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Expression of the MHC class III genes

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The second (C2) and fourth (C4) components of complement and factor B (B) are coded for by genes within the major histocompatibility complex (MHC). These proteins are synthesized in liver and in extrahepatic mononuclear phagocytes. The isolation of complementary DNA probes corresponding to each of these proteins now permits analysis of the molecular mechanisms controlling expression of the class III MHC genes.

Genetic control of *C4* gene expression has been examined in two model systems. A defect in post transcriptional processing of C4-specific RNA accounts for a failure to generate mature C4 mRNA in homozygous deficients of a C4 deficient guinea-pig strain. On the other hand, a quantitative difference in the amounts of mature C4 liver mRNA accounts for the genetic variation in C4 levels observed among several mouse strains.

The maturation of monocytes to macrophages results in changes in biosynthesis of the MHC class III products; for example, a significant increase in rate of secretion of C2 and B is noted in human monocytes during the first 3 d in culture and the proportion of C2-producing cells is greater in freshly isolated macrophages than in monocytes. Macrophages demonstrate selective increases in factor B and C2 mRNA characteristic of specific tissues. In the guinea-pig macrophage, *C4* gene expression is regulated by a selective feedback mechanism induced by extracellular native C4. The C4 binds to the macrophage cell surface mediating a change in transcription or, less likely, a change in stability of C4 mRNA. Regulation of C4 synthesis in the mouse macrophage is accomplished by mechanisms that are independent of this feedback control but the murine cells also display separate mechanisms for regulation of C4 and factor B-specific mRNA levels. Resident and elicited macrophages from either mouse or guinea-pig differ with respect to expression of the class III MHC gene products.

These studies form the basis for evaluating the molecular regulation of inflammation, maturation of mononuclear phagocytes and the genetic variants and deficiencies of complement proteins.

The recognition of genetic deficiencies and polymorphisms of the major histocompatibility (MHC) class III gene products, the fourth (C4) and second (C2) components of complement and factor B of the alternative complement pathway, has prompted studies of the structure and expression of MHC class III genes. This has provided insight into molecular mechanisms that regulate inflammation.

The fourth component of complement

The fourth component of complement (C4) is a serum glycoprotein composed of three disulphide-linked polypeptides (Schreiber & Muller-Eberhard 1974). Synthesis of the 185000 Da single chain precursor of C4, pro-C4 (Hall & Colten 1977; Roos *et al.* 1978) is directed by a polyadenylated mRNA of approximately 5 kilobases (kb) (Whitehead *et al.* 1983;

Ogata *et al.* 1983). The beta chain is the amino terminal subunit, the alpha chain the central subunit and the gamma chain the carboxyterminal segment of pro-C4 (Goldberger *et al.* 1980; Goldberger & Colten 1980; Parker *et al.* 1980; Karp *et al.* 1981). These chains are separated in the precursor protein by arginine-rich intersubunit linking peptides (Whitehead *et al.* 1983; Ogata *et al.* 1983; M. C. Carroll, unpublished) similar to the linking peptides between the beta and alpha subunits of pro-C3 (Domdey *et al.* 1982) and pro-C5 (Lundwall *et al.* 1984) precursors of the third and fifth complement components. Post synthetic processing of pro-C4 involves proteolysis and most likely, excision of the linking peptides. Additional post synthetic changes in alpha chain include sulphation (Karp 1983) modification of residues within the region of the thiolester site and glycosylation of alpha and beta chains (Roos *et al.* 1980; Matthews *et al.* 1982). Finally, extracellular modification of alpha chain results in a change in apparent size of approximately 4000 Da, presumably owing to loss of a carboxyterminal peptide of uncertain length (Chan *et al.* 1983). This modification may include removal of all or a residual portion of the linking peptide and perhaps other residues at the carboxyterminus of alpha chain. Direct sequence analysis of post synthetic intermediates will be required to establish the precise molecular events accounting for this observation.

Additional variation in subunit size, in this instance based on genetic factors, has been detected in human (Roos *et al.* 1982; Mauff *et al.* 1983) and mouse (Roos *et al.* 1978; Carroll & Capra 1979; Ferreira *et al.* 1980; Natsuume-Sakai *et al.* 1980) serum C4. The alpha chain of C4 derived from one of the human C4 loci (C4A) is apparently 2000 Da heavier than the alpha chain product of the other locus (C4B). These differences in alpha chain size may reflect the primary structural alterations that account for the known differences in specific haemolytic activity of C4A and C4B (Awdeh & Alper 1980). A structural variant in human beta chain has also been recognized (Mauff *et al.* 1983) but this variant is found in C4 proteins apparently derived from both loci.

In the mouse, structural polymorphisms of C4 have been detected for each of the subunits and, in particular strains bearing the S^{w7} haplotype, differences in C4-specific haemolytic activity have been correlated with the structural variant (Atkinson *et al.* 1980). A C4-like protein (Slp) which lacks haemolytic activity is also found in certain strains. Expression of the gene coding for Slp is under hormonal control (Passmore & Shreffler 1970). Slp is also synthesized as a single chain precursor which is processed in a manner similar to that described for C4 (Parker *et al.* 1979). Recently, the organization of the murine complement genes within the S region of the major histocompatibility complex has been revealed (figure 1) using cDNA probes for the class III genes and a series of overlapping cosmid clones. The initial results indicated that genes for C2 (D. D. Chaplin, personal communication), factor B and two 'C4-like' genes designated *C4x* and *C4y* are oriented in the same direction (5′–3′) within a region of 250–300 Kb (Chaplin *et al.* 1983).

Identification of the second of the two C4-like genes (*C4y*) as C4 was accomplished by demonstrating synthesis and secretion of haemolytically active C4 by murine L cells transfected with a cosmid that encompassed the entire coding sequence plus 5′ flanking sequences (Chaplin *et al.* 1984). Similar experiments using cosmids corresponding to the *C4x* gene, thus far have not resulted in expression of this gene in the transfected cells as indicated by the failure to detect mRNA specifically hybridizing with the C4 cDNA probe in cell lysates. A number of possible explanations for this result have been considered. Among them is the possibility that if *C4x* is Slp, androgen stimulation may be required for its expression. Alternatively, it is possible

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that insufficient 5′ flanking sequences were included in the particular cosmid used, thus excluding information critical for even constituative expression. In any event, the failure to detect a third 'C4-like' gene in screening the cosmid library makes it likely, though at the present time still speculative, that *C4x* corresponds to the Slp gene. Additional studies using Slp-specific cDNA probes or synthetic oligonucleotides will be required before a definitive assignment of Slp to the *C4x* locus can be made.

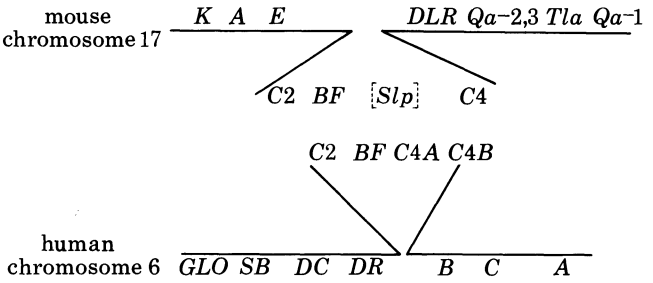

FIGURE 1. The order of the class III genes for mouse and human are given in 5′–3′ orientation. The relative position is known with respect to the other MHC genes but the orientation is not, since cosmids overlapping with the loci adjacent to C2 or C4 (mouse) and C4B (human) have not been identified. The *Slp* locus is in dotted brackets to indicate the lack of direct evidence for the position of this gene.

The mouse MHC class III genes are therefore oriented in a sequence similar to their human counterparts (Carroll *et al.* 1984), supporting further the suggestion based on the structure and function of the proteins that the *C4A* gene corresponds to but has drifted less than the *Slp* gene. The human *C4B* locus would then be analogous to the *Ss* gene which specifies synthesis of functional mouse C4. The high degree of fidelity in transcription, post transcriptional processing, translation, post synthetic modification and secretion of C4 in cells transfected with the C4 gene has considerable implications for the further analysis of the mechanisms for genetic and microenvironmental regulation of MHC class III gene expression. Some of the specific questions that will be pursued using this strategy are described in the balance of this review.

REGULATION OF C4 BIOSYNTHESIS

Newell *et al.* (1982) have demonstrated, using functional and immunochemical methods, biosynthesis of murine C4 in short term primary cultures of mouse-resident peritoneal macrophages. The rate of C4 secretion declined rather rapidly within the first few hours in culture, although total protein synthesis remained approximately constant and there was an apparent increase in factor B synthesis. In the initial study both feedback inhibition and instability of secreted C4 were ruled out as possible explanations for the striking decrease in C4 production as a function of time in culture. More recently it has also been possible to exclude changes in secretion rate or postsynthetic processing of the precursor protein as mechanisms accounting for the decreasing rates of C4 production and to demonstrate that the change in synthetic rate is a function of the concentration of specific C4 mRNA per cell (Sackstein *et al.* 1984). In addition, during the course of these experiments we detected a reciprocal increase in steady state levels of factor B mRNA, indicating that the change in cellular C4 mRNA levels cannot simply be the result of a limitation of the *in vitro* culture conditions. The most likely explanation for these findings is a change in rates of transcription of C4 and factor B RNA

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during the time the macrophages are in culture but the signal or signals directing this change are not known. Alternative explanations include changes in post transcriptional processing or stability of the specific C4 and factor B mRNA.

Substantial species specific differences in the mechanisms for regulating *C4* and *Factor B* gene expression in primary macrophage cultures have been noted and will be described later. In the murine system, elicited or activated macrophages generated in the peritoneal cavity by injection with oil, casein, thioglycolate, concanavalin A or lipopolysaccharide produce markedly less C4 even in the initial few hours in culture when compared with the amount of C4 produced by resident peritoneal macrophages (Newell & Atkinson 1983). That is, approximately 3–10 times more C4 is synthesized and secreted in the resident cells as in the elicited cell populations. This phenomenon is the result of control of transcription, post transcriptional processing or, less likely, stability of the specific C4 mRNA because again the steady state C4 mRNA levels correlate with the changes in synthesis of pro-C4 in the various cell types (Sackstein *et al.* 1984).

Histocompatibility linked genetic control of the serum levels of a protein designated Ss was discovered in inbred mouse strains approximately 20 years ago by Shreffler & Owen (1963). Subsequently this protein was shown to be C4 (Demant *et al.* 1973); the difference between high and low Ss strains amounts to 10–20 fold in levels of C4 as assessed functionally and immunochemically. Only recently has any insight into the basis for this variation been obtained. Initial work comparing the biosynthesis of C4 in peritoneal macrophages obtained from high and low C4 strains failed to demonstrate a difference in the rate of synthesis either during the initial period in culture or at later time periods when the amount of C4 synthesized in both cell types decreased (Newell *et al.* 1982). These results suggested either (i) that the mechanisms regulating serum levels of C4 in the various strains are present only in liver, not in macrophages, or (ii) that catabolism of C4 in the strains with low serum levels is markedly greater than in strains with high levels. Evidence for the former hypothesis was obtained from two lines of investigation. First, Rosa & Shreffler (1983) showed that primary hepatocyte cultures produced C4 at rates that corresponded to the strains from which the liver was obtained. Rates of synthesis and absolute amounts of C4 produced by the liver cell cultures correlated with the differences in serum C4 levels. In separate studies a parallel difference in content of liver C4 specific mRNA was noted (Ogata *et al.* 1983; Chaplin *et al.* 1983) indicating that the regulation of C4 mRNA levels in the hepatocyte differs from that in the macrophage from high and low C4 strains, perhaps owing to differences in rates of transcription. It should be possible with the use of methods for measuring transcription *in vitro* to determine the basis for this tissue specific genetically determined regulation of C4 gene expression.

As noted above, important differences in regulation of C4 synthesis by murine and guinea-pig macrophages have been observed. In guinea-pigs, the *C4* gene is also within the MHC (Bitter-Suermann *et al.* 1977) but in contrast to human and murine species, evidence for only a single C4 gene has been obtained, thus far. Bitter-Suermann *et al.* (1981) showed that the genes for C2 and factor B are also MHC linked in the guinea pig, but, in this species, the order of the MHC class III genes is not known. The sensitivity and specificity of the haemolytic assay for guinea-pig C4 has permitted analysis of C4 biosynthesis on a single cell level in this species. The results of studies using this approach indicate that differences in tissue-specific regulation of C4 synthesis may even be more complicated than was appreciated from investigations of murine C4 biosynthesis. The method for detection of C4-producing cells involves a modification

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of the approach used by Jerne & Nordin (1963) for quantification of antibody-synthesizing cells. C4-specific haemolytic plaques are generated by overlaying macrophage monolayers with an agarose gel containing sensitized erythrocytes and the plaques are developed using C4 deficient serum or partially purified complement reagents lacking the C4 protein (Alpert *et al.* 1983). C2-specific plaque-forming cells are generated in a similar manner but in this case, with the use of C2 deficient serum for developing the plaques. Approximately 10% of bone marrow

TABLE 1. SECRETION OF COMPLEMENT PROTEINS BY MONONUCLEAR PHAGOCYTES

		proportion of complement plaque forming cells (%)					
				macrophage			
species	complement	bone marrow	blood monocyte	peritoneal	spleen	breast	lung
human	C2	n.d.	1.0–7.5†	n.d.	n.d.	47	5.5
guinea-pig	C2	0	10.7	44.0	46.3	n.d.	2.4
guinea-pig	C4	9.8	11.3	46.5	44.5	n.d.	43.9

† Proportion increases with time in culture from 1.0% on day 1 to maximum of 7.5% at one week or longer.
n.d., Not determined.

derived adherent mononuclear phagocytes and a comparable proportion of the circulating monocytes produce C4 haemolytic plaques. About 40–50% of the macrophages derived from bronchoalveolar wash (lung macrophages), peritoneal cavity and spleen generate plaque-forming cells. The proportion of cells producing C4 and those producing C2 varied from one tissue site to another and at different stages in maturation of the mononuclear phagocytic series of cells (table 1).

Beller & Ho (1982) and Beller & Unanue (1981) have shown that surface Ia antigen, a class II MHC gene product, is expressed on a subpopulation of mouse peritoneal macrophages and that expression of Ia is a function of maturation and specific stimulus. Several years ago, Barber *et al.* (1976) found C4 protein on the cell membrane of a subpopulation of guinea-pig peritoneal and alveolar macrophages and further that the surface bound C4 antigen was probably derived from fluid phase C4 (Barber & Berkholder 1978). These observations, coupled with studies showing changes in rates of C4 biosynthesis in tissue culture prompted a detailed investigation of the cellular regulation of C4 biosynthesis in guinea-pig peritoneal macrophages.

Initially Auerbach *et al.* (1983) confirmed that about 20–25% of the peritoneal macrophage population bears surface C4 antigen but it was clear that the surface C4-bearing cell population was not stable, since incubation of the cells in medium containing or lacking preformed fluid phase C4, altered the proportion of C4 surface positive cells. The ability to separate cells displaying surface C4 from those lacking surface C4 with the use of the fluorescence-activated cell sorter permitted a correlation of the presence of surface C4 antigen with functional characteristics of the cells; namely the secretion of C2 and C4 (table 2). The C4-bearing cell population constituted the majority of peritoneal macrophages that secrete haemolytically active C4. However within 12 h, secretion of C4 was markedly decreased in this cell population. The C4 surface negative cell population initially contained relatively few C4 secreting cells but this proportion increased when the macrophages were cultured in fresh medium or in C4 deficient conditioned medium. In contrast, when C4 purified from plasma or conditioned medium containing C4 was added to the culture, the cells failed to produce C4. These and other experiments suggested a relation between the binding of C4 derived from the medium

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to the cell surface and secretion of C4 by a subpopulation of peritoneal macrophages. It should be noted that under the conditions of these experiments, the presence or absence of fluid phase or surface cell bound C4 antigen did not affect the rate of C2 secretion per cell nor the proportion of C2 producing cells in the macrophage cell population. The majority of the C4 antigen contained on the cell surface was native C4, although trace amounts of antigenically detectable C4 peptides were present. Further work will be required to define precisely the C4 binding site and the specificity of interaction with the cell surface acceptor sites.

TABLE 2. SECRETION OF C4 AND C2 BY GUINEA-PIG MACROPHAGES AS A FUNCTION OF CELL MEMBRANE BOUND C4

	proportion of complement plaque-forming cells					
	0 h		12 h			
macrophage subpopulations			conditioned medium		fresh medium	
	C4	C2	C4	C2	C4	C2
$C4S^+$	94	38	0	40	3	42
CrS^-	4.6	41	5.0	41	38	40

$C4S^+$ Cells bearing surface C4 antigen at time 0.
$C4S^-$ Cells lacking surface C4 antigen at time 0.

Further studies (Auerbach *et al.* 1984*a*) demonstrated that binding of C4 protein to the cell surface results in specific inhibition of C4 biosynthesis and that this correlates with a decrease in the steady state level of C4 mRNA. Inhibition of C4 synthesis (i) was accomplished with both serum derived and macrophage derived C4 (the former probably of hepatocyte origin), (ii) was independent of haemolytic activity of the C4 since methylamine-inactivated C4 protein inhibited biosynthesis of C4 at concentrations equal to that of untreated, fully active, C4 protein and (iii) the inhibitory effect was reversible in that depletion of C4 from the medium resulted in an increase in C4 mRNA and new synthesis of C4 within two hours. The specific decrease of C4 mRNA in cells incubated in the presence of extracellular C4 was not accompanied by a change in factor B specific mRNA or total RNA content. Hence these data suggest that feedback inhibition of C4 biosynthesis is regulated at the level of transcription or post transcriptional processing. It is unlikely that specific mRNA stability has been altered, but this possibility has not been rigorously excluded.

The direct regulation of C4 production by macrophages as a function of the presence of extracellular C4 would have important consequences in the evolution of an inflammatory response. That is, it would provide a mechanism for controlling constitutive secretion of C4 in resting macrophages as well as a basis for repletion of C4 following complement consumption or diffusion from a site of inflammation. The direct control of C4 synthesis would thus not depend on nor necessarily correlate with the plasma concentrations of C4, but would reflect the local concentration of extracellular C4 in the tissue environment.

Apart from these differences between the guinea-pig and murine systems, this observation is consistent with the demonstration that macrophages from high and low C4 strains produce comparable amounts of C4. Hence, in the murine system as well, macrophage production of C4 is under controls distinct from those which determine plasma levels of the corresponding protein. Administration of pharmacological agents such as cortisone acetate or cyclophosphamide likewise specifically alter biosynthesis of C4 by tissue macrophages without affecting serum levels (Pennington *et al.* 1979). Similarly, induction of a chronic inflammatory disease

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in the guinea-pig lung induces an increase in both C2 and C4 secretion by the bronchoalveolar macrophage without recruitment of a specific subpopulation of complement producing cells or a change in serum complement levels (Alpert *et al.* 1984).

Genetic deficiency of the fourth component of complement in guinea-pigs is inherited as an autosomal codominant trait which results in absence of C4 (assessed either antigenetically or functionally) in the plasma of homozygous deficient animals (Ellman *et al.* 1970). Cells and tissues from C4 deficient guinea-pigs which are in other respects normal, do not synthesize C4 (Colten & Frank 1972). Somatic cell hybrids generated by fusing C4-deficient peritoneal macrophages and HeLa cells yield clones capable of synthesizing and secreting functionally active *human* C4 (Colten & Parkman 1972). This effect is mediated by a soluble factor which elutes from Sephadex G-100 at a position corresponding to a 45000 Da marker. The macrophage derived factor specifically induces production of C4 in HeLa cells without an effect on total protein synthesis (Colten 1972). Production of the factor and the response to it is inhibited by low concentrations of actinomycin D and cyclohexamide. The HeLa cells are responsive to the factor only after entering the early S phase of the cell cycle (Colten 1974). Small amounts of this factor are detectable in normal guinea-pig macrophages but not in other cells. Moreoever, no primary or established cell line other than HeLa is responsive to the factor.

Initial cell free biosynthetic studies suggested that mRNA from homozygous C4 deficient animals could direct synthesis of a heterogeneous mixture of C4 polypeptides (polysome bound C4 precipitable material) but not intact pro-C4 (Hall & Colten 1978). In subsequent studies, however, we did not detect these polysome bound C4 polypeptides (Whitehead *et al.* 1983). A human C4 cDNA clone which encompassed the entire gamma chain coding region (Whitehead *et al.* 1983) was used to pursue this question further. This clone specifically hybridizes with a 5 kb mRNA in normal guinea-pig liver. In the C4 deficient guinea-pig liver, a low abundance 7 kb mRNA species specifically hybridized with the C4 cDNA clone, but no mature C4 mRNA was detected. We suggested that this 7 kb C4 mRNA species might be an intermediate precursor of mature C4 mRNA; that is, that C4 deficiency in the guinea-pig is due to a post transcriptional processing defect. Direct studies of post transcriptional processing of C4 mRNA, a detailed structural analysis of the mutant *C4* gene and functional studies of genomic clones in transfected cells will be required to define fully the basis of this genetic deficiency. Obviously, a similar approach can now be applied to the analysis of complement deficiencies in humans and other species.

The second component (C2) and factor B

Like C4, C2 is also synthesized in hepatocytes and in cells of the monocyte–macrophage series. Studies of extrahepatic synthesis of C2 in guinea-pig and human mononuclear phagocytes during maturation (Alpert *et al.* 1983; Cole *et al.* 1982) revealed changes in the proportion of complement-producing cells and in rates of synthesis (see table 1). For instance, no C2 producing cells are detected in guinea-pig bone marrow but 10% of the blood monocyte population are C2 plaque-forming cells (Alpert *et al.* 1983). In different tissues the proportion of macrophages producing C2 varies from about 45% in spleen and peritoneum to 2.5% in lung. The subpopulation of cells producing C2 characteristic of each tissue remains quite constant for several days in culture; that is, is independent of the aforementioned regulatory signals that modulate C4 synthesis.

The fact that the complement-producing lung macrophages synthesize C2 at a rate 5–10

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times the rate of synthesis in complement-producing peritoneal macrophages offsets the 20-fold difference in proportion of C2 producing cells, so that the absolute amount of C2 generated from equal numbers of unfractionated peritoneal and lung macrophages is comparable. An unresolved question raised by these observations is whether specific subpopulations of monocytes migrate to different tissues or whether regulatory signals characteristic of a given tissue provide local control of C2 gene expression.

Induction of an inflammatory response leads to increases in local C2 production by two mechanisms; (i) an increase in macrophage cell number and (ii) a 2–3 fold increased rate of synthesis per cell. This increased rate of C2 synthesis per cell is the result of an increase in C2 mRNA but probably reflects a nonspecific effect on transcription and translation since total cellular RNA and protein secreted are increased in the inflammatory cell exudate (Auerbach *et al.* 1984*b*). The proportion of C2 producing cells in such an inflammatory exudate is similar to that in a resting cell population, that is, there is no evidence for selective recruitment of complement-producing cells (Cole *et al.* 1980).

Human mononuclear phagocytes also display characteristic differences in C2 and factor B synthesis as a function of maturation, tissue site and response to stimuli that elicit an inflammatory exudate. These were analysed in detail initially by Einstein *et al.* (1976) following the development of a method for prolonged culture of human monocytes. Biologically active C2 is secreted by monocytes in tissue culture for several months and the rate of C2 synthesis per cell varies as a function of the length of time the cells are maintained *in vitro*, that is, the rate of C2 synthesis at two months is approximately three times the rate during the second week of culture. Moreover, little or no C2 is secreted during the first 3–6 d. Similar kinetics of factor B biosynthesis have also been observed (Whaley 1980; Beatty *et al.* 1981; Cole *et al.* 1982).

Several manipulations of the culture conditions significantly modulate baseline rates of C2 secretion by human monocytes. For example, the initial lag in C2 biosynthesis can be abolished by the addition of a soluble product of antigen stimulated lymphocytes to the culture medium (Littman & Ruddy 1977) and decreased C2 biosynthesis is effected by the addition of Fc fragment of IgG, concanavalin A (Passwell *et al.* 1980) or serum-treated immune complexes (Whaley *et al.* 1983). In the latter instance, the possibility was raised that inhibition of biosynthesis was mediated via interaction of an immune complex bound C3 fragment with one of the several C3 receptors known to be present on the monocyte cell membrane. Certain lipids also inhibit C2 biosynthesis in human monocytes (Kolski & Strunk 1981) and guinea-pig macrophages (Strunk *et al.* 1979). Although it is clear that the effect of lipid is independent of the type of free fatty acid, the mechanism accounting for this mode of regulation of C2 biosynthesis and its physiological importance is as yet unknown.

Synthesis of C2 and factor B is initiated without lag and rates of synthesis are several times greater in human macrophages isolated from breast milk or bronchoalveolar lavage than in monocytes harvested from the peripheral blood of the same individuals (Cole *et al.* 1982, 1983). In addition, the ratio of C2 to factor B secreted by the macrophage differs from the relative rates of secretion of each of these components in the monocyte even after prolonged culture. That is, secretion of haemolytically active C2 exceeds factor B by 3.5 fold and 7 fold in milk and bronchoalveolar macrophages respectively, whereas the ratio of C2 to factor B secreted by the monocytes is constant (C2/B = 1) for several weeks *in vitro*. The factors regulating C2 and factor B synthesis as a function of stage of maturation of human mononuclear phagocytes have been investigated to some extent. Differences in proportion of C2-producing

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cells among mononuclear phagocyte populations, similar to those observed in the guinea-pig have been recognized (table 1). Changes in rates of post translational modification; secretion, or stability of the extracellular products have been excluded as significant factors accounting for the modulation of C2 and factor B biosynthesis in the human monocyte and macrophage. Finally, preliminary studies using specific cDNA probes for factor B (Woods *et al.* 1982) and C2 (Woods *et al.* 1984) suggest that the regulation is exerted at a pretranslational level, that is, is either the result of transcriptional control or post-transcriptional processing (F. S. Cole, H. S. Auerbach, G. Holdberger, and H. R. Colten, unpublished) but the details remain to be elucidated.

It has not been possible to investigate biosynthesis of C2 in human hepatocytes as a result of both ethical constraints and technical problems but it is clear that liver is the origin of most, if not all, C2 found in plasma. The availability of a well differentiated hepatoma derived cell line HepG2 has provided a useful model for investigation of this issue. Three intracellular forms of C2 protein have been identified; an 84000 Da species which after further modification is secreted (within 1–2 h) as native C2 plasma protein and two lower molecular mass species (79000 and 70000 Da) that remain cell associated for at least 12 h (Perlmutter *et al.* 1984). Further studies established that the cell-associated C2 proteins were not proteolytic products of the 84000 Da species, that all three forms of C2 protein contain similar carbohydrate moieties and that only the 84000 Da form is susceptible to cleavage by C1s. Cell free biosynthesis directed by mRNA from HepG2 or from normal human liver generated three primary translation products which correspond to unglycosylated forms of the three intracellular C2 protein species. Multiple forms of intracellular C2 protein have also been detected in *Xenopus* oocytes injected with normal human liver or HepG2 mRNA (Colten *et al.* 1983). The functional importance and the molecular events accounting for the cell associated C2 is now under investigation.

Conclusion

The work cited in this review underscores the importance of species and tissue-specific regulation of class III MHC genes. Furthermore, modulation of extrahepatic production of C2, factor B and C4 reflects maturation and differentiation of cells in the monocyte macrophage series. Additional control of synthesis of these proteins is exerted during the course of an inflammatory response. These have implications for both homoeostasis of complement (C4) tissue levels under resting conditions and for repletion of C4 following consumption resulting from activation of the classical pathway. The recent isolation of suitable probes for the class III MHC genes has facilitated measurement of changes in transcription and translation associated with these biological phenomena and has yielded preliminary data on the molecular basis of genetic variants and deficiencies of the complement proteins.

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