MICROBIOLOGY—1985

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PREFACE

This volume is the 12th in the annual *Microbiology* series for the American Society for Microbiology and the first with me as Editor of the series without the aid of David Schlessinger, the prior Editor. A few changes have been made in hopes of building on the past success of the series and strengthening and expanding the material. First, I have introduced a standing group of associate editors with individual responsibilities in different areas. These are Peter F. Bonventre, University of Cincinnati Medical Center, Cincinnati, Ohio (Medical Microbiology and Immunology); Josephine A. Morello, University of Chicago, Chicago, Ill. (Clinical Microbiology and Virology); Sondra Schlesinger, Washington University School of Medicine, St. Louis, Mo. (Virology); Simon D. Silver, Washington University, St. Louis, Mo. (Genetics and Molecular Biology of Industrial Microorganisms); and Henry C. Wu, Uniformed Services University of the Health Sciences, Bethesda, Md. (Biology, Genetics, and Ultrastructure of Microbes). We have selected interesting and important offerings from the ASM Annual Meeting, ICAAC, and the ASM Conferences to provide material in the forefront of research for this volume. The format has been changed somewhat so that, in addition to extended abstracts, there is a large proportion of minireviews. These latter offerings are intended to provide material of general and lasting interest both for the specialist and for those wishing to learn more about a general area, while the extended abstracts will ensure something of specific tehnical interest for everyone. We would all welcome suggestions and proposals from readers and prospective participants in the series.

It is my pleasure to have undertaken this work, starting last year with David Schlessinger and continuing now with the Editorial Committee. We look forward to continued growth and evolution of the *Microbiology* series over the next decade.

LORETTA LEIVE
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Medical Microbiology and Immunology

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A. Complement System and Host Defense Against Infection

Introductory Note

The papers presented in this symposium concern the opsonic function of the complement system. The complement system originated to prevent microbial invasion, and the primary mechanism through which it accomplishes this task is opsonization. Opsonization is from the Greek word "opsōnein" and means "to prepare for the (dinner) table." In terms of host defense, it means that the foreign invader is prepared (coated) such that it is more easily ingested. C3b, iC3b, and, to a lesser extent, C4b and possibly C5b are the opsonic complement proteins.

There are receptors for C3b/C4b and iC3b on phagocytic cells, and the interaction between these fragments (ligands) and receptor proteins accounts for the phenomenon of opsonization. C3 deficiency is associated with severe and recurrent pyogenic infections, attesting to the critical importance of this molecule in host defense. The role of complement receptors on nonphagocytic cells is not so clear but probably relates to the processing of immune complexes. Through a cooperative set of interactions, these receptors for complement fragments promote the clearance, processing, and degradation of foreign materials.

In this symposium, this phenomenon of opsonization is reviewed. During the past 5 years exciting advances have occurred in this area. The symposium begins with a discussion of the biology of C3 and C4. Then R. P. Levine reviews the function of the internal thioester bond within C3. It is through cleavage of this bond that C3b and C4b can become covalently attached to cell surfaces. J. A. Winkelstein provides a concise review of complement deficiency states. These experiments of nature most clearly point out the critical role of the complement system in host defense against infection. The last two papers in the symposium summarize the recent explosion of information relative to the structure and function of complement receptors for C3 and C4 and their fragments.

To summarize, the phenomenon of opsonization via the classical complement pathway consists of antibody recognizing a foreign antigen and binding to it, complement activation, covalent attachment of C3b and C4b to the foreign material, interaction of receptors on leukocytes with these ligands, processing, and, in some cases, ingestion of the foreign material—a very efficient and fascinating way of dealing with microbes.

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The Complement System: Biology of the Opsonic Components

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Complement was first described by Ehrlich and Morgenroth in 1899 as a serum substance which completes the action of antibody (15). Over the past 85 years, the complement system has been demonstrated to be a complex self-assembling cascade composed of at least 20 serum glycoproteins (see 28 and 39 for detailed reviews). Deficiencies of almost all the complement components have been reported and are associated with clinical presentations ranging from connective tissue diseases to life-threatening infections (1). The complement system functions as an extracellular effector pathway by mediating opsonization or lysis and by promoting the inflammatory response. Membrane-bound fragments serve as ligands for receptors on erythrocytes and leukocytes to facilitate the clearance and phagocytosis of antigens. Such interactions form the basis for the pathophysiology of many immune complex-mediated and autoimmune diseases.

In this series of papers, selected recent developments in the study of the structure and function of the opsonic components of the complement system are reviewed. Emphasis is placed on the biology of the opsonic components, C3 and C4, the consequences of complement deficiency states, and the receptors for these two components and their degradation fragments. We present here a brief overview of the complement system before discussing the biology of C3 and C4.

CLASSICAL AND ALTERNATIVE COMPLEMENT PATHWAYS

The classical pathway is the primary humoral mediator of antigen-antibody reactions and is activated by immune complexes of the immunoglobulin G or M type (reviewed in 39). Interaction with the Fc portion of immunoglobulin G (subclasses 1, 2, and 3) or immunoglobulin M induces a conformational change in C1q resulting in the activation of C1r (see Fig. 1). C1r activates C1s via proteolysis, and C1s, in turn, cleaves C4 to yield C4a and C4b and C2 to yield C2a and C2b. C4b binds C2a to form the classical pathway C3 convertase ($\overline{C4b2a}$). The alternative pathway is evolutionarily older and represents an antibody-independent system (28). Activation of the alternative complement is not clearly understood, but is related to the chemical composition of cell surfaces (see legend to Fig. 1 and references 28 and 34). Factors B, D, and C3 are the early components of the alternative pathway and interact to form the alternative pathway convertase ($\overline{C3bBb}$). Although the two pathways utilize different sets of early components, both convertases cleave C3 into C3a and C3b and share the terminal components (C5 to C9 or membrane attack complex).

C3b and C4b are modified by a number of proteolytic enzymes and cofactors. C3b is degraded by the regulatory enzyme C3b/4b inactivator (or I) and its cofactor H to give rise to C3d,g and C3c (see Fig. 2). C4b undergoes a similar degradation scheme with I and C4-binding protein (C4-bp). These regulatory proteins (i.e., C4-bp, H, and I) provide a means for limiting the spread of complement activation as the cleaved proteins are unable to continue the complement cascade. However, C3b and C4b and their degradative fragments bind to cellular receptors and in so doing promote the inflammatory response and the removal of foreign material.

GENETICS

C4 is an ~200,000-dalton glycoprotein composed of three disulfide-linked subunits (18, 37, 41). The α, β, and γ subunits have M_rs of ~93,000, 78,000, and 33,000, respectively. Located within the major histocompatibility complex are two structural genes for C4 (reviewed in 14, 42, 42a). These duplicated genes (designated C4A and C4B in humans) encode two structurally and functionally distinct, but closely related, glycoproteins. Using isoelectric focusing, 13 and 22 structural variants have been detected for the C4A and C4B loci, respectively (24). These structural variants are thought to be due to amino acid differences in the C4d (α2) fragment (5, 8). In standard hemolytic assays employing sheep erythrocytes, the C4B gene product is hemolytically more efficient than the C4A gene product (19). In addition to α-chain variants, Mauff and colleagues have recently demonstrated β-chain polymorphism that segregates independently of α-chain polymorphism (25).

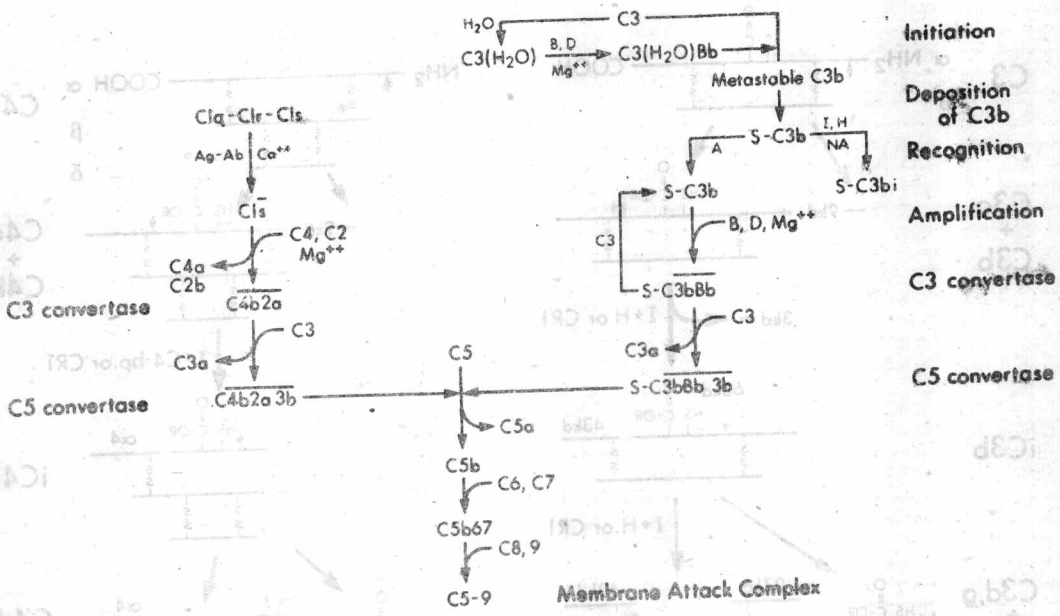

FIG. 1. Classical and alternative pathways of the complement system. Classical pathway: see text for discussion. Alternative pathway: the initial event of the alternative pathway is hypothesized to be a low-grade spontaneous generation of a C3 molecule with a hydrolyzed thioester bond, designated as $C3(H_2O)$. $C3(H_2O)$ mimics C3b and binds factors B and D in the presence of Mg^{2+} to generate the fluid phase C3 convertase, $C3(H_2O)Bb$. $C3(H_2O)Bb$ can then cleave native C3 to generate a metastable C3b molecule. Metastable C3b may randomly attach to surrounding "receptive" surfaces (5). On activating surfaces, the surface-bound C3b molecule is amplified in a C3b-dependent positive feedback loop by binding factors B and D to generate the alternative pathway C3 convertase, $\overline{C3bBb}$. On nonactivating surfaces (NA), the control proteins (I and H) cleave C3b and thereby inactivate the molecule.

Additional polymorphic variation has been found at the DNA level (44). Hence, C4 displays an unusually high degree of polymorphism.

C3 is an ~185,000 M_r glycoprotein composed of two disulfide-linked chains (reviewed in 16). The α and β chains of C3 have M_rs of ~115,000 and 78,000, respectively. There is one structural gene for C3. Two common electrophoretic variants, C3S (slow) and C3F (fast), and several rare variants have been identified (2).

Like the two C4 genes, C2 and B are structurally and functionally related complement components that probably also arose by gene duplication. B, C2, C4A, and C4B make up the class III genes of the major histocompatibility complex and tend to be inherited as a unit, termed complotype (3, 7). Several groups have suggested that these complotypes may play a pathogenetic role in major histocompatibility complex-associated diseases (e.g., 21-hydroxylase-deficiency congenital adrenal hyperplasia) (44). Moreover, an association of systemic lupus erythematosus with deficiencies of C4 and C2 suggests that lack of a classical pathway may predispose one to immune complex-mediated diseases (1). The underlying mechanism is probably secondary to an inefficient clearance mechanism since these individuals cannot activate the classical pathway to C3.

BIOSYNTHESIS

The liver is the primary site of synthesis of most complement proteins, including C3, C4, and C5. These three evolutionarily related proteins are synthesized as single-chain polypeptide precursors, designated pro-C3, pro-C4, and pro-C5, respectively (26, 27, 33; reviewed in 16 and 42a). The NH_2- to COOH-terminal order of the three subunits is β-α-γ in pro-C4 and β-α in pro-C3 and pro-C5. Pro-C4 requires two intracellular proteolytic cleavages (i.e., at the β-α and α-γ junctions) to yield the three-subunit molecule (Fig. 3). The DNA sequences of both mouse and human C3 and C4 and human C5 indicate that the subunit junctions have intervening sequences rich in basic amino acids which are excised as the precursor molecule is converted to the multisubunit molecule (5, 14, 16, 32, 43; A. B. Lundwall, R. A. Wetsel, K. Torsten, A. S. Whitehead, D. Woods, R. C.

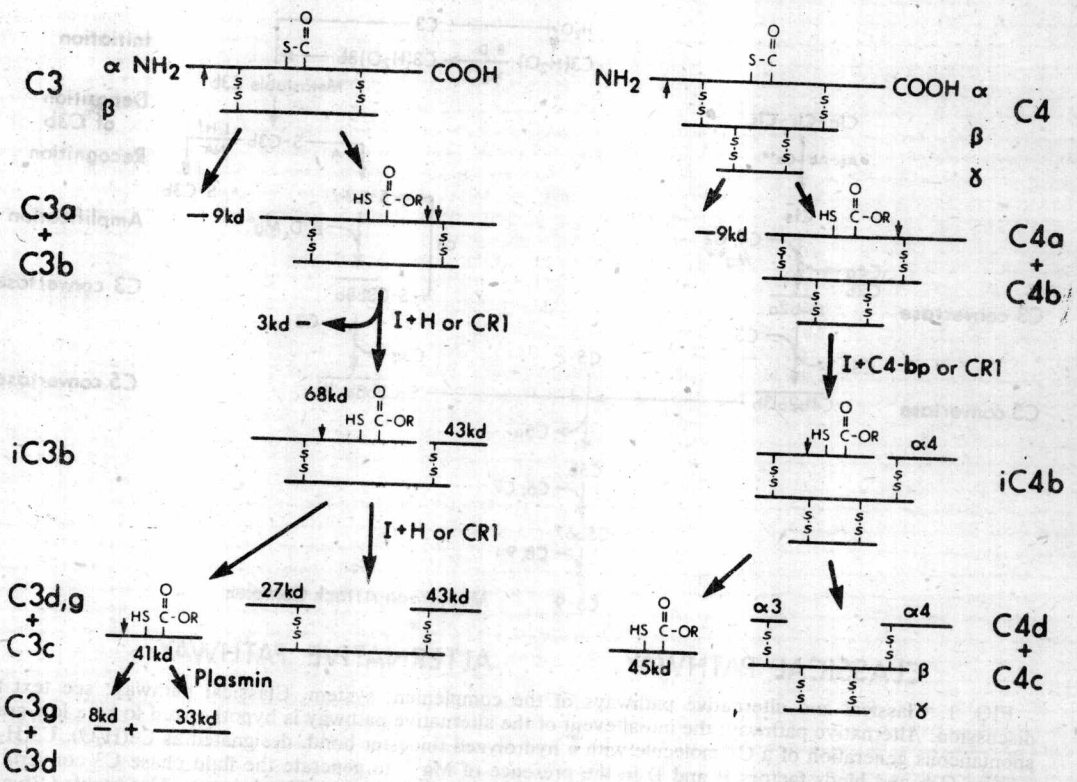

FIG. 2. Degradation of C3b and C4b by regulatory enzymes and cofactors I, C4-bp, and H. C3b and C3(H_2O), generated by the classical or alternative pathway convertases or by hydrolysis of the thioester bond, can be degraded by the regulatory enzymes, C3b/C4b inactivator (I) and factor H. In the presence of H or the C3b/C4b receptor (CR1), factor I initially cleaves at two sites (small arrows) on the α chain to release an ~3,000 M_r peptide from C3b. The remaining ~184,000 M_r iC3b molecule is composed of the β-chain disulfide linked to α-chain fragments with M_rs of ~68,000 and 43,000. The ~68,000 M_r α-chain fragment is further cleaved to give rise to an ~41,000 M_r C3d,g molecule and an ~27,000 M_r α-chain fragment. The latter fragment and the ~43,000 α-chain fragment remain disulfide linked to the β chain to form an ~143,000 M_r C3c molecule. CR1 and I appear to be responsible for this second cleavage under physiologic conditions. The C3d,g molecule is bonded through the thioester bond to the substrate. Noncomplement proteases further cleave an ~8,000 M_r C3g fragment from the NH_2 terminus of the C3d,g fragment to yield C3d. C4b undergoes a similar degradation scheme by C4-binding protein (C4-bp) and I. The α′ chain of C4b is cleaved into three fragments, designated α2, α3, and α4 (reviewed in 31). Whereas α2 or the C4d fragment is an ~44,000 M_r molecule that is released from the C4b molecule, α3 and α4 remain covalently bound to the β and γ chains through disulfide bonds to form the C4c molecule. The NH_2- to COOH-terminal order of the three fragments in the α′ chain is α3-α2-α4 (38).

Ogden, H. C. Colten, and B. F. Tack, Fed. Proc. 43:1492, 1984). For example, in pro-C4 the sequence of Arg-Lys-Lys-Arg appears just NH_2 terminal to the α chain, and the sequence of Arg-Arg-Arg-Arg appears just NH_2 terminal to the γ chain (5, 43). Porter and colleagues have suggested that these sequences may act as recognition sites for an endopeptidase with trypsin-like specificity followed by a carboxypeptidase B-like exopeptidase to yield the multichain molecules (5).

Failure of cleavage at either one of the two subunit junctions in pro-C4 will give rise to two-subunit, incompletely processed C4 molecules (see Fig. 3). Recent studies demonstrated that two C4-related glycopeptides with M_rs of ~168,000 and 125,000 are secreted in lesser quantities than, but with similar kinetics to, the three-subunit C4 molecule (9, 10; reviewed in reference 42a). Structural analyses indicate that the ~168,000 and ~125,000 M_r molecules represent the uncleaved βα and αγ peptides, respectively (9). Both βα and αγ peptides are not present on nonreduced gels, and hence the remaining cleaved γ and β chains are disulfide linked to their respective uncleaved subunits. These incompletely processed molecules are secreted in substantial quantities (10 to 40%) in culture by murine hepatocytes and macrophages and HEpG2 cells, but together make up only

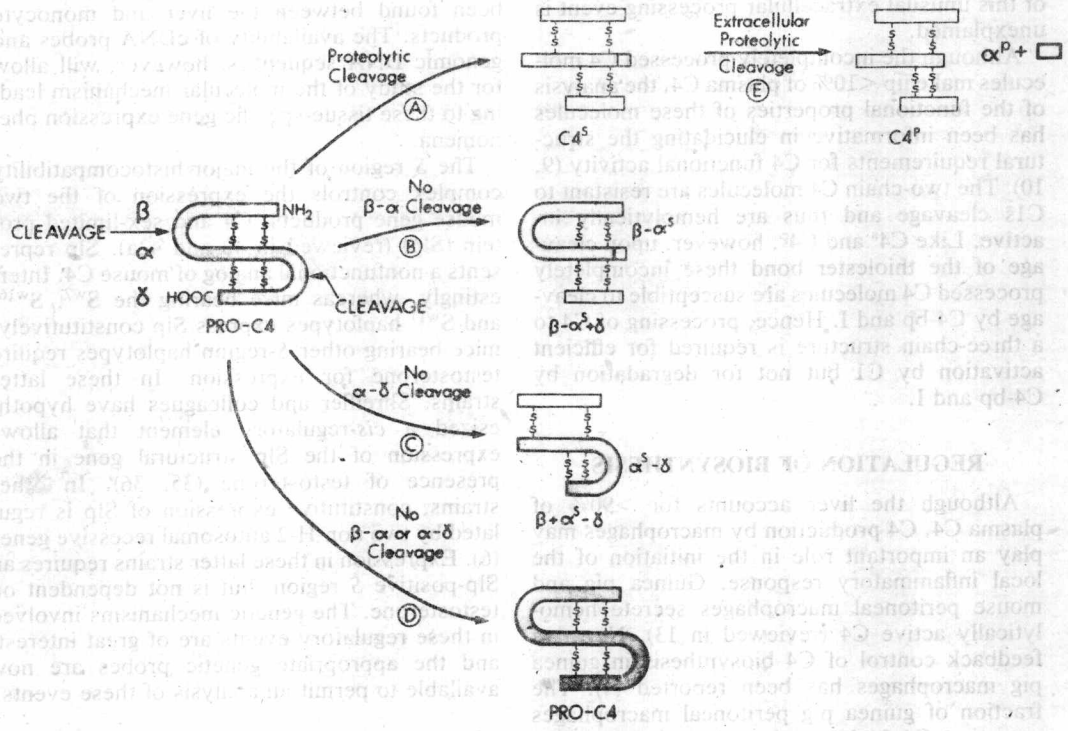

FIG. 3. Model of C4 processing (modified from 9). See text for discussion.

~4% of the circulating C4. This difference may be accounted for by a shortened half-life in vivo.

Murine macrophages and hepatocytes secrete various amounts of the two incompletely processed molecules (reviewed in reference 42a). The ratio of C4($\beta\alpha+\gamma$) to C4($\beta+\alpha\gamma$) is determined by their H-2 haplotypes (i.e., the *S* region of the mouse major histocompatibility complex where the C4 structural genes are located). The *cis*-dominant expression of these fragments in F_1 mice suggests that this incomplete cleavage of pro-C4 is due to an alteration in the structure of the C4 molecule rather than due to an *S*-region-linked protease. A mutation in the arginine-rich regions at the $\beta\alpha$ and $\alpha\gamma$ junctions may give rise to these inefficiently cleaved pro-C4 molecules.

Single-chain C3 and C4 molecules with M_rs of ~185,000 and ~200,000, respectively, are also secreted by HEpG2 cells (9, 26, 27). Structural analyses indicate that these molecules represent an extracellular form of pro-C4 and pro-C3. Pro-C4 is also found in plasma (~3% of the total C4) (17).

EXTRACELLULAR PROCESSING

C4 is secreted as a three-subunit molecule, designated C4ˢ ("s" for the secreted form of C4) (11, 23; reviewed in reference 42a). C4ˢ has an α chain with an M_r ~5,000 greater than that of the α chain of the predominant plasma form of C4, designated C4ᴾ (11, 23). C4ᴾ and C4ˢ constitute ~85% and 8%, respectively, of plasma C4 in mice and humans. The remaining 7% of plasma C4 is composed of incompletely processed C4 molecules (see above). The ~5,000 M_r difference between the secreted (C4ˢ) and major plasma (C4ᴾ) forms is in the ~20,000 M_r COOH-terminal fragment of the α chain of C4ˢ and is not due to carbohydrate. Upon secretion, an ~5,000 M_r propeptide is cleaved from the COOH-terminal portion of the α chain of C4ˢ to yield C4ᴾ. Three other evolutionarily related proteins, α_2 macroglobulin, C3, and C5, do not undergo this type of extracellular proteolytic processing (A. Chan, unpublished data).

FUNCTIONAL PROPERTIES OF C4ˢ AND C4-RELATED MOLECULES

Presumably, the extracellular processing of C4ˢ to C4ᴾ is of physiologic importance. However, data indicate that C4ˢ has functional properties similar to those of C4ᴾ, including: (i) an intact thioester bond, (ii) susceptibility to $\overline{C1s}$ cleavage, (iii) similar hemolytic efficiencies, and (iv) ability of C4bˢ and iC4ˢ to be degraded by C4-bp and I (10). Thus, the biologic significance

of this unusual extracellular processing event is unexplained.

Although the incompletely processed C4 molecules make up <10% of plasma C4, the analysis of the functional properties of these molecules has been informative in elucidating the structural requirements for C4 functional activity (9, 10). The two-chain C4 molecules are resistant to C1s cleavage and thus are hemolytically inactive. Like $C4^s$ and $C4^p$, however, upon cleavage of the thiolester bond these incompletely processed C4 molecules are susceptible to cleavage by C4-bp and I. Hence, processing of C4 to a three-chain structure is required for efficient activation by C1 but not for degradation by C4-bp and I.

REGULATION OF BIOSYNTHESIS

Although the liver accounts for >90% of plasma C4, C4 production by macrophages may play an important role in the initiation of the local inflammatory response. Guinea pig and mouse peritoneal macrophages secrete hemolytically active C4 (reviewed in 13). Negative feedback control of C4 biosynthesis in guinea pig macrophages has been reported (4). The fraction of guinea pig peritoneal macrophages secreting C4 is inversely proportional to the quantity of C4 present in culture. This inhibitory effect is specific for C4 and does not affect the secretion of other proteins, including C2. A decrease in C4 mRNA parallels this C4 inhibitory effect, and therefore regulation occurs at a pretranslational level. Such negative feedback control has not been demonstrated in mice, but activated peritoneal macrophages (by adherence or elicitation by pro-inflammatory agents) shut down their synthesis of C4 (29).

Another regulatory event controlled at the pretranslational level is the 10- to 20-fold differences in serum C4 levels among different mouse strains (reviewed in reference 42a). These quantitative differences are not due to alterations in C4 catabolism (30). Primary hepatocyte cultures from C4-high strains synthesize more C4 than cultures from C4-low strains (40). These differences are also paralleled by differences in hepatocyte C4 mRNA levels (12). Interestingly, macrophages from C4-high and C4-low strains synthesize similar amounts of hemolytically active C4 (30) and have similar levels of C4 mRNA (R. Sackstein and H. R. Colten, Fed. Proc. **43**:1747, 1984). Therefore, this regulatory element appears to be tissue specific.

Another tissue-specific phenomenon is the secretion of C3 by human peripheral blood monocytes (reviewed in 16). Monocytes secrete antigenically detectable, but functionally inactive, C3. To date, no structural differences have been found between the liver and monocyte products. The availability of cDNA probes and genomic DNA sequences, however, will allow for the study of the molecular mechanism leading to these tissue-specific gene expression phenomena.

The *S* region of the major histocompatibility complex controls the expression of the two mouse gene products, C4 and sex-limited protein (Slp) (reviewed in 42 and 42a). Slp represents a nonfunctional analog of mouse C4. Interestingly, whereas mice bearing the S^{w7}, S^{w16}, and S^{w17} haplotypes express Slp constitutively, mice bearing other *S*-region haplotypes require testosterone for expression. In these latter strains, Shreffler and colleagues have hypothesized a *cis*-regulatory element that allows expression of the Slp structural gene in the presence of testosterone (35, 36). In other strains, constitutive expression of Slp is regulated by two non-H-2 autosomal recessive genes (6). Expression in these latter strains requires an Slp-positive *S* region, but is not dependent on testosterone. The genetic mechanisms involved in these regulatory events are of great interest, and the appropriate genetic probes are now available to permit an analysis of these events.

OLIGOSACCHARIDE STRUCTURE AND FUNCTION

Oligosaccharide moieties have been demonstrated to be important in the function of glycoproteins. Recent studies of mouse C4 have demonstrated an association between carbohydrate structure and functional activity. Mouse C4 has several complex oligosaccharides on the α chain and a high-mannose oligosaccharide on the β chain (20–22). The γ chain is not glycosylated. The ~7,000 M_r difference between the α chains of the hemolytically active C4 molecule and the nonfunctional Slp molecule is due to two additional carbohydrate moieties on the latter (20, 22). Furthermore, the ~5,000 M_r difference between the C4 α chains of the $H\text{-}2^{w7}$ haplotype (which has about one-third the hemolytic efficiency) and other C4 alleles correlates with the absence of an oligosaccharide residue on the carboxyl-terminal portion of the $H\text{-}2^{w7}$ C4 α chain (21).

Recent work has demonstrated a similar carbohydrate structure for human C4 (A. C. Chan and J. P. Atkinson, J. Immunol., in press). Although the C4A and C4B gene products differ in their hemolytic efficiencies (19), no carbohydrate difference has been detected between the two molecules (19; Chan and Atkinson, in press). Whereas both the α and β chains of human C3 are glycosylated, only the α chain of mouse C3 is glycosylated (16). Among the vari-