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# Increased production of the third component of complement (C3) by monocytes from patients with systemic lupus erythematosus

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## SUMMARY

We measured *in vitro* C3 production by peripheral blood monocytes from patients with systemic lupus erythematosus (SLE), and found it to be significantly greater than that from normal controls. We also found that monocytes from SLE patients with active disease produced a markedly larger amount of C3 than those from SLE patients with inactive disease. Production of C3 by monocytes correlated with serum levels of anti-dsDNA antibodies and inversely correlated with serum C3 levels in SLE patients. Serial measurement of C3 in the culture supernatant from each SLE patient showed that C3 production by monocytes fell in parallel with a decrease of disease activity. The effect of corticosteroids was ruled out as there was no relation between the level of C3 production by monocytes and the dose of prednisolone. This seems to be the first study in which the C3 production was assayed at a cellular level in SLE patients, and this study suggests that the local C3 production is increased in SLE patients.

**Keywords** C3 complement production monocytes systemic lupus erythematosus

## INTRODUCTION

Activation of complement system by immune complexes plays a central role in the manifestation of systemic lupus erythematosus (SLE) (Fauci, Haynes & Katz, 1978). In SLE, serum levels of complement components are usually decreased in patients with active disease (Lange, Wasserman & Slobody, 1960; Schur & Sandson, 1968; Ueda, Kusaba & Yanase, 1983). The decreased levels of these proteins are considered to be due to the combination of increased consumption and decreased or normal production. Findings of decreased or normal production of complement components in SLE are based on the results of *in vivo* experiments using radiolabelled complement proteins (Alper & Rosen, 1967; Sliwinski & Zvaifler, 1972; Ruddy *et al.*, 1975; Charlesworth *et al.*, 1989). Regulation of complement production in SLE patients is poorly understood as there are few studies at the cellular level.

The third component of complement (C3) is one of the most important complement components, since it serves as the substrate for C3-converting enzymes of both alternative pathway, C3bBb, and classical pathway, C4b2a, and since its cleavage generates many of the complement-dependent activities (Colten, Ooi & Edelson, 1979). C3 is synthesized by monocytes, macrophages (Einstein *et al.*, 1977), hepatocytes (Alper & Rosen, 1976), fibroblasts (Senger & Hynes, 1978) and endothelial cells (Ueki *et al.*, 1987).

In the present study, in order to define the production of C3 at a cellular level in SLE, we measured *in vitro* C3 production by peripheral blood monocytes from SLE patients using ELISA. Unexpectedly, our results suggest that the production of C3 by monocytes is increased in patients with SLE.

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## MATERIALS AND METHODS

*Subjects*

We studied 21 patients with SLE (10 active and 11 inactive) and 11 healthy adults matched for age and sex. All of the patients met the revised American Rheumatism Association Criteria for the diagnosis of SLE (Tan *et al.*, 1982). Three of the 10 patients with active SLE had never been on corticosteroids and the other seven patients with active SLE and all 11 patients with inactive SLE were treated with prednisolone (10–60 mg/day). Activity of the disease was estimated on the basis of lupus activity criteria count (LACC) proposed by Urowitz *et al.* (1984).

*Preparation of human monocytes*

Mononuclear cells were isolated from heparinized peripheral blood by density gradient centrifugation on Ficoll–Conray. After washing in 10 mM phosphate-buffered saline (PBS), the cells were suspended in RPMI 1640 (Nissui, Tokyo, Japan) with 10% heat-inactivated (56°C, 2 h) fetal calf serum (FCS) (Sera Lab, Sussex, UK) in a tissue culture plate and incubated for 1 h

at 37°C to allow for adherence of the monocytes. Non-adherent cells were removed and adherent cells were gently detached from the plate with a rubber policeman. The adherent cells were >90% monocytes, as determined by Wright–Giemsa staining and phagocytosis of latex beads, and were viable >95% by trypan blue exclusion.

*Monocyte cultures*

Monocytes were incubated in 1 ml of RPMI 1640 with 10% heat-inactivated FCS at a final concentration of $1 \times 10^6$ cells/ml at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. A sample (100 μl) of culture supernatant was removed on days 1, 3, 5 and 7 for assay of C3, and replaced with 100 μl of fresh culture medium.

*Quantification of C3*

C3 was assayed by the method of Ueki *et al.* (1987) with some modification, using ELISA. Briefly, the wells in microtitre plates were coated with goat anti-human C3 serum (Cappel, Cochranville, PA). After washing, purified C3 (Cordis Lab., Miami, FL) or culture supernatants were added, and the preparations were incubated. The plates were treated with rabbit anti-human C3 IgG (MBL, Nagoya, Japan) horseradish peroxidase (HRP) labelled goat anti-rabbit IgG (Cappel), and ABTS (Wako Co., Tokyo, Japan) as a substrate for HRP. The enzyme activity was read under an autoreader (SLT-Lab Instruments, Austria). The values of the supernatants were compared with a standard curve obtained from purified C3.

*Assay for serum C3, C4, $CH_{50}$ and antibody to dsDNA*

Serum C3, and C4 were measured by laser nephelometry, $CH_{50}$ by a modification of Mayer's method. Anti-dsDNA antibodies were assayed by radioimmunoassay kits (Amersham International, Amersham, UK).

*Statical analysis*

The Wilcoxon rank sum test was used to determine the statistical significance. Correlations between individual parameters were tested by Spearman's rank correlation of the coefficient.

## RESULTS

*C3 production by monocytes from SLE patients*

Monocytes from active and inactive SLE patients and healthy controls were incubated without any stimulation for 7 days, and C3 concentrations in culture supernatants were assayed. As shown in Fig. 1, monocytes produced a measurable C3 by day 1, and produced increasing amount of C3 until day 7. Although there were no significant differences in the three groups on day 1, C3 production by monocytes from patients with active SLE was markedly higher than that from patients with inactive SLE and healthy controls after day 3 ($P<0.01$). C3 production by monocytes from patients with inactive SLE was significantly higher than production by control monocytes on day 7 ($P<0.05$). Figure 2 shows C3 production by monocytes from each subject on day 7. Monocytes from patients with active SLE produced $86.5 \pm 15.3$ ng/$10^6$ cells per ml (mean ± s.e.m.) of C3, a level markedly higher than that from those subjects with inactive SLE ($26.2 \pm 6.4$) and from healthy controls ($8.7 \pm 2.2$).

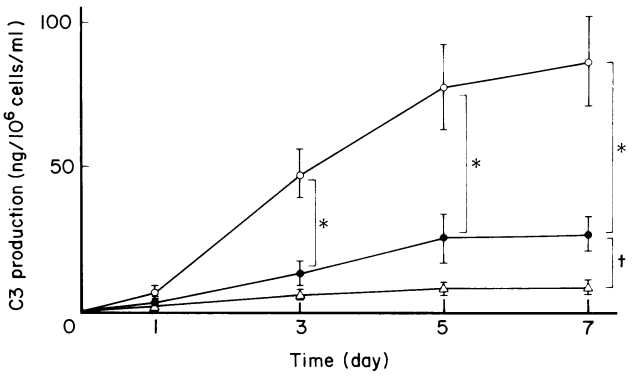


**Fig. 1.** Kinetics of C3 production by monocytes ($1 \times 10^6$/ml) from patients with active (○), inactive (●) systemic lupus erythematosus and healthy controls (△). Mean ± s.e.m. * $P<0.01$; † $P<0.05$.

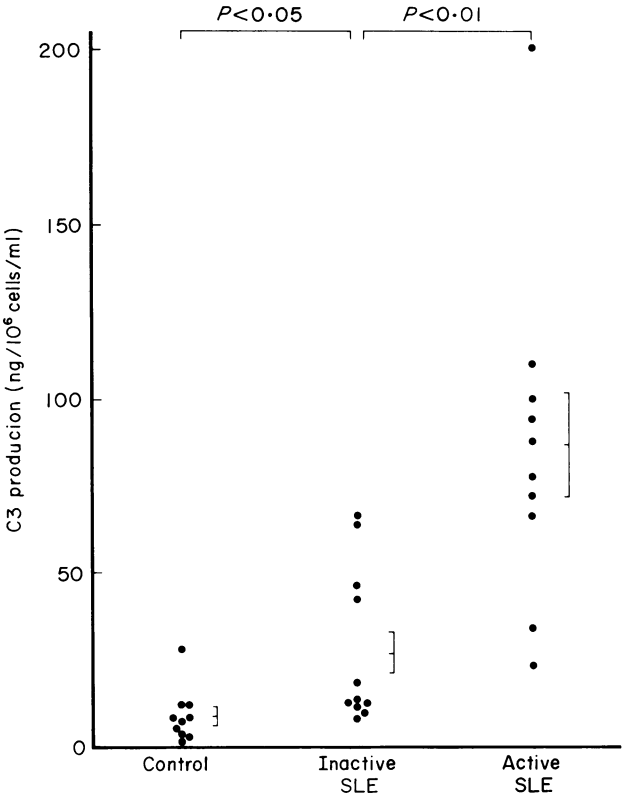


**Fig. 2.** C3 production by monocytes ($1 \times 10^6$/ml) from patients with active and inactive systemic lupus erythematosus (SLE) and from healthy controls on day 7.

*Correlation of C3 production by monocytes with several parameters related to SLE*

For a precise study on the high production of C3 by SLE monocytes, the correlation between C3 production by monocytes and serum levels of complement and anti-dsDNA antibody in SLE was examined. Interestingly, an inverse correlation was found between C3 production by monocytes and serum C3 levels ($r_s = -0.493$, $P<0.05$) (Fig. 3). There was no significant correlations between C3 production by monocytes and serum

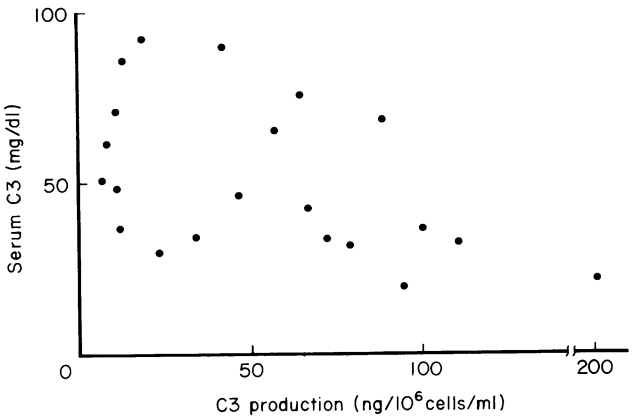


**Fig. 3.** Correlation of C3 production by monocytes on day 7 with serum C3 levels in patients with systemic lupus erythematosus. $r_s = -0{\cdot}493$; $P < 0{\cdot}05$.

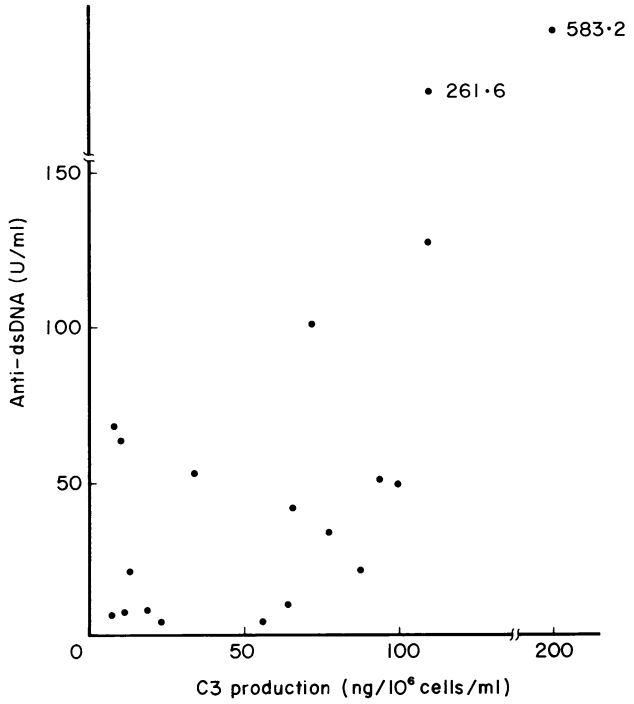


**Fig. 4.** Correlation of C3 production by monocytes on day 7 with serum levels of anti-dsDNA antibodies in patients with systemic lupus erythematosus. $r_s = 0{\cdot}457$; $P = 0{\cdot}06$.

$CH_{50}$ and C4 (data not shown). Levels of anti-dsDNA antibody weakly correlated with C3 production by monocytes ($r_s = 0{\cdot}457$, $P = 0{\cdot}06$) (Fig. 4). To determine whether corticosteroids had any effect on C3 production by monocytes in SLE, correlations between the dose of prednisolone and C3 production were examined. As shown in Fig. 5, there was no significant correlation. No increased C3 production by monocytes was noted in a healthy volunteer on 10 mg/day of prednisolone for more than 2 weeks (data not shown).

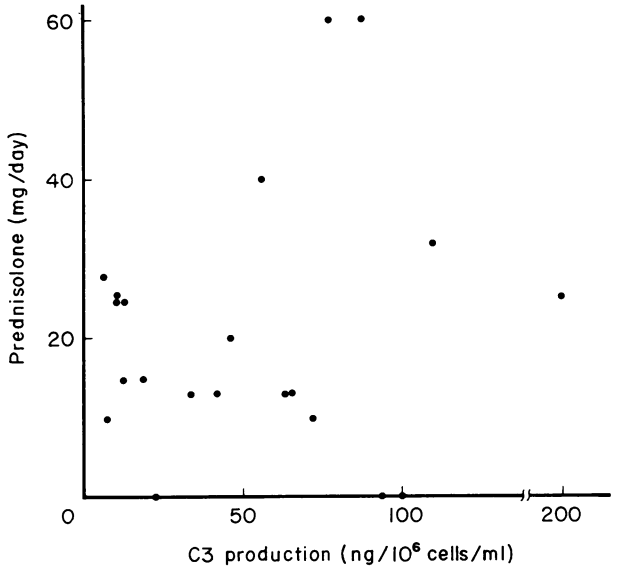


**Fig. 5.** Correlation of C3 production by monocytes on day 7 with dose of prednisolone in patients with systemic lupus erythematosus $r_s = 0{\cdot}011$; $P = 0{\cdot}96$.

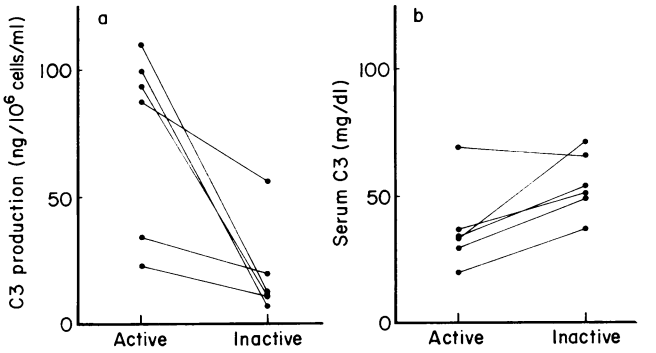


**Fig. 6.** (a) C3 production by monocytes and (b) serum C3 levels in the same six systemic lupus erythematosus patients with changes in the disease activity. The period between active and inactive stages ranges from 3 to 6 months.

*Serial assay of C3 production by monocytes from individual patients*

To define whether C3 production by monocytes would be altered with the disease activity in individual patients with SLE, C3 production by monocytes in the active period was compared with that in the inactive period after 3–6 months in six SLE patients. As shown in Fig. 6a, C3 production fell in parallel with a decrease in the disease activity in all patients. Conversely, the serum C3 levels increased in five of six patients (Fig. 6b).

## DISCUSSION

Hypocomplementaemia is found in patients with active SLE. The mechanism is thought to be due to the accelerated consumption of complement, i.e. the activation of complement system (Lange *et al.*, 1960; Schur & Sandson., 1968). This

proposal is supported by the finding that there is a co-existence of reduced levels of complement components and increased levels of the complement cleavage products in the sera of SLE patients (Essen & Isomaki, 1985; Hopkins *et al.*, 1988). However, little is known of the production of complement components in SLE patients. Sliwinski & Zvaifler (1972) injected radioactive labelled C3 intravenously and noted a decreased synthesis of C3 in SLE patients with hypocomplementaemia. The results, however, were not based on the direct synthetic rate of C3, but were derived from the catabolic rate. In addition, it is not known whether intravenously administered radiolabelled purified C3 is catabolysed in a same fashion as natural C3. Other investigators, using a similar method, predicted that C3 production is decreased or at most normal in SLE patients (Alper & Rosen, 1967; Ruddy *et al.*, 1975; Charlesworth *et al.*, 1989).

To our knowledge, this is the first study in which the C3 production was assayed at a cellular level in SLE patients. Unexpectedly in discord with previously reported *in vivo* results, our study demonstrated an increased C3 production *in vitro* by peripheral blood monocytes from SLE patients. In addition, we found that monocytes from patients with active SLE produced markedly more C3 than those from patients with inactive SLE. Support for this was obtained by evidence of a correlation between C3 production and the serum levels of anti-dsDNA antibodies. Moreover, serial measurements of the C3 production in each SLE patient showed that C3 production by monocytes was reduced in parallel with the decrease of disease activity of SLE. It should be noted that the C3 production by monocytes inversely correlated with the serum levels of C3. C3 production by monocytes may well parallel the C3 consumption in SLE patients.

C3 is synthesized by hepatocytes (Alper & Rosen, 1976), fibroblasts (Senger & Hynes, 1978) and endothelial cells (Ueki *et al.*, 1987) as well as monocytes and macrophages. It is not known, however, what amount of C3 each cell synthesizes in a resting state and in response to certain stimulations, and how the C3 production by these cells is mutually regulated *in vivo*. Passwell *et al.* (1988) found an increased extra-hepatic expression of C3, factor B, C2 and C4 mRNA in murine lupus nephritis. In humans, Ceulaer, Papazoglou & Whaley (1980) described an increased synthesis of complement components by monocytes and synovial fluid macrophages from patients with rheumatoid arthritis. It is possible, therefore, that the C3 production by monocytes and macrophages is enhanced at affected local sites in SLE patients. The response of other C3-producing cells is unknown and might be differently regulated from that of monocytes and macrophages if the total C3 production is not increased, as suggested by *in vivo* studies. Hence the increased C3 production by monocytes may not be reflected in the serum C3 levels in SLE.

The mechanism of increased C3 production by monocytes in SLE patients is unclear. Some cytokines regulate the complement production. Interleukin-1 (IL-1) and tumour necrosis factor (TNF) upregulate C3 synthesis at pre-translational level in human hepatocytes (Perlmutter & Colten, 1986; Perlmutter *et al.*, 1986a, 1986b). In SLE patients, however, various functional defects in monocytes and T cells have been described and it has been demonstrated that the production of cytokines including IL-1, TNF and interleukin-2 (IL-2) by these cells is impaired (Linker-Israeli *et al.*, 1983; Katayama *et al.*, 1983; Phillips *et al.*, 1985; Shirakawa, Yamashita & Suzuki, 1985). Production of C3 and other cytokines by monocytes may thus be independently regulated. The effect of corticosteroids was ruled out as there was no relation between the amount of C3 production and dose of corticosteroids, and an increased C3 production was observed in three patients before they were administered with steroids. Moreover, C3 production by monocytes from a healthy control on prednisolone daily was not increased.

There are some possible mechanisms to explain the increased C3 production by monocytes in SLE patients. Immune complexes, activators of the complement system, may stimulate C3 production by monocytes in SLE (Fauci *et al.*, 1978). The increased complement cleavage products found in sera of patients with active as well as inactive SLE (Hopkins *et al.*, 1988) may stimulate C3 production by monocytes, as monocytes have complement receptors (Fearon, Kaneko & Thomson, 1981). The reduced level of serum complements may stimulate C3 production by monocytes to maintain certain levels of C3. In guinea pig peritoneal macrophages, fluid phase extracellular C4 inhibits C4 production at the pre-translational level (Auerbach *et al.*, 1984).

We present novel findings of increased C3 production by monocytes from SLE patients. The precise mechanism and regulation of C3 production remain to be elucidated.

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## REFERENCES

ALPER, C.A. & ROSEN, F.S. (1967) Studies of the in vivo behavior of human C'3 in normal subjects and patients. *J. clin. Invest.* **46,** 2021.

ALPER, C.A. & ROSEN, F.S. (1976) Genetics of the complement system. *Adv. hum. Genet.* **7,** 141.

AUERBACH, H.S., BAKER, R.D., MATTHEWS, W.J. JR & COLTEN, H.R. (1984) Molecular mechanism for feedback regulation of C4 biosynthesis in guinea pig peritoneal macrophage. *J. exp. Med.* **159,** 1750.

CEULAER, C.D., PAPAZOGLOU, S. & WHALEY, K. (1980) Increased biosynthesis of complement components by cultured monocytes, synovial fluid macrophages and synovial membrane cells from patients with rheumatoid arthritis. *Immunology*, **41,** 37.

CHARLESWORTH, J.A., PEAKE, P.W., GOLDING, J., MACKIE, J.D., PUSSELL, B.A., TIMMERMANS, V. & WAKEFIELD, D. (1989) Hypercatabolism of C3 and C4 in active and inactive systemic lupus erythematosus. *Ann. rheum. Dis.* **48,** 153.

COLTEN, H.R., OOI, Y.M. & EDELSON, P.J. (1979) Synthesis and secretion of complement proteins by macrophages. *Ann. NY Acad. Sci.* **332,** 482.

EINSTEIN, L.P., HANSEN, P.J., BALLOW, M., DAVIES, A.E., DAVIS, J.S., ALPER, C.A., ROSEN, F.S. & COLTEN, H.R. (1977) Biosynthesis of complement (C3) in vitro by monocytes from both normal and homozygous C3-deficient humans. *J. clin. Invent.* **60,** 963.

ESSEN, R.V. & ISOMAKI, H. (1985) Immunonephelometric quantitation of complement split product(C3d) plasma levels in rheumatic disease. *Scand. J. Rheumatol.* 14, 421.

FAUCI, T.Y., HAYNES, B.F.& KATZ, P. (1978) The spectrum of vasculitis. *Ann. intern. Med.* **89,** 660.

FEARON, D.T., KANEKO, I. & THOMSON, G.G. (1981) Membrane distribution and adsorptive endocytosis by C3b receptors on human polymorphonuclear leukocytes. *J. exp. Med.* **153,** 1615.

HOPKINS, P., BELMONT, H.M., BUYON, J., PHILLIPS, M., WEISSMANN, G. & ABRAMSON, S.B. (1988) Increased levels of plasma anaphylatoxins in systemic lupus erythematosus predict flares of the disease and may elicit vascular injury in lupus cerebritis. *Arthritis Rheum.* **31,** 632.

KATAYAMA, S., CHIAS, D., KNUTSON, D. & BARNETT, E.V. (1983) Decreased Fc receptor avidity and degradative function of monocytes from patients with systemic lupus erythematosus. *J. Immunol.* **131,** 217.

LANGE, K., WASSERMAN, E. & SLOBODY, L.B. (1960) Significance of serum complement levels for diagnosis and prognosis of acute and subacute glomerulonephritis and lupus erythematosus disseminatus. *Ann. intern. Med.* **53,** 636.

LINKER-ISRAELI, M., BAKKE, A.C., KITRIDOU, R.C., GENDLER, S. GILLIS, S. & HOROWITZ, D.A. (1983) Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus (SLE). *J. Immunol.* **130,** 2651.

PASSWELL, J. SCHREINER, G.F., NONAKA, M., BEUSCHER, H.U.& COLTEN, H.R. (1988) Local extrahepatic expression of complement genes C3, factor B, C2 and C4 is increased in murine lupus nephritis. *J. clin. Invest.* **82,** 1676.

PERLMUTTER, D.H. & COLTEN, H.R. (1986) Molecular immunology of complement biosynthesis: a model of single-cell control of effector-inhibitor balance. *Annu. Rev. Immunol.* **4,** 231.

PERLMUTTER, D.H., DINARELLO, C.A., PUNSAL, P.I. & COLTEN, H.R. (1986a) Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. *J. clin. Invest.* **78,** 1349.

PERLMUTTER, D.H., GOLDBERGER, G., DINARELLO, C.A., MIZEL, S.B. & COLTEN, H.R. (1986b) Regulation of class III major histocompatibility complex gene products by interleukin 1. *Science*, **232,** 850.

PHILLIPS, R., LOMNITZER, R., WADEE, A.A. & ROBSON, A.R. (1985) Defective monocyte function in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **34,** 69.

RUDDY, S., CARPENTER, C.B., CHIN, K.W., KNOSTMAN, J.N., SOTER, N.A., GOTZE, O., MULLER-EBERHARD, H.J. & AUSTEN, K.F. (1975) Human complement metabolism: an analysis of 144 studies. *Medicine*, **54,** 165.

SCHUR, P.H. & SANDSON, J. (1968) Immunological factors and clinical activity in systemic lupus erythmatosus. *N. Engl. J. Med.* **278,** 533.

SENGER, D.R. & HYNES, R.O. (1978) C3 component of complement secreted by established cell lines. *Cell*, **15,** 375.

SHIRAKAWA, F., YAMASHITA, U. & SUZUKI, H. (1985) Decrease in HLA-DR-positive monocytes in patients with systemic lupus erythematosus. *J. Immunol.* **134,** 3560.

SLIWINSKI, A.J. & ZVAIFLER, N.J. (1972) Decreased synthesis of the third component of complement(C3) in hypocomplementemic systemic lupus erythematosus. *Clin. exp. Immunol.* **11,** 21.

TAN, E.M., COHEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25,** 1271.

UEDA, A., KUSABA, T. & YANASE, T. (1983) Determination of the second component of complement(C2) by electroimmunoassay in sera from patients with systemic lupus erythematosus. *Clin. exp. Immunol.* **53,** 217.

UEKI, A., SAI, T., OKA, H., TABATA, M., HOSOKAWA, K. & MOCHIZUKI, Y. (1987) Biosynthesis and secretion of the third component of complement by human endothelial cells *in vitro*. *Immunology*, **61,** 11.

UROWITZ, M.B., GALDMAN, D.D., TOZMAN, E.C.S. & GOLDSMITH, C.H. (1984) The lupus activity criteria count. *J. Rheumatol.* **11,** 783.