MOLECULAR MECHANISM FOR FEEDBACK REGULATION OF C4 BIOSYNTHESIS IN GUINEA PIG PERITONEAL MACROPHAGE

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The fourth component of complement is a three chain glycoprotein of 200,000 mol wt that functions in the classic complement pathway (1). The gene for C4, located within the major histocompatibility complex (MHC) (2–5), gives rise to a mature mRNA of ~5 kilobases (kb) (6) which directs synthesis of ProC4, a single chain precursor of the native protein (7). C4 is synthesized in the liver and in cells of the monocyte/macrophage series (8). Previous studies (9) established that the fraction of guinea pig peritoneal macrophages secreting hemolytically active C4 was inversely proportional to the concentration of fluid phase C4. This effect correlated with the binding of native C4 to the macrophage cell surface, and surface C4 binding had no effect on the proportion of macrophages secreting C2, another MHC-linked complement protein. Those studies demonstrated the inhibitory effect of fluid phase C4 by monitoring functional C4 secretion by single cells and they did not delineate whether the inhibitory effect is on synthesis, postsynthetic processing, secretion, or catabolism; i.e., they did not define the mechanism of feedback inhibition of C4 secretion. The availability of a C4 cDNA probe and methods for immunochemical detection of ProC4 synthesis makes it possible to address this question.

In this report we provide evidence that fluid phase extracellular C4 specifically inhibits its own production by affecting the transcription or posttranscriptional modification of C4 mRNA, leading to a net decrease of C4 mRNA without a change in total cellular RNA or RNA specific for factor B. No additional effect on translation, postsynthetic processing, secretion, or catabolism was demonstrated.

Materials and Methods

Media. Dulbecco's modified Eagle's medium with 4.5 g/l glucose, Dulbecco's modified Eagle's medium lacking methionine, medium 199 (M199), fetal bovine serum (FBS),

This work was supported by grants HL32361, HD17461, and AI21167 from the U. S. Public Health Service. H. Auerbach is the J. W. Kay Fellow in Cystic Fibrosis.

1 Abbreviations used in this paper: BSA, bovine serum albumin; dHIFBS, dialyzed, heat-inactivated FBS; DOC, sodium deoxycholate; eff mol, effective molecule; FBS, fetal bovine serum; M199, medium 199; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
glutamine, penicillin (10,000 U/ml), streptomycin (10,000 µg/ml), Hanks' balanced salt solution (HBSS), and 10× HBSS without calcium, magnesium, and bicarbonate were purchased from Microbiological Associates, Walkersville, MD or Gibco Laboratories, Grand Island, NY. FBS was heat inactivated at 56°C for 2 h and was dialyzed against distilled water followed by normal saline (dHIFBS) for use in experiments in which [³⁵S]methionine was used.

Washed elicited (PE) and resident macrophage cell suspensions were adjusted to concentrations of 0.5–4 × 10⁶ cells/ml and adherent monolayer cultures were prepared as previously described (10). To prepare conditioned media, monolayers (2 × 10⁷ cells per dish) were maintained in 5 ml of supplemented M199 for 24 h. The media were then removed and cleared of debris by centrifugation for 15 min at 600 g. These conditioned media (C4S) were assayed for C4 content and stored at −70°C until use. Conditioned media lacking C4 (C4D) were prepared exactly as described for C4S media with macrophage monolayers from guinea pig genetically deficient in C4 (11). In some experiments, C4 hemolytic activity was ablated in the conditioned media by adding methylene (final concentration 1 M) at 37°C for 2 h followed by extensive dialysis against HBSS and then M199.

Hemolytic Assay of C2 and C4. Portions of macrophage media were removed from the culture dishes at timed intervals and frozen immediately at −90°C until assayed for functional C2 and C4. The source and preparation of reagents for the hemolytic assays of C2 and C4 as well as their specificity and sensitivity have been described (12). Estimates of complement production were calculated by dividing the number of effective molecules per milliliter of medium by the concentration of DNA in the cell lysate.

Fluorometric Assay of DNA. The number of macrophages in each culture dish was determined using a previously described modification of the method of Kissane and Robins (13). One microgram of DNA is equivalent to 1.0–1.5 × 10⁶ cells. Radiolabeling, immunoprecipitation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were performed as previously described (14). Briefly, macrophage monolayers were pulsed for a suitable interval with 10% dHIFBS containing 250 µCi/ml [³⁵S]-methionine (~1,000 µCi/mmol; New England Nuclear, Boston, MA). The cells were lysed by freeze-thawing with a solution of phosphate-buffered saline (PBS) (pH 7.5) containing 100 mM KCl, 1% Triton X-100 (Sigma Chemical Co., St. Louis, MO), 0.5% sodium deoxycholate (DOC) (Sigma Chemical Co.), 2 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma Chemical Co.), and 10 mM EDTA (lysis buffer). The cells and lysates were centrifuged at 100,000 g for 2 h at 4°C, then stored at −90°C. Extracellular media and cell lysates were thawed and centrifuged at 10,000 g for 5 min at 4°C just before analysis. Total radiolabeled proteins were assessed by trichloroacetic acid precipitation of 1 µl of lysates and media (15). Each sample was then diluted to yield a final concentration of 0.5% Triton X-100, 0.5% SDS, 0.25% DOC, and 5 mM EDTA in PBS (pH 7.4; ionic strength, 0.15) to which a previously determined amount of antiserum was added, and the mixtures were incubated overnight at 4°C. Fixed Staphylococcus aureus (IgSorb; Enzyme Center, Boston, MA), sonicated and prewashed three times with a solution of 1% Triton X-100, 1% SDS, 0.5% DOC, and 5 mg/ml bovine serum albumin (BSA) in PBS (pH 7.4) was added to each sample and incubated for 1 h at 4°C. The pellets (10,000 g for 1 min) were washed six times with 1 ml of detergent solution lacking BSA. Precipitates were dissolved in 15 µl of sample buffer (0.05 M Tris, pH 6.8, 1% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.01% bromphenol blue), centrifuged (10,000 g for 2 min), and subjected to SDS-PAGE (16). Gels were fixed, dried, and exposed to x-ray film (Kodak XAR-5) at −70°C.

Antisera. Antisera to guinea pig C2 and C4 were raised in homozygous C2- or C4-deficient animals by previously described methods (17). An IgG fraction of goat anti-human factor B that cross-reacts with guinea pig factor B was purchased from Atlantic Antibodies, Scarborough, ME. Rabbit anti-guinea pig C4 was prepared as previously
described (9). Specificity of antisera were tested by the inhibition of complement-mediated lysis, by Ouchterlony analysis, and immunoelectrophoresis.

Isolation of RNA. Adherent monolayers in 100-mm petri dishes were washed three times with HBSS and then lysed with 3 ml of 4 M guanidinium thiocyanate (Fluka A. G., Buchs, Switzerland), 25 mM sodium citrate, and 0.5% sodium-N-laurylsarcosinate (ICN K&K Laboratories Inc., Plainview, NY). The cells were freed from the dish with a rubber policeman and the cell lysate was drawn through needles of decreasing size: 18, 21, and 23 gauge. The guanidinium lysate was layered onto a 2-ml 5.7 M cesium chloride (Bethesda Research Laboratories, Gaithersburg, MD) cushion and centrifuged in a Beckman SW50.1 rotor (Beckman Instruments, Inc., Fullerton, CA) for 17 h at 55,000 rpm at 20°C. The RNA pellet was washed twice with cold 70% ethanol, then dissolved in 200 µl sterile double-distilled water, reprecipitated with ethanol, and dissolved in water. RNA content was determined at 260 nm. Yields were in the range of ~5–10 µg RNA/10⁸ cells.

cDNA Clones. The C4 clone, pC4AL1, encompasses the α-γ linking peptide, the γ chain, the 3′ untranslated region, and a 16 nucleotide poly-A tail. Factor B cDNA clone, pBfA10, isolated (18) from the same human adult liver library as the C4 clone, is 1.6 kb and spans the entire Bb fragment plus more than half of Ba. It includes the complete 3′ untranslated region and the poly-A tail. Both probes cross-hybridize with guinea pig RNA.

Dot Hybridization. The method used was a modification of a previously described technique (19). 50 µl of RNA in distilled water was diluted in 30 µl of 1.5 M NaCl, 1.5 M sodium citrate, pH 7.0, and 20 µl 57% formaldehyde, heated to 60°C for 15 min, and then applied to nitrocellulose paper in 4-mm dots under suction on a minifold apparatus (Schleicher & Schuell, Inc., Keene, NH). The nitrocellulose paper was baked for 90 min at 80°C under vacuum. Prehybridization, hybridization with a ^32P-labeled cDNA probe, and autoradiograph analysis were carried out as described for Northern blot analysis (see below). C4-specific RNA could be detected in a sample containing <5 µg total RNA from guinea pig macrophages.

Detection of Specific mRNA by Northern Blot Analysis. 10–50 µg total RNA was dissolved into 20 µl solution of 50% deionized formamide, 0.0625% formaldehyde, 20 mM 3(N-morpholino)propanesulfonic acid (MOPS) (Sigma Chemical Co.), 5 mM sodium acetate, and 1 mM EDTA, and were heated for 5 min at 60°C. Gels for separation of RNA were poured from 1% agarose containing 2.2 M formaldehyde (20). RNA was visualized under ultraviolet illumination, then transferred to nitrocellulose paper (21) and baked at 80°C for 90 min at reduced pressure.

Prehybridization and Hybridization. Prehybridization and hybridization solutions were 75 mM NaCl, 75 mM sodium citrate, 5× Denhardt’s solution (22), 25 mM phosphate, 50% deionized formamide (vol/vol), 0.5 mg/ml herring sperm DNA, and 0.1% SDS. To the hybridization solution was added 10% dextran sulfate. All procedures were conducted at 42°C. The cDNA probes were ^32P-labeled by nick translation and free ^32P cleared with spin dialysis. The probes were boiled for 5 min and incubated with the nitrocellulose paper at 42°C overnight. The nitrocellulose paper was then washed in 0.15 M NaCl, 0.15 M sodium citrate, pH 7.0 (1× SSC) with 0.1% SDS at 50°C, dried, and an autoradiograph performed.

Purification of Guinea Pig C4. Functionally active guinea pig C4 was isolated from plasma using a modification of the method of Gigli (24). Guinea pigs were bled into benzamidine and EDTA (final concentration, 10 mM) and then PMSF (2 mM) was added to the plasma. EDTA (final concentration, 20 mM) was added and the pH was adjusted to 8.6. The plasma was applied to DEAE Sephadex. The starting material contained 90 mg/ml protein and the C4 titer was 1.02 × 10⁶. Starting buffer was 0.01 M Tris HCl, 0.1 M NaCl, 0.01 M benzamidine, 0.02 M EDTA, 0.02% NaNO₃, and 0.001 M PMSF, pH 8.6, and elicted with a linear gradient from 0.1 to 0.5 M NaCl. C4-active fractions were pooled and concentrated, dialyzed against 0.02 M phosphate, 1 M NaCl, 10 mM benzamidine, 10 mM EDTA, 0.02% NaNO₃, 0.001 M PMSF, pH 7.0 and applied to Sephadex
Results

Effect of Extracellular C4 on Production of Functional C4

Effect of cell density. Macrophage monolayers were established in 15-mm Linbro wells (Linbro Chemical Co., Hamden, CT) at cell densities ranging from 0.5 to $4.0 \times 10^6$ and aliquots of medium were assayed for C2 and C4 activity at timed intervals up to 30 h. Measurement of DNA at each time demonstrated no significant decrease during the experimental period. The amount of functionally active C4 per microgram DNA detected in culture media was inversely proportional to cell number (Fig. 1). Cell density had no effect on net functional C2 produced per cell ($20 \pm 5$ effective molecules/µg DNA). An effect of total cell number, cell density, or direct cell-cell interaction was ruled out by plating varying ratios of C4-deficient and C4-sufficient cells to yield cultures each with the same number of total cells ($2 \times 10^6$). The amount of active C4 was solely a function of the number of C4-producing cells; i.e., the presence of C4-deficient cells in the cultures had no effect on net C4 secretion (Fig. 2).

Conditioned media purified C4. To determine if the inhibition of C4 secretion was due to substrate limitation or a nonspecific product, conditioned media were...
obtained from C4-deficient (C4D) and conventional (C4S) guinea pigs. C4S conditioned medium contained $7.5 \times 10^{10}$ effective molecules/ml; none were detected in medium from C4D cultures. C4S media, when incubated with C4-deficient macrophage monolayers or at 37°C in the absence of cells for 8 h, lost <5% of its hemolytic activity. Newly established C4S peritoneal macrophage monolayers were fed with these conditioned media or supplemented fresh M199 and sampled for C2 and C4 content and DNA. Total protein secretion, C2 secretion, and cell survival in culture were unaffected by exposure to methylamine-inactivated C4S medium. Net secretion of hemolytically active C4 was inhibited 50% in cells fed with C4S conditioned active (nine separate experiments) or inactivated conditioned media. Purified C4 (70% native C4 and 30% C4b; specific activity, $1.2 \times 10^{11}$ effective molecules [eff mol]/μg protein) was inactivated with methylamine. In a preliminary experiment, 1.5 μg ($7 \times 10^{10}$ eff mol/ml) inhibited C4 production by 40%. Net secretion of functional C4 was a direct function of input of methylamine-inactivated purified C4 (Fig. 3). Active C2 measured in the same medium appeared to be less as well, but further experiments demonstrated that although methylamine-inactivated C4 had no effect on the C4 hemolytic assay, it inhibited the C2 assay by 35%. In addition, precipitation of C4 from medium containing methylamine-inactivated C4 with rabbit anti-C4 and staph A (IgSorb) led to an apparent increase in functional C2 from $1.8 \times 10^8$ to $5.24 \times 10^8$ eff mol C2/ml. Medium from cells cultured in the absence of exogenous C4 contained $5.4 \times 10^8$ eff mol C2/ml.

**Effect of Extracellular C4 on Biosynthesis of ProC4 and Secretion of C4**

*Kinetics of inhibition and release.* Macrophage monolayers were incubated in either fresh, C4D, or C4S medium for 6 h in the presence of 250 μCi [35S]-methionine per milliliter. Monolayers were lysed with a 1% Triton mixture at timed intervals and examined for the presence of radiolabeled ProC4 and C2 by immunoprecipitation and SDS-PAGE. Lysates from cells incubated in C4S me-
FIGURE 3. Effect of methylamine-inactivated purified C4 on secretion of functional C4 by guinea pig macrophages. After 8 h of incubation, the highest concentration of methylamine-treated C4 reduced C4 hemolytic activity by ~75%; C2 hemolytic activity was reduced by 30%. Unlike C4, C2 inhibition was demonstrated to be an artifact of the detection system in mixing experiments that showed a decrease in C2 activity in the presence of methylamine-treated C4. C2 titers returned to baseline (corrected C2) when methylamine-inactivated C4 was removed from the medium before assaying for C2 activity.

FIGURE 4. Reversibility of C4 suppression: SDS-PAGE (7.5%) of intracellular ProC4 immunoprecipitated from lysates of macrophages incubated in C4 conditioned medium containing 250 μCi [35S]methionine per milliliter (suppressed). Cells were washed extensively, then (B) refed with either C4 conditioned (suppression) or fresh (release) media, both containing [35S]methionine. The recovery of an identical amount of ProC4 from a duplicate lysate of the (B) suppression 4-h time point using twice the amount of antibody (2X) indicates that the continued suppression of ProC4 biosynthesis by fluid phase C4 is not a result of the blocking of antibody by the presence of unlabeled C4.

dium demonstrated the most intense band at 2–4 h and no detectable radiolabeled ProC4 by 6 h (Fig. 4), while increasing amounts of ProC4 were detected in controls throughout the 6 h. After 6 h, the cells were washed and then refed with either C4D or C4S media containing [35S]methionine. C4 biosynthesis resumed within 1–2 h after the removal of C4, whereas continued suppression of C4 biosynthesis was observed in the presence of medium containing exogenous C4. In some experiments, C4 was precipitated from lysates with twice the amount
of antibody. Lane 2x of Fig. 4 demonstrates no increase in recoverable radiolabeled ProC4 using twice the antibody amounts. Autoradiographs from nine experiments were subjected to densitometric quantitation of the amount of inhibition at 6 h. Mean inhibition was 84% ($P < 0.005$). Fig. 5 shows the lack of inhibition of radiolabeled C2 biosynthesis from macrophages incubated in C4D, fresh, or C4S medium.

Secreted $[^{35}S]$methionine-labeled C4 was decreased by 85% (analyzed by densitometry) at 6 h in extracellular medium from cells maintained in the presence of 1.5 $\mu$/ml purified C4 and cells incubated in fresh medium, while cells incubated in C4S secreted 70% less C4 than controls. Trichloroacetic acid-precipitable, $^{35}S$-labeled protein and radiolabeled C2 from cells incubated in fresh, C4S-, or exogenous C4-containing medium were all similar, as were DNA from duplicate monolayers.

Serial immunoprecipitation of C4 was then performed to exclude the possibility that the exogenous preformed unlabeled C4 antigen blocked detection of radiolabeled C4. No further labeled C4 was immunoprecipitated (Fig. 6). Finally, pulse chase experiments ruled out a block in secretion of C4 by cells incubated in C4S conditioned medium (data not shown).

**Effect of Extracellular C4 on C4-specific, B-specific, and Total Cellular RNA**

To test whether the effect of extracellular C4 on C4 biosynthesis was exerted at a translational or pretranslational level, monolayers incubated in 100-mm tissue culture dishes with either C4S or fresh medium were lysed at timed intervals with 3 ml guanidinium thiocyanate per dish and RNA was isolated. The result of a dot hybridization assay is shown in Fig. 7. The kinetics of disappearance of the specific C4 mRNA parallel the kinetics of inhibition of biosynthesis of ProC4. Factor B–specific RNA was unchanged even in cells incubated with medium containing C4 (Fig. 7). Finally, when monolayers incubated in either C4S or fresh media for 6 h were then washed and refed with fresh media and RNA was isolated at 0, 2, and 4 h, dot hybridization showed the reappearance of specific C4 mRNA within 2 h (Fig. 7B). RNA isolated from partially suppressed macrophage populations (4 h incubation in C4S) was similar in size to controls.

![Figure 5. 9% SDS-PAGE of C2 immunoprecipitated from media of macrophages incubated in fresh, C4D, or C4S media.](image-url)
Figure 6. SDS-PAGE (7.5%) of [35S]methionine-labeled C4 secreted from macrophages incubated in the presence of either purified C4 (A), C4S conditioned medium (B), or fresh medium (C) for 6 h. Densitometry scanning shows that lane A is 15% of control (C), and B is 70% of control. Lanes D, E, and F are sequential immunoprecipitations with antiserum to C4 of A, B, and C, respectively, showing that all labeled C4 was initially immunoprecipitated.

Figure 7. Dot hybridization using human C4 and factor B cDNA probes: Equal amounts of total RNA isolated from macrophages incubated in either C4-containing or fresh media were placed on 4-mm nitrocellulose spots and RNA specific for C4 and factor B was detected by complementary DNA C4 and factor B probes. (A) Shows the disappearance of C4-specific RNA by 4 h from macrophages incubated in C4 conditioned media (suppressed) as compared with cells incubated in control (unsuppressed) media, while factor B RNA remains detectable throughout 4 h in either media. (B) Demonstrates the reappearance of C4-specific RNA within 2 h after macrophages were washed extensively and placed in fresh medium.

Discussion

This report describes the biochemical mechanism that accounts for a regulatory role of fluid phase C4 in its own production by guinea pig peritoneal macro-

when analyzed on Northern blots (Fig. 8). These data suggest that suppression of C4 biosynthesis is a pretranslational event.
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FIGURE 8. Northern blot analysis of equivalent amounts of RNA isolated from guinea pig macrophages at time 0 (A) or incubated for 4 h in either control (B) or C4S conditioned (C) media. Lanes D, E, and F correspond to lanes A, B, and C visualized under ultraviolet light with ethidium bromide staining.

phages. Such direct regulation of C4 production by macrophages would have important consequences in the evolution of the inflammatory response by controlling the constitutive secretion of C4 in resting macrophages as well as by providing a basis for the repletion of C4 after complement consumption or the removal of C4 from a site of inflammation. This direct control would not depend on nor necessarily reflect plasma concentrations of C4 but rather the tissue concentration of C4. Examples of local control of complement synthesis without change in serum levels have been reported. For instance, Pennington et al. (24) reported specific inhibition of C2 and C4 biosynthesis by guinea pig bronchoalveolar macrophages after intraperitoneal instillation of cortisone acetate or cyclophosphamide, whereas serum levels for these proteins were normal. Chronic Pseudomonas aeruginosa pneumonia induced by intratracheal inhalation of an antigen-oil bead suspension in the guinea pig leads to a specific increase in C2 and C4 secretion by isolated bronchoalveolar macrophages without the recruitment of increasing proportions of complement-secreting cells or the alteration of serum complement levels (25).

Direct feedback control of protein biosynthesis has been reported for another protein (26). The amino terminal extension peptides of the procollagen molecule have been shown to inhibit the synthesis of collagen from fetal calfskin and human fibroblast cultures, while native collagen has no effect on its own production (26). The biochemical mechanism for collagen feedback inhibition is yet to be described.

Initial experiments suggested that C4 production per cell was inversely related to the number of cells plated. Cell density alone, substrate limitation, or nonspecific product inhibition did not account for the selective decrease in C4 synthesis and secretion. Direct evidence that C4 inhibits its own production was obtained with the use of guinea pig C4 purified from plasma. These results indicated that both macrophage-derived C4 and C4 from plasma (presumably liver derived in
analogy with other species) were capable of inhibiting C4 synthesis. Hence, in
tissue, either local C4 production or the influx of plasma C4 resulting from
increased vascular permeability associated with inflammation would decrease
macrophage production of C4.

The isolated guinea pig plasma C4 used in these experiments was >95% pure
as assessed by SDS-PAGE, and its hemolytic activity (1.05 × 10^{13} eff mol/mg
protein) compared favorably to purified C4 reported elsewhere (23, 27). Fur-
thermore, the amount of purified C4 required to effect 50% inhibition, 1.9 ×
10^{10} eff mol/ml (0.02 μg/ml), was similar to the amount of C4 in the conditioned
media. Both are within a range of C4 likely to be present in tissues.

Methylamine alters the thiolester site of C4 and renders it hemolytically
inactive without affecting the structural integrity of the molecule (27). Analysis
of the purified C4, as well as C4 in conditioned medium, showed a loss of all
hemolytic activity, while it appeared identical to pretreated C4 on SDS-PAGE.
The methylamine-treated C4 inhibited biosynthesis of C4 by macrophages at
concentrations equal to pretreated C4, demonstrating that the effect was not
dependent on hemolytic activity nor on an intact thiolester site. Initial experi-
ments with methylamine-treated C4 showed an effect on C2 detection (50% 
decrease by hemolytic assay). Two experiments indicated that this effect is an
artifact of the assay system. Depletion of methylamine-treated C4 from the
medium before measurement of the C2 function restored the C2 to control
levels. Mixing experiments confirmed that at these concentrations methylamine-
treated C4 inhibited the hemolytic assay for C2 without affecting the C4 hemo-
lytic assay.

The fact that conditioned medium harvested from normal but not C4-deficient
macrophage cultures also inhibited C4 biosynthesis makes it unlikely that the
effect was due to a contaminant in the purified C4 preparation or C4 altered
during purification. Further studies will be required to define the precise ligand
and ligand binding site that modulates feedback inhibition of C4 biosynthesis.

We have demonstrated that C4 specifically inhibits C4 biosynthesis and does
not inhibit the synthesis of other proteins or affect guinea pig macrophages
nonspecifically. Cell viability, synthesis of total protein, and the synthesis of C2,
are all unaffected by C4 in the culture medium. Furthermore, the inhibitory
effect is reversible. The kinetics of postsynthetic processing of the C4 protein is
also unaffected by exogenous C4. The kinetics of [^{35}S]methionine incorporation
into the precursor protein ProC4, the stability of the ProC4 molecule, the
secretion of native C4, and the stability of the secreted C4 were identical in
suppressed and control cultures. Evidence of a specific decrease in C4 mRNA
suggests an effect on transcription or posttranscriptional processing of C4-specific
RNA. The decrease of C4 mRNA in cells incubated in the presence of C4 was
not accompanied by a decrease in factor B-specific mRNA or total RNA.

Taken together the data in this report define the molecular basis for local
control of a component of the inflammatory response. In addition they provide
an approach to study the mechanisms of macrophage activation and response to
other microenvironmental modulations.
Summary

Previous reports have shown that regulation of local extrahepatic production of complement may not reflect the regulation of plasma concentrations of the corresponding proteins and, further, that alteration of the tissue microenvironment can affect local macrophage protein synthesis. This report describes the molecular basis for control of the biosynthesis and secretion of a class III major histocompatibility complex gene product, the fourth component of complement (C4), from guinea pig macrophages by extracellular native C4 protein. The effect is specific for C4 synthesis, since production of C2 and total secreted protein was unaffected by fluid phase C4. C4 synthesis by extracellular C4 is regulated at a pretranslational level, without an effect on posttranslational proteolytic cleavage, glycosylation, or secretion. Specific C4 and factor B cDNA probes were used to demonstrate, by dot hybridization and Northern blot analysis, a decrease in messenger RNA coding for C4 that paralleled the inhibition of C4 biosynthesis, while the amount of total RNA and mRNA specific for factor B remained constant. Inhibition of C4 biosynthesis and the disappearance of mRNA encoding C4 occurred between 4 and 6 h after exposure of the macrophages to biologically active or methylamine-inactivated C4 protein.

These data demonstrate that regulation of C4 biosynthesis by guinea pig macrophages serves as a model for the study of the molecular mechanisms of macrophage activation as well as the control of production of a component of the inflammatory response.

Received for publication 14 November 1983 and in revised form 13 March 1984.

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