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Modulation of monocyte complement synthesis by lymphocytes and lymphocyte-conditioned media

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SUMMARY

Culture supernatants from mitogen- and antigen-stimulated human peripheral blood lymphocytes (PBL), stimulated synthesis of the second complement component (C2) by human monocytes, but not as effectively as the stimulated PBL themselves, which adhered to the monocytes and caused marked spreading. In contrast to PBL, lymphocytes isolated from the synovial membranes (SML) of patients with rheumatoid arthritis and their culture supernatants were able to stimulate C2 synthesis without exposure to mitogens or antigens. Depletion of B and T populations showed that T cells were responsible for stimulation of C2 synthesis. Further studies of synthesis rates of C2, C3 factor B (B), C1 inhibitor, and properdin (P) were undertaken, and it was found that lymphocytes and their supernatants increased synthesis of C2, B and C1 inhibitor, and reduced synthesis of C3 and P. This profile of activity was identical to that produced by the addition of recombinant γ-intereron (rIFN-γ) to the cultures. Furthermore the addition of a monoclonal antibody to rIFN-γ to cultures abrogated the effects of rIFN-γ, and almost completely reversed the effects of lymphocytes and their supernatants. Thus it appears that γ-interferon is the lymphocyte product which is responsible for the modulation of monocyte complement synthesis. The results of studies with synovial membrane lymphocytes raise the possibility that this process occurs *in vivo*. Monocyte C2 had a higher specific functional activity (SpFA) than serum C2 isolated from serum or C2 produced by HepG2 cells. Monocyte C2 formed a C3 convertase which had a longer half-life than that found with both serum C2 or HepG2 C2. Thus monocyte C2 behaves like oxidized C2. Monocytes exposed to rIFN-γ, lymphocytes or lymphocyte-conditioned medium (LCM) produced C2 which had an even higher SpFA. Although antibody to IFN-γ prevented any increase in C2 synthesis in monocyte cultures containing lymphocytes or LCM, C2 SpFA was still increased. Thus a second lymphocyte product is responsible for this 'oxidation' effect. This production of 'oxidized' C2 by monocytes and further 'oxidation' by the action of either lymphocytes or γ-interferon might play a significant role in the perpetuation of complement activation at sites of inflammation.

Keywords complement synthesis γ-interferon monocytes

INTRODUCTION

Human monocytes in culture synthesize a number of complement components including the second (C2) and third (C3) components, factor B (B), properdin (P) and C1-inhibitor (Einstein, Schneeberger & Colten, 1976; Whaley 1980; Strunk *et al.*, 1983; Yeung-Laiwah *et al.*, 1984). Macrophages, isolated from the synovial fluids of patients with rheumatoid arthritis, synthesize greater quantities of C2 and B than do monocytes from the same individuals (De Ceulaer, Papazoglou & Whaley, 1981). The factors which stimulate complement synthesis by macrophages are still being defined, but as the supernatants of cultures of antigen-stimulated lymphocytes have been shown to contain a potent stimulator of monocyte C2 synthesis (Littman & Ruddy 1977, 1979) it is probable that in the chronically inflamed rheumatoid joint a lymphocyte product is responsible for the high synthesis rates of C2 and B by synovial fluid macrophages. This study was undertaken to define more closely the role of lymphocytes and their secretory product, γ-interferon (IFN-γ) in the regulation of monocyte complement synthesis.

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MATERIALS AND METHODS

Reagents

Hanks' balanced salt solution (HBSS), RPMI 1640 (Flow Laboratories), Dulbecco's calcium- and magnesium-free phosphate-buffered saline (PBS), Dulbecco's modified Eagles medium (DMEM), fetal calf serum (FCS), L-glutamine, pokeweed mitogen (PWM), antibiotic/antimycotic solution contain-

ing penicillin, streptomycin and fungizone (GIBCO-BRL), the dye H33258 (Hoechst Pharmaceuticals), collagenase, trypsin, deoxyribonuclease (Sigma), phytohaemagglutin (PHA; Wellcome Diagnostics), Concanavalin A (ConA; ICN Biomedicals Ltd) and streptokinase/streptodornase (Varidase, SK/SD; Lederle U.K. Ltd.), were obtained from commercial sources. Freeze-dried *E. coli*-derived recombinant γ-interferon (rIFN-γ; Immuneron, BG8201, lot no. 10M05) was a gift from Biogen SA, Geneva, Switzerland. This preparation had a specific activity of 14 iru/ng and a pyrogen concentration of 0·4 ng/dosage unit (100 μg). The freeze-dried material was stored at 2°C and reconstituted with sterile water to give a concentration of 100 μg ($1{\cdot}4 \times 10^6$) units/ml before use. A monoclonal antibody to rIFN-γ was a gift from Dr Chi-Ming Liang (Biogen Research Corp., Cambridge, MA). This IgG1 antibody had a neutralization titre of 1:100000. Human AB serum (ABS) was supplied by the Scottish Blood Transfusion Service (Law Hospital, Carluke, Lanarkshire, UK) and was heat-inactivated for 2 h at 56°C. C2 was purified from fresh frozen plasma (Ruddy & Austen 1967).

Monocyte culture

Method 1. Monocyte monolayers were prepared in Linbro multiwell tissue culture plates from the discarded buffy coats of blood bank donations (Lappin *et al.*, 1984) and cultured in RPMI-ABS (RPMI containing 10% ABS) (1 ml) at 37°C in a humidified atmosphere. After 3 days the supernatants were removed and culture continued in either RPMI-FCS (RPMI containing 20% heat-inactivated FCS) (those cultures to which rIFN-γ or peripheral blood lymphocyte-conditoned medium (PBLCM) would be added) or RPMI-ABS (those cultures to which peripheral blood lymphocytes (PBL) would be added). The supernatants were again replaced two days later when rIFN-γ, PBLCM or PBL were added to the cultures. This day was termed day 0. Samples of culture supernatants (250 μl) were removed on days 1, 3 and 5. On day 7 the culture was terminated by the removal of the entire supernatant, washing the monolayers in RPMI and then lysing them in 200 μl SDS 0·05% (w/v), for DNA determination (Cesarone *et al.*, 1979). In experiments in which lymphocytes were added to monocyte cultures, the DNA determination was performed on a replicate monolayer which had not had PBL added.

Method 2. For studies of the effects of rheumatoid arthritis synovial membrane lymphocytes (SML), venous blood was taken from patients 24 h prior to surgery. Monocyte monolayers were prepared as described in method 1 and cultured in RPMI-ABS. Monolayers prepared by either of these procedures consisted of over 95% monocytes as assessed by morphology under phase-contrast, phagocytosis of latex particles and staining for non-specific esterase.

Preparation of peripheral blood lymphocyte-conditioned medium (PBLCM)

The non-adherent cells from the mononuclear leucocyte suspensions used for the preparation of monocyte monolayers were washed in HBSS, resuspended to $2{\cdot}5 \times 10^6$/ml in RPMI-ABS and incubated at 37°C with an optimal concentration of mitogen (PHA; Con-A; PWM) or antigen (SK/SD). After 16 h the cultures were centrifuged and the cells washed in RPMI-1640 to remove mitogen or antigen and resuspended to their original volume in RPMI-ABS. The cultures were continued for a further 48 h at 37°C and after centrifugation (400 *g* for 10 min at 4°C) the supernatants were harvested and dialysed against RPMI while the cell pellets were washed in RPMI and resuspended in RPMI-ABS. PBL and PBLCM were added to cultures of monocytes from the same donor. In all experiments the occurrence of lymphocyte transformation was checked by measuring the incorporation of ^{3}H-thymidine into DNA during a 16 h incubation of 1×10^5 cells (Bender & Prescott, 1962).

Isolation of lymphocytes from synovial membrane (SML)

Synovial membrane was removed from patients with rheumatoid arthritis undergoing synovectomy or joint replacement surgery. The tissue was washed with Dulbecco's calcium- and magnesium-free PBS and cut into 1–2 mm^3 fragments which were then washed in PBS before being suspended in HBSS containing collagenase (0·02% w/v) and deoxyribonuclease (0·01% w/v) and incubated for 3 h at 37°C in a shaking water bath. The suspension was centrifuged (400 *g* for 10 min at room temperature), and the supernatant containing debris was discarded. The pellet was resuspended in PBS, washed three times and then re-incubated in PBS containing trypsin (0·05% w/v) and EDTA (0·02% w/v) for 1 h at 37°C. The cells were separated from undigested fragments by filtration through sterile gauze, centrifuged (400 *g* for 10 min at room temperature), washed twice with DMEM containing 10% ABS and finally resuspended in this medium. Adherent cells were removed by adherence to Petri dishes. The non-adherent cells, which were over 95% lymphocytes as assessed by morphology and staining for T and B markers (see below), were washed once and resuspended to $2{\cdot}5 \times 10^6$/ml in RPMI-ABS and incubated at 37°C for 48 h under the same conditions as were described for PBL. The culture supernatant was harvested by centrifugation, dialysed against RPMI and then added to cultures of monocytes from the same individual. The SML were washed and added to a replicate series of cultures.

Separation of lymphocyte subpopulations

Lymphocyte preparations were depleted of T cells or B cells by means of a panning procedure, using a pan-T-cell murine monoclonal antibody (RFT11) to remove T cells and a cocktail of three antibodies (RFB4, RFB6, RFB7) to remove B cells, (kindly supplied by Professor George Janossy, Royal Free Hospital, London). Following culture with mitogens or antigens as described above, PBL were washed in ice-cold RPMI and then incubated for 30 min at 0°C with fresh RPMI containing either T or B cell monoclonal antibodies. Following washing in ice-cold RPMI, the cells were then layered onto Petri dishes which had been coated with the IgG fraction of rabbit anti-mouse globulin and incubated for a further 30 min at 0°C. The non-adherent cells were removed and layered onto a second antibody-coated Petri dish for a further 30 min at 0°C. The non-adherent cells were then washed in RPMI, resuspended in RPMI-ABS and added to cultures of monocytes from the same individual. In some experiments they were incubated at 37°C in RPMI-ABS for a further 48 h and the supernatants were harvested, dialysed and added to monocyte monolayers. SML were depleted of T or B populations using the same procedure.

In all experiments the efficiency of the panning procedure was assessed by the immunoperoxidase staining of cytocentrifuge smears using the pan-T monoclonal antibody and the

cocktail of B cell monoclonal antibodies. In each case the enriched population was contaminated by no greater than 5% of that which had been depleted.

Measurement of complement components

C2, C3, B, P and C1-inhibitor levels in culture supernatants were measured by ELISA procedures (Lappin *et al.*, 1986). The rate of accumulation of each component in the culture supernatant was linear over the period of study. Secretion rates for each component were determined from the difference in concentrations between days 3 and 5. C2 functional activity and the rate of decay of the C3 convertase, C4b2a, formed with monocyte or serum C2, were measured haemolytically using EAC14 cells (Whaley, 1985). C2 levels were also measured in the supernatants of cultures of human hepatocellular carcinoma cell line, HepG2.

RESULTS

A comparison of the effects of LCM and lymphocytes on C2 synthesis

In preliminary experiments the following points were established. (i) The supernatants from mitogen and antigen-stimulated PBL cultures (PBLCM) stimulated C2 synthesis, PHA being approximately three-fold more potent than ConA, PWM or SK/SD. The supernatants from untreated PBL cultures had little or no effect on C2 synthesis. (ii) The addition of mitogen- or antigen-stimulated PBL to monocyte cultures resulted in a dramatic increase in stimulation of C2 synthesis, far greater than that observed with the PBLCM. This was observed with all three mitogens and SK/SD, but again the greater effect was produced by PHA. In most experiments untreated PBL did not affect C2 synthesis and in the minority of experiments in which it occurred, the degree of stimulation was no greater than 10%. (iii) Phase-contrast examination of the monocyte cultures to which PBL had been added showed marked spreading of the monocytes, with PBL adhering to their surfaces. (iv) Removal of lymphocytes which did not adhere to monocytes did not reduce the levels of C2 in the cultures. (v) SMLCM or SML stimulated C2 synthesis without exposure to mitogens or antigens.

For further experiments PBL were stimulated with PHA. The stimulatory effects of PBLCM and PBL were compared by adding different numbers of PBL to replicate monocyte cultures and adding the volume of PBLCM from the same culture, which contained that number of cells. Significant stimulation of C2 synthesis (75%) was produced by 10^3 PBL while the PBLCM from 10^5 cells produced a similar degree of stimulation (Fig. 1a). The degree of stimulation increased with the number of PBL added to the cultures. Similar results were obtained with SML and SMLCM medium (Fig. 1b).

The lymphocyte population responsible for stimulation of C2 synthesis

In these experiments the number of intact PBL or SML added to each culture was 10^5. The number of cells from preparations which had been depleted of a population was adjusted so that the number of the remaining cells in each population was equivalent to that in the original intact cell preparations. B-depleted, PHA-transformed PBL still stimulated C2 synthesis, whereas T-depleted preparations did not (Fig. 2a). Similar results were observed with SML (Fig. 2b). Phase-contrast microscopic studies of the cultures showed that both T and B cells adhered to monocytes. The degree of monocyte spreading produced by T-enriched cells was greater than that occurring in cultures to which B-enriched PBL were added.

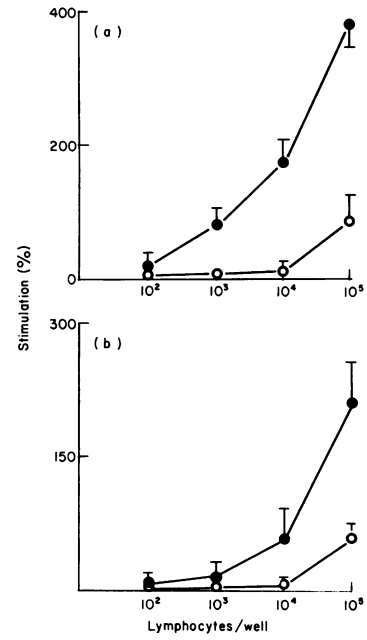

Fig. 1. The effects of PHA-treated peripheral blood lymphocytes (PBL) and PHA-treated lymphocyte-conditioned medium (PBLCM) on the production of C2 by human monocytes (a) and the effects of synovial membrane lymphocytes (SML) and synovial membrane lymphocyte-conditioned medium (SMLCM) on the production of C2 (b). Each point represents the mean and SEM of three experiments. Supernatant (○); lymphocytes (●).

Identification of the factor which stimulates C2 synthesis

Like rIFN-γ, PBL, SML, PBLCM and SMLCM stimulated synthesis of C2, B, and C1-inhibitor but reduced synthesis of C3 and P (Hamilton *et al.*, 1987; Lappin and Whaley, 1987) (Figs 3 and 4). In the presence of antibody to rIFN-γ (100 units/ml), rIFN-γ failed to produce any changes in the synthesis of the complement components studied, and PBL, SML, PBLCM and SMLCM had little effect on their synthesis rates (Figs 3 and 4).

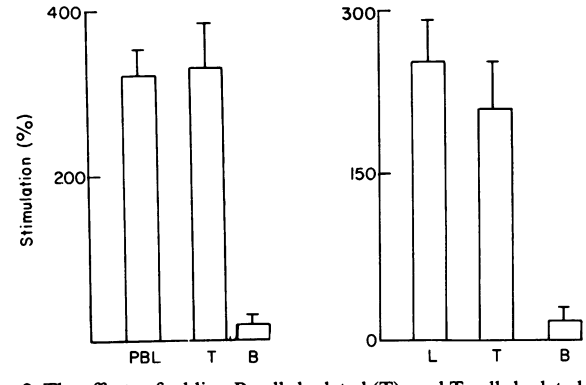

Fig. 2. The effects of adding B-cell-depleted (T), and T-cell-depleted (B) PHA-treated PBL on C2 production (Left). The effects of T-cell-depleted and B-cell-depleted SML on C2 production are shown on the right. The results are expressed as the mean of two determinations from a single experiment.

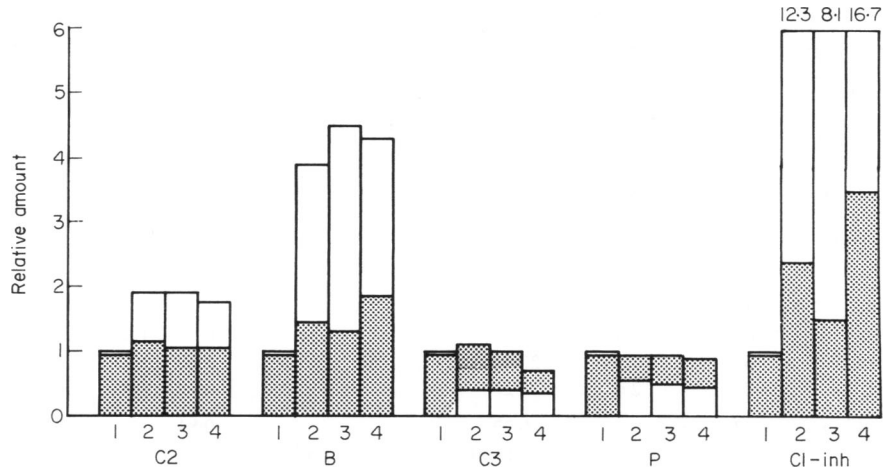

Fig. 3. Comparison of the effects of recombinant γ-interferon (rIFN-γ), PHA-treated peripheral blood lymphocyte supernatants (PBLCM) and PHA-treated peripheral blood lymphocytes (PBL 10^4 cells) added in the presence of (100 units) antibody to rIFNγ (▩) or absence of antibody to rIFNγ (□) on the synthesis of C2, B, C3, P and C1-inh. (Control synthesis rates (mol/cell/min) mean ± standard error of the mean; C2 48 ± 3; B 18 ± 5; C3 1264 ± 234; P 900 ± 42; C1-inh; 266 ± 27. Control cultures (1); rIFN-γ-treated cultures (2); PBLCM-treated cultrues (3); PBL-treated cultures (4). The results are expressed as the mean of four experiments.

Effect of recombinant γ-interferon (rIFN-γ) lymphocyte-conditioned media and lymphocytes on the specific functional activity of C2

The specific functional activity (SpFA) of C2 in control monocyte supernatants was $3{\cdot}52 \times 10^7$ effective molecules/ng compared with $0{\cdot}71 \times 10^7$ for serum C2 and $0{\cdot}84 \times 10^7$ for C2 produced by HepG2 cells (Table 1). The half-life ($T_{\frac{1}{2}}$) of the C3 convertase, C4b2a, formed with monocyte C2 was 9·2 min at 30°C, whereas those formed with serum C2 and C2 produced by HepG2 cells had half-lives of 3·5 min. The SpFAs for C2 from cultures of normal monocytes which had been treated with rIFN-γ, PBLCM and PHA-transformed PBL were increased approximately two-fold and the half-lives of C4b2a formed with C2 from these monocyte cultures were more prolonged (Table 1). The concentrations of C2 protein in cultures to which the monoclonal antibody to rIFN-γ had been treated with rIFN-γ, PBLCM, SMLCM, PBL or SML were similar to those in the control culture, showing that C2 synthesis was unaltered. However, antibody to rIFN-γ reduced levels of C2 haemolytic activity to control levels only in those cultures treated with rIFN-γ. Levels of C2 haemolytic activity in those exposed to PBL, SML, PBLCM or SMLCM remained above levels seen in the control cultures. Thus, the C2 SpFAs in these cultures were increased and the $T_{\frac{1}{2}}$ of C4b2a remained unduly prolonged (Table 1). Untransformed PBL produced a small increase in C2 synthesis but the SpFA of the C2 and the half-life of C4b2a formed with the C2 were the same as those in control monocytes (data not shown). Purified C2 which had been oxidized with I_2/KI (Polley & Müller-Eberhard 1967) formed a C3 convertase with increased stability (Fig. 5). In contrast the half-life of

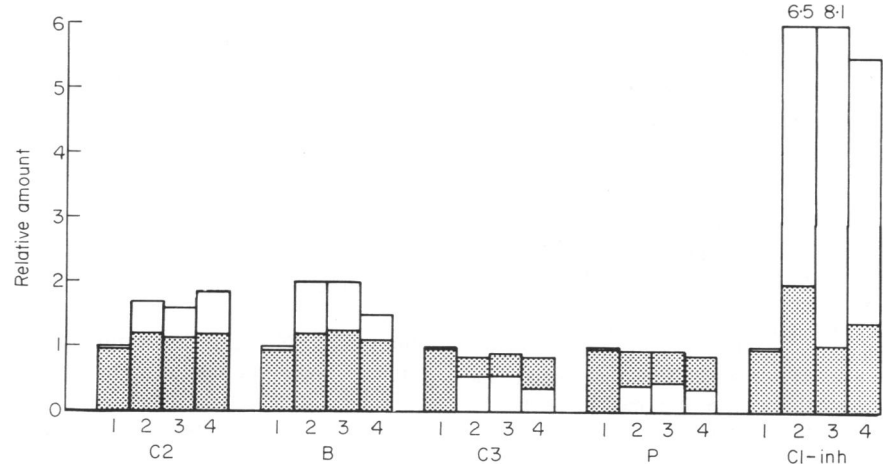

Fig. 4. Comparison of the effects of recombinant γ-interferon (rIFN-γ), synovial membrane lymphocyte culture medium (SMLCM) and synovial membrane lymphocytes (SML, 10^4 cells) added in the presence of (100 units) antibody to rIFN-γ (▩) or absence of antibody (□) to rIFN-γ on the synthesis of C2, B, C3, P and C1-inh. Control synthesis rates (mol/cell/min) mean: C2 48; B 31; C3, 991; P 855; C1-inh 428. Control untreated cultures (1); rIFN-γ-treated cultures (2); SMLCM-treated cultures (3); SML-treated cultures (4). The results are expressed as the mean of two experiments performed in duplicate.

Table 1. Characteristics of monocyte C2

Anti-IFN-γ	C2 protein* (−/+)		C2 activity† (−/+)		SpFA‡ (−/+)		Stability of C4b2a§ (−/+)	
Control monocytes	2·3	2·2	8·1	8·4	3·52	3·81	9·2	9·4
+ rIFN-γ	4·3	2·6	24·3	10·8	5·65	4·14	13·5	10·5
+ PBLCM	4·1	2·4	34·2	19·9	8·34	7·96	13·4	11·8
+ PBL	3·8	2·5	32·5	24·0	8·55	7·69	13·6	11·5
RA monocytes	2·3	2·2	7·9	7·9	3·43	3·59	9·0	9·0
+ rIFN-γ	3·6	2·9	24·0	11·0	6·67	4·21	13·5	10·0
+ SMLCM	3·6	2·9	23·4	15·6	6·50	5·63	13·0	12·5
+ SML	4·2	2·6	27·2	15·6	6·48	4·90	13·0	12·6
Hep G2 cells	0·33		0·27		0·84		3·5	
Serum C2	20000 (ng/ml)		14000 (e.m. ($\times 10^{-7}$)/ml)		0·71		3·5	
Purified C2	46100 (ng/ml)		35000 (e.m. ($\times 10^{-7}$)/ml)		0·76		3·5	

Characteristics of C2 produced by monocytes from normal individuals (control) in the absence or presence of recombinant γ-interferon (rIFN-γ 10 ng/ml), autologous peripheral blood lymphocyte conditioned medium (PBLCM; 400 μl/well) or autologous peripheral blood lymphocytes transformed with PHA (PBL; 10^4/well) and C2 produced by monocytes from a patient with rheumatoid arthritis (RA) in the absence and presence of rIFN-γ (10 ng/ml), autologous synovial membrane lymphocyte conditioned medium (SMLCM 400 μl/well) or autologous synovial membrane lymphocytes (SML; 10^4/well). (−/+) denotes the presence or absence of antibody to recombinant γ-interferon (anti-IFN-γ; 100 units/ml). Also shown are the characteristics of C2 produced by Hep G2 cells, serum C2 and purified C2. The number of lymphocytes used in this experiment was adjusted to give a similar response to that produced by undiluted conditioned media (PBLCM or SMLCM).

* ng/μg DNA; † e.m. ($\times 10^{-7}$) μg DNA; ‡ e.m. ($\times 10^{-7}$) ng C2; § $T_{\frac{1}{2}}$ min at 30°C.

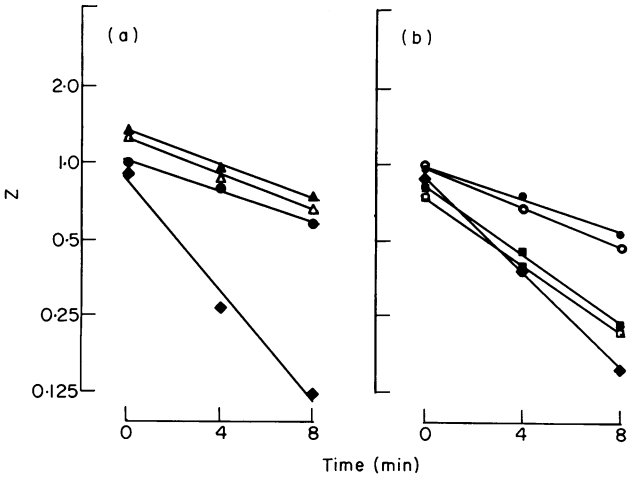

Fig. 5. The half-life at 30°C of C3 convertase (C4b2a) prepared with: (a) C2 purified from serum (◆, $T_{\frac{1}{2}}$ = 2·6 min), purified C2 oxidized using KI/I_2 (●, $T_{\frac{1}{2}}$ = 10·5 min), monocyte C2 (△, $T_{\frac{1}{2}}$ = 8·7 min) and monocyte C2 oxidized using KI/I_2 (▲, $T_{\frac{1}{2}}$ = 10·5 min). (b) C2 purified from serum (Experiment 2, ◆, $T_{\frac{1}{2}}$ = 3·3 min), purified C2 (100 units/ml) incubated for 2 h in control monocyte cultures (○, $T_{\frac{1}{2}}$ = 7·2 min), in control culture medium (□, $T_{\frac{1}{2}}$ = 4·6 min), in rIFN-γ-treated monocyte cultures (●, $T_{\frac{1}{2}}$ = 9·2 min) and rIFN-γ-treated monocyte culture medium (■, $T_{\frac{1}{2}}$ = 5·0 min). Each assay was performed in duplicate.

C4b2a formed with monocyte C2 which had been oxidized with I_2/KI was only slightly increased (10·5 min) compared with untreated monocyte C2 (8·7 min) (Fig. 5). When purified C2 was added to control or rIFN-γ-treated monocyte cultures, incubated at 37°C for 2 h, and subsequently tested by haemolytic assay it was found to form C4b2a with a prolonged half-life (7·2 min and 9·2 min respectively). Formation of C4b2a with C2 which had incubated with monocyte culture supernatant was only slightly prolonged.

DISCUSSION

The stimulation of monocyte C2 production by lymphokine-containing culture supernatants was first documented by Littman & Ruddy (1977) who later showed that the activity was a T lymphocyte product (Littman & Ruddy 1979). As γ-interferon (IFN-γ) accounts for most, if not all, of the macrophage activating content of lymphokine preparations (Nathan *et al.*, 1983) and as γ-interferon has been shown to stimulate C2 synthesis (Strunk *et al.*, 1985; Hamilton *et al.*, 1987) it seemed probable that IFN-γ was the factor in mitogen- or antigen-stimulated lymphocyte supernatants which stimulated monocyte C2 synthesis.

We have shown that T cells were responsible for increased C2 synthesis, that the effects of PBLCM and SMLCM on the synthesis of C2, B, C1-inhibitor, C3 and P were the same as those of rIFN-γ, and that antibody to rIFN-γ abrogated these effects. These findings indicate that IFN-γ is the lymphokine which exclusively modulates synthesis of C2, B, P, C3 and C1-inhibitor. The finding that antigen- or mitogen-stimulated lymphocytes had more potent effects on complement synthesis than the supernatants is probably explained by the continued production of IFN-γ by lymphocytes and/or the greater efficiency of this lymphokine when it is secreted directly onto the monocyte surface. At sites of chronic inflammation, where lymphocytes and macrophages are in intimate contact (Unanue,

1981), it is possible that this paracrine effect of IFN-γ is important. The results of our experiments with rheumatoid arthritis synovial membrane lymphocytes show that within the rheumatoid synovial membrane, and probably at other sites of chronic inflammation, the local production of IFN-γ by activated T lymphocytes appears to play a major role in the modulation of local complement synthesis.

Monocyte C2 behaves differently from serum C2 and C2 produced by the hepatocellular carcinoma cell line, HepG2, by having a higher SpFA (units/ng protein), and forming a C3 convertase (C4b2a), which is more stable ($T_{\frac{1}{2}}$ 9·2 min at 30°C) compared with that formed with C2 purified from serum (3·5 min) or C2 produced by HepG2 cells (3·5 min). Thus, monocyte C2 behaves like oxidized serum C2 (Polley & Müller-Eberhard, 1967). Although it has not been formally proven, it is likely that C2 which has been synthesized by monocytes has been oxidized by the secretory oxygen products of these cells, unlike C2 produced by HepG2 cells. This conclusion is supported by the observation that purified C2 which had been incubated with monocyte cultures formed a more stable C3 convertase than that formed with the untreated C2. Furthermore, since the C3 convertase formed with monocyte C2 which had been oxidized with I_2/KI only showed only a minor prolongation in its half-life, it is likely that monocyte C2 has already been oxidized.

As IFN-γ stimulates the secretion of macrophage oxygen products (Nathan *et al.*, 1983) it seems likely that the increased SpFA of C2 and the stability of C4b2a which occur in the presence of cytokine are due to further oxidation. Although anti-rIFN-γ virtually prevented any increase in synthesis of C2 in cultures to which PBL, SML, PBLCM or SMLCM had been added, the SpFA of C2 in these culture supernatants, and the stability of C4b2a did not return to the values seen in control monocyte cultures. These findings argue that another lymphocyte product is responsible for much of their effects on C2 activity.

The presence of 'oxidized' C2 at sites of inflammation will increase the efficiency of C3 activation by the classical pathway and may influence the outcome of the inflammatory response.

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