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Modulation of mRNA expression and secretion of C1q in mouse macrophages by anti-inflammatory drugs and cAMP: evidence for the partial involvement of a pathway that includes cyclooxygenase, prostaglandin E_2 and adenylate cyclase

P. K. E. TRINDER, D. FAUST, F. PETRY & M. LOOS *Institute of Medical Microbiology and Hygiene, Johannes-Gutenberg University, Hochhaus/Augustusplatz, Mainz, Germany*

SUMMARY

Isolated BALB/c mouse thioglycollate-elicited (inflammatory) peritoneal macrophages release at least 10 times more C1q than do isolated resident peritoneal macrophages. Addition of non-steroidal anti-inflammatory drugs (NSAID) to thioglycollate-elicited macrophages in culture inhibited the release of C1q and reduced levels of C1q-specific mRNA. Contrastingly, the NSAID were found to enhance C1q-specific mRNA levels in resident macrophages, although no increase in C1q levels secreted was observed. This suggests that the response of macrophages to NSAID, with respect to C1q synthesis, reflects the developmental stage of the macrophage. The gold salt auranofin (AFN) was found to enhance markedly C1q synthesis at both transcriptional and secretory levels in thioglycollate-elicited macrophages whilst, conversely, AFN reduced mRNA levels in resident macrophages. This indicates that AFN and the NSAID may work via the same or similar biochemical pathway, but with opposing effects. The glucocorticoid hydrocortisone (HC) greatly enhanced C1q-specific mRNA levels in both thioglycollate-elicited and resident macrophages, although no parallel increases in C1q secreted were observed. The data on inhibition of C1q biosynthesis by NSAID in thioglycollate-elicited macrophages are supported by the enhancement of C1q biosynthesis following addition of prostaglandin E_2 (PGE_2) or dibutyryl cyclic AMP (dBcAMP) to the cultures. From these experiments, it is concluded that C1q biosynthesis is controlled, at least in part, by a pathway involving cAMP.

INTRODUCTION

Inflammatory processes, such as those associated with rheumatoid arthritis, inextricably involve the activation of complement,[1,2] most commonly through the initiation of the classical pathway of complement via C1, the first component of the complement system. C1 is comprised of a calcium-dependent $C1r_2C1s_2$ tetramer and a collagen-like subunit, C1q.[3] The intact C1q molecule is made up of 18 polypeptide chains—six A, six B and six C chains. These interlink to form a hexamer consisting of six globular heads, each of which is attached to a collagen-like arm with the arms meeting to form a central stalk. Activation of C1 is usually via the Fc-binding globular heads of C1q,[4] but can also occur in the intact C1q molecule via binding to the C1q A chain,[5] as is the case with lipopolysaccharide (LPS) and the acute-phase protein C-reactive protein.[6]

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Correspondence: Dr M. Loos, Institute of Microbiology and Hygiene, Johannes-Gutenberg University, Hochhaus/Augustusplatz, 55101 Mainz, Germany.

With the exception of C1q, the majority of complement components are synthesized in the liver.[7] Evidence suggests, however, that as well as being a source of many other complement components, macrophages provide the main site of C1q synthesis.[7,8] The fact that C1q is synthesized by a cell intimately involved in the inflammatory process,[1,9,10] a cell that, following activation, is involved in the ingestion and destruction of both foreign (e.g. bacteria) and altered self particles and cells and in the presentation of antigens to T cells, led us to investigate the synthesis and secretion of this molecule by resident and inflammatory macrophages from BALB/c ANN mice. This is of particular interest as it is via C1q that immune complexes created as part of the humoral immune response activate the classical complement pathway. Work by Minta *et al.*[11] has revealed that several of the major non-steroidal anti-inflammatory drugs (NSAID) inhibit both the classical and alternative complement pathways in human sera, *in vitro*, by direct interaction with one or more complement components. In the study presented here, we have investigated the effects of several anti-inflammatory drugs on the synthesis and secretion of C1q by resident and inflammatory macrophages from BALB/c ANN mice. Furthermore, we have used

the cyclo-oxygenase product prostaglandin E_2 (PGE_2) and dibutyryl cyclic AMP (dBcAMP), a stable and cell permeable analogue of cAMP, to assess further the role of a pathway involving cyclo-oxygenase, PGE_2 and adenylate cyclase in modulation of C1q synthesis and secretion by mouse inflammatory macrophages.

MATERIALS AND METHODS

Reagents

Brewer's thioglycollate broth was obtained from Oxoid Ltd (Wesel, Germany). Cell growth (CG) cell culture medium was from Camon Ltd (Wiesbaden, Germany) and consisted of Iscove's modified Dulbecco's medium supplemented with bovine serum albumin (BSA) 1 mg/ml, human transferrin 32 μg/ml and soya bean lipids 157 μg/ml, and was buffered with HEPES and $NaHCO_3$, pH 7·2. Gentamicin (Gibco/BRL Ltd, Eggenstein, Germany), at a final concentration of 0·5%, was added before use. Türk's solution was purchased from E. Merck (Darmstadt, Germany). Acetylsalicylic acid (ASS), indomethacin (IM), naproxen (NAP) and hydroco-Hisone (HC) were kindly provided by Hoechst AG (Wiesbaden, Germany), and auranofin (AFN) was a kind gift from Smith-Kline Beecham Ltd (Munich, Germany). dBcAMP was purchased from Serva Ltd (Heidelberg, Germany). Phenol was from Appligene Ltd (Heidelberg, Germany), agarose and dextran sulphate were from Pharmacia Ltd (Freiburg, Germany), and (α^{32}P)-dCTP was from Amersham Buchler (Braunschweig, Germany). An enzyme-linked immunosorbent assay (ELISA) kit from ELISA Technologies (Lexington, KY, USA), for quantifying levels of PGE_2, was obtained through Wak-Chemie Medical GmbH (Bad Homburg, Germany). Monospecific polyclonal antibody against mouse C1q was raised by serial intramuscular injection of a Freund's incomplete adjuvant/mouse C1q emulsion. C1q was purified from mouse serum as previously described by Stemmer & Loos,[12] and the purity of the C1q preparation was confirmed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) (data not shown). Monospecificity of the antibody for C1q was confirmed by Western blotting with mouse serum and purified C1q (data not shown). Unless specified, all other reagents were from Sigma Ltd (Munich, Germany). For cell cultures, all reagents were of tissue culture grade, otherwise high purity molecular biology reagents were used.

Mice

Specific pathogen-free inbred BALB/c ANN mice were obtained from the institute's breeding unit (originally from ZFV, Hannover, Germany).

Macrophage culture

Eight to 10-week-old female BALB/c ANN mice were injected intraperitoneally (i.p.) with 1 ml 3% thioglycollate broth. Peritoneal cells were harvested 4 days later, by peritoneal lavage with 5 ml of cold (~4°) CG medium, without anticoagulant. For harvesting of resident macrophages, mice were removed from the institute's animal breeding unit immediately before cell harvesting. The cells were spun down (350 *g*) and erythrocytes lysed by brief addition of sterile water before addition of excess CG medium. After a further spin (350 *g*) cells were resuspended in CG medium at a concentration of $2{\cdot}5 \times 10^5$ macrophages/ml (cell counts were determined by phase-contrast microscopy using a Neubauer haemocytometer after staining cells with Türk's solution), and plated in 2 ml/mm well in 12-well (23 mm diameter) culture plates (Costar, Bodenheim, Germany). After incubation in an humidified atmosphere at 37° with 5% CO_2 for 3 hr, non-adherent cells were removed by washing vigorously three times with CG medium. The resulting monolayer cultures were found to consist of >95% macrophages (as determined by morphology and non-specific esterase staining). Cells were washed once more with CG medium before addition of experimental substances in 2 ml CG medium/well. The number of macrophages removed by the washes was determined, and averaged 20% (1×10^5) of the original culture density for both resident and thioglycollate-elicited inflammatory macrophages, thus the final cell count/well after removal of non-adherent cells was $\sim 4 \times 10^5$ ($\pm\ 2 \times 10^4$).

C1q antigenic assay

Quantities of macrophage C1q secreted into culture supernatants were assessed by sandwich ELISA on microtitre plates (Immunosorb, Nunc Ltd, Wiesbaden, Germany). Monospecific polyclonal goat IgG against mouse C1q was used as the trapping antibody, and the same antibody, biotinylated, was used together with avidin–horseradish peroxidase conjugate and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) substrate as a detection system. Absorbance at 405 nm was measured on an Anthos Labtec microplate (Anthos Labtec Instruments, Salzburg, Austria) reader using Mikrotek software (Microtek Laborsysteme, Overath, Germany). Purified C1q from mouse serum was used as standard.

C1q haemolytic assay

Haemolytic activity of C1q present in culture supernatants was assessed using an assay described by Golan *et al.*,[13] except that in place of human C1q the culture supernatants contained mouse C1q. Briefly, 100 μl of sheep erythrocytes ($1{\cdot}3 \times 10^8$ cells/ml) sensitized with rabbit anti-erythrocyte antibodies and carrying guinea-pig C4 (EAC4), were incubated with 100 μl of cell culture supernatant (dilution series) and 100 μl human C1r/C1 s (10 molecules/cell). After washing, the cells were incubated with 100 μl guinea-pig C2 (50–100 molecules/cell) before addition of 1 ml of EDTA-treated guinea pig-serum (diluted 1 : 50). Cell lysis was determined by comparing absorbance of the supernatant at 412 nm with that of a water-lysed (100% lysis) control (100 μl EAC4 cells + 1200 μl H_2O). Purified mouse serum C1q was used as a standard.

PGE_2 quantification

PGE_2-containing cells and culture supernatants were harvested as described by Mackenzie *et al.*[14] and assayed for PGE_2 by ELISA as described in the manufacturer's instructions (ELISA Technologies). Briefly, following incubation of thioglycollate-elicited inflammatory macrophages with the relevant anti-inflammatory drug [ASS, NAP, IM, AFN or the glucocorticoid hydrocortisone (HC)], culture supernatants were removed and stored at −20° for assay at a later date. The remaining plated cells were extracted with 1 ml/well methanol at −20° overnight, following which the cell debris-free supernatant was vacuum dried (Speedvac; Bachofer GmbH, Reutlingen, Germany) to remove methanol, and reconstituted in phosphate-buffered saline (PBS), pH 7·4 (K = 15 mS; K = conductivity in millisiemens), before assay by ELISA. The PGE_2 ELISA is a

competition ELISA, whereby PGE_2 in the sample competes with an enzyme conjugate for a limited number of binding sites on the antibody-coated plate. Wells precoated with antibody were incubated (1 hr, room temperature) with sample or standard (PGE_2 solution) mixed with diluted enzyme conjugate. After washing with the buffer provided, the bound enzyme conjugate was detected by addition of K-Blue Substrate®. The reaction was stopped by the addition of 1 M HCl, and the absorbance at 450 nm was measured by using a Titertek Multiscan MCC/340 ELISA reader.

cDNA probes

A single-stranded antisense cDNA probe from the mouse C1q A chain was prepared by primer extension of full-length M13 subclones followed by restriction enzyme digestion and separation on alkaline denaturing gels containing 30 mM NaOH and 2 mM EDTA.[8]

Preparation of cDNA probes for hybridization

Labelling of cDNA probes with (α^{32}P)-dCTP was carried out using the Megaprime DNA labelling system (Amersham Buchler, Braunschweig, Germany), according to the protocols provided. Free counts were removed using spun columns[15] and the activity of the ^{32}P-labelled cDNA determined using a β-counter (Hewlett-Packard, Waldbronn, Germany).

RNA preparation and Northern blotting

Each assay used 4×10^6 macrophages (eight wells of a 12-well culture plate). Total cellular RNA was isolated using the single-step acid guanidinium thiocyanate–phenol–chloroform method of Chomczynski & Sacchi.[16] Total RNA yield from resident macrophages was found to be < 20% of that obtained with the same number of inflammatory macrophages (DNA yield was similar for both sets of cells). RNA (10 μg/well) was denatured and separated by electrophoresis in a 1% agarose–formaldehyde gel;[15] even loading of the gel was checked by ethidium bromide staining (Fig. 2). Internal controls were not used as both α-actin- and cyclophilin-specific mRNA were found to be influenced by several of the substances used in these cells (data not shown). After electrophoresis the RNA was blotted by capillary transfer[15] onto Hybond N nylon membranes (Amersham Buchler, Braunschweig, Germany). Membranes were baked at 80° for 2 hr and prehybridized for 4–6 hr at 42° in 50 mM Tris–HCl, pH 7·4, 1 M NaCl, 10 × Denhardt's solution,[15] 1·0 mg/ml tetrasodium pyrophosphate × 10 H_2O, 100 mg/ml dextran sulphate, 1 mg/ml SDS, 100 μg/ml denatured salmon sperm DNA and 50% v/v deionized formamide. Denatured ^{32}P-labelled cDNA (ssDNA) probes were added to the prehybridization buffer (minimum of 4×10^5 counts/ml) and hybridized to the blots overnight at 42°. After washing blots extensively with 2 × SSC/1% SDS at 65°, blots were finally washed with 0·2 × SSC/1% SDS and exposed to X-ray film (XAR-5; Eastman Kodak, Rochester, NY) at −70°, for periods varying from 24 hr to 72 hr depending on the activity of the probe and the amount bound to the blot. Densities of the bands obtained were compared using a Herolab Densitometer with EASY plus (Rev. 2.06) software (Herolab GmbH, Wierloch, Germany).

RESULTS

Cultured peritoneal macrophages obtained from mice injected i.p. with thioglycollate broth (thioglycollate-elicited inflammatory macrophages) and from mice obtained from the institute's breeding unit (specific pathogen-free) immediately before use (resident macrophages), were treated with the NSAID ASS, NAP and IM, the steroidal anti-inflammatory drug HC or the gold salt auranofin AFN. Levels of C1q secreted/released by the cells into the culture medium were determined over a 8-day period, and levels of C1q-specific mRNA were determined at 48 hr. As a rule, inflammatory macrophages (50–60 ng C1q/ml culture medium) were found to secrete over 10 times more C1q than resident (non-inflammatory) macrophages (< 5 ng C1q/ml culture medium). It is worth noting here that resident peritoneal macrophages adhered to culture plastic considerably less than inflammatory macrophages. Resident macrophages were less stretched, more rounded and smaller than inflammatory macrophages in culture, and extreme care was needed in the removal of non-adherent cells (so as to ensure that total cell counts remained similar to those of inflammatory macrophages). Generally, levels of C1q secreted by both resident and inflammatory macrophages, with or without test substance, peaked at day 1 and then remained more or less level for the rest of the experiment. In order to simplify comparison, the data presented here represent the state of events at 48 hr of culture. With the exception of the gold salt AFN (reduced cell viability by day 4 in the presence of 10^{-5} M or 10^{-6} M AFN), none of the treatments had a significant effect on cell viability (data not shown). Furthermore, in additional control experiments, no effect on the haemolytic activity of C1 or C1q could be observed with any of the test substances, at the concentrations used (data not shown).

Resident macrophages

Cells were cultured in the presence of 10^{-5}–10^{-8} M ASS, NAP, IM, HC or AFN in culture medium. The levels of C1q measured in culture supernatants from resident macrophages treated with any one of the above drugs ranged from 2·0 to 3·8 ng/ml at 48 hr (data not shown). Although in some cases a reduction in C1q secretion was observed, and with one AFN concentration a small increase, no statistically significant dose-dependency was observed, since these values were too close to the detection limit of the ELISA system used and therefore the error (±0·8 to ±3·1 ng/ml) was too great for any reliable conclusions to be drawn.

NSAID (ASS, NAP, IM) reduce levels of C1q secreted by inflammatory macrophages

The effect of ASS was to suppress levels of C1q secreted at all doses of ASS tested (10^{-5}–10^{-8} M). This suppression was found to be dose-dependent (Fig. 1), reductions being apparent within 24 hr and remaining constant throughout the 8-day culture period. NAP and IM also suppressed the levels of C1q secreted, and the doses tested (10^{-5}–10^{-8} M) were found to be inhibitory in a dose-dependent manner (Fig. 1).

HC, predominantly induces a decrease in levels of C1q released by inflammatory macrophages

Inflammatory macrophages cultured in the presence of 10^{-5}–10^{-7} M HC were found to release slightly less C1q into the

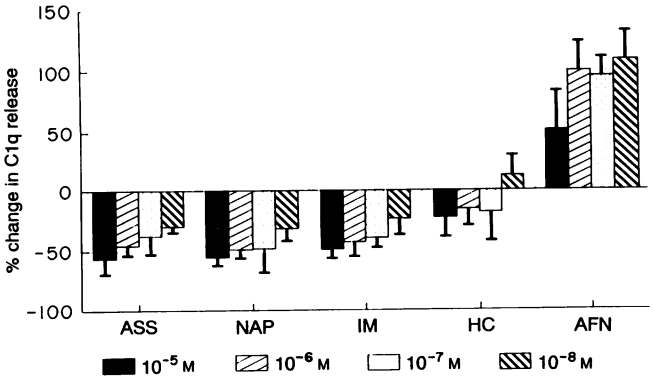

Figure 1. Effect of anti-inflammatory drugs on the secretion of C1q by BALB/c mouse thioglycollate-elicited peritoneal macrophages. C1q released is expressed as percentage change over controls (0) at 48 hr. Experimental details are as described in the Materials and Methods.

culture medium than controls within the first 48 hr of incubation (Fig. 1). In comparison, 10^{-8} M HC brought about an increase in levels of C1q present in the culture supernatant. At all HC doses used, the variation in values of C1q-secretion obtained was found to be relatively large.

The gold salt AFN dramatically increases levels of C1q released by inflammatory macrophages

Inflammatory macrophages cultured in the presence of the gold salt AFN, exhibited large increases in levels of C1q released into the culture medium (Fig. 1). It should be noted here that by day 4 cell viability at higher concentrations (10^{-5} and 10^{-6} M) was severely reduced (data not shown).

Levels of PGE_2 produced by inflammatory macrophages following incubation with the various test substances correlate with observed changes in C1q secretion

Each of the five anti-inflammatory drugs was tested for its ability to reduce levels of PGE_2 produced by the cells (Fig. 2). PGE_2 concentrations were found to correlate well with the changes observed in C1q secretion: ASS, NAP and IM all brought about significant reductions in PGE_2 levels, whilst HC had little, if any, effect and AFN significantly increased PGE_2 levels.

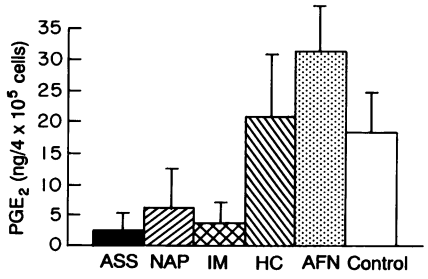

Figure 2. Effect of anti-inflammatory drugs on production of PGE_2 by thioglycollate-elicited peritoneal macrophages. PGE_2 production is expressed as ng/4 × 10^5 cells present at 48 hr, and is the product of PGE_2 released into the culture supernatant and that present intracellularly (extracted overnight with methanol, see the Materials and Methods).

C1q-specific mRNA levels are altered by incubation of resident macrophages with the various test substances

The Northern blot (autoradiograph) is shown in Fig. 3a. Concentrations of test substances were: ASS, NAP, IM and HC 10^{-5} M, AFN 10^{-7} M. Densitometer measurement of the bands revealed that C1q-specific mRNA produced by resident macrophages incubated with any of the three of the NSAIDs increased C1q-specific mRNA levels (Table 1). The steroid HC also greatly increased levels of C1q-specific mRNA. Only the addition of the gold salt AFN brought about a reduction in C1q-specific mRNA levels.

C1q-specific mRNA levels present in inflammatory macrophages following incubation with the various test substances only partially correlate with changes in levels of secreted C1q

The Northern blot is represented in Fig. 3b. Of the three NSAID, ASS produced the biggest reduction in C1q-specific mRNA levels compared to controls (Table 1). NAP appeared to have no effect on levels of C1q-specific mRNA, whilst IM reduced mRNA levels. The steroid HC brought about a large increase in C1q-specific mRNA levels, as did the gold salt AFN.

dBcAMP and PGE_2 both increase C1q secretion by inflammatory macrophages

To characterize further the effects seen when inflammatory (thioglycollate-elicited) macrophages were treated with the NSAID ASS, NAP and IM, inflammatory macrophages were treated in culture with different concentrations of dBcAMP, an analogue of cAMP exhibiting increased stability and good membrane permeability, or PGE_2, a cyclo-oxygenase product that activates adenylate cyclase.

The effect of dBcAMP on cultured inflammatory macrophages was to produce a dose-dependent increase in the levels of C1q protein released into the culture supernatant (Fig. 4a). The haemolytic activity of the culture supernatants (expressed as the number of effective molecules C1q/ml) was found to agree well with the observed increase in C1q protein present in the culture supernatant (Fig. 4b).

Addition of PGE_2 also led to a dose-dependent increase in the release of C1q from the cells (Fig. 5a) compared to controls. Furthermore, when inflammatory macrophages were incubated for 24 hr with 10^{-5} M IM and at 24 hr 10^{-6} M PGE_2 was added, the inhibitory effect of IM alone could be overcome (Fig. 5a). Again, these changes in levels of C1q protein secreted were parallelled by corresponding changes in haemolytic activity of the culture supernatants (Fig. 5b). In a further experiment, cells were incubated in the presence of 10^{-5} M dBcAMP and 10^{-6} M PGE_2 together, resulting in an increase in C1q release from the cells that was less than additive (Fig. 6). Cells were also incubated in the presence of dBcAMP and IM together, but this combination, at the doses applied, was found to be lethal for the cells, with cell

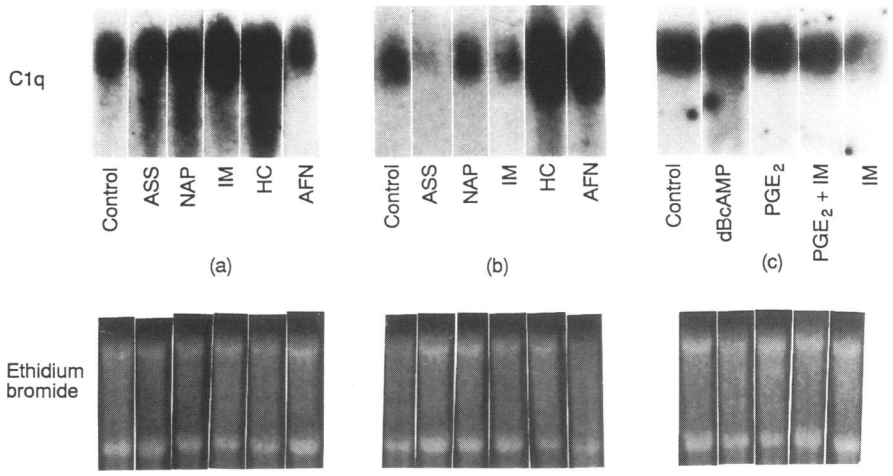

Figure 3. Effect of anti-inflammatory drugs, dBcAMP and PGE_2 on expression of C1q-specific mRNA, as determined by Northern blotting. Experimental details are as described in the Materials and Methods. (a) Resident BALB/c peritoneal macrophages; (b) and (c) Thioglycollate-elicited peritoneal macrophages.

viability being reduced by >90% within 24 hr (data not shown).

Changes in C1q-specific mRNA levels following incubation with dBcAMP or PGE_2 correlate with changes in C1q secretion by inflammatory macrophages

The Northern blot is presented in Fig. 3c. Within 48 hr, cells treated with dBcAMP and PGE_2 had increased levels of C1q-specific mRNA (Table 1). In comparison, IM reduced C1q-specific mRNA levels compared to controls. Interestingly, addition of PGE_2 at 24 hr to cultures treated for 24 hr previously with IM resulted in levels of C1q-specific mRNA comparable to those of untreated controls by 48 hr.

Table 1. Effect of anti-inflammatory drugs, dBcAMP and PGE_2 on the expression of C1q-specific mRNA in BALB/c mouse peritoneal macrophages*

	Change over controls (1·0)	
Substance	Resident macrophages	Thioglycollate-elicited macrophages
ASS 10^{-5} M	1·5†	0·1
NAP 10^{-5} M	2·0	1·0
IM 10^{-5} M	2·7	0·6
dBcAMP 10^{-4} M	ND	1·4
PGE_2 10^{-6} M	ND	1·25
IM 10^{-5} M	ND	0·6
IM + PGE_2	ND	0·95
HC 10^{-5} M	3·7	6·1
AFN 10^{-8} M	0·7	4·3

* Experimental details are as described in the Materials and Methods.

† Autoradiographs were analysed by densitometry. Values represent the *x*-fold changes in mRNA levels compared to those of untreated control cells, which were assigned a value of 1·0.

ND, not done.

DISCUSSION

Various workers have shown that the level of complement synthesis is dependent upon the state of activation of the macrophage and its tissue localization (summarized in ref. 17). However, apart from the finding that human monocytes secrete no C1q and monocyte-derived macrophages do,[18] little has been published with respect to the control mechanisms involved in C1q biosynthesis. Our observation that mouse resident macrophages secrete less than one-tenth as much C1q as do mouse inflammatory (thioglycollate-elicited) macrophages correlates well with the previously observed levels of C1q in monocytes and monocyte-derived macrophages.[18] Inhibitors of collagen biosynthesis, such as 2,2′-dipyridyl and 3,4-dehydro-

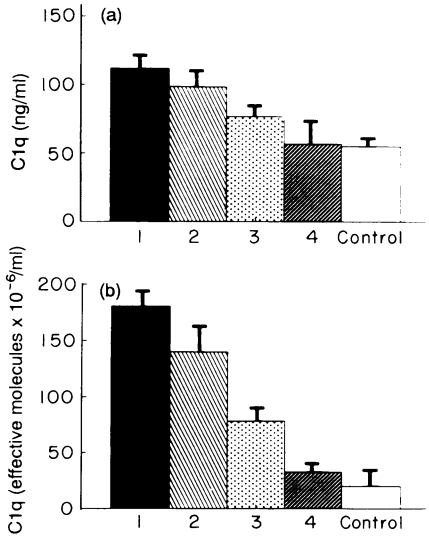

Figure 4. Enhanced C1q secretion in thioglycollate-elicited peritoneal macrophages cultured in the presence of dBcAMP. Experimental details are as described in the Materials and Methods. (a) C1q protein (ng/ml) released at 48 hr, as determined by ELISA. (b) C1q haemolytic activity released at 48 hr (effective molecules C1q × 10^{-6}/ml). 1, 10^{-4} M; 2, 10^{-5} M; 3, 10^{-6} M; 4, 10^{-7} M.

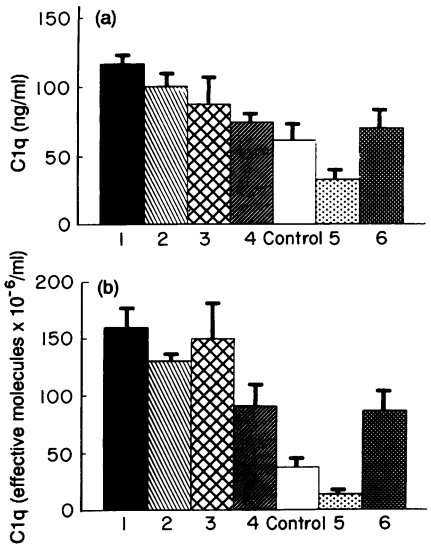

Figure 5. Enhanced C1q secretion in thioglycollate-elicited peritoneal macrophages cultured in the presence of PGE_2. Experimental details are as described in the Materials and Methods. (a) C1q protein (ng/ml) released at 48 hr, as determined by ELISA. (b) C1q haemolytic activity released at 48 hr (effective molecules C1q $\times 10^{-6}$/ml). 1, 10^{-6}M; 2, 10^{-7}M; 3, 10^{-8}M; 4, 10^{-9}M; 5, 10^{-5}M IM; 6, 10^{-5}M IM + 10^{-6}M PGE_2.

DL-proline, have been used to study post-transcriptional modification of C1q. Studies by Müller *et al.*[19] have shown inhibition by 2,2'dipyridyl, a blocker of prolyl and lysyl hydrolases and an iron chelator, of liberation of haemolytically active C1q from guinea-pig macrophages to be reversible, indicating that post-translational hydroxylation is necessary before C1q can be secreted by the cell. Similarly, incorporation of 3,4-dehydro-DL-proline, a proline anologue that cannot be hydroxylated, was found to block release of C1q from guinea-pig macrophages.[20]

All three of the NSAID were found to be inhibitory with respect to C1q secretion by inflammatory macrophages whereby, although the drug NAP was found to have little effect on C1q-specific mRNA levels, both ASS and IM produced significant reductions in C1q-specific transcription, suggestive of pretranslational inhibition of C1q synthesis. Interestingly, however, in resident macrophages, C1q-specific mRNA levels were found to be increased following incubation

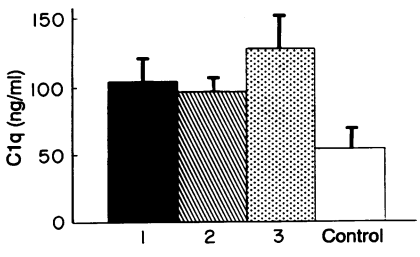

Figure 6. Effect of simultaneous addition of dBcAMP and PGE_2 to thioglycollate-elicited peritoneal macrophages in culture on C1q secretion (C1q protein in ng/ml) released at 48 hr. 1 10^{-5}M dBcAMP; 2, 10^{-7}M PGE_2; 3, 10^{-5}M dBcAMP + 10^{-7}M PGE_2.

with any one of the three NSAID, despite there being a small but non-significant reduction in levels of C1q secreted. Again, this suggests that the stage of development of the macrophage greatly influences its response to NSAID treatment, at least in respect to C1q synthesis. In a manner converse to the NSAID, the gold salt AFN was found to increase greatly the synthesis of C1q, both at the transcriptional level and with regard to secretion, in inflammatory macrophages. In resident macrophages C1q-specific mRNA levels were found to be reduced following treatment with AFN. This again is a reversal of what was observed with the NSAID, suggesting that AFN and the NSAID may work via the same or a similar biochemical pathway, but with opposing effects. Little is known about the mode of action of AFN and other gold salts, other than that they are supposed to inhibit macrophage function and thereby selectively suppress inflammatory responses.[21] Gold salts have been used with varying success in the long-term treatment of chronic inflammatory processes.

The glucocorticoid HC was found to enhance greatly C1q-specific mRNA levels in both inflammatory and resident macrophages, although these increases were not paralleled by corresponding increases in levels of C1q secreted. HC has been reported to act, with respect to inflammatory processes, by inducing release of a phospholipase A_2 (PLA_2) inhibitory glycoprotein, termed lipomodulin.[22] This inhibition of PLA_2 has a negative effect on the release of arachidonic acid, thereby slowing production of arachidonic acid metabolites such as leukotrienes (lipo-oxygenase pathway) and prostaglandins (cyclo-oxygenase pathway). This is interesting as the NSAID also affect prostaglandin synthesis, but rather than interacting with PLA_2 they inhibit cyclo-oxygenase activity directly. Furthermore, there is evidence that NSAID also inhibit cAMP-dependent protein kinase activity.[23] The partial involvement of a prostaglandin-dependent (cyclo-oxygenase) pathway in the synthesis of C1q is substantiated by the fact that exogenous PGE_2 has a positive effect on both transcription of C1q-specific mRNA and on the post-translational levels of C1q secreted by inflammatory macrophages, whereas the NSAID IM reduced these parameters (and also reduced intracellular levels of PGE_2, which could then be restored to control levels by addition of exogenous PGE_2). Furthermore, the fact that dBcAMP was also able to increase both C1q-specific mRNA levels and C1q secretion by inflammatory macrophages supports the involvement of a cAMP-dependent pathway in C1q biosynthesis. This pathway can be only partially inhibited by IM, as even at high doses of IM both transcription of C1q-specific mRNA and secretion of C1q were reduced by only 50%. This is presumably due to the presence of other pathways involving adenylate cyclase and cAMP.

With regard to the role of cAMP-dependent pathways in C synthesis by mononuclear phagocytes, virtually all studies to date have involved human monocytes, and in this context C1q has not been studied. Lappin & Whaley[24–26] were able to show inhibition of C2, C3, C4, C5, factor B, properdin, factor H and C3b inactivator synthesis by mechanisms involving cAMP accumulation, whilst synthesis of these C components was enhanced by direct inhibition of adenylate cyclase using α_1-adrenergic receptor agonists, or by inhibition of cyclo-oxygenase, thereby reducing adenylate cyclase activation by E-type prostaglandins.

In conclusion, it is possible to up-regulate the synthesis of

haemolytically active C1q by mouse peritoneal macrophages, at a pretranslational level, by activation of a cyclic AMP-dependent pathway. Partial inhibition of C1q synthesis can be achieved by abrogation of cyclo-oxygenase activity using NSAID. The inhibition of C1q biosynthesis by NSAID correlates with their established anti-inflammatory activity, with less C1q being available for binding to immune complexes, and a reduction in activation of the classical complement pathway.

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