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Comparative Biology of the α_2 -Macroglobulin-Based Immune System

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1 Introduction

Most multicellular organisms are subject during their lives to a nearly continual challenge by pathogenic microorganisms and multicellular parasites. In response, a variety of immune processes have evolved to suppress pathogens that have invaded the body. Some of these immune processes are phylogenetically unique. Perhaps the best-known example is the induced antibody response based on VDJ gene rearrangement of the immunoglobulin heavy and light chain genes, which apparently is present only in the vertebrates (Marchalonis and Schluter 1990). Other immune systems, such as the prophenoloxidase system (Söderhäll and Smith 1986), lysozyme (Engström et al. 1985), the pentraxins (Tennent et al. 1993), and α_2 -macroglobulin (Armstrong and Quigley 1995), are of more ancient origin, because they are present in modern representatives of diverse animal phyla. This chapter reviews the role of the α_2 -macroglobulin system in immunity.

α_2 -Macroglobulin is the signature member of a protein family that includes C3, C4, and C5 (Tack 1983; Sottrup-Gensen 1987), which are important components of the vertebrate complement system, pregnancy-zone protein (Christensen et al. 1989; Devriendt et al. 1991), which is an acute phase protein of mammals,

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and the α_2 -macroglobulin homologues found in arthropods (Armstrong and Quigley 1995) and mollusks (Armstrong and Quigley 1992; Bender et al. 1992; Thøgersen et al. 1992). Members of the α_2 -macroglobulin family share peptide sequence identity (Spycher et al. 1987; Sottrup-Jensen et al. 1990a; Thøgersen et al. 1992) and most members possess a unique reactive internal thiol ester domain that is discussed in more detail later. The α_2 -macroglobulins are soluble proteins that may be present at high concentration in the plasma⁴ and that are also found in the whites of the eggs of birds and reptiles (Ikai et al. 1983, 1990; Nagase et al. 1983).

The first function ascribed to plasma α_2 -macroglobulin was the binding and inhibition of proteases. The protease inhibitory mechanism of α_2 -macroglobulin is unique amongst enzyme inhibitors, because it involves the physical entrapment

Effect of Limulus Plasma Inhibitor on
Trypsin Hydrolysis of ^{14}C -Casein

Effect of Limulus Plasma Inhibitor on
Trypsin Hydrolysis of BAPNA

Fig. 1. Presence in *Limulus* plasma of a protease inhibitor that eliminates the caseinolytic activity of trypsin (*above*); but fails to inhibit the activity of trypsin against the low molecular mass amide substrate BAPNA (N^α -benzoyl-DL-arginine *p*-nitroanilide; *below*). Plasma was cleared of hemocyanin by ultracentrifugation and is referred to as HSS (high-speed supernatant). Hydrolysis of [^{14}C]-casein (*tp*) was determined by the release of acid-soluble [^{14}C]. Hydrolysis of BAPNA was determined by the increase in optical absorbance at 410 nm

⁴ Members of the α_2 -macroglobulin family of proteins comprise almost 5% of the plasma proteins of mammals, and α_2 -macroglobulin is the third most abundant protein in the plasma of the American horseshoe crab, *Limulus polyphemus*.

Table 1. Comparison of α_2 -macroglobulin with active site protease inhibitors

<i>α_2-Macroglobulin</i>		<i>Active site inhibitors</i>
Inhibits the proteolytic activity of proteases without inhibiting the hydrolysis of low molecular mass amide or ester substrates		Inhibits activity of target proteases against polypeptide and low molecular mass substrates
Reacts with endopeptidases of diverse catalytic mechanisms and substrate specificities		Reacts with a narrow spectrum of related proteases
Shields bound proteases from antibodies and high molecular mass active site inhibitors		Bound proteases remain reactive with antibodies
Presence of a unique internal reactive thiol ester group		Internal thiol ester is found only in proteins of the α_2 -macroglobulin family

of the protease molecule in a molecular cage that forms a stearic barrier that prevents contact of the entrapped protease molecule with protein substrates and leaves the active site of the enzyme intact (Barrett and Starkey 1973; Starkey and Barrett 1977). Ester and amide substrates of the bound protease that are small enough to diffuse into the α_2 -macroglobulin cage are readily hydrolyzed (Fig. 1; Bieth et al. 1970; Starkey and Barrett 1977). The protease-inhibitory mechanism of α_2 -macroglobulin stands in marked contrast to all other protease inhibitors, which bind to the active site and inhibit the activity of the target protease against both protein and low molecular mass amide and ester substrates (Laskowski and Kato 1980; Travis and Salvesen 1983). This is one of several diagnostic properties (Table 1) that initially allowed us to characterize a protease inhibitory activity that we identified in the plasma of the American horseshoe crab, *Limulus polyphemus*, as the first known invertebrate homologue of α_2 -macroglobulin (Quigley et al. 1982; Quigley and Armstrong 1983). Both the presence and function of the internal thiol ester group and the nature of the physical reorganization of α_2 -macroglobulin during interaction with proteases have attracted the attention of physical biochemists. The physiological functions of α_2 -macroglobulin have attracted the attention of immunologists and cell biologists.

2 Biochemistry of α_2 -Macroglobulin

The plasma α_2 -macroglobulins are monomers, dimers, and tetramers of a 180–185 kDa peptide, with the dimeric form (Fig. 2) being the most widely distributed (Starkey and Barrett 1982; Armstrong et al. 1991). All other protease inhibitors interact directly with the active site of the enzyme and inactivate its activity

Fig. 2 Scanning transmission electron microscopy of freeze-dried, unstained, uncoated α_2 -macroglobulin. **A** A low magnification view of a field of molecules; **B** a high magnification view of three α_2 -macroglobulin molecules. The elongated structure of the individual α_2 -macroglobulin molecules is consistent with a dimeric organization. The *cylindrical structure* in **A** is a tobacco mosaic virus particle, which was added to the sample as an internal calibration standard

against proteins and against low molecular mass ester and amide substrates (Laskowsky and Kato 1980; Travis and Salvesen 1983). Retention of the esterolytic and amidolytic activities of α_2 -macroglobulin-bound proteases can be shown by their continued ability to hydrolyze low molecular mass substrates small enough to diffuse into the α_2 -macroglobulin cage.

The enfolding of the targeted protease by the α_2 -macroglobulin polypeptide chain is initiated by a proteolytic cleavage of α_2 -macroglobulin at a specialized region, the bait region (Harpel 1973; Hall and Roberts 1978; Sottrup-Jensen et al. 1981, 1989). Most forms of α_2 -macroglobulin subsequently undergo a significant compaction as a manifestation of the trapping process (Barrett et al. 1979; Bjork and Fish 1982; Gonias et al. 1982; Armstrong et al. 1991). All active-site protease inhibitors are limited in the range of proteases that they inhibit (Laskowsky and Kato 1980; Travis and Salvesen 1983), but α_2 -macroglobulin is unique amongst protease inhibitors for its ability to interact with endopeptidases of diverse catalytic mechanisms and substrate specificities (Starkey and Barrett 1977). The bait region contains an ensemble of peptide bonds that provide in the aggregate at least one suitable cleavage site for most endopeptidases. This is the basis for the broad spectrum of reactivity of α_2 -macroglobulin against all manner of proteases

This internal thiol ester is unique to the members of the α_2 -macroglobulin family of proteins (Tack 1983; Sottrup-Jensen 1987). Prior to reaction with proteases, the thiol ester is protected from reaction with most nucleophilic substrates. However, small amines such as methylamine and ammonium can access the thiol ester and react to form covalent derivatives of the glutamyl residue (Fig. 3). This destroys the subsequent ability of the molecule to react with proteases (Barrett et al. 1979; Bjork and Fish 1982; Armstrong and Quigley 1987). Following the normal reaction with proteases, the thiol ester is exposed and the glutamyl residue reacts with nucleophiles in its immediate environment (Fig. 3; Tack et al. 1980; Sottrup-Jensen et al. 1980). Reaction with ϵ -amino and hydroxyl residues on the reacting protease allows α_2 -macroglobulin to establish covalent γ -glutamyl isopeptide bonds linking the protease to the peptide bearing the thiol ester domain (Fig. 3; Sottrup-Jensen et al. 1990b; Chen et al. 1992). C3 and C4 of the vertebrate complement system also experience activation of the internal thiol ester following proteolytic cleavage. Apparently, the thiol ester of C3 and C4 is directed away from the proteolytic cleavage site, because in this case, the isopeptide bonds link with external proteins and carbohydrate moieties, rather than with the reacting protease (Law and Levine 1977). The isopeptide bonding of C3 and C4 to external acceptors is essential for attachment to target particles such as invading microbes. Covalent binding of C3 marks foreign particles as targets for immune destruction by cytotoxicity and phagocytosis (Law and Reid 1988). Monomeric forms of plasma α_2 -macroglobulin, such as α_1 inhibitor-3, also require isopeptide bonding, this time with the reacting protease, to bind protease (Enghild et al. 1989a). Although α_2 -macroglobulin cannot bind proteases if its thiol ester has been reacted with methylamine (Steinbuch et al. 1968; Swenson

Fig. 4. Pore-limit polyacrylamide gel electrophoresis of human and *Limulus* α_2 -macroglobulin. Unreacted tetrameric human α_2 -macroglobulin (lane 1) shows a slower mobility in the gel than trypsin-reacted human α_2 -macroglobulin (lane 2). Similarly, unreacted dimeric *Limulus* α_2 -macroglobulin (lane 3) shows a slower mobility than after reaction with trypsin (lane 4), chymotrypsin (lane 5), or methylamine (lane 6).

and Howard 1979; Nielsen et al. 1994), the physiological function of thiol ester-mediated isopeptide bonding of multimeric forms of plasma α_2 -macroglobulin is less clear, because variant forms of α_2 -macroglobulin that lack the thiol ester, such as chicken ovomacroglobulin, bind proteases perfectly well (Kitano et al. 1982; Nagase and Harris 1983; Feldman and Pizzo 1984; Nielsen et al. 1994), and proteases such as pancreatic elastase, which lacks ϵ -amino groups, and that consequently is unable to establish isopeptide bonds with the γ -carbonyl of the activated thiol ester is, nevertheless, successfully bound by multimeric forms of α_2 -macroglobulin (Salvesen et al. 1981; Van Leuven et al. 1981). Also, *Limulus* α_2 -macroglobulin establishes isopeptide bonds exclusively within the peptide chains of α_2 -macroglobulin itself, and fails to bond covalently with the enfolded protease (Quigley et al. 1991).

The refolding of α_2 -macroglobulin following cleavage at the bait region results in a significant compaction of the molecule that has been documented as

Fig. 5. Gel-filtration chromatography of unreacted and chymotrypsin-reacted α_2 -macroglobulin. Samples of unreacted and chymotrypsin-reacted *Limulus* α_2 -macroglobulin were applied to a TSK G4000SW gel permeation column and eluted with 0.05 M phosphate buffer. The protease-reacted sample was significantly retarded, indicative of a compaction of the molecule. The calibration bars above the figure indicate the elution positions of native and chymotrypsin-reacted tetrameric (human α_2 M and ovomacroglobulin) and dimeric (hagfish α_2 M) forms of α_2 -macroglobulin. The column was further calibrated with tetrameric, dimeric, and monomeric ovomacroglobulin. The dimeric forms of α_2 -macroglobulin (e.g., *Limulus* and hagfish α_2 -macroglobulin) show a more pronounced compaction as estimated by the separation of the elution profiles of native and reacted forms than do the tetrameric varieties

an increase in electrophoretic mobility by pore-limit electrophoresis (Fig. 4; Barrett et al. 1979; Armstrong et al. 1991), by a retardation of the molecule by gel filtration chromatography (Fig. 5; Armstrong et al. 1991), by electron microscopy (Fig. 6; Armstrong et al. 1991; Delain et al. 1992), and by a variety of physical techniques including small angle X-ray scattering (Arakawa et al. 1994). Interestingly, dimeric forms of α_2 -macroglobulin characteristically experience a more

Fig. 6A-D. Transmission electron microscopy of individual molecules of negatively stained *Limulus* α_2 -macroglobulin. A, B Low-magnification views, respectively, of fields of native and chymotrypsin-reacted *Limulus* α_2 -macroglobulin molecules. C, D High-magnification views, respectively, of individual molecules of native (C) and chymotrypsin-reacted (D) *Limulus* α_2 -macroglobulin. The native molecule of *Limulus* α_2 -macroglobulin is relatively extended and resembles a butterfly, whereas the chymotrypsin-reacted molecule is significantly more compact.

profound compaction than tetrameric α_2 -macroglobulin (Fig. 5; Armstrong et al. 1991). Characterization at the atomic level of the configurational changes of proteolyzed α_2 -macroglobulin is not yet available. Recent success in crystallizing the protein indicates that the high-resolution picture available from X-ray diffraction analysis is possible (Andersen et al. 1991, 1994).

3 Physiological Function of α_2 -Macroglobulin

As emphasized previously, one of the principal functions of α_2 -macroglobulin is to bind and inhibit a diverse array of endopeptidases that appear in the body fluids (Sottrup-Jensen 1987). Proteases play important roles in a variety of immune processes, including blood clotting and clot resolution (Furie and Furie 1992; Iwanaga et al. 1992), complement activation (Reid and Porter 1981), inflammation (Cohn 1975; Haverman and Janoff 1978), and tissue remodeling (Werb 1993). Proteases, whether of endogenous or exogenous origin, have the potential for serious destructive effects on the surrounding tissues after their release into the tissue spaces. Proteases contribute to a variety of pathological conditions such as tumor dissemination (Testa and Quigley 1990) and a variety of degenerative connective-tissue disorders, which are directly traceable to the activities of proteases present in the wrong places and at the wrong times (Perlmutter and Pierce 1989). Additionally, proteases are important agents facilitating the invasion of parasites (Breton et al. 1992; McKerrow et al. 1993). The unique ability of α_2 -macroglobulin to react productively with all manner of endopeptidases enables it to back up the array of more specialized active-site inhibitors that may be present in the circulation and to capture endopeptidases for which the animal may lack a reactive active-site inhibitor. For example, the horseshoe crab appears to lack circulating active-site inhibitors of serine proteases (Quigley and Armstrong 1983). Instead, in this organism, α_2 -macroglobulin serves as the principal barrier to the persistence of serine proteases in the blood (Melchior et al. 1995).

In mammals (Van Leuven 1984) and in *Limulus* (Melchior et al. 1995), the interaction of proteases with α_2 -macroglobulin initiates a receptor-mediated clearance pathway that rapidly removes the α_2 -macroglobulin-protease complex from the plasma. In mammals, α_2 -macroglobulin is but one of a diverse array of protease inhibitors in the plasma (Laskowski and Kato 1980; Travis and Salvesen 1983), whereas in *Limulus* it is only plasma inhibitor involved in protease clearance (Quigley and Armstrong 1983; Melchior et al. 1995). Both in mammals and in *Limulus*, the cell surface receptors ignore native unreacted α_2 -macroglobulin, but rapidly bind protease-reacted α_2 -macroglobulin. The conformational changes of α_2 -macroglobulin following proteolysis of the bait region result not only in an enfolding of the protease molecule, but also expose a previously hidden recognition domain close to the carboxyl terminus of the α_2 -macroglobulin chain that is recognized by the cell surface receptor (Sottrup-Jensen et al.

1986; Van Leuven et al. 1986; Enghild et al. 1989b; Thomsen and Sottrup-Jensen 1993). The clearance time of active proteases introduced into the circulation of mammals is short, typically 5–10 min (Ohlsson 1971). Clearance times are longer in *Limulus*, with half clearance times of 20–25 min, but this appears primarily to be a consequence of a more sluggish circulation of the blood in that animal and a consequently slower initial mixing of the injected protease with the blood (Melchior et al. 1995). In mammals, clearance is primarily to hepatocytes (Davidson et al. 1985; Van Dijk et al. 1992; Ogata et al. 1993), whereas the blood cells are the principal cells involved in clearance in *Limulus* (Melchior et al. 1995). Both in *Limulus* and in mammals, the receptor-bound α_2 -macroglobulin-protease complex is internalized and degraded in secondary lysosomes (Van Leuven 1984; Melchior et al. 1995). Based on these observations, it has been suggested that one of the primary functions of α_2 -macroglobulin is to serve as a protease carrier molecule with the capabilities of binding endopeptidases of all possible enzymatic mechanisms and from all possible endogenous and exogenous sources to thereby render the bound proteases sensitive to removal from the body fluids by the receptor-mediated endocytotic pathway. In this sense, α_2 -macroglobulin is thought to serve as a broad-spectrum recognition system for the identification and marking of proteases for their subsequent endocytosis and destruction in secondary lysosomes.

The mammalian cell surface receptor for α_2 -macroglobulin has recently been identified as low-density lipoprotein receptor-related protein (LRP), a transmembrane protein related to the well-characterized low-density lipoprotein receptor (Kristensen et al. 1990; Strickland et al. 1990). Interestingly, this protein is a receptor for a variety of dissimilar ligands including plasminogen activator/plasminogen activator inhibitor-1 complexes, lipoprotein lipase, lactoferrin, *Pseudomonas* exotoxin A, and apolipoprotein E-rich chylomicron remnant (Hussain et al. 1991; Moestrup et al. 1993; Nykjaer et al. 1993; Andreasen et al. 1994). LRP is, like α_2 -macroglobulin itself, an ancient protein that was present prior to the great evolutionary radiation that established the divergent deuterostome and protostome superphyla because it has been found in modern representatives of lineages that diverged at the time of the Precambrian radiation, notably in vertebrates and the nematode, *Caenorhabditis elegans* (Yochem and Greenwald 1993). It has not been established whether invertebrate homologues of LRP participate in the clearance of α_2 -macroglobulin-protease complexes, but a protein has been identified in detergent extracts of *Limulus* blood cells that has recognition capabilities that makes it a likely candidate for the α_2 -macroglobulin receptor (Aimes et al. 1995).

In addition to its activity as a protease-binding molecule, α_2 -macroglobulin is also capable of binding a variety of other proteins, most notably several of the peptide mitogens (James 1990; Borth 1992; Gonias 1992). In some cases, the binding of peptide mitogens to α_2 -macroglobulin has been shown to require the activation of the thiol ester and involves the establishment of a disulfide linkage via the exposed thiol of the cysteinyl residue of the activated thiol ester (Borth and Luger 1989). The binding of cytokine growth factors has proven interesting

because certain cytokines have been found to be complexed with α_2 -macroglobulin in the serum of mammals, suggesting that the interaction is physiologically significant (Huang et al. 1984; O'Connor-Court and Wakefield 1987). Perhaps binding to α_2 -macroglobulin represents one of the several buffering systems that regulate and restrain cytokines. In some situations, the binding to α_2 -macroglobulin inhibits the mitogenic activities of the growth factor and in other cases it does not (Borth 1992). Also, it must be remembered that serine proteases, which are primary targets of α_2 -macroglobulin, are potent mitogens, which interact with a dedicated G-protein-coupled surface receptor (Vu et al. 1991). Although the best-described interaction of the different growth factors with the target cells is via specific cytokine receptors (Sporn and Roberts 1990), the frequent association of circulating cytokines and mitogenic proteases with α_2 -macroglobulin established the possibility that productive mitogenic interaction might also occur via the LRP/ α_2 -macroglobulin receptor. Thus, when exploring the functions of α_2 -macroglobulin in invertebrates, it is important to extend our vision beyond the specifics of α_2 -macroglobulin-protease interaction and to consider the possibility that proteases may not be the only important molecules whose functions are modulated by binding to α_2 -macroglobulin. Indeed, as will be described below, protease-reacted forms of α_2 -macroglobulin function as negative regulators of a plasma-based cytolytic system in *Limulus*.

One of the important immune defense strategies of higher animals is to kill invading pathogenic organisms by inducing their cytolysis in the blood. In vertebrates, cytolysis is mediated by the complement system, which is comprised of an ensemble of serially activated effector proteins and a number of receptors and regulatory proteins (Law and Reid 1988). The key event in the activation of the mammalian complement system is the covalent binding of C3 to the surfaces of target cells, thereby marking them for destruction by cytolysis and phagocytosis. Binding involves the covalent bonding of the γ -carbonyl of the glutamyl residue of the reactive internal thiol ester of the C3 molecule with hydroxyl and amino residues at the surface of the target particle (Law and Levine 1977).

Invertebrates also have plasma- or hemocyte-based cytolytic systems (Canicatti 1990), but none have been shown unequivocally to resemble the vertebrate complement system at the molecular level. The plasma cytolytic system of the horseshoe crab, *Limulus polyphemus*, shows a superficial similarity to the vertebrate system because it employs members of two protein families involved in the vertebrate complement system, namely, α_2 -macroglobulin and a member of the pentraxin protein family (Engchild et al. 1990; Armstrong et al. 1993). As mentioned previously, C3 and α_2 -macroglobulin are members of the same family of proteins, based on peptide sequence homology and the presence of the reactive thiol ester (Tack 1983; Sottrup-Jensen 1987). Inactivation of the thiol ester of C3 by treatment with methylamine inactivates the cytolytic activity of vertebrate plasma and a similar inactivation of α_2 -macroglobulin by methylamine treatment significantly reduces the cytolytic activity of *Limulus* plasma (Engchild et al. 1990; Armstrong et al. 1993). Activity is restored to methylamine-treated *Limulus* plasma by addition of *Limulus* α_2 -macroglobulin (Fig. 7).

HEMOLYTIC ACTIVITY OF *LIMULUS* PLASMA

Fig. 7. Cytolytic activity of *Limulus* plasma that has been depleted of hemocyanin by ultracentrifugation ($120\,000 \times g$, 16 h). Washed sheep erythrocytes were incubated with increasing amounts of *Limulus* plasma at room temperature for 4 h, and the extent of hemolysis was determined by measuring the quantity of hemoglobin released into the incubation buffer (optical absorbance at 412 nm). Untreated plasma (filled squares) shows a dose-responsive hemolytic activity that is diminished in plasma pretreated with methylamine (open square) to inactivate α_2 -macroglobulin. The hemolytic activity of methylamine-treated plasma is restored by addition of $2\,\mu\text{M}$ *Limulus* α_2 -macroglobulin (circle).

The initial interpretation of these observations was that α_2 -macroglobulin plays an essential role as an effector of cytolysis, perhaps similar to the role of C3 in the mammalian complement pathway (Engild et al. 1990). This, however, does not appear to be the case. The *Limulus* cytolytic system does not show the absolute requirement for α_2 -macroglobulin in the manner that the vertebrate system does for C3 (Armstrong et al. 1993). Instead, the actual cytolytic protein of the *Limulus* system is a member of the pentraxin protein family, namely, the lectin limulin (Srimal et al. 1993; Armstrong et al. 1994a). In mammals, members of the pentraxin protein family can activate the classical pathway of the complement system (Miyazawa and Inoue 1990; Jiang et al. 1991). Highly purified limulin is cytolytic alone at physiological concentrations and limulin-free plasma is without cytolytic activity (Armstrong et al. 1994a). Unactivated *Limulus* α_2 -macroglobulin does not increase the cytolytic activity of limulin. Instead, protease- or methylamine-activated forms of α_2 -macroglobulin function in the *Limulus* cytolytic system as inhibitors of the cytolytic activity of limulin (Armstrong et al. 1994a). Although *Limulus* α_2 -macroglobulin does not serve as a functional equivalent of C3, the inhibitory role of protease-activated α_2 -macroglobulin may

be of importance in restraining the cytolytic activities of limulin to prevent unwanted damage to host tissues. It is to be remembered that the vertebrate complement system includes a spectrum of regulatory factors that serve exactly that function. Thus, *Limulus* employs molecular homologues to components of the mammalian complement system in its immune defense, but without their serving as exact functional homologues.

4 Evolutionary Considerations

Systems of immunity can be characterized as adaptive or innate. The former are characterized by inducibility and selectivity for the inducing challenge and often have the property of immunologic memory. The latter are expressed constitutively and are of broader reactivity. Probably the best-characterized immune system is the adaptive, immunoglobulin-based immune system of vertebrates. In many people's minds, immunology is solely about the study of the immunoglobulin-based immune system and functionally connected systems such as the complement system. In truth, serious compromise of the adaptive antibody-based immune system of vertebrates is typically followed by illness and death, often at the hands of an indolent pathogen that would be of little threat to the uncompromised individual (Waldman 1988). Although characteristic immunoglobulin protein motifs are of ancient origin, being present in invertebrates and vertebrates (Grenningloh et al. 1990), the system of immunoglobulin gene rearrangement to construct a large number of immune proteins from a small number of genes is unique to vertebrates (Marchalonis and Schluter 1990). That the immunoglobulin-based immune system has not been unambiguously identified in an invertebrate suggests that this system evolved after the Precambrian divergence of the evolutionary lineages leading to the vertebrates and the diverse invertebrate phyla. In this context, it is interesting that invertebrates survive, often in highly septic environments, without this particular system of immunity. Although the life cycle of many invertebrates is completed in only weeks or months, individuals of other species may live for many years. 17-Year cicadas live for 17 years, horseshoe crabs live at least 20 years, lobsters may live for 50 or more years, and freshwater clams live for 80–100 years (Comfort 1961; Finch 1990)—and without antibodies or lymphocytes. It is clearly possible to fashion a highly effective immune system without resorting to the complex and sometimes self-destructive antibody-based system that we see in the vertebrates. Instead, invertebrates depend mostly on a variety of innate immune systems to defend against pathogenic parasites.

Interestingly, several elements of the innate immune system are common to invertebrates and vertebrates. α_2 -Macroglobulin is certainly an ancient component of internal defense because it is present in all classes of vertebrates (Starkey and Barrett 1982; Osada et al. 1986), mandibulate and chelicerate arthropods (Armstrong and Quigley 1995), and cephalopod, gastropod, and

bivalve mollusks (Armstrong and Quigley 1992; Thøgersen et al. 1992). The forms of α_2 -macroglobulin found in invertebrates share many of the unique features of vertebrate α_2 -macroglobulin including the presence of the reactive internal thiol ester (Armstrong and Quigley 1987; Spycher et al. 1987; Sottrup-Jensen et al. 1990a), the ability to interact with proteases of various catalytic mechanisms (Quigley and Armstrong 1985), and an interaction that involves the physical envelopment of the protease molecule by α_2 -macroglobulin that leaves the enzymatic site intact (Quigley and Armstrong 1983, 1985; Armstrong et al. 1985; Armstrong and Quigley 1987). Because the vertebrate, arthropod, and molluscan lineages diverged in evolution approximately 550 million years ago (Sepkoski 1978; Runnegar 1982), the presence of α_2 -macroglobulin in modern representatives of these three classes of animals indicates its antiquity and demonstrates that the molecule must have evolved prior to the Precambrian evolutionary radiation of the modern phyla of higher animals. The preservation of this unique molecule for so extended a period of evolution in forms as distinctively different as arthropods, mollusks, and vertebrates indicates that it plays an essential role in the survival of higher animals.

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