Regulatory Roles of Tμ and Tγ Cells in the Collaborative Cellular Initiation of the Extrinsic Coagulation Pathway by Bacterial Lipopolysaccharide

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Abstract

The Shwartzman reaction is a classic biologic response in which the coagulation system is activated in vivo. Cellular initiation of the extrinsic coagulation protease cascade can be mediated by one or more limbs of the lymphoid response to diverse biological stimuli. The T cell-instructed monocyte and macrophage responses that have been implicated are mediated by a number of different cellular pathways and are elicited not only by antigens and allogeneic cells but also by other stimuli such as immune complexes and the lipid A moiety of bacterial lipopolysaccharide (LPS). The latter response has been implicated in the pathogenesis of the disseminated intravascular coagulation associated with bacterial infection. In the rapid collaborative cellular pathway response to LPS, we have described a relatively rigorous requirement for T helper cells in induction of the biosynthesis of tissue factor and Factor VII by monocytes. To elucidate potential regulatory aspects of this cellular procoagulant response, we provide the first evidence for the existence of T suppressor cells for the cellular procoagulant response to LPS by the rapid T cell-instructed pathway. Human peripheral blood lymphocytes were separated by cytoaffinity into Fcγ-positive and Fcμ-positive cells and were characterized for their functional properties in the procoagulant response. Tμ cells mediated the monocyte response, consistent with their identity with instructor cells. Tγ cells suppressed the response of monocytes to LPS in the presence of Tμ cells, suggesting that they possess suppressor function for this response. The Tγ suppressor cells required stimulation by LPS to express their suppressor function and they exerted their suppressive effect directly on the monocyte. The existence and participation of LPS-responsive T suppressor cells on the cellular procoagulant response in vitro add a new dimension to the complexity of the rapid pathway of the collaborative cellular procoagulant response and may be important in the pathogenesis of disseminated intravascular coagulation.

Introduction

The recognitive repertoire of the lymphoreticular system to diverse biological stimuli is extensive. Once triggered by stimuli, specific T cells can elicit specific differentiated functions in a variety of effector cells such as the resting B lymphocyte, the precytolytic T cell, and the monocyte or macrophage. Fibrin deposition, the product of selective induction of certain T cell instructed monocyte responses, is a recognized feature of a variety of immunologic tissue lesions among which the lesions of hyperacute renal allograft rejection (1), delayed type hypersensitivity reactions (DTH)[1] (2, 3), and the synovial lesions of rheumatoid arthritis (4) may be examples. Anticoagulant therapy inhibits the DTH reaction both in animals (5) and in humans (6). Induration of the DTH lesion is a result of fibrin deposition as evidenced by the absence of induration in congenital afibrinogenemic individuals (3).

Peripheral blood mononuclear cells (PBM) as well as other lymphoid populations initiate and propagate the extrinsic coagulation protease cascade, both in vitro and in vivo, in response to a variety of stimuli (7, 8) including endotoxin (9, 10), complement fragments (11), lectins (12), antigens (13–15), viruses (16, 17), immune complexes whether soluble (18, 19) or cell surface-associated (20), and allogeneic stimulation (21–23). Biosynthesis of the tissue factor molecule (24) as well as of Factor VII (25) is observed in the human response to lipopolysaccharide (LPS). An independent protease capable of cleaving prothrombin to thrombin and designated monocyte prothrombinase has been described in response to certain classes of lipoproteins (26) and in the mouse to a variety of stimuli (27). We as well as others have shown that monocytes are the cellular source of procoagulant activity (PCA), and T lymphocytes are necessary for the full and rapid collaborative induction of these monocyte PCA responses (28–30). Two general T cell dependent cellular pathways have been suggested, namely direct cellular contact between T cells and monocytes as exemplified by the rapid type I response to LPS and immune complexes (28–31), and a slower indirect type II response mediated by lymphokines (8, 23, 32, 33).

We have shown the existence of T helper cells for the LPS-driven monocyte PCA responses which were of the Leu 3a and 9.3 monoclonal antibody marker class by fluorescent activated cell sorting (34, 35). In this study we extend analyses of the regulatory T cells for the type I response to LPS (7) to provide the first evidence for the unanticipated existence of LPS responsive T suppressor cells. Moretta et al. (36, 37) showed that T lymphocytes possess surface Fc receptors for IgM (Tμ) and IgG (Tγ). In a pokeweed mitogen-driven system

Portions of this work were presented at the annual meeting of the American Association of Immunologists, Chicago, IL, 1983. This is publication IMM 3587 from the Scripps Clinic.

Dr. Levy received a Medical Research Council of Canada Scholarship. Dr. Schwartz is a Fellow of the John A. Hartford Foundation.

Received for publication 13 November 1984.

J. Clin. Invest.

0021-9738/85/08/0548/08 $1.00
Volume 76, August 1985, 548–555

1. *Abbreviations used in this paper:* DTH, delayed type hypersensitivity reactions; EA-IgG, ox erythrocytes coated with specific IgG antibody; EA-IgM, ox erythrocytes coated with specific IgM antibody; LPS, lipopolysaccharide; nSRBC, neuraminidase-treated sheep erythrocytes; PBM, peripheral blood mononuclear cells; PCA, procoagulant activity; Tμ, T lymphocyte surface Fc receptors for IgM; Tγ, T lymphocyte surface Fc receptors for IgG.

of polyclonal immunoglobulin (Ig) synthesis by B lymphocytes, T cells with Fc receptors for IgG (Tγ) deliver a suppressor signal, whereas those with Fc receptors for IgM (Tμ) provide the requisite helper function. This system has been used as an in vitro analytical model for analysis of regulatory cells and has been related to antigen-driven responses. In the present studies we show that the Tμ subset of T cells is capable of supporting the induction of monocyte PCA by LPS. Furthermore, Tγ cells suppress the induction of monocyte PCA of the tissue factor type. The existence of such a regulatory network for a nonantigenic response, supports the hypothesis that regulatory T cells have evolved to provide recognitive functions of broad specificity and to couple such recognitive events to the appropriate effector cells. This role may be of potential significance in the variation of susceptibility of mouse strains to endotoxin in eliciting disseminated intravascular coagulation, and in the possibility that the great variation in severity of clinical disseminated intravascular coagulation may be attributable in part to regulatory variables imposed by T suppressor cells.

Methods

Cells. PBM were isolated from heparinized venous blood of normal healthy fasting volunteers by centrifugation over Ficoll-Hypaque as previously described (38). Viability of cells was >98% as assessed by trypan blue dye exclusion; and recovery of cells was >80%. After isolation, the cells were resuspended in complete media consisting of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 25 μg/ml aureomycin, grade II (Sigma Chemical Co., St. Louis, MO), 2 mM glutamine, and 10% fetal calf serum (Flow Laboratories, Inc., McLean, VA) or in serum-free medium. PBM were separated into predominantly T and non-T cell populations using neuraminidase-treated sheep erythrocytes (nSRBC) (38). Equal volumes of 5% nSRBC in RPMI and PBM were resuspended at 1×10^7 cells/ml in serum-free media and incubated for 10 min at 37°C. The cells were centrifuged at 475 *g* for 5 min and were incubated at ambient temperature (23°C) for 60 min. The cells were resuspended gently and 5.0 ml fractions were layered onto 3.0 ml Ficoll-Hypaque (1.074 g/cm^3, Ficoll, Pharmacia Fine Chemicals, Piscataway, NJ; Hypaque, Winthrop Laboratories, Sterling Drug Co., Aurora, Ontario) and the gradients were centrifuged at 1,200 *g* for 15 min. Cells at the interface were <1% nSRBC positive. They were washed and resuspended at 1×10^6 cells/ml. Monocytes were separated by cytoadherence to plastic dishes as previously described (29) and were >94% esterase-positive and >97% latex-positive. Monocyte preparations used in these studies were minimally responsive to LPS for the PCA response in the absence of T cells, and were thus competent to analyse the type I PCA response.

The nSRBC-positive T cells were collected at the bottom of the Ficoll-Hypaque gradient and the nSRBC were lysed by incubation at 37°C for 10 min with 0.14 M NH_4Cl_2, 0.015 M Tris, pH 7.2. These T cell preparations were >98% nSRBC-positive. These T enriched cells were subfractionated after culture overnight at 2×10^6 cells/ml in complete RPMI medium in plastic tissue culture dishes (Falcon Plastics, Oxnard, CA). The T cells were washed and resuspended at 1.5×10^7/ml in serum-free medium and mixed with 7.5% ox erythrocytes coated with specific IgG antibody (EA-IgG). The preparation and isolation of IgG and IgM rabbit antibodies to ox erythrocytes has been described previously (38). 2-ml fractions were layered over 3.0 ml Ficoll-Hypaque, the gradients were incubated for 10 min at 37°C and at room temperature (23°C) for 90 min, and were centrifuged at 1,200 *g* for 10 min. The EA-IgG rosette positive cells were recovered at the bottom of the gradient (Tγ cells). The cells were collected, the EA-IgG were lysed with NH_4Cl_2-Tris buffer, and the cells were washed three times in complete RPMI. The nonrosetted interface cells were washed once in serum-free RPMI, resuspended at 1.5×10^7 cells/ml, mixed with an equal volume of 7.5% ox erythrocytes coated with specific IgM antibody (EA-IgM), incubated at 37°C for 10 min, then layered over Ficoll-Hypaque and incubated at 4°C for a further 90 min. After centrifugation, the EA-IgM positive cells were recovered. The EA-IgM were lysed and the Tμ cells were resuspended in serum-free medium. Tμ and Tγ cells possessed helper and suppressor capacity, respectively, for pokeweed mitogen-driven Ig synthesis (38).

PCA response in vitro. PBM at 1×10^6 cells/ml and monocytes at 1×10^5 cells/ml were added to 16-mm diam 24-well plates in complete RPMI and stimulated with 10 μg/ml LPS (*E. coli* 0111 B4 isolated by the butanol method and kindly provided by Dr. David Morrison, Emory University, Atlanta, GA) as previously described (25, 29) and provided comparable results. In analyses performed in serum-free medium, LPS was employed at 10 ng/ml. The cells were cultured for 6–8 h at 37°C in a 5% CO_2 atmosphere. After culture, samples of viable cells or cells disrupted by freeze-thawing and sonication were assayed in duplicate for their ability to accelerate the spontaneous clotting time of normal pooled citrated human plasma in a modified one-stage recalcification assay as previously described (29). In brief, the assay consisted of 0.1 ml of test sample or rabbit brain thromboplastin positive control (Difco Laboratories, Inc., Detroit, MI), and 0.1 ml of citrated normal fasting human plasma. To initiate the reaction, 0.1 ml of $CaCl_2$ (25 mM) was added and the clotting time was recorded. The rabbit brain thromboplastin standard at 36 mg dry mass was assigned a value of 100,000 mU. There was no activity present in the RPMI or fetal calf serum or LPS. As with previous studies, spontaneous clotting times of 180±20 s were observed and rabbit brain thromboplastin at 1,000 mU/ml gave a time of 50±3 s, and the coefficient of variation of PCA assays was 8% (29). PCA was characterized using congenital factor-deficient plasmas. The PCA was dependent on the presence of Factor VII, Factor X, Factor V, and prothrombin typical of tissue factor.

Monoclonal antibody markers. Cell populations were typed by standard indirect immunofluorescence (39) on three preparations from the same donor used in the presented data for T cell subsets. Monoclonal antibodies OKT3, OKT4, OKT8, and OKM1 were used.

Other media and other solutions were tested for the presence of endotoxin by the limulus assay (E. Toxate, Sigma Chemical Co., St. Louis, MO). LPS was undetectable in reagents and media to the lower limits of the assay <1 ng/ml.

Results

T cell populations. The total number of PBM, lymphocytes, monocytes, T cells, Tμ, Tγ, and T null cells was determined in the blood samples of the eight normal volunteers used in this study (Table I). There was <20% variation in the number of PBM isolated and the monocytes comprised ~12% of the PBM as judged by nonspecific esterase staining and phagocytosis. The number of Tγ, Tμ, and T null cells was relatively consistent with less than a 12% coefficient of variation for normal subjects on all days in which they were studied.

Basal PCA. PBM expressed very little PCA, as intact viable cells and total cellular PCA after disruption was quite low, suggesting little activation during isolation of the cells (Table II). This basal activity was equal to or within a threefold increase of the spontaneous PCA of the PBM analyzed immediately after isolation from the blood of the normal volunteers. To confirm the cellular source of the PCA, the cells were separated by adherence to plastic plates and PCA of nonadherent and adherent cells was assayed. As previously observed, the adherent cells were the source of >60% of the viable (surface) PCA and greater than 76% of the total PCA of PBM. There was a small amount of basal activity, both surface and total, in the nonadherent fractions, which could be accounted

Table I. Cell Populations of PBM

Cell population	Cell no.*	Percent of PBM*
	×10⁶ per ml blood	
PBM	1.42±0.25	100
B cells‡	0.21±0.05	14.7
Monocytes§	0.17±0.03	11.8
T cells‖	1.02±0.15	71.9
Tγ	0.18±0.03	12.7¶
Tμ	0.71	69.6¶
Tnull	0.10	9.8¶

* Based on the average of eight normal controls.
‡ Nonadherent, sheep erythrocyte rosette negative, esterase, and latex negative cells.
§ >96% esterase positive, >98% latex positive adherent cells.
‖ Esterase negative, nonadherent, >96% nSRBC positive.
¶ Percent of T cells.

for by contamination with nonspecific esterase-positive monocytes as well as rare nonspecific esterase-negative nonadherent cells, which are positive for PCA by plaque assay (28).

LPS-stimulated PCA. PBM were incubated at 1×10^6 cells per ml of complete RPMI medium with 10 μg/ml LPS for 6 h, then assayed for viable and total cellular PCA (Table III). An 11.3-fold increase in viable PCA was observed, and >90% of this activity was localized to the esterase-positive adherent cell population (monocytes). No increase in PCA above basal levels was detected in the nonadherent esterase-negative cells (lymphocytes). Total PCA increased 25-fold over control (basal) activity and, after separation of PBM into adherent, esterase-positive (monocytes) and nonadherent, esterase-negative (lymphocytes) cells, the majority of activity (>89%) was localized to the monocytes. No increase in PCA was found in the nonadherent cells (Table III). Induced PCA was dependent on the presence of Factors VII, X, V, and prothrombin in assay plasmas consistent with identity as tissue factor.

T cell requirement for expression of monocyte PCA. When 1×10^5 adherent monocytes were stimulated with 10 μg/ml endotoxin in the absence of lymphocytes for 6 h and assayed for PCA, no increase in PCA was observed. Similarly, when 4×10^5 T lymphocytes were stimulated with endotoxin in the absence of monocytes and assayed after a 6-h incubation, no increase in PCA was observed (data not shown). However, when 1×10^5 monocytes were stimulated with LPS in the presence of 4.0×10^5 autologous T lymphocytes, there was a 25-fold increase of monocyte PCA. No further increase was noted as the T cell/monocyte ratio was increased to 10:1. In contrast, no increase in monocyte PCA was observed when 4×10^5 non-T lymphocytes were added to 1×10^5 monocytes and the cells were stimulated with LPS (Table IV).

To determine whether a specific subset of T cells was responsible for the LPS-induced monocyte PCA, T cells were separated by cytoadherence and monocytes were stimulated with 10 μg/ml LPS in the presence of increasing numbers of Tγ, Tμ, or T null cells. No increase in PCA was noted when 1×10^5 monocytes were stimulated with LPS in the presence of 4×10^5 Tγ or T null cells (Table V). In contrast, monocytes stimulated with 4×10^5 Tμ cells exhibited a 48-fold increase in PCA.

The various lymphocyte populations were also characterized for expression of selected markers (Table VI). Whereas the T and Tμ populations possessing helper function were predominantly $T4^+$, significant numbers of $T8^+$ cells were present as well as small numbers of $M1^+$ large granular lymphocytes. In contrast, Tγ cells contained mostly $M1^+$ cells, of which most seemed to be large granular lymphocytes and few were $T8^+$. There was no direct PCA expression by LPS stimulation of these T cell fractions and rare esterase positive monocytes or Tμ helper cells. Variable numbers of Tμ cells were added to 1×10^5 monocytes and the cells were stimulated with LPS (Fig.

Table II. Basal Procoagulant Activity

Cell population	PCA	
	Viable cells	Disrupted cells (total)
	mU per 10⁶ cells	*mU per 10⁶ cells*
PBM	70±25	150±20
Lymphocytes*	35±10	45±10
Monocytes‡	470±35	1,150±200

* Lymphocytes were <1% esterase positive and did not ingest latex particles.
‡ Monocytes were >92% esterase positive and >94% latex positive.

Table III. Cellular Source of Procoagulant Activity Induced by LPS

Cell population*	PCA	
	Viable cells	Total cellular content
	mU per 10⁶ cells	*mU per 10⁶ cells*
PBM	800±50	3,750±250
Lymphocytes	40±15	55±20
Monocytes	950±300	29,500±450

* Cells were stimulated with 10 μg/ml LPS and then separated into subpopulations by cytoadherence. The monocytes were >94% esterase positive and the lymphocytes were nonadherent and <1% esterase positive.

Table IV. Cellular Requirement for LPS-induced Monocyte PCA

LPS-stimulated cell*	Total cellular PCA
	mU per 10⁶ monocytes
—	1,000
Lymphocytes‡	24,685
T cells§	25,500
Non-T cells‖	1,200

* 4×10^5 of the designated cells were incubated with 1×10^5 monocytes and 10 μg/ml LPS in complete medium for 6 h at 37°C, 5% CO_2.
‡ Lymphocytes were nonadherent and <1% esterase positive.
§ T lymphocytes were defined as cells rosetting with nSRBC.
‖ Non-T lymphocytes did not rosette with nSRBC.

Table V. T Cell Subpopulations and Induction of Monocyte PCA by LPS

LPS-stimulated cell*	Total cellular PCA
	mU per 10^6 monocytes
—	950
T cells	28,500
Tμ	47,100
Tγ	750
T null	850

* Stimulating lymphocytes (4×10^5) were incubated with 10 μg/ml of LPS for 30 min, washed, and added to 1×10^5 monocytes. After 6 h, cellular PCA was analyzed.

1). The Tμ cells were removed and the monocytes were assayed for total cellular PCA. At a Tμ to monocyte ratio of 0.3:1, a threefold increase in monocyte PCA was observed which increased to twenty-fold at a ratio of 0.7:1. The PCA reached a plateau of 48 times basal activity at a Tμ to monocyte ratio of 1.5:1, and there was no further increase as the ratio approached 4:1 (Fig. 1). No increase in monocyte PCA was seen when monocytes were incubated with increasing numbers of T null or Tγ cells and stimulated with LPS.

Role of Tγ cells in the induction of monocyte PCA by LPS. Because monocytes expressed a larger increment in PCA when they were triggered by LPS-stimulated Tμ cells than with LPS-stimulated unfractionated lymphocytes or T lymphocytes, the existence of a suppressor cell population capable of modulating the collaborative PCA response was explored. Increasing numbers of Tγ cells were incubated with LPS for 30 min, washed three times, and then added to 1.5×10^5 Tμ cells. These cells were stimulated again with LPS to assure triggering of the Tμ cells (29), and then added to 1×10^5 monocytes for 30 min to achieve collaborative induction of monocyte PCA. The lymphocytes were then removed and the monocytes were incubated for a further 4–6 h to permit biosynthesis of the PCA. When compared with monocytes triggered with LPS-stimulated Tμ cells alone, there was a marked attenuation of PCA induction in monocytes triggered with Tμ cells to which Tγ cells had been added. At a Tγ/Tμ ratio of 4:1, the monocyte PCA declined by 20% to 40,500 mU/10^6 monocytes.

Table VI. Monoclonal Antibody Markers on T Cell Populations

Cell population	$T3^+$	$T4^+$	$T8^+$	$M1^+$
	%	%	%	%
Lymphocytes	82	48	22	12
T	90	58	34	4
Non-T	2	1	2	14
Tμ	80	50	19	6
Tγ	8	4	2	18
T null	75	52	15	12

Data was derived from three separate analyses of donor G.L. by immunofluorescence as described in Methods. Lymphocytes were nonadherent cells after isolation of PBM and were <2% nonspecific esterase positive. T cells and subsets were isolated by described cytoadherence methods.

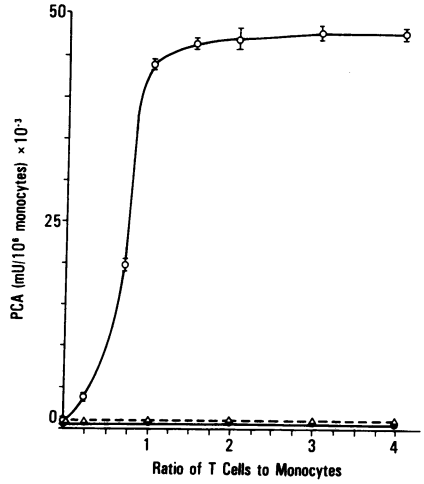

Figure 1. Collaborative T cell requirements for the induction of monocyte procoagulant activity by LPS. Monocytes (1×10^5) were incubated in 1 ml of complete medium with increasing numbers of Tμ (○), Tγ (●), or T null (△) cells, stimulated with 10 μg/ml LPS for 6 h, then assayed for PCA.

A maximum suppression of 68% was observed at a Tγ/Tμ ratio of 7.5:1 (Fig. 2).

Cellular target of suppression. To determine whether the site of action of the Tγ suppressor cell was the Tμ instructor cell or the monocyte, 7.5×10^5 LPS-triggered Tγ cells were: (*a*) added to 1×10^5 Tμ instructor cells, stimulated with LPS for 30 min, washed three times, and then added to 1×10^5 monocytes, or (*b*) added directly to monocytes. The latter was included to assess a direct effect on the monocyte. After 30-min incubation, the lymphocytes were removed from the adherent monocytes by vigorous washing and LPS-triggered Tμ instructor cells were added. Full suppression of monocyte PCA was observed when LPS-triggered Tγ cells were added directly to the monocytes, removed, and the response of the monocytes to LPS-triggered Tμ cells was assessed. The effect was comparable to that observed when LPS-triggered Tγ cells were first added to Tμ cells, then triggered with LPS and added to monocytes (data not shown). LPS induction of Tγ suppressor activity was required because when the monocytes were incubated for 30 min with Tγ cells that had not been triggered by LPS, washed free of Tγ cells, and incubated with LPS-triggered Tμ cells, no suppression of the induction of monocyte PCA was observed.

Next, the cellular interactions were analyzed in the context of the dominance of the effect over time of each of the regulatory T cell populations. We first examined the duration of suppression in the presence of triggered Tγ cells (Fig. 3 *A*).

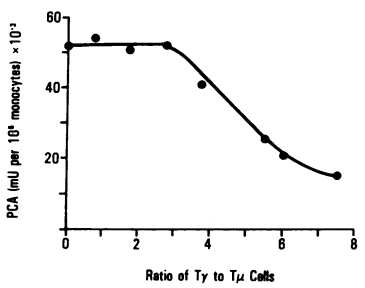

Figure 2. The effect of Tγ cell activity on the induction of monocyte PCA by LPS in the presence of Tμ cells. Monocytes (1×10^5) were incubated with mixtures of Tγ and 1.5×10^5 Tμ cells and stimulated with 10 μg/ml LPS. PCA was assayed 6 h later.

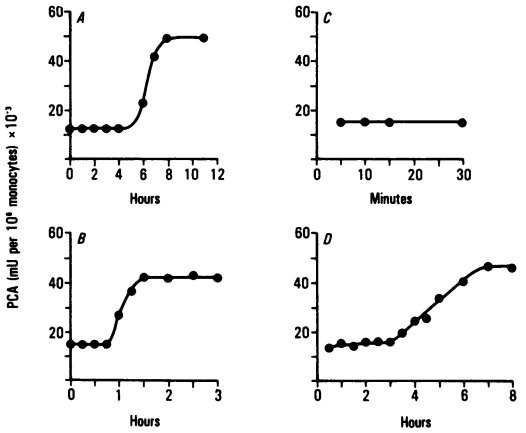

Figure 3. Dissection and kinetics of Tγ suppression of the collaborative monocyte PCA response to LPS. (*A*) Duration of suppression in the presence of Tγ cells. To 1×10^5 monocytes in 1 ml of complete medium were first added 7.5×10^5 LPS-triggered Tγ cells. Next, 1.5×10^5 LPS-triggered Tμ cells were added at the indicated intervals. The PCA response was assayed 6 h after the addition of the Tμ cells. (*B*) Interval of susceptibility to suppression. Monocytes (1×10^5) were first incubated with LPS-triggered 1.5×10^5 Tμ cells for 30 min to achieve the collaborative induction. Then 7.5×10^5 LPS-stimulated Tγ cells were added at the indicated intervals. The PCA response was assayed 6 h after the initial induction with Tμ cells. (*C*) Minimum interval for the Tγ suppressive effect. Monocytes (1×10^5) were incubated with 7.5×10^5 LPS-stimulated Tγ cells for 5–30 min. The Tγ cells were removed and the monocytes were induced with 1.5×10^5 LPS-stimulated Tμ cells. Monocytes were incubated for an additional 6 h and assayed for PCA. (*D*) Duration of suppression after the Tγ effect. To 1×10^5 monocytes were added 2.5×10^5 LPS-triggered Tγ cells. 30 min later they were removed by vigorous pipetting and 1.5×10^5 triggered Tμ cells were added at the indicated intervals. The PCA response was assayed 6 h after addition of the triggered Tμ cells.

Tγ cells were incubated with LPS for 30 min, washed free of LPS, and added to monocytes. After addition of these triggered Tγ cells, the monocytes were refractory to the subsequent PCA inductive effects of triggered Tμ cells for 4–6 h in the continuous presence of Tγ cells. However, the suppression declined with time and monocytes eventually recovered full responsiveness to LPS-triggered Tμ instructor cells. This was presumed to represent both the sum of the decline of Tγ suppressive activity and the recovery of monocyte responsiveness, which is confirmed below.

In the experiments illustrated in Fig. 3 *B*, the temporal duration after induction during which Tγ cells can suppress the monocyte PCA response was characterized. Monocytes were first stimulated by LPS-triggered Tμ cells, then LPS-triggered Tγ cells were added at various times thereafter. Suppressive effects were observed only if the Tγ cells were added to the monocytes within 1 h of exposure of the monocytes to triggered Tμ cells. This suggests that suppression can be imposed for as long as 1 h after communication of the Tμ instructor cell with the monocyte based on prior evidence that communication between the instructor T cell and the monocyte is completed within 15 min (28).

To determine the interactive kinetics between Tγ cells and monocytes for full suppression of PCA induction, the experiments as represented in Fig. 3 *C* were performed. We added 7.5×10^5 LPS-triggered Tγ cells to 1×10^5 monocytes for intervals of 5–30 min. The T cells were removed thoroughly by vigorous pipetting followed by microscopic confirmation of the absence of lymphocytes. The monocytes then were incubated with 4×10^5 LPS-triggered Tμ cells. Full suppression of the monocyte PCA response was observed with exposure to Tγ cells for as little as 5 min, indicating: (*a*) that the monocyte can be suppressed rapidly (<5 min), and (*b*) that the target monocyte can be suppressed so that either it does not perceive the subsequent message from triggered Tμ cells or it simply can not execute the appropriate cellular response.

Finally, to characterize the duration of the Tγ suppression of the monocyte per se (Fig. 3 *D*), LPS-triggered Tγ cells were incubated with monocytes for 5 min and removed. The suppressed monocytes were washed to remove the Tγ cells, and LPS-triggered Tμ instructor cells were added. Monocytes were refractory to the Tμ effects for 2–3 h after exposure to the effects of LPS-triggered Tγ cells. Subsequently, the monocytes recovered and were able to respond with a normal PCA response when stimulated by LPS-triggered Tμ cells. To support the specificity of the cellular suppressor effect, no similar suppressor cell activity was found in the T null cell population (data not shown). Because the Tγ were removed after a 5-min interaction with the monocytes, the problem of continuing suppression of the monocyte response and of suppression of the added Tμ instructor cells was obviated. The more prolonged refractory period observed in Fig. 3 *A* was interpreted as the result of the longer duration of the Tγ suppressor capacity when these cells remained with the monocytes. We estimated that triggered Tγ cells retained suppressor activity for ~2 h after LPS exposure. This was obtained by subtraction of the half recovery time of 5 h for monocytes from the half recovery time of 7 h for monocytes in the presence of triggered Tγ cells.

Discussion

Activation of coagulation in the vascular system is associated with a variety of clinical syndromes that follow from the consumption of coagulation factors and the local deposition of fibrin (40–42) among which gram-negative bacterial septicemia is a classic example. The underlying Shwartzman reaction has been pathogenetically related to some of the diverse clinical features of disseminated intravascular coagulation or consumption coagulapathy (43–45). One underlying postulated mechanism has been that cellular elements of the blood initiate the coagulation cascade (9, 10, 24, 46). This has been borne out; and it is now clear that initiation of coagulation can be unequivocally attributed to the monocyte (28). One limb of the lymphoid response to biologic stimuli including antigens involves an initial recognition by T cells, followed by the collaborative T cell-mediated induction of selected effector functions of cells of the monocyte-macrophage lineage. These T cell-instructed monocyte responses have been implicated as central to the Shwartzman reaction (9, 10, 47), delayed-type hypersensitivity reactions (2–6, 48), and the pathogenesis of murine viral hepatitis (16, 17). Furthermore, it has been identified as a component of the response to allogeneic cells (21–23), a response that may be responsible for the vascular injury associated with and implicated as critical to first set graft rejection (49) and hyperacute organ graft rejection.

It has been established that the human monocyte can initiate the extrinsic coagulation protease cascade as a result of induced synthesis and expression of tissue factor on its surface (24, 50). It is apparent also that human monocytes can be induced by T helper cells to synthesize Factor VII (25), the initial enzyme in the extrinsic pathway. Although this topic is of some controversy, a requirement for T cell collaboration has been implicated in the response of cells of the monocyte lineage to LPS (28, 34, 35). The T instructor cell requirement is even more evident for the response to allogeneic cells (22, 23) and antigens (15). We have recently provided evidence for a unifying hypothesis of the existence of four cellular pathways by which monocytes and macrophages are induced to the procoagulant phenotype, three of which are T cell-instructed (7).

The present studies provide the first demonstration that a subset of T lymphocytes, Tμ defined by Fc receptors for IgM, can be triggered by LPS to induce human monocyte PCA of tissue factor type. This is consistent with previous analysis indicating that Leu 3a and 9.3 positive helper T cells also possess this functional capacity (34, 35). However, the LPS-driven pathway I is not the same as the antigen-driven pathway II and such markers provide only limited correlation with functional properties. The use of T cell Fc receptors provides an independent set of markers that need not precisely coincide with monoclonal antibody markers as shown in Table VI and previously reported by Reinherz et al. (39). In contrast, neither Tγ nor T null cells were effective, though the latter contained many T4 cells, comparable with the Leu 3a marker.

We describe for the first time the ability of Tγ cells to suppress the lymphoid PCA response to LPS. Whereas it would be expected that suppressor T cells for responses to antigen and allogeneic cells would exist, suppression of a response to LPS, which is mediated by the lipid A moiety of this molecule (10, 50), was unanticipated. Kinetic and other aspects of suppression of the response were characterized. First, the Tγ cells were not constitutively suppressive. They must be triggered as exemplified by the requirement that they be exposed to LPS. The triggering of the Tγ cells was rapid; it occurred within 30 min, similar to that of collaborative helper T cells as described previously, and it did not require DNA or protein synthesis (data not shown). Previously, we showed that relatively large numbers of T lymphocytes were required for the full expression of monocyte PCA in the type I response. Maximal PCA occurred at a T lymphocyte to monocyte ratio of 4:1. With further purification of T lymphocytes to Tμ and Tγ subsets, we found that the absolute requirement of lymphocytes for generation of monocyte PCA remained; however, the number of specific (Tμ) lymphocytes required as reflected by the ratio of helper cell to monocyte decreased. An increase in monocyte PCA was observed at a Tμ to monocyte ratio of 0.3 with maximal PCA at a ratio of 1.5. Therefore, separation of T cells by the Fc receptor further enriched for the T helper cell function responsible for induction of monocyte PCA. Additionally, stimulation by Tμ cells resulted in a greater increase in monocyte PCA than was observed when monocytes were stimulated by lymphocytes or unfractionated T cells. Subfractionation of T cells by reference to the Leu 3a marker did not enhance comparably the magnitude of the response relative to the unfractionated T cells (34), suggesting nonconcordance of the T cell subpopulations, which is also borne out by monoclonal antibody typing in Table VI. This led us to postulate the presence of a second subset of T lymphocytes that could suppress the monocyte PCA response.

The exact site of action of Tγ suppressor cells is not yet thoroughly delineated; however, our experiments suggested that the suppressor effect was imposed directly on the monocyte. The possibility of direct suppression of Tμ triggering or effector function was not, however, excluded as a second or auxiliary event. Monocytes first incubated with unstimulated Tγ cells and washed free of these cells, could be stimulated by LPS-triggered Tμ cells with no reduction in the PCA response. In contrast, monocytes that were first incubated with LPS-triggered Tγ cells and then stimulated with LPS-triggered Tμ cells did not produce a PCA response. In addition, they remained refractory to LPS-triggered Tμ helper cells for at least 2 h. The observation that large numbers of Tγ cells were required to fully suppress the response suggests that either these cells functioned poorly as suppressors of monocyte PCA, or more likely that there were few of the specific LPS-responding cells within the Tγ subset.

Separation of T lymphocytes by the functional Fc receptor method has met with some criticism. Reinhertz et al. (51) and others (52, 53) have reported that some of the cells within the Tμ population have different cell surface markers as evidenced by the use of monoclonal antibodies. OKT4 monoclonal antibody reacted with 60% of the T cell population and these cells were required for the optimal development of the cytotoxic cell in cell-mediated lympholysis, induction of B cell differentiation, proliferation, and immunoglobulin synthesis in a pokeweed mitogen-driven system and the production of helper factors (39). Reinherz et al. (51) observed partial enrichment of Tμ cells within the OKT4 subset, but less than a precise correlation of the cell subpopulations defined by monoclonal antibodies and those defined by Fc receptors. Furthermore, isolation of either OKT4$^+$ (helper) or OKT5$^+$ (cytolytic/suppressor) cell subsets provided no significant segregation relative to the Tγ population in their hands. We observed similar discordance in the present study. Other investigators have demonstrated that Tμ cells, when placed in culture, may lose the Fcμ receptor and later express Fcγ receptors (54), indicating the dynamic characteristics of the Fc receptor-defined cells. However, functional segregation of cells on the basis of Fc receptor expression by those experienced with the methods has led to isolation of cell populations that are specifically functional in immune response systems in vitro (36–38). We find that the helper cell population of T cells required for the induction of monocyte PCA consistently and solely falls within the Tμ subset and therefore must be a relatively constant and stable fraction of these cells. Information from the present and prior analyses of the instructor cell for the type I PCA response (34, 35) permits the conclusion that their phenotype is Leu 3a$^+$9.3$^+$Fcμ^+.

The observation that a relatively large number of Tγ cells was required for suppression is compatible with the existence of only a small proportion of LPS responsive suppressor cells available within the Tγ population. However, because we do not know the functional effectiveness of these cells, analysis at the clonal level will provide the best information. Abnormalities in both numbers and function of Tμ and Tγ cells have been observed in patients with a variety of clinical disorders (55–57). Whether aberrations in T subsets functional in the PCA response may occur is not known yet. The evidence that this unusual lymphoid response is subject to regulation by both

helper and suppressor T cells suggests that differences in the number and activation of these regulatory T cell subsets in different individuals and in disease may play a significant role in the clinical severity of diseases associated with endotoxinemia.

Acknowledgments

The authors are grateful to Lai Sum Fung for his excellent technical assistance and to Esther Kroecker and Lois Hill for preparation of the manuscript.

These studies were supported in part by a grant from the Medical Research Council of Canada and grant PO1 HL-16411 from the National Heart, Lung, and Blood Institute.

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