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Research Article

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Regulatory Roles of T μ and T γ 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 Fc γ -positive and Fc μ -positive cells and were characterized for their functional properties in the procoagulant response. T μ cells mediated the monocyte response, consistent with their identity with instructor cells. T γ cells suppressed the response of monocytes to LPS in the presence of T μ cells, suggesting that they possess suppressor function for this response. The T γ 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,

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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 (T μ) and IgG (T γ). In a pokeweed mitogen-driven system

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; T μ , T lymphocyte surface Fc receptors for IgM; T γ , T lymphocyte surface Fc receptors for IgG.

of polyclonal immunoglobulin (Ig) synthesis by B lymphocytes, T cells with Fc receptors for IgG (T γ) deliver a suppressor signal, whereas those with Fc receptors for IgM (T μ) 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 T μ subset of T cells is capable of supporting the induction of monocyte PCA by LPS. Furthermore, T γ 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 μ g/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/cm³, 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 NH₄Cl₂, 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 (T γ cells). The cells were collected, the EA-IgG were lysed with NH₄Cl₂-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 T μ cells were resuspended in serum-free medium. T μ and T γ 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 μ g/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% CO₂ 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 CaCl₂ (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 \pm 20 s were observed and rabbit brain thromboplastin at 1,000 mU/ml gave a time of 50 \pm 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, T μ , T γ , 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 T γ , T μ , 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* |
|-----------------|----------------------------|-----------------|
| | $\times 10^6$ per ml blood | |
| PBM | 1.42 \pm 0.25 | 100 |
| B cells‡ | 0.21 \pm 0.05 | 14.7 |
| Monocytes§ | 0.17 \pm 0.03 | 11.8 |
| T cells | 1.02 \pm 0.15 | 71.9 |
| T γ | 0.18 \pm 0.03 | 12.7¶ |
| T μ | 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 μ g/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 μ g/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,

Table II. Basal Procoagulant Activity

| Cell population | PCA | |
|-----------------|-----------------------|-------------------------|
| | Viable cells | Disrupted cells (total) |
| | mU per 10^6 cells | mU per 10^6 cells |
| PBM | 70 \pm 25 | 150 \pm 20 |
| Lymphocytes* | 35 \pm 10 | 45 \pm 10 |
| Monocytes‡ | 470 \pm 35 | 1,150 \pm 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 10^6 cells | mU per 10^6 cells |
| PBM | 800 \pm 50 | 3,750 \pm 250 |
| Lymphocytes | 40 \pm 15 | 55 \pm 20 |
| Monocytes | 950 \pm 300 | 29,500 \pm 450 |

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

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 μ g/ml LPS in the presence of increasing numbers of T γ , T μ , 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 T γ or T null cells (Table V). In contrast, monocytes stimulated with 4×10^5 T μ 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 T μ 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, T γ 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 T μ helper cells. Variable numbers of T μ cells were added to 1×10^5 monocytes and the cells were stimulated with LPS (Fig.

Table IV. Cellular Requirement for LPS-induced Monocyte PCA

| LPS-stimulated cell* | Total cellular PCA |
|----------------------|---------------------------|
| | mU per 10^6 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 μ g/ml LPS in complete medium for 6 h at 37°C, 5% CO₂.

‡ 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 |
| T μ | 47,100 |
| T γ | 750 |
| T null | 850 |

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

1). The T μ cells were removed and the monocytes were assayed for total cellular PCA. At a T μ 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 T μ 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 T γ cells and stimulated with LPS.

Role of T γ cells in the induction of monocyte PCA by LPS. Because monocytes expressed a larger increment in PCA when they were triggered by LPS-stimulated T μ 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 T γ cells were incubated with LPS for 30 min, washed three times, and then added to 1.5×10^5 T μ cells. These cells were stimulated again with LPS to assure triggering of the T μ 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 T μ cells alone, there was a marked attenuation of PCA induction in monocytes triggered with T μ cells to which T γ cells had been added. At a T γ /T μ ratio of 4:1, the monocyte PCA declined by 20% to 40,500 mU/10⁶ 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 |
| T μ | 80 | 50 | 19 | 6 |
| T γ | 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 non-adherent cells after isolation of PBM and were <2% nonspecific esterase positive. T cells and subsets were isolated by described cytoadherence methods.

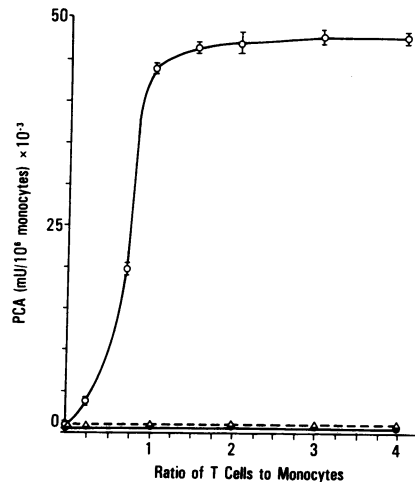


Figure 1. Collaborative T cell requirements for the induction of monocyte procoagulant activity by LPS. Monocytes (1×10^5) were incubated in 1 ml of complete medium with increasing numbers of T μ (○), T γ (●), or T null (△) cells, stimulated with 10 μ g/ml LPS for 6 h, then assayed for PCA.

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

Cellular target of suppression. To determine whether the site of action of the T γ suppressor cell was the T μ instructor cell or the monocyte, 7.5×10^5 LPS-triggered T γ cells were: (a) added to 1×10^5 T μ 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 T μ instructor cells were added. Full suppression of monocyte PCA was observed when LPS-triggered T γ cells were added directly to the monocytes, removed, and the response of the monocytes to LPS-triggered T μ cells was assessed. The effect was comparable to that observed when LPS-triggered T γ cells were first added to T μ cells, then triggered with LPS and added to monocytes (data not shown). LPS induction of T γ suppressor activity was required because when the monocytes were incubated for 30 min with T γ cells that had not been triggered by LPS, washed free of T γ cells, and incubated with LPS-triggered T μ 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 T γ cells (Fig. 3 A).

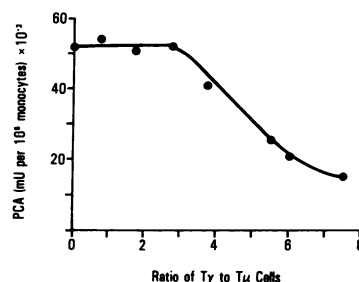


Figure 2. The effect of T γ cell activity on the induction of monocyte PCA by LPS in the presence of T μ cells. Monocytes (1×10^5) were incubated with mixtures of T γ and 1.5×10^5 T μ cells and stimulated with 10 μ g/ml LPS. PCA was assayed 6 h later.

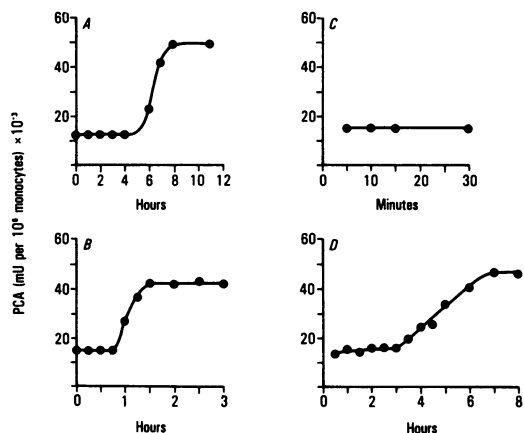


Figure 3. Dissection and kinetics of T γ suppression of the collaborative monocyte PCA response to LPS. (A) Duration of suppression in the presence of T γ cells. To 1×10^5 monocytes in 1 ml of complete medium were first added 7.5×10^5 LPS-triggered T γ cells. Next, 1.5×10^5 LPS-triggered T μ cells were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

T γ cells were incubated with LPS for 30 min, washed free of LPS, and added to monocytes. After addition of these triggered T γ cells, the monocytes were refractory to the subsequent PCA inductive effects of triggered T μ cells for 4–6 h in the continuous presence of T γ cells. However, the suppression declined with time and monocytes eventually recovered full responsiveness to LPS-triggered T μ instructor cells. This was presumed to represent both the sum of the decline of T γ 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 T γ cells can suppress the monocyte PCA response was characterized. Monocytes were first stimulated by LPS-triggered T μ cells, then LPS-triggered T γ cells were added at various times thereafter. Suppressive effects were observed only if the T γ cells were added to the monocytes within 1 h of exposure of the monocytes to triggered T μ cells. This suggests that suppression can be imposed for as long as 1 h after communication of the T μ 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 T γ 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 T γ 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 T μ cells. Full suppression of the monocyte PCA response was observed with exposure to T γ 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 T μ cells or it simply can not execute the appropriate cellular response.

Finally, to characterize the duration of the T γ suppression of the monocyte per se (Fig. 3 D), LPS-triggered T γ cells were incubated with monocytes for 5 min and removed. The suppressed monocytes were washed to remove the T γ cells, and LPS-triggered T μ instructor cells were added. Monocytes were refractory to the T μ effects for 2–3 h after exposure to the effects of LPS-triggered T γ cells. Subsequently, the monocytes recovered and were able to respond with a normal PCA response when stimulated by LPS-triggered T μ 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 T γ 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 T μ 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 T γ suppressor capacity when these cells remained with the monocytes. We estimated that triggered T γ 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 T γ 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 coagulopathy (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, T_{μ} 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 T_{γ} 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 T_{γ} 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 T_{γ} cells were not constitutively suppressive. They must be triggered as exemplified by the requirement that they be exposed to LPS. The triggering of the T_{γ} 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 T_{μ} and T_{γ} subsets, we found that the absolute requirement of lymphocytes for generation of monocyte PCA remained; however, the number of specific (T_{μ}) lymphocytes required as reflected by the ratio of helper cell to monocyte decreased. An increase in monocyte PCA was observed at a T_{μ} 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 T_{μ} 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 T_{γ} 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 T_{μ} triggering or effector function was not, however, excluded as a second or auxiliary event. Monocytes first incubated with unstimulated T_{γ} cells and washed free of these cells, could be stimulated by LPS-triggered T_{μ} cells with no reduction in the PCA response. In contrast, monocytes that were first incubated with LPS-triggered T_{γ} cells and then stimulated with LPS-triggered T_{μ} cells did not produce a PCA response. In addition, they remained refractory to LPS-triggered T_{μ} helper cells for at least 2 h. The observation that large numbers of T_{γ} 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 T_{γ} subset.

Separation of T lymphocytes by the functional Fc receptor method has met with some criticism. Reinherz et al. (51) and others (52, 53) have reported that some of the cells within the T_{μ} 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 T_{μ} 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 T_{γ} population in their hands. We observed similar discordance in the present study. Other investigators have demonstrated that T_{μ} cells, when placed in culture, may lose the Fc μ receptor and later express Fc γ 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 T_{μ} 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⁺Fc μ ⁺.

The observation that a relatively large number of T_{γ} cells was required for suppression is compatible with the existence of only a small proportion of LPS responsive suppressor cells available within the T_{γ} 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 T_{μ} and T_{γ} 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 endotoxemia.

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