# Detection of Human Factor B Biosynthesis in Culture Supernatants and Cell Lysates by ELISA (43178)

MASAMITSU MITANI, ROBERT A. GOOD, AND NOORBIBI K. DAY
*All Children's Hospital, University of South Florida, St. Petersburg, Florida*

*Abstract.* **We report here on the development of a simple, sensitive, convenient, and quantitative enzyme-linked immunosorbent assay for human factor B, a protein of the alternative complement pathway, by the sandwich method using goat anti-human factor B antibody. The assay described herein is reproducible and highly specific for human factor B. The assay was used to determine biosynthesis (cell lysates or extracts) and secretion (supernatants) of human factor B using human monocyte cell line U937. Phorbol myristate acetate strongly enhanced (10- to 20-fold) biosynthesis of factor B by U937. The combination of a sensitive enzyme-linked immunosorbent assay and phorbol myristate acetate enabled us to use a microculture system.** [P.S.E.B.M. 1991, Vol 196]

Complement (C) proteins are mainly produced by liver cells. Extrahepatic cells also are known to synthesize C components (1–3). Investigation of extrahepatic C biosynthesis may be crucial to understand the role of C in local sites during injury or infection. Among the major candidates for C synthesis by extrahepatic cells include monocytes/macrophages, fibroblasts, endothelial cells, and astrocytes. The most common cells or cell lines used are of the monocyte/macrophage or hepatocyte series. Almost all components of C (classical and alternative pathways) including component(s) of the membrane attack complex (C5–C9) have been shown to be synthesized *in vitro* (3). The human monocyte cell line U937 has been most commonly used.

The methods used in general for the biosynthesis of C components include both functional (hemolytic) and immunochemical assays. Autoradiography of immunoprecipitated C proteins of cell lysates or supernatants which have been previously subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is also widely used. Others have included radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) to determine production of C components *in vitro*.

Received June 4, 1990. [P.S.E.B.M. 1991, Vol 196]
Accepted September 20, 1990.

0037-9727/91/1962-0188$2.00/0


Factor B, a component of the alternative pathway of the C system, is responsible, with C3 and factor D, for the initiation and the positive feedback amplification of the alternative C pathway (4). It has been shown that factor B biosynthesis is enhanced by lipopolysaccharide, interferons ($\alpha$-interferon and $\gamma$-interferon), and interleukin 1 using standard autoradiograph analysis (5, 6), a method which is tedious, time consuming, and not very sensitive.

To monitor the production of C *in vitro* by cells, the ideal system should be simple, sensitive, specific, quantitative, reproducible, capable of detecting active and inactive C proteins, and be able to distinguish *de novo* synthesis from preformed proteins. In this study, we have developed a sensitive ELISA for measurement of human factor B in order to quantify its biosynthesis by U937 cells. Further by ELISA, we have studied the effect of (phorbol 12-myristate 13-acetate) (PMA) on the production of factor B by U937 cells.

## Materials and Methods

**Cell Line.** The human monocyte-like, histiocytic lymphoma cell line, U937, was obtained from the American Type Culture Collection (Rockville, MD) and maintained *in vitro* as suspension cultures in RPMI 1640 medium supplemented with 10% heat-inactivated (56°C, 1 hr) fetal bovine serum, 2 m*M* L-glutamine. 100 units of penicillin/ml and 100 $\mu$g of streptomycin/ml (GIBCO, Grand Island, NY) at 37°C in a humidified atomosphere of 10% $CO_2$-90% air.

**Reagents for ELISA.** Goat anti-human factor B serum and its chromatographically purified IgG anti-

body (Atlantic Antibodies, Scarborough, ME), bovine serum albumin, Cohn fraction V (Calbiochem-Behring Corp., La Jolla, CA), and *p*-nitrophenyl phosphate disodium (Sigma, St. Louis, MO) as phosphatase substrate were used.

Conjugation of alkaline phosphatase (ALP; Sigma) to goat anti-human factor B serum was done by using glutaraldehyde (Sigma) according to the method of Voller *et al.* (7).

The assay buffers were 0.05 *M* phosphate buffer (pH 7.4) containing 0.15 *M* NaCl (PBS) and 0.05% Tween 20 (Sigma) in PBS. The coating buffer was 0.05 *M* carbonate-bicarbonate buffer (pH 9.7). The substrate buffer was 10% diethanolamine buffer (pH 9.8).

**C Components, Antibodies, and Antimetabolites.** Purified human C components, C1–C9, were obtained from Cordis Laboratories, Miami, FL, and were tested for functional purity in standard hemolytic assays by Diamedix Corp., Miami, FL. Purified human C7 and C8 were obtained from Cytotech, San Diego, CA, and they were tested both for functional purity in standard hemolytic assays and for biochemical purity by PAGE. Human factor B, factor B-depleted human serum, monoclonal antibodies to human C7, C8, factor H, factor I, Ba fragment of factor B (Ba), and Bb fragment of factor B (Bb) were obtained from Cytotech. Calibrated normal human serum (NHS) was obtained from Atlantic Antibodies. Mitomycin C, cycloheximide, and actinomycin D were obtained from Sigma and used as inhibitors of DNA, RNA, and protein synthesis, respectively.

**ELISA for Human Factor B.** Many combinations of reagents, plates, and variables of time and concentration were tested to establish a satisfactory procedure. In this report, only the optimized basic procedure is described. All washes were three times with 0.05% Tween/PBS using Ultrawash-II (Dynatech Laboratories, Inc., Alexandria, VA). All incubations were for 2 hr at room temperature unless otherwise stated. The wells of 96-well flat bottom microtiter plates (Nunc-Immuno Plate I; Nunc, Roskilde, Denmark) were coated with 75 μl of goat anti-human factor B IgG antibody (1/4000; 40 μg/ml) in coating buffer and left overnight at 4°C. After washing, 250 μl of 1% bovine serum albumin PBS was added to each well and incubated to block the unbound sites. After a second washing, 75 μl of standards or samples were applied to each well in duplicate and incubated for 2 hr. Following another washing, 100 μl of 1/500 ALP-conjugated goat anti-human factor B serum were added and incubated at room temperature. The plates were again washed and 100 μl of phosphatase substrate (1 mg/ml) in 10% diethanolamine buffer were added. The optical density (OD) of samples was then measured at a test wavelength of 410 nm and a reference wavelength of 450 nm using an MR 650 Microplate Reader (Dynatech) every 30 min for 1 hr. The data presented in Results show the mean amounts of factor B synthesized in cell lysates or secreted in the culture supernatants by $10^6$ cells in the original cultures.

Artificial colorimetric change at the edge or corner of the plates (edge effects) was sometimes observed. Therefore, we avoided use of outer wells for test samples.

**Biosynthesis of Human Factor B.** U937 cells were washed three times and resuspended in serum-free RPMI 1640 medium supplemented with 2 m*M* L-glutamine, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. The cells were dispensed at a final concentration of $1 \times 10^6$ cells/ml into 60- × 15-mm plastic dishes (Corning Glass Works, Corning, NY) in a final volume of 10–15 ml in a macroculture system, or into 96-well flat-bottom culture plates (Linbro, McLean, VA) in a final volume of 200 μl in a microculture system. The cultures were stimulated with 10 ng of PMA/ml (Sigma) for 4 days unless otherwise mentioned at 37°C in a 10% $CO_2$-90% air atmosphere. In some experiments, 4 α-phorbol 12,13-didecanoate (4α-PDD; Sigma) was used as a biologically inactive chemical phorbol ester analogue of PMA (8, 9). PMA and 4α-PDD were dissolved at 100 μg/ml in 95% ethanol and stored in aliquots at −80°C.

**Preparation of Cell Lysates and Culture Supernatants.** Following incubation, cell cultures were centrifuged and cell supernatants were sterilized by filtration using a 0.2-μm Millipore filter (Millipore Corp., Bedford, MA). In some experiments, supernatants were then concentrated using an Immersible CX-10 ultrafilter (Millipore) with a molecular weight cutoff of 10,000 or a Minicon B-15 concentrator (Amicon, Lexington, MA) with a molecular weight cutoff of 15,000. The concentrated samples were then filtered. The cell pellets were lysed by addition of 0.5 ml of lysing buffer consisting of 0.5% Nonidet P40, 10 m*M* Tris-HCl, 0.5 *M* NaCl (pH 7.3), and 2 m*M* phenylmethylsulfonyl fluoride, and left overnight at 4°C. Cell extracts or lysates were centrifuged, then sterile filtered. Samples were stored at 4°C and used within 1 month.

**Immunoprecipitation, SDS-PAGE, and Autoradiography of Factor B.** Immunoprecipitation of $^{35}S$-labeled human factor B in U937 cell lysates or supernatants by goat anti-human factor B IgG antibody, SDS-PAGE, and autoradiography was carried out according to the method reported previously (6). Methylated $^{14}C$-labeled molecular weight marker proteins were obtained from NEN Research Products (Du Pont, Wilmington, DE).

## Results

**Standardization of ELISA for Human Factor B.** To select the proper plate and to determine the optimal concentrations of reagents, several kinds of microtiter plates were coated with varying dilutions of goat anti-

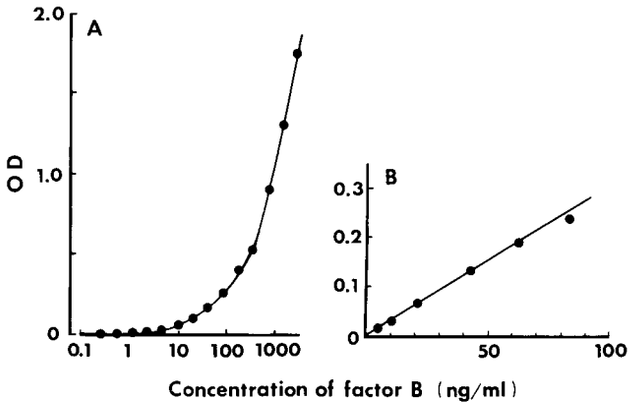


**Figure 1.** Standard curves obtained by ELISA using optimally diluted goat anti-human factor B IgG and ALP-conjugated goat anti-human factor B antibodies. OD was measured at 60 min of reaction time. Purified human factor B was applied at the concentration of 0–2500 ng/ml (A) and 0–84 ng/ml (B).

**Table I.** Specificity of ELISA for Factor B

| Group | Sample | OD at sample concentration (ng/ml) of | | | |
|---|---|---|---|---|---|
| | | 1 | 10 | 100 | 1000 |
| 1 | Factor B | 0.009 | 0.060 | 0.279 | 0.729 |
| 2[a] | C1 | 0.001 | 0.001 | 0.001 | 0.003 |
| | C2 | 0.004 | 0.004 | 0.001 | 0.011 |
| | C3 | 0.001 | 0.002 | 0.000 | 0.002 |
| | C4 | 0.002 | 0.000 | 0.000 | 0.004 |
| | C5 | 0.003 | 0.003 | 0.005 | 0.003 |
| | C6 | 0.001 | 0.003 | 0.005 | 0.015 |
| | C7 | 0.001 | 0.005 | 0.125 | 0.640 |
| | C8 | 0.001 | 0.005 | 0.008 | 0.075 |
| | C9 | −0.001 | −0.001 | 0.000 | 0.006 |
| 3[b] | C7 | 0.001 | 0.003 | 0.009 | 0.012 |
| | C8 | 0.001 | 0.002 | 0.001 | 0.001 |
| 4[c] | Factor B-depleted NHS | −0.003 | −0.001 | −0.001 | 0.001 |

[a] Functionally pure C components were used.
[b] Both functionally and biochemically pure C components were used.
[c] NHS depleted of factor B was obtained from Cytotech, and diluted 1/200,000, 1/20,000, 1/2,000, and 1/200, respectively.

human factor B antibody and ALP-conjugated goat anti-human factor B (1/500 to 1/20,000, respectively) were tested. The OD of standard human factor B samples of known concentration was measured every 15 min for 1 hr. The higher the concentrations of both antibodies used, the more rapid were the reactions observed (data not shown). We chose Nunc-Immuno Plate I, a 1/4,000 dilution for goat anti-human factor B coating antibody, a 1/500 dilution for ALP-conjugated goat anti-human factor B antibody, and 1 hr of reaction time for optimal conditions. Standard curves, which were obtained using the optimal concentrations of antibodies described above, are shown in Figure 1. The standard curve was almost linear at least between 0 and 50 ng/ml after 1 hr of reaction time. Consequently, samples were serially diluted 2- or 3-fold, and the factor B concentration was determined in this range.

It is important to include a standard curve for each 96-well plate to avoid the influence of minute variation of each plate due to the high sensitivity of the method used.

**Specificity of ELISA for Human Factor B.** The specificity of this ELISA method was first tested (Table I). As shown when functionally purified components (C1–C9) were tested, only C7 and C8 were detected at

**Table II.** Additional Evidence of Specificity of ELISA for Factor B

| Coating antibody against[a] | OD at factor B concentration (ng/ml) of | | |
|---|---|---|---|
| | 10 | 100 | 1000 |
| Factor B | 0.077 | 0.617 | 1.589 |
| Ba | 0.026 | 0.221 | 0.721 |
| Bb | 0.003 | 0.154 | 0.547 |
| C7 | 0.004 | 0.003 | 0.006 |
| C8 | 0.007 | 0.004 | 0.007 |
| Factor H | 0.002 | 0.002 | 0.006 |
| Factor I | 0.001 | 0.002 | 0.006 |
| None[b] | 0.000 | 0.004 | 0.007 |

[a] Plates were coated with the antibodies listed above (1/1500) and incubated overnight. Purified factor B was added next. After washing the plates, ALP-conjugated goat anti-human factor B antibody (1/500) was added.
[b] Plates were coated with no antibody.

**Table III.** Application of Heat-Treated Samples to ELISA for Factor B

| Sample | Treatment | OD at a sample concentration (ng/ml) of | | |
|---|---|---|---|---|
| | | 10 | 100 | 1000 |
| NHS | — | 0.099 | 0.507 | 1.003 |
| | 50°C, 1 hr | 0.040 | 0.357 | 0.850 |
| | 56°C, 1 hr | 0.012 | 0.123 | 0.558 |
| Factor B | — | 0.091 | 0.475 | 0.891 |
| | 50°C, 1 hr | 0.010 | 0.114 | 0.685 |
| | 56°C, 1 hr | 0.007 | 0.080 | 0.515 |

**Table IV.** Biosynthesis of Factor B Driven by PMA

| Experiment | Stimulator | Conc. (ng/ml) | Factor B production in | |
|---|---|---|---|---|
| | | | Supernatants (ng/$10^6$ cells) | Lysates (pg/$10^6$ cells) |
| 1 | PMA | 0 | <0.5 | <10 |
| | | 1 | 3.9 ± 0.4 | 72 ± 3 |
| | | 10 | 10.6 ± 0.5 | 137 ± 4 |
| | | 100 | 9.0 ± 1.0 | 128 ± 3 |
| | | 500 | 4.0 ± 0.5 | 64 ± 1 |
| 2 | — | | 0.8 ± 0.2 | 23 ± 3 |
| | 4αPDD | 10 | 1.1 ± 0.1 | 23 ± 2 |
| | PMA | 10 | 14.9 ± 0.8 | 152 ± 5 |

a concentration of 100 and 1000 ng/ml, respectively, in this system. However, when both functionally and biochemically purified C7 and C8 were tested, no reaction was observed. No reaction was present when factor B-depleted serum was used (Table I). When plates were coated with antibody to other C complements rather than factor B, ALP-conjugated anti-factor B antibody did not react in these plates (Table II).

**Effect of Heat on Factor B Determination by ELISA.** Factor B has been reported to be very labile to heat at 50°C (10). In the present experiments when factor B or NHS was heated at 50°C for 56°C for 1 hr, the reactivity (OD) was reduced by about 50% (Table III).

**Application of ELISA for Factor B to Detect Biosynthesis of Factor B *In Vitro*.** U937 cells were cultured with 1–500 ng of PMA/ml for 4 days, and the amount of factor B in culture supernatants and cell lysates was determined by ELISA (Table IV, Experiment 1). The optimal concentration of PMA for induction of factor B production by U937 cells was shown to be 10–100 ng/ml. A chemical analogue of PMA, 4$\alpha$-PDD, did not enhance the biosynthesis of factor B (Table IV, Experiment 2).

Kinetics of PMA-driven factor B production by U937 cells was next examined in both culture supernatants and cell lysates (Fig. 2). The production of factor B was first detected on Day 1 in cell lysates from U937 cells stimulated with PMA. Factor B detected in lysates increased, peaked on Day 4, and decreased thereafter (closed triangle). On the other hand, factor B in the supernatants was detected on Day 3, rapidly increased, and reached a plateau on Day 5 (closed circle). However, a higher quantity of factor B was detected in the supernatants when compared with the lysates. In the case of unstimulated U937 cells, factor B production was also observed. However the amount of factor B detected was very low, in cell lysates after 3

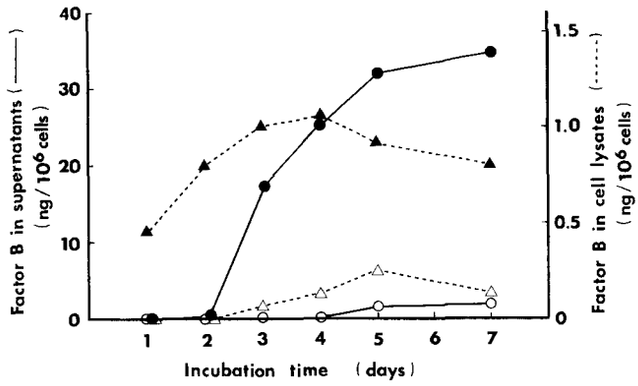


**Figure 2.** Kinetics of factor B biosynthesis in PMA-stimulated U937 cells. U937 cells were cultured with (●, ▲) or without (○, △) 10 ng of PMA/ml for 1 to 7 days. The concentration of factor B in the cell lysates and culture supernatants was determined by ELISA.

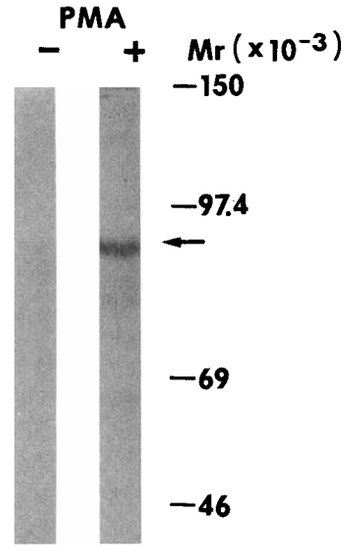


**Figure 3.** Autoradiography of 12% SDS-PAGE of U937 cell lysates metabolically labeled with [$^{35}$S]methionine and immunoprecipitated with goat anti-human factor B IgG. U937 cells were incubated with 10 ng of PMA/ml for 3 days. Molecular weight markers are indicated. PMA-induced factor B biosynthesis is indicated by an arrow.

(open triangle) days and in culture supernatants after 5 days (open circle).

Enhancement of biosynthesis of factor B by PMA was also shown by SDS-PAGE and autoradiography of [$^{35}$S]methionine-labeled, immunoprecipitated factor B protein from intracellular lysates of U937 cells incubated with PMA for 4 days (Fig. 3). Intracellular factor B was synthesized as a single chain 91,500-Da polypeptide and had lower molecular mass than factor B present in plasma or supernatants, as reported previously (5).

**Effect of Antimetabolites on Biosynthesis of Factor B.** The effect of antimetabolites on the biosynthesis of factor B induced by PMA was examined by following three separate treatments with mitomycin C, actinomycin D, and cycloheximide as inhibitors of DNA, RNA, and protein synthesis, respectively (Table V). U937 cells were pretreated with each antimetabolite for 1 hr, washed three times, and cultured with PMA for 4 days (pretreatment experiments). Cells were cultured with PMA and each antimetabolite together for 4 days (co-culture experiments). Cells were cultured with PMA for 4 days and each antimetabolite was pulsed for the last 1 day (pulse experiments). Factor B synthesis was inhibited by all three antimetabolites used both in supernatants and lysates. However, pretreatment with cycloheximide was not so effective to inhibit biosynthesis of factor B as co-culture experiment. This suggested that cycloheximide reversibly inhibited the factor B biosynthesis.

## Discussion

A method, which to date has not been described, was developed for the determination, quantitation, and

**Table V.** Effect of Antimetabolites on the Biosynthesis of Factor B Induced by PMA

| PMA | Antimetabolite | Factor B | | | |
|---|---|---|---|---|---|
| | | No treatment | Pretreatment[a] | Co-culture[b] | Pulse[c] |
| − | — | 0.5[d] (40)[e] | | | |
| + | — | 8.5 (596) | | | |
| + | Mitomycin C (20 μg/ml) | | 1.4 (120) | 0.1 (16) | 5.1 (288) |
| + | Cycloheximide (10 μg/ml) | | 5.9 (368) | <0.1 (16) | 5.1 (288) |
| + | Actinomycin D (10 μg/ml) | | <0.1 (32) | <0.1 (8) | 6.4 (394) |

[a] Cells were pretreated with each antimetabolite for 1 hr, washed three times, and cultured with PMA (10 ng/ml) for 4 days.
[b] Cells were cultured with PMA and each antimetabolite together for 4 days.
[c] Cells were cultured with PMA for 4 days. Each antimetabolite was pulsed for the last 1 day.
[d] The value shows the mean amount of factor B detected in the supernatants (ng/$10^6$ cells).
[e] Value in the parentheses shows the mean amount of factor B detected in cell lysates (pg/$10^6$ cells).

biosynthesis of factor B using U937 cells. This assay is quick, simple to perform, easy to detect, sensitive, reliable, specific for factor B, and utilizes commercially available reagents. Optimal doses and conditions giving maximum sensitivity were first determined. With appropriate conditions, the smallest amount of factor B detected was 1 ng/ml and standard curve was linear at <50 ng/ml (Fig. 1B). It is known that U937 cells produce additional biologic substances including prostaglandin $E_2$ and several C components (11, 12). Using the reagents we established for factor B ELISA, no cross-reactivity with any of the C components described above, with the exception of prostaglandin $E_2$ (which we have not tested), was seen. Evidence that our method was specific for factor B is as follows: (i) This assay did not detect both functionally and immunochemically purified complement components (C1–C9) including C7 and C8, which were detected if only functionally purified components were used (Table I). (ii) By this ELISA no reaction was observed with factor B-depleted NHS (Table I). (iii) ALP-conjugated goat anti-human factor B antibody did not react with the proteins that were captured by antibodies to factor B-unrelated antigens (Table II). Furthermore, both Ba and Bb fragments of factor B were detected and the method could be modified to detect specifically either Ba or Bb fragment by coating plates with monoclonal antibody to Ba or Bb, respectively (Table II). This assay also detected both active and heat-inactive factor B (Table III).

Biosynthesis of factor B was also demonstrated in the culture supernatants and cell lysates of PMA-stimulated U937 cells. The optimal concentration of PMA was 10–100 ng/ml, and biologically inactive phorbol ester analogue 4α-PDD (8, 9) had no enhancing effect (Table IV). Kinetic studies of PMA-induced factor B biosynthesis showed that factor B synthesis was first detected in U937 cell lysates on Day 1 and increased with the peak of Day 4, and that factor B secreted into supernatants was detected on Day 3, increased rapidly, and reached the plateau on Day 5 (Fig. 2). In unstimulated U937 cells, a very small amount of factor B was detected in cell lysates after 3 days and in culture supernatants after 5 days of incubation. PMA shortened the lag phase of factor B synthesis and strongly increased the amount of factor B produced and secreted by U937 cells. These data of kinetics showed that factor B detected in cell lysates and culture supernatants was synthesized *de novo*, but not preformed. This was also confirmed by the experiments using antimetabolites (Table V). PMA-induced factor B biosynthesis was inhibited by co-culture or pretreatment with mitomycin C, cycloheximide, or actinomycin D. Pretreatment of U937 with cycloheximide inhibited PMA-driven factor B biosynthesis to a lesser extent than co-culture experiment, suggesting that cycloheximide inhibited the synthesis of factor B and its effect was reversible as has been suggested by others (13).

Synthesis of C *in vitro* can also be investigated using incorporation of radiolabeled amino acids into specific proteins with sizes and subunit structures identical to the correspoding proteins in plasma. The determination of C synthesis using these methods can be estimated by a variety of methods, including autoradiography of immunoprecipitated proteins previously subjected to SDS-PAGE or determining incorporation of radioactive isotopes into synthesized specific proteins. Using the former method, we have confirmed the biosynthesis of factor B detected by ELISA (Fig. 3).

It is not clear whether PMA-enhanced factor B production is the result of cell maturation induced by PMA (14, 15) or specific induction of factor B synthesis. Our studies do not address the above questions. We can however estimate PMA-induced factor B production in the cell culture supernatants directly, without first concentrating the supernatants. The report of the development of a sensitive method for factor B would be useful to investigate the regulation, mechanism(s), and properties of factor B biosynthesis in a microculture system.

This study was supported by Grants CA40931 and A122360-03

from the national Institute of Health and by Grants from the Newland and the Eleanor Dana Foundation.

1. Colten HR. Complement synthesis. In: Day NK, Good RA, Eds. Biological Amplification Systems in Immunology. New York: Plenum Publishing Co., p47, 1977.
2. Colten HR, Strunk RC, Perlmutter DH, Cole FS. Regulation of complement protein biosythesis in mononuclear phagocytes. In: Cohn ZA, Ed. Ciba Foundation Symposium 118, Biochemistry of Macrophages. Chichester: Pitman, p141, 1986.
3. Johnson E, Hetland E. Mononuclear phagocytes have the potential to synthesize the complete functional complement system. Scand J Immunol **27**:489, 1988.
4. Pangburn MK, Müller-Eberhard HJ. The alternative pathway of complement. Springer Semin Immunopathol 7:163, 1984.
5. Perlmutter DH, Strunk RC, Goldberger G, Cole FS. Regulation of complement proteins C2 and factor B by interleukin-1 and interferon-gamma acting on transfected L cells. Mol Immunol **23**:1263, 1986.
6. Sutton MBSJ, Strunk RC, Cole FS. Regulation of the third component of complement and factor B in cord blood monocytes by lipopolysaccharide. J Immunol **136**:1366, 1986.
7. Voller A, Bidwell DE, Bartlett A. Enzyme immunoassays in diagnostic medicine. Theory and practice. Bull World Health Organ **53**:55, 1976.
8. Yip YK, Pang RHL, Oppenheim JD, Nachbar MS, Henriksen D, Zerebeckyj-Eckhardt I, Vilcek J. Stimulation of human gamma interferon production by diterpene esters. Infect Immun **34**:131, 1981.
9. Kraft AS, Anderson WB. Phorbol esters increase the amount of $Ca^{2+}$, phospholipid-dependent protein kinase associated with plasma membrane. Nature **301**:621, 1983.
10. Goodkofsky I, Lepow IH. Functional relationship of factor B in the properdin system to C3 proactivator of human serum. J Immunol **107**:1200, 1971.
11. Nichols WK. LPS stimulation of complement (C3) synthesis by a human monocyte cell line. Complement **1**:108, 1984.
12. Barnum SC, Volanakis JE. In vitro biosynthesis of complement protein D by U937 cells. J Immunol **134**:1799, 1985.
13. Beatty DW, Davis AE III, Cole S, Einstein LP, Colten HR. Biosynthesis of complement by human monocytes. Clin Immunol Immunopathol **18**:334, 1981.
14. Rovera G, Santoli D, Damsky C. Human promyelocytic leukemis cells in culture differentiate into macrophage-like cells when treated with a phorbol ester. Proc Natl Acad Sci USA **76**:2779, 1979.
15. Minta JO, Pambrun L. In vitro induction of cytologic and functional differentiation of the immature human monocytelike cell line U-937 with phorbol myristate acetate. Am J Pathol **119**:111, 1985.