation with LPS must be compatible only at the I-A subregion of the major histocompatibility complex (38). Whether this same requirement pertains to this response to allogeneic stimuli in the human is a topic of current investigation.

In the present study the direct requirement for T cells is demonstrated for the first time. This is a reasonable expectation, since it would not be readily anticipated that allogeneic signals would directly induce the monocyte. The low PCA responses of purified monocytes to allogeneic stimulation demonstrate that only small amounts of PCA can be generated via such a direct pathway or that a slight contamination of responding monocytes with T cells is able to support a suboptimal PCA response. A T cell requirement for optimal monocyte PCA

response has been demonstrated for a variety of responses (reviewed in reference 20) and is consistent with the general hypothesis for this pathway that T cells serve as the recognition unit and the monocyte or macrophage as the effector unit.

The alloantigenic stimulation of monocytes resulting in enhanced expression of procoagulant molecules may represent an important link between the immune system and the coagulation pathways, the former being responsible for recognition and the latter for effector function. In clinical transplantation, the tissue lesions of the predominantly vascular (humoral) type of renal allograft rejection often display extensive vascular thrombosis and deposition of fibrin at perivascular locations (6). Circulating blood monocytes or tissue-bound mononuclear phagocytes, described as major constituents of cellular infiltrates in rejected renal allografts (39), may have a role in these clotting processes through allogeneic activation of their procoagulant pathways. This concept is supported by the findings of Hattler et al. (40) who evaluated the PCA in mononuclear blood cells recovered from a rejected renal allograft and demonstrated PCA significantly higher than that in corresponding cells in the peripheral blood. In their recent report, Halloran et al. (41) observed elevated PCA levels in PBM in patients with rejecting renal transplants. In addition, in rejected first-set skin allotransplants, intravascular thrombosis has been a common event according to some studies, such as by Henry et al. (7). In a more recent report, Dvorak et al. (42) have described a less frequent occurrence of thrombotic phenomena but, nevertheless, suggest a contribution of this mechanism to infarction of allografts. In these studies, vascular alterations in skin transplants were accompanied by dermal fibrin accumulation.

Summary

The recognition of alloantigens by human lymphoid cells initiates a collaborative cellular pathway that rather rapidly induces in adherent cells (monocytes) the synthesis and expression of cell surface tissue factor, the initiating cofactor of the extrinsic coagulation pathway. This response was vigorous, generating tissue factor to a level nearly comparable to the response to endotoxin. However, it was temporally discordant with characterized lymphoid procoagulant responses to endotoxin, virus, and immune complexes in that it reached a maxima at 48 h, well after these other responses but clearly much faster than the well recognized proliferative responses to allogeneic stimulation. Using the Daudi lymphoblastoid B cell line, the allogeneic response could be fully elicited in a dose-dependent fashion within 18 h. The induction of monocyte tissue factor required collaboration with T lymphocytes, in accord with previously described T cell-instructed monocyte responses. HLA-DR was implicated as the allogeneic signal by the ability of two monoclonal antibodies to completely block, in a dose-dependent fashion, the induction of this pathway. Notably, the allogeneic procoagulant response was quantitatively discordant with respect to the allogeneic proliferative response, suggesting differences in specificity.

This relatively rapid response may be applicable to typing of determinants in the major histocompatibility complex that are not equivalently identified by alternative analyses, and may be significant in tissue transplantation. The cellular

pathway, linking allogeneic recognition with induction of a monocyte response that initiates the coagulation pathway, represents a further example of the linkage between these biologic systems, and is consistent with a pathogenetic role in allograft rejection by the promotion of vascular thrombosis and interstitial fibrin accumulation.

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