Absence of Monocyte Procoagulant Activity during the Immune Response to Influenza Virus

MARGARET MATHIES† AND NANCY HOGG*

Macrophage Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England

Received 9 August 1988/Accepted 13 December 1988

The ability of mononuclear phagocytes (MPh) to manifest procoagulant activity (PCA) resulting in the formation of fibrin is thought to be a key MPh effector function in tissue repair. The present study addresses the question of whether monocyte PCA is confined to tissue hypersensitivity reactions or is a general correlate of all immune responses. We show here that PCA is not the obligate outcome when the immune system is stimulated. In particular, under in vitro conditions in which a mitogen (phytohemagglutinin) or an antigen (purified protein derivative of tuberculin) elicits good PCA responses, incubation with influenza virus does not result in the generation of PCA, although other parameters of response to the virus appear to be intact. Moreover, influenza virus can cause suppression of PCA when cultures are stimulated with either phytohemagglutinin, purified protein derivative of tuberculin, or endotoxin, conditions which would otherwise result in good PCA responses. This lack of PCA persists throughout the culture period and is not caused by an effect of influenza virus on the viability of either MPh or leukocytes in general.

The ability to generate procoagulant activity (PCA) is an effector function of mononuclear phagocytes (MPh) (8, 10). This monocyte PCA has been identified as thromboplastin, or tissue factor, an agent which initiates the extrinsic pathway of coagulation (9, 19, 37, 48), culminating in the polymerization of fibrin (20). PCA is generated in MPh through major histocompatibility complex (MHC)-restricted T-cell collaboration (28, 42, 43) partly mediated by PCA-specific lymphokines (14, 40). Reports indicate that it can also be induced by the direct interaction of mitogens or antigen-antibody complexes with MPh (8, 10, 44). In vivo, the laying down of fibrin at sites of tissue immune responses has been recognized for many years. For example, fibrin deposits have been observed in models of delayed-type hypersensitivity (36), in kidney allografts (17), and in tumors (7), with a direct association of the fibrin with MPh in each situation (5, 6, 15, 16, 22). It is speculated that this type of monocyte coagulation may be an important component of both wound healing and tissue repair. This raises the question of whether activation of monocyte PCA is the result only of tissue delayed-type hypersensitivity responses (12) or whether it occurs generally in all types of immune activation. We have begun to investigate this question in vitro by comparing the PCA generated by human monocytes in response to a mitogen (phytohemagglutinin [PHA]) and a delayed-type hypersensitivity-type antigen (purified protein derivative of tuberculin [PPD]) (9, 48) with the PCA generated by influenza virus, a stimulus known to provoke both cellular and humoral immune responses in humans.

MATERIALS AND METHODS

Cells and cell culture. Peripheral blood mononuclear cells (PBMs) were obtained from the blood of normal volunteers by Ficoll-Hypaque density centrifugation ($\delta = 1.09$). The cells were washed twice (viability, >95%) and adjusted to a concentration of 2×10^6 viable cells per ml in RPMI 1640 (Flow Laboratories Ltd., Irvine, Scotland) supplemented

with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–1 mM sodium pyruvate–2 mM glutamine–10% type AB human serum. For the proliferation assay, 0.2 ml of medium containing 4×10^5 PBMs and 10 μ l of an appropriate dilution of antigen or mitogen were incubated in 96-well round-bottomed microdilution plates (no. 76-013-05; Flow Laboratories). For the PCA assay, 1 ml of the cell suspension and 10 μ l of an appropriate dilution of the antigen or mitogen were incubated at 37°C in 12-by-75-mm plastic tissue culture tubes (no. 2058; Becton Dickinson Labware, Oxnard, Calif.) for various time periods as indicated.

Antigens, mitogens, and other reagents. Concentrations of antigens and mitogens were selected on the basis of optimal proliferative responses. PHA (Leucoagglutinin; Pharmacia, Inc., Piscataway, N.J., or Difco Laboratories, Detroit, Mich.) was used at a final concentration of 10 μ g/ml; PPD was obtained from the Ministry of Agriculture (Fisheries and Food, Central Veterinary Laboratory, Surrey, England), reconstituted as recommended, and used at a final dilution of 1/2,500 (25 μ g/ml); and Formalin-inactivated influenza virus strain A/Tex/1/77 was obtained from Alan Rosenthal (Merck Sharpe & Dohme Research Laboratories, Rahway, N.J.) and used at 10 μ g/ml, which represented 5 hemagglutinating units per ml. The same doses of PHA, PPD, and influenza virus used to stimulate T-cell proliferation were used to stimulate the production of PCA. Concentrations 5-fold greater or 10-fold less showed no significant differences in the levels of PCA induced.

The above reagents, the standard and low-endotoxin tissue culture media (Flow Laboratories), and added supplements were all tested for the presence of endotoxin by the *Limulus* assay (E-toxate; Sigma Chemical Co., Poole, Dorset, England). The standard tissue culture medium was estimated to contain more than 8 ng of endotoxin per ml; the low-endotoxin tissue culture medium, PHA, PPD, and influenza virus all contained less than 20 pg of endotoxin per ml and were used in the reported experiments except where indicated. When tissue culture medium low in endotoxin (<20 pg/ml) was used, very low amounts of PCA were generated in the absence of added mitogen or antigen. An average of 22.8 ± 13.5 U of PCA was measured in cell sonic

* Corresponding author.

† Present address: Joint Science Department, The Claremont Colleges, 11th and Dartmouth Avenue, Claremont, CA 91711.

extracts from day 1 to 6 of the culture period. This is in contrast to the much higher background levels observed by ourselves and others (44) when tissue culture medium high in endotoxin was used.

A standard preparation of human brain thromboplastin was obtained from the Coagulation Unit, University College Hospital, London, England. The human factor-deficient plasmas were obtained as follows: plasmas deficient in either factor II, VII, VIII, or IX were obtained from George King Bio-Medical Inc., Overland Park, Kans.; plasmas deficient in factor VII or X were obtained from Diagnostic Reagents Ltd., Thame, England; and plasma from two patients deficient in von Willebrand's factor was obtained from the Haematology Unit, Great Ormond St. Hospital, London, England.

Proliferation assay. After the appropriate optimal incubation period, which was 2 to 3 days for PHA-stimulated cultures and 5 to 6 days for PPD- or influenza virus-stimulated cultures, 100 μ l of culture fluid was removed from each well and replaced with 100 μ l of fresh RPMI 1640 containing 7% type AB human serum and 1 μ Ci of [3 H]thymidine (Amersham Corp., Arlington Heights, Ill.). After a further 4-h incubation period, the cells were harvested and counted by liquid scintillation. Samples were always run in quadruplicate.

In vitro anti-influenza virus antibody production. The procedures for the in vitro production and detection of anti-influenza antibody have been previously described in detail (27, 49). Briefly, PBMs stimulated with influenza virus at 0.5 hemagglutinating units per ml were cultured for 6 days in round-bottomed 96-well microdilution trays in RPMI 1640 containing 10% fetal calf serum (FCS). The cells were then washed and recultured in medium containing 5% FCS for a further 18-h period, after which supernatants were collected and assayed for anti-influenza virus antibody by using an enzyme-linked immunosorbent assay.

Assay for PCA. Although the PCA of whole-cell suspensions may be the most relevant in terms of biological function, clotting activities of supernatants, whole-cell suspensions, and sonic extracts were tested in all experiments. Supernatant fluids contained little activity during incubation periods of up to 6 days, during which time cell viability remained high. Because of a previous report in the literature indicating the presence of an inhibitory agent in the culture fluids (13), some experiments were done in which the cells were suspended in their own culture fluid rather than in fresh medium. The results of the two methods of suspension were always comparable, and no evidence was found in any of our experiments for the existence of an inhibitor of PCA in the culture supernatants (data not shown).

After the designated incubation period (varying from 1 to 6 days), the supernatant culture fluids were withdrawn, and the cells were suspended in 1 ml of RPMI 1640 lacking serum. Samples of suspended cells were sonicated for 30 s at an amplitude of 6 μ m with a sonicator (Soniprep 150; MSE Scientific Instruments, Crawley, England). Duplicate samples of supernatant fluid, cell suspension, and sonic extract were tested for procoagulant activity in a standard one-step recalcification assay as follows. One hundred microliters of the sample or of a thromboplastin standard was added to 100 μ l of citrated plasma in a glass tube (3 by 3/8 in. [ca. 8 by 1 cm]) and prewarmed at 37°C for 30 s. The clotting reaction was initiated by the addition of 100 μ l of 25 mM CaCl_2 . The tubes were incubated at 37°C with frequent tipping, and the time required to form a visible fibrin clot was recorded. A standard curve was drawn by plotting the log of known

dilutions of human brain thromboplastin against the clotting time in seconds, and the clotting times of experimental samples were converted to units by assigning the undiluted thromboplastin standard an arbitrary value of 10^7 U/ml. Clotting times normally ranged from approximately 25 s (1:25 dilution of thromboplastin standard) to 150 s (10^{-6} dilution of thromboplastin or RPMI 1640 control). In a second method of data analysis, the data were fitted to a computerized linear regression curve, allowing experimental clotting times to be converted into units of PCA activity. The linear regression analysis was programmed with the interactive mathematical modeling tool Mlab (24). The two methods of computation gave comparable results within 10%. Samples were done in duplicate, and a new standard curve was established for each experiment.

Immunofluorescence assay for viability of monocytes and total PBMs. Each sample tube containing 2×10^6 cells per ml was centrifuged and suspended in 60 μ l of medium containing 2% FCS. Samples of 20 μ l plus appropriate dilutions of monoclonal antibodies specific for myeloid cells (monoclonal antibody 44, used as tissue culture supernatant [32]; anti-Leu M3, 1:20 [Becton Dickinson, Monoclonal Center Inc.]) were incubated on ice for 30 min. Control cultures included medium containing 2% FCS or a monoclonal antibody (5.5) specific for a cytoplasmic antigen (N. Hogg, unpublished data). Cells were washed three times with cold medium, suspended in 50 μ l of rhodamine-conjugated goat anti-mouse immunoglobulin (1:100; Cappel Laboratories, Downingtown, Pa.), and incubated on ice for a further 30 min. After being washed two or three times, cells were suspended in 50 μ l of culture medium and mixed with either trypan blue or 5 drops of an acetone solution containing a few grains of fluorescein diacetate (2). The cell suspensions were then examined by using fluorescence microscopy. Monocytes were identified by rhodamine fluorescence, and lymphocytes were identified by the lack of it. Viable cells could be distinguished by their ability to exclude trypan blue or, in the fluorescein diacetate test, by their ability to metabolize this compound and then exhibit green fluorescence.

RESULTS

Inhibition of leukocyte PCA by influenza virus. In an attempt to determine whether leukocyte PCA is activated in response to different kinds of immune stimulation, PBMs isolated from normal volunteers were incubated in culture with influenza virus (strain A/Tex/1/77), PHA (a T-cell mitogen), and PPD (the antigen associated with tuberculin sensitivity reactions). After 6 days of culture, samples of each cell suspension (supernatants, intact cells, and sonicated cells) were assayed to determine the amount of PCA which had been formed. Figure 1 shows the PCA present both in whole cells and in cell sonic extracts of a typical experiment with PBMs from three individuals who gave a proliferative response to PPD (and PHA). The culture of PBMs for 6 days in the presence of PHA or PPD resulted in a significant increase in the amount of PCA over that which was formed when cells were incubated in medium alone. Although the amount of intracellular PCA detected was always greater than that of membrane-bound PCA, the proportions did not substantially differ with the method of stimulation. For example, the percentage of PCA detected in whole cells compared with sonicated cells was $21.2 \pm 14\%$ in PPD-stimulated cultures, $34.5 \pm 20\%$ in PHA-stimulated cultures, $25.6 \pm 15.4\%$ in influenza virus-stimulated cultures, and $16.6 \pm 7\%$ for PBMs incubated in medium alone (n

FIG. 1. Procoagulant activity of PBMs (2×10^6 /ml) cultured in medium alone or with PHA ($10 \mu\text{g/ml}$), PPD ($25 \mu\text{g/ml}$), or influenza virus (FLU) ($10 \mu\text{g/ml}$ [5 hemagglutinating units per ml]) for 6 days. The PCAs of intact cells and cell sonic extracts from three individuals were compared and were calculated as units $\pm$ standard deviation per 2×10^5 PBMs.

= 31). Unexpectedly, the presence of influenza virus in concentrations which were known to stimulate a substantial proliferative response (see below) either failed to cause an increase in PCA or actually suppressed the background level of PCA present when cells were cultured in medium alone.

Further characterization of the inhibition of PCA by influenza virus: no interference in the thromboplastin assay by virus or cell culture products. It seemed possible that the lowered clotting activity seen in the cultures containing influenza virus might be due to direct interference with the clotting reaction either by the virus itself or by some inhibitory agent present in the cultures. To test for this, samples of the influenza virus preparation (see Materials and Methods) and cell sonic extracts from the influenza virus-incubated cultures were mixed with samples of either the brain thromboplastin standard or sonic extracts of PHA-stimulated cells and were shown to have no direct inhibitory effect on the clotting process, i.e., the clotting times were not prolonged by the presence of these sonic extracts (data not shown). We thus concluded that influenza virus exerts its inhibitory effect at the level of the development of PCA in the cell culture and not simply on the clotting reaction per se.

No toxic effect of virus on leukocytes. The fact that PCA levels failed to develop in cultures containing influenza virus might also have been explained if the virus were exerting a toxic effect on the cultured cells. Because monocytes are the cells which actively produce PCA, but T cells are known to contribute inducing factors, it was of interest to assess the viability of both cell types throughout the incubation period. Samples from influenza virus-containing, PPD-containing, and control cultures were tested prior to incubation (time 0) and after 1, 2, and 6 days of culture. Both monocytes (which

were specifically labeled with monoclonal antibodies 44 and Leu M3) and lymphocytes (not labeled with these monoclonal antibodies) continued to fluoresce brightly when stained with fluorescein diacetate throughout the culture period, indicating that they remained viable in all cultures for at least 6 days. In addition, total leukocyte viability remained high during the same period in all cultures tested, whether by trypan blue exclusion or the fluorescein diacetate method.

Lack of correlation of "flu effect" with other parameters of immune function. Previous studies have indicated that there is wide variation among individuals in the ability of their PBMs to mount an in vitro response to influenza virus (33). We wished to determine whether the depressive effect of the virus on PCA formation is a general phenomenon or whether it is correlated with a low immune response to influenza virus and was thus observed in our study because we had, by chance, happened to select only low responders as subjects.

An experiment to test this point is shown in Table 1. The PBMs from seven individuals were simultaneously incubated in culture for 6 days and tested for cell proliferation, levels of anti-influenza virus antibody, and ability to generate procoagulant activity. Three general patterns of response occurred. Two individuals (subjects 1 and 2) gave a high proliferative response and high titers of antibody against influenza virus ($>30 \text{ ng/ml}$), another two (subjects 3 and 4) also gave a high proliferative response but were low antibody responders, and the remaining three individuals (subjects 5, 6, and 7) were low responders in terms of both T-cell thymidine incorporation and in vitro antibody production. In this experiment the PBMs from these seven individuals were also cultured with or without influenza virus for 6 days in medium containing 8 ng of endotoxin per ml and assayed for

TABLE 1. Lack of correlation between influenza virus-specific immune responses and generation of PCA

Subject no.	PBM proliferation (cpm) ^a		Stimulation index	Antibody titer (ng/ml) ^b	PCA (U/2 × 10 ⁵ sonicated cells) ^c		% Inhibition of PCA
	+Flu	-Flu			-Flu	+Flu	
1	51,804	1,152	45.0	79.4	779.0	117.5	84.9
2	22,763	1,352	16.8	30.2	1,674.0	35.0	97.9
3	27,477	529	51.9	3.7	2,708.5	133.0	95.1
4	37,014	545	67.9	6.3	229.5	76.5	66.7
5	10,175	937	10.9	8.1	1,260.0	94.5	92.5
6	7,712	568	13.6	6.3	1,831.0	60.5	96.7
7	1,680	327	5.1	5.8	342.0	34.0	90.1

^a The proliferation of 4 × 10⁵ PBMs cultured in the presence (+Flu) or absence (-Flu) of influenza virus (at 5 hemagglutinating units per ml for 6 days) was assessed by measuring [³H]thymidine incorporation.

^b Amount of anti-influenza virus antibodies made by 4 × 10⁵ PBMs on day 7 of response to influenza virus.

^c PCA of 2 × 10⁵ PBMs cultured for 6 days in the presence (+Flu) or absence (-Flu) of influenza virus.

PCA. The depressive effect of the virus on PCA occurred in all seven cases, although there was some variation in the percentage of depression because of the differences in the amount of PCA generated in the cultures in the presence of endotoxin. We conclude from this experiment that the inhibition of PCA by influenza virus is shown by the PBMs of all individuals, regardless of their ability to mount an *in vitro* immune response to influenza virus. The fact that these cells proliferated and produced antibody in the presence of influenza virus further suggests that its effect on PCA is not simply due to a generalized inactivation of cells resulting from agglutination, fusion, or other membrane alterations.

Effect of influenza virus on monocyte PCA which is stimulated by other antigens and mitogens. In order to test whether the presence of influenza virus would inhibit the increase in PCA caused by other agents, PBMs were cultured in the presence of PHA or PPD with and without the addition of influenza virus (Table 2). The addition of influenza virus was found to depress the normal amount of PCA formed in response to PPD by 95%. Similarly, influenza virus had a depressing effect on the formation of PCA in PHA-stimulated cultures, although the effect was much more variable. Preliminary studies using live virus of other strains of influenza A (Chile and Mississippi) indicated a similar pattern of depression (data not shown).

Time course of PCA induction and inhibition by influenza virus. The inhibition of monocyte PCA by influenza virus was examined over the course of 6 days. A typical experiment is illustrated in Fig. 2. Although the influenza virus inhibition of both PPD and PHA responses was evident within the first 24 h in culture, the extent of inhibition was

TABLE 2. Depression by influenza virus of PCA stimulated by other agents (cell sonic extracts, day 6)^a

PCA stimulant	PCA (U/2 × 10 ⁵ sonicated cells)		% PCA decrease
	-Flu	+Flu	
PPD (n = 12)	1,952 ± 1,087	86 ± 50	95 ± 2
PHA (n = 12)	2,336 ± 934	626 ± 368	70 ± 16

^a PBMs (2 × 10⁵) were cultured for 6 days in the presence of PPD or PHA alone (-Flu) or in the presence of PPD or PHA plus influenza virus (+Flu).

FIG. 2. Time course of PCA induction and inhibition by influenza virus. The samples represented were exposed to medium alone, influenza virus (FLU), PPD, PPD plus influenza virus (PPD + FLU), PHA, or PHA plus influenza virus (PHA + FLU). The data shown are from an experiment typical of three which were carried out by using different donors. The PCA was calculated as units per 2 × 10⁵ PBMs.

more striking later in the incubation period, e.g., on days 4 to 6. The generally heightened responsiveness evident on day 1 compared with day 2 has been observed by others (48) and probably resulted from nonspecific activation of monocytes during harvesting and preparation for culture.

Nature of the PCA. Given the multiplicity of interacting factors known to be involved in clotting, it was of interest to try to define the nature of the substance(s) produced in these PBM cultures which resulted in the enhanced clotting activity. Plasmas deficient in various blood clotting factors were tested with pooled PHA- and PPD-stimulated cell sonic extracts from day-5 or -6 cultures. The brain thromboplastin standard was used to assess the activity of the extrinsic pathway, and tissue culture medium was used to control for the reactions of the intrinsic pathway. The results of a typical experiment (Table 3) indicate that the detected PCA had a partial dependence on an exogenous source of factor VII and an absolute dependence on factor X.

DISCUSSION

Mononuclear phagocytes have essential functions in several aspects of the immune response. One function which

TABLE 3. Requirement of monocyte PCA for known coagulation proteins

Plasma deficient in factor:	Clotting time (s) with:		
	Tissue culture medium (intrinsic pathway)	Brain thromboplastin ^a (extrinsic pathway)	Cell sonic extract ^b
None ^c	160.5	42.5	42.5
VII	151	124	75
VIII	>900	44	45
von Willebrand's	>300	53	57
IX	>900	50	45
X	>900	>900	>900

^a Brain thromboplastin standard diluted 1:600 to provide activity comparable with that of the cell sonic extract.

^b 2 × 10⁵ PPD- and PHA-stimulated cells per sample.

^c Normal human plasma.

has been recently identified is the production by stimulated monocytes of procoagulants, i.e., factors which activate the clotting reaction. In an attempt to ascertain whether this particular MPh activity is associated with all immune responses which are dependent on accessory cells or whether it is selectively induced, we compared the ability of PHA, PPD, and influenza virus to elicit monocyte PCA in vitro. Both PHA and PPD were found to induce high levels of a PCA which showed complete dependence on factor X but only partial dependence on factor VII. These findings confirm previous reports (18, 43) and can be explained by the observations that stimulated monocytes not only make thromboplastin or tissue factor (10, 19) but also synthesize (47) and bind (3) factor VII/VIIa. Such activated cells are thus capable of assembling on the membrane the initial components of the extrinsic coagulation pathway which, by interaction with factors X, II, and fibrinogen from plasma, are able to generate fibrin (21, 46). However, in contrast to the PCA stimulated by PHA and PPD, we observed a failure of PCA induction in cultures responding to influenza virus. This flu effect was able to override the PCA-inducing activities of other activating agents. Thus, when cultures were incubated with PHA and PPD along with influenza virus, the expected PCA was either severely or completely depressed.

As induction of PCA generally appears to require MHC-restricted collaboration with T cells (19, 20, 48), it is possible that the lack of PCA in influenza virus-incubated cultures could be due to an effect by virus on T cells in the form of generation of suppression or inhibition of help. However, there was no correlation in this study between the lack of PCA and differences in levels of influenza-stimulated T-cell proliferation or in T-cell help assessed at the level of antibody production (Table 1). These findings confirm the many reports of adequate humoral and cellular responses to influenza virus observed both in vitro (26, 33) and in vivo (34, 35). It is possible that there was a failure on the part of influenza virus-activated T cells to produce the PCA-specific lymphokine(s) (40, 43). However, murine influenza-specific T cells have been shown to make such PCA-inducing activity (41).

In general, influenza virus did not affect the proliferative response to PHA which normally appeared 3 days before the optimum proliferation induced by influenza virus itself (M. Mathies and B. Lum, unpublished observations). Thus, the virus did not appear to affect T-cell responses or the accessory cell activities of monocytes upon which T-cell responses depend.

The most likely explanation for the PCA inhibitory effect is a direct interaction of virus with monocytes, which are known to be targets of infection by live influenza virus (18, 33). For example, endotoxin, which acts directly on monocytes to generate PCA (44), has its effect blocked by influenza virus. Preliminary experiments in which separated cell populations were individually incubated with influenza virus in the presence of endotoxin reinforce this conclusion (data not shown). A simple explanation for the lack of PCA might be that exposure to virus affected the viability of the monocytes. However, neither monocytes nor PBMs showed any decline in viability during the experiments.

The functioning of MPh appears to be affected in a selective manner. Thus, accessory cell activity remained intact, as immune responses to influenza virus could be generated in vitro (26, 33) and proliferation initiated by PHA was not significantly depressed. However, numerous MPh functions have been reported to become depressed when monocytes are exposed to live influenza virus. Others have

found that live influenza virus, but not inactivated virus (4), causes decreases in (i) mitogen responses (4, 31, 39), (ii) immunoglobulin production (4), (iii) the ability of monocytes to phagocytose (11, 31), and (iv) the response to chemotactic signals (11, 23). It appears that such infection has a broad effect on monocyte-differentiated functions while not affecting viability (4). The cleavage of 5'-terminal fragments from host cell mRNAs by viral core proteins (25) provides a possible mechanism by which influenza virus could prevent the expression of newly induced monocyte functions. This activity does not require viral replication, and whether it proceeds to some degree with inactivated virus is not known. If so, the generation of PCA is one differentiated function of monocytes which is extremely sensitive to viral exposure. It would be interesting to know whether there is a relationship between a lack of PCA and exposure to other viruses which preferentially infect human macrophages, such as measles virus (4), poliovirus (45), and cytomegalovirus (38).

Lack of PCA is not characteristic of all viral infections, as the level of response to mumps virus in humans (13), West Nile virus (1), and hepatitis virus in mice (29, 30) has been directly correlated with the level of PCA. The amount of PCA made in response to infection with murine hepatitis virus MHV.3 depends on the strain of mouse (29). Mice which succumb to MHV.3 infection are those which generate high levels of PCA, and there are indications that the microthrombi formed are a key factor in the pathology of the infection (30). The laying down of fibrin may serve as a useful early event in the repair of damage during tissue immune responses, but a role for such an activity within the peripheral circulation, inevitably leading to obstruction, is not apparent. Further studies are required to elucidate the kinds of immune responses which are accompanied by the induction of PCA and to understand the physiological significance of this phenomenon.

ACKNOWLEDGMENTS

This work was supported by the Imperial Cancer Research Fund. We are extremely grateful to Linda Wilkinson (Coagulation Unit, University College Hospital, London, England) for donating to us many coagulation factor-deficient plasmas and for assistance with the recalcification assay, to Jonathan Lamb (MRC TB Unit, Hammersmith Hospital, London, England) for performing the anti-influenza virus antibody enzyme-linked immunosorbent assays, to Chris Rawlings (Imperial Cancer Research Fund) for devising the Mlab program for linear regression analysis, and to Kim Richardson and Sarah Murdoch for preparation of the manuscript.

LITERATURE CITED

1. Allan, J. E., and P. C. Doherty. 1986. Stimulation of helper/delayed type hypersensitivity T cells by flavivirus infection: determination by macrophage procoagulant assay. *J. Gen. Virol.* 67:39-46.
2. Beverley, P. C. L. 1986. Human leucocyte subpopulations, p. 58.1-58.15. In D. M. Weir (ed.), *Handbook of experimental immunology*. Cellular immunology, 4th ed. Blackwell Scientific Publications, Ltd., Oxford.
3. Broze, G. J. 1982. Binding of human factor VII and VIIa to monocytes. *J. Clin. Invest.* 70:526-535.
4. Casali, P., G. P. A. Rice, and M. B. A. Oldstone. 1984. Viruses disrupt functions of human lymphocytes. Effects of measles virus and influenza virus on lymphocyte-mediated killing and antibody production. *J. Exp. Med.* 159:1322-1337.
5. Colvin, R. B., R. A. Johnson, M. C. Mihm, and H. F. Dvorak.

1973. Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. *J. Exp. Med.* **138**:686-698.
6. Dvorak, A. M., A. B. Connell, K. Proppe, and H. F. Dvorak. 1978. Immunologic rejection of mammary adenocarcinoma (TA3-St) in C57B1/6 mice: participation of neutrophils and activated macrophages with fibrin formation. *J. Immunol.* **120**:1240-1248.
7. Dvorak, H. F., S. Orenstein, A. C. Carvalho, W. Hallowell, W. H. Churchill, A. M. Dvorak, S. J. Galli, J. Feder, A. M. Bitzer, J. Rypysc, and P. Giovenco. 1979. Induction of a fibrin-gel investment: an early event in line 10 hepatocarcinoma growth mediated by tumor-secreted products. *J. Immunol.* **122**:166-174.
8. Edgington, T. S., G. A. Levy, B. S. Schwartz, and D. S. Fair. 1981. A unidirectional pathway of lymphocyte-instructed macrophage and monocyte function characterized by the generation of procoagulant monokines, p. 173-196. *In* W. O. Weigle (ed.), *Advances in immunopathology*. Elsevier/North-Holland Publishing Co., New York.
9. Edwards, R. L., and F. R. Rickles. 1980. The role of human T cells (and T cell products) for monocyte tissue factor generation. *J. Immunol.* **125**:606-609.
10. Edwards, R. L., and F. R. Rickles. 1988. The role of monocyte tissue factor in the immune response. *Lymphokine Rep.* **1**:181-210.
11. Gardner, I. D., and J. W. M. Lawton. 1982. Depressed human monocyte function after influenza infection *in vitro*. *RES J. Reticuloendothel. Soc.* **32**:443-448.
12. Geczy, C. L., E. Farram, D. K. Moon, P. A. Meyer, and I. F. C. McKenzie. 1983. Macrophage procoagulant activity as a measure of cell-mediated immunity in the mouse. *J. Immunol.* **130**:2743-2749.
13. Geczy, C. L., and P. A. Meyer. 1982. Leukocyte procoagulant activity in man: an *in vitro* correlate of delayed-type hypersensitivity. *J. Immunol.* **128**:331-336.
14. Gregory, S. A., R. S. Kornbluth, H. Helin, H. G. Remold, and T. S. Edgington. 1986. Monocyte procoagulant inducing factor: a lymphokine involved in the T cell-instructed monocyte procoagulant response to antigen. *J. Immunol.* **137**:3231-3239.
15. Hancock, W. W. 1984. Analysis of intragraft effector mechanisms associated with human renal allograft rejection: immunohistological studies with monoclonal antibodies. *Immunol. Rev.* **77**:61-84.
16. Hancock, W. W., R. H. Lord, A. J. Colby, T. Diamantstein, F. R. Rickles, C. Dijkstra, N. Hogg, and N. L. Tilney. 1987. Identification of IL 2R⁺ T cells and macrophages within rejecting rat cardiac allografts, and comparison of the effects of treatment with anti-IL 2R monoclonal antibody or cyclosporin. *J. Immunol.* **138**:164-170.
17. Hattler, B. G., R. E. Rocklin, P. A. Ward, and F. R. Rickles. 1973. Functional features of lymphocytes recovered from a human renal allograft. *Cell. Immunol.* **9**:219-234.
18. Helin, H., and T. S. Edgington. 1983. Allogeneic induction of the human T cell instructed monocyte procoagulant response is rapid and elicited by HLA-DR. *J. Exp. Med.* **158**:962-975.
19. Hetland, O., A. Brovold, R. Holme, G. Gaudernack, and H. Prydz. 1985. Thromboplastin (tissue factor) in plasma membranes of human monocytes. *Biochem. J.* **228**:737-743.
20. Hogg, N. 1983. Human monocytes are associated with the formation of fibrin. *J. Exp. Med.* **157**:473-485.
21. Hogg, N. 1983. Human monocytes have prothrombin cleaving activity. *Clin. Exp. Immunol.* **53**:725-730.
22. Hopper, K. E., C. L. Geczy, and W. A. Davies. 1981. A mechanism of migration inhibition in delayed type hypersensitivity reactions. I. Fibrin deposition on the surface of elicited peritoneal macrophages *in vivo*. *J. Immunol.* **126**:1052-1058.
23. Kleinerman, E. S., C. A. Daniels, R. P. Polisson, and R. Synderman. 1976. Effect of virus infection on the inflammatory response: depression of macrophage accumulation in influenza-infected mice. *Am. J. Pathol.* **85**:373-382.
24. Knott, G. D. 1979. Mlab—a mathematical modeling tool. *Comp. Programs Biomed.* **10**:271-280.
25. Krug, R. M. 1985. The role of RNA priming in viral and trypanosomal mRNA synthesis. *Cell* **41**:651-652.
26. Lamb, J. R., D. D. Eckels, M. Phelan, P. Lake, and J. N. Woody. 1982. Antigen-specific human T-lymphocyte clones: viral specificity of influenza virus immune clones. *J. Immunol.* **128**:1428-1432.
27. Lamb, J. R., J. N. Woody, R. J. Hartzman, and D. D. Eckels. 1982. *In vitro* influenza virus-specific antibody production in man: antigen-specific and HLA-restricted induction of helper activity mediated by cloned human T lymphocytes. *J. Immunol.* **129**:1465-1470.
28. Levy, G. A., and T. S. Edgington. 1980. Lymphocyte cooperation is required for amplification of macrophage procoagulant activity. *J. Exp. Med.* **151**:1232-1244.
29. Levy, G. A., J. L. Leibowitz, and T. S. Edgington. 1981. Induction of monocyte procoagulant activity by murine hepatitis virus type 3 parallels disease susceptibility in mice. *J. Exp. Med.* **154**:1150-1163.
30. Levy, G. A., P. J. MacPhee, L. S. Fung, M. M. Fisher, and A. M. Rappaport. 1983. The effect of mouse hepatitis virus infection on the microcirculation of the liver. *Hepatology* **3**:964-973.
31. Lewis, D. E., B. E. Gilbert, and V. Knight. 1986. Influenza virus infection induces functional alterations in peripheral blood lymphocytes. *J. Immunol.* **137**:3777-3781.
32. Malhotra, V., N. Hogg, and R. B. Sim. 1986. Ligand binding by the p150.95 antigen of U937 monocytic cells: properties in common with complement receptor type 3 (CR3). *Eur. J. Immunol.* **16**:1117-1123.
33. McMichael, A. J., F. M. Gotch, D. W. Dongworth, A. Clark, and C. W. Potter. 1983. Declining T-cell immunity to influenza, 1977-82. *Lancet* **ii**:762-764.
34. Mitchell, D. M., P. Fitzharris, R. A. Knight, and G. C. Schild. 1982. Kinetics of specific *in vitro* antibody production following influenza immunization. *Clin. Exp. Immunol.* **48**:491-498.
35. Mitchell, D. M., A. J. McMichael, and J. R. Lamb. 1985. The immunology of influenza. *Br. Med. Bull.* **41**:80-85.
36. Nelson, D. S. 1965. The effects of anticoagulants and other drugs on cellular and cutaneous reactions to antigen in guinea-pigs with delayed-type hypersensitivity. *Immunology* **9**:219-234.
37. Prydz, H., and A. C. Allinson. 1978. Tissue thromboplastin activity of isolated human monocytes. *Thromb. Haemostasis* **39**:582-591.
38. Rice, G. P. A., R. D. Schrier, and M. B. A. Oldstone. 1984. Cytomegalo-virus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. *Proc. Natl. Acad. Sci. USA* **81**:6134-6138.
39. Roberts, N. J., and R. T. Steigbigel. 1978. Effect of *in vitro* virus infection on response of human monocytes and lymphocytes to mitogen stimulation. *J. Immunol.* **121**:1052-1058.
40. Ryan, J., and C. L. Geczy. 1986. Characterization and purification of mouse macrophage procoagulant inducing factor. *J. Immunol.* **137**:2864-2870.
41. Schiltknecht, E., G. L. Ada, and T. J. Braciale. 1984. Macrophage procoagulant-inducing activity of influenza-specific effector T cells. *Cell. Immunol.* **89**:342-354.
42. Schwartz, B. S. 1985. Antigen induced monocyte procoagulant activity. Requirement for antigen presentation and histocompatibility leukocyte antigen DR molecules. *J. Clin. Invest.* **76**:970-977.
43. Schwartz, B. S., P. J. Reitnauer, J. A. Hank, and P. M. Sondel. 1985. A human T cell clone that mediates the monocyte procoagulant response to specific sensitizing antigen. *J. Clin. Invest.* **76**:1279-1282.
44. Semeraro, N., A. Biondi, R. Lorenzet, D. Locati, A. Mantovani, and B. Donati. 1983. Direct induction of tissue factor synthesis by endotoxin in human macrophages from diverse anatomical sites. *Immunology* **50**:529-535.
45. Soontiens, F. J. C. J., and J. van der Veen. 1973. Evidence for a macrophage-mediated effect of poliovirus on the lymphocyte response to phytohemagglutinin. *J. Immunol.* **111**:1411-1419.
46. Tracy, P. B., M. S. Rohrbach, and K. G. Mann. 1983. Functional prothrombinase complex assembly on isolated monocytes and lymphocytes. *J. Biol. Chem.* **258**:7264-7267.
47. Tsao, B. P., D. S. Fair, L. K. Curtiss, and T. S. Edgington. 1984.

- Monocytes can be induced by lipopolysaccharide-triggered T lymphocytes to express functional factor VII/VIIIa protease activity. *J. Exp. Med.* **159**:1042–1057.
48. **van Ginkel, C. J. W., W. P. Zeijlemaker, L. A. M. Stricker, J. I. H. Oh, and W. G. van Aken.** 1981. Enhancement of monocyte thromboplastin activity by antigenically stimulated lymphocytes: a link between immune reactivity and blood coagulation. *Eur. J. Immunol.* **11**:579–583.
49. **Zanders, E. D., C. M. Smith, and R. E. Callard.** 1981. A micromethod for the induction and assay of specific *in vitro* antibody responses by human lymphocytes. *J. Immunol. Methods* **47**:333–338.