haustion and extensive replacement of thymus stem cells in mammals is wanting (*14*). Our position is that the thymus, at least in the frog, is an organ in which lymphopoiesis is genuinely self-sustaining. The thymus is the stem cell source of the diverse populations of peripheral antigen-reactive lymphocytes (T and B cells). Given the initially large indigenous population of stem cells in the developing thymus, there is no compelling reason to suppose that the functional thymus loses its reserve of undifferentiated, or immature, lymphoid cells. We suggest, therefore, that there is a self-perpetuating population of lymphoid stem cells in the intact thymus, and that such a resident population is neither replenished nor replaced by immigrant stem cells from the bone marrow. The important implication is that normal thymic involution in aging frogs is associated with a progressive impairment of the capacity to mount immune responses.

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4. The formation of the second polar body is suppressed when the egg, 5 minutes after insemination, is subjected to hydrostatic pressure of 5000 lb/in² (350 kg/cm²) for 6 minutes. The resulting embryo is triploid. One set of chromosomes is paternal (the contribution of the sperm nucleus) and two sets are maternal (the normal maternal haploid contribution and the second polar body) [S. Dasgupta, *J. Exp. Zool.* **151**, 105 (1962)].
5. Stages of embryonic development of the frog are described in W. Shumway, *Anat. Rec.* **78**, 139 (1940). At the tail-bud stage (stage 17), there is no differentiation of blood cells.
6. The anterior and posterior halves are immunologically unresponsive to each other inasmuch as each half is essentially a massive, tolerance-inducing graft.
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8. In the strictest interpretation of the experimental results, the only unconditional conclusion is that the spleen and bone marrow are colonized by lymphoid cells from the anterior portion of the chimeric embryo, not necessarily the thymus gland. It is reasonable, however, to implicate the thymus gland as the specific source of the colonizing lymphoid cells [see also (*2*)].
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12. The conventional monophyletic concept is that the precursors of lymphoid, myeloid, and erythroid cell lines are derived from a single population of pluripotential stem cells. In anurans, however, it appears that lymphocytes and erythrocytes have different origins, the former arising in situ from elements in the thymic rudiment itself and the latter originating from blood-borne elements in the ventral blood islands. Our data thus favor the polyphyletic theory that recognizes two or more separate types of blood stem cells [see also J. G. Hollyfield, *Dev. Biol.* **14**, 461 (1966)].
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Neisseria gonorrhoeae and *Neisseria meningitidis*: Extracellular Enzyme Cleaves Human Immunoglobulin A

Abstract. *The gonococcus and meningococcus, which infect human mucosal surfaces, elaborate a highly specific proteolytic enzyme which cleaves the immunoglobulin A1 subclass of the principal mucosal antibody, immunoglobulin A (IgA). The susceptible Pro-Thr bond lies in a unique region of the IgA heavy chain; the IgA2 subclass, lacking this peptide bond, is enzyme resistant.*

Neisseria gonorrhoeae and *Neisseria meningitidis* are gram-negative diplococci that are strictly pathogenic for human beings in whom they cause gonorrhea and meningitis, respectively. In the initial stages of infection these microorganisms colonize mucosal surfaces and penetrate the epithelial membrane. In human beings the mucosal surfaces lining the respiratory, alimentary, and urogenital tracts have an elaborate immune system characterized by the secretion of antibody of the immunoglobulin A (IgA) class (*1*). Secretory IgA is synthesized by immunocompetent cells lying in the lamina propria of the mucosa beneath the epithelium, and these cells respond with antibody production to many microbial and macromolecular antigens present at the mucosal surface (*1*). The situation following gonorrheal infection is somewhat paradoxical, however, for although antigonococcal antibody of the secretory IgA type appears in the urethral secretions of patients recovering from infection, reinfection with the gonococcus may occur despite this seemingly adequate immunologic response (*2*). We report here that clinical isolates of *N. gonorrhoeae* and four groups of *N. meningitidis* grown in in vitro culture elaborate extracellular, highly specific, proteolytic enzymes that cleave human serum and secretory IgA to yield Fcα and Fabα fragments. This appears to be the first identification of extracellular enzymes from the pathogenic *Neisseria*, and their specificity for human IgA suggests that they may play a role in neisserial infections.

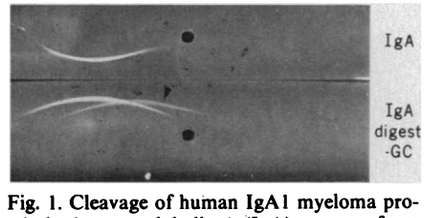

Fig. 1. Cleavage of human IgA1 myeloma protein by immunoglobulin A (IgA) protease from *Neisseria gonorrhoeae* (immunoelectrophoresis). The top well contains the purified, intact IgA; the bottom well shows the same protein following exposure to gonococcal enzyme (*GC*) for 6 hours at 37°C. The antiserum in the trough is unabsorbed goat antiserum to human IgA. The arrow indicates the Fcα fragment, which shows a precipitin reaction of immunologic nonidentity with the other fragment, Fabα. The anode is to the right.

Clinical isolates of *N. gonorrhoeae* (Kellogg colony types 1, 2, 3, and 4) and *N. meningitidis* (groups A, B, C, and Y) were examined for enzyme production. The organisms were inoculated into Difco GC base supplemented with Isovitalex (BBL, Cockeysville, Md.) and incubated, with gentle swirling, for 24 hours at 37°C under an atmosphere of expired air introduced by blowing through a sterile pipette plugged with cotton. The culture was centrifuged, the bacterial mass discarded, and proteins precipitated from the clarified supernatant by the addition of solid ammonium sulfate to a final concentration of 60 percent. The precipitate from 100 ml of supernatant was resuspended in 4 or 5 ml of phosphate buffer (*p*H 7.5, 0.05*M*), dialyzed against the same buffer, and examined for enzyme activity by incubation at 37°C with human IgA myeloma proteins at a concentration of 10 mg/ml in phosphate buffer. Serum IgA protein substrates of both subclasses, IgA1 and IgA2, were isolated from the plasma of patients with multiple myeloma, and colostral secretory IgA was isolated from human milk obtained 1 month postpartum (*3*). Proteolytic cleavage of IgA was detected by cellulose acetate electrophoresis, immunoelectrophoresis in which rabbit antiserums of known specificity were used, and by disc gel electrophoresis in 5 percent polyacrylamide gels, *p*H 9.5; these methods have been described previously in detail (*4*). Bacterial culture medium that had been incubated without the addition of bacterial inoculum was similarly treated to serve as a control.

Culture filtrates of the four colony types of *N. gonorrhoeae* and all four groups of *N. meningitidis* contained enzyme that rapidly cleaved all six human myeloma IgA proteins of the IgA1 subclass to yield two large fragments; a typical digest examined by electrophoresis is shown in Fig. 1. The IgA fragments were identified as Fabα and Fcα by criteria previously outlined (*4*, *5*), including electrophoretic mobility, reactivity with antiserums specific for light or heavy chains, and approximate molecular weight as determined by molecular sieve chromatography. Neither the gonococcal nor meningococcal enzymes were able to cleave any of a group of four human myeloma proteins of the IgA2 subclass. In addition, the enzymes could not

cleave human immunoglobulin G, M, or E paraproteins, mouse and dog IgA myeloma proteins, pooled rabbit serum IgA, or a variety of other standard protease substrates including gelatin, casein, bovine hemoglobin, and collagen. The oxidized β-chain of insulin, ordinarily susceptible to all other well-characterized microbial extracellular neutral proteases (*6*), was also not cleaved by the neisserial enzymes. The enzymes readily cleaved human secretory IgA to yield typical Fabα and Fcα fragments. We have not yet determined the relative susceptibility of the IgA1 and IgA2 subclasses in secretory IgA. Both enzymes were fully inhibited in the presence of 50 mm of ethylenediaminetetraacetate, indicating a heavy metal requirement for the expression of catalytic activity.

The *Neisseria* strains were examined for IgA protease production as an extension of earlier work with human oral *Streptococcus sanguis* species (*4, 7*). Among streptococci, IgA protease has been identified only in those of Lancefield group H, other strains being negative (*8*). *Streptococcus sanguis* IgA protease cleaves the IgA1 heavy chain at a Pro-Thr peptide bond in a stretch of amino acids composed entirely of prolyl, threonyl, and seryl residues as shown in Fig. 2 (*9, 10*). This enzyme also is inactive against IgA2 proteins, presumably because the threonyl component of the enzyme-sensitive peptide bond is included in a 13-residue amino acid deletion characteristic of the IgA2 hinge region (Fig. 2). To determine whether the gonococcal enzyme cleaved a bond similar to that attacked by the streptococcus, the Fcα fragment was purified (*4*) from a gonococcal digest of human IgA1 myeloma protein and subjected to limited NH_2-terminal amino acid sequencing by using Edman degradation on a Beckman sequencer (*11, 12*). The sequence obtained was as follows: -Thr-Pro-Ser-Pro-Ser-Cys-Cys-His-Pro-Arg-. Alignment of this sequence with that of the IgA1 heavy chain (*10*) localized the site of cleavage to a Pro-Thr peptide bond in the hinge region (Fig. 2). The gonococcal-sensitive Pro-Thr lies in a region of the IgA1 hinge that is deleted in the IgA2 molecule, affording a likely explanation for the insensitivity of the IgA2 subclass to this enzyme, as shown in Fig. 2. Of interest was that the Pro-Thr peptides cleaved by the gonococcal and streptococcal enzymes were different. Figure 2 also shows that each cleaves a Pro-Thr peptide bond in only one of the duplicated segments of the hinge. Surprisingly, neither enzyme is active against the Pro-Thr split by the other, since the sequence data on both the *S. sanguis* and gonococcal Fcα preparations were homogeneous.

These findings demonstrate that two distinct genera of microorganisms elaborate highly active proteolytic enzymes with specificity for human IgA proteins of the IgA1 subclass. The resistance of IgA2 proteins can be attributed to the absence of the susceptible peptide bonds resulting from the deletion of the IgA2 hinge region. Despite the proximity of the two susceptible Pro-Thr bonds to each other and the similarity of their surrounding residues, each enzyme is unusually selective, which indicates that specificity is highly sensitive to substrate conformation or is dependent on the carbohydrate bound to the IgA1 hinge (*13*). The suggestion has been made that the hinge is under separate genetic control and represents a segment of DNA, possibly of episomal origin, which has been integrated into the genes coding for immunoglobulins (*10, 14*). Support for this contention comes from a comparison of the

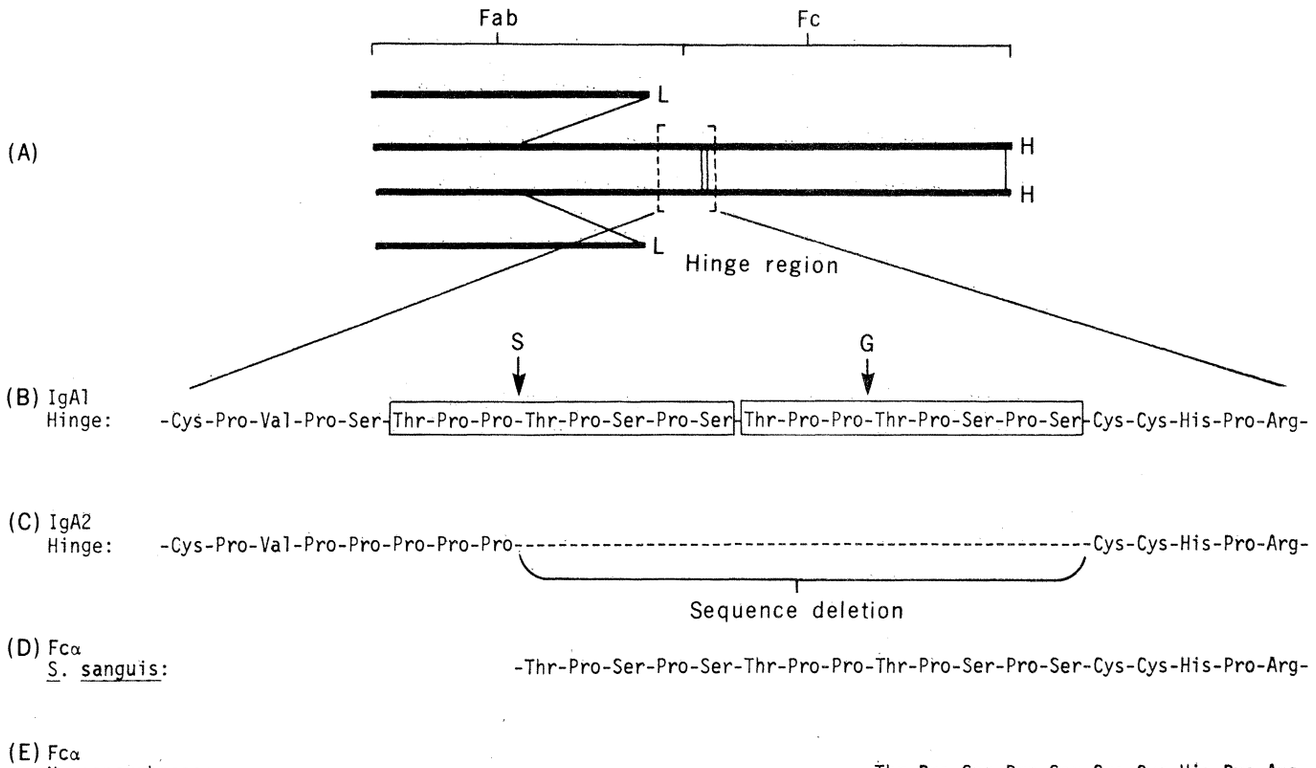

Fig. 2. The hinge region of human immunoglobulin A and the cleavage products of the two extracellular bacterial enzymes. (A) Schematic diagram of the human IgA1 monomer (molecular weight, about 170,000) showing heavy (*H*) and light (*L*) polypeptide chains linked by disulfide bridges, and the Fab (antigen binding) and Fc regions of the molecule. The enzyme sensitive hinge region, in brackets, is expanded below. (B) Amino acid sequence of the IgA1 hinge region (*10*). The characteristic sequence duplication is shown in blocks, and the arrows indicate the Pro-Thr bond cleaved by the *S. sanguis* (*S*) and *N. gonorrhoeae* (*G*) enzymes, respectively. (C) The hinge region of a human IgA2 protein aligned with IgA1 (above) showing the sequence deletion characteristic of the IgA2 subclass. The deletion, which begins at threonine and thus involves both peptide bonds susceptible to the bacterial enzymes, affords an explanation for the enzyme resistance of IgA2 molecules. (D and E) Limited NH_2-terminal sequences of the IgA1 Fc fragment produced by the *S. sanguis* and *N. gonorrhoeae* enzymes, respectively. Alignment with the IgA1 sequence above localizes the bond cleaved by each enzyme and illustrates the specificity each has for only one of the Pro-Thr bonds in the duplicated segments.

heavy chain sequences of human IgA and IgM, which exhibit progressively increasing homology from the first to the last (COOH-terminal) constant region domains interrupted by the hinge region, where there is virtually no homology.

In human beings, only 10 percent of serum IgA but up to 50 percent of colostral IgA is of the IgA2 subclass (*15*), data which have led us to suggest (*4*) that the relative enrichment of secretions with the IgA2 subclass could confer selective advantage through its resistance to microbial IgA protease. This hypothesis is strengthened by the findings reported here that two unrelated microorganisms produce enzymes of nearly identical specificity for the hinge region of IgA1 proteins. An alternative interpretation would be that the IgA1 subclass evolved from IgA2 and that microorganisms encountering secretory IgA1 underwent mutation manifested by IgA protease production. This issue cannot be resolved with the data available.

If *N. gonorrhoeae* elaborates this IgA-destructive enzyme when infecting human beings, the production of the enzyme may be of clinical importance. Since this agent can infect individuals having apparently adequate secretory (urethral) antibody (*2*), one may speculate that IgA protease may allow the organisms to resist immune attack. However, the resistance of secretory IgA2 subclass proteins to the enzyme would raise theoretical objections to such a concept although the relative concentrations of the two subclasses has not been reported for the human urogenital tract, and thus the dominance of IgA2 at such mucosal sites must be considered an unproved assumption.

Finally, among the gonococcal colony forms, types 1 and 2, but not types 3 and 4, are regularly pathogenic in human volunteers (*16, 17*). As enzyme production has been found with all four colony types we cannot attribute pathogenicity to enzyme alone. We have not, however, excluded significant differences in the level of enzyme produced among the types, nor do we know whether all the enzymes are similarly active under the prevailing physiological conditions at susceptible mucosal surfaces.

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Latimeria, the Living Coelacanth, Is Ovoviviparous

Abstract. *Dissection of a specimen of* Latimeria chalumnae *in the American Museum of Natural History revealed that it is a gravid female containing five advanced young, averaging 317.8 millimeters long. Each has a large yolk sac with no apparent connection to the surrounding oviducal wall. We conclude that* Latimeria *is ovoviviparous.*

Latimeria chalumnae Smith is the only living representative of a distinctive and once widespread group of lobe-finned fishes (*Coelacanthini*) first known from rocks of Devonian age and long believed to have become extinct in the Late Cretaceous. The capture of a living representative off the coast of South Africa in 1938 triggered an extensive search for additional specimens. It was not until 1952, however, that a second specimen was collected, this time off the Comoro Islands. Since then, more than 80 specimens have been taken by Comorean fishermen, at the rate of three or four a year.

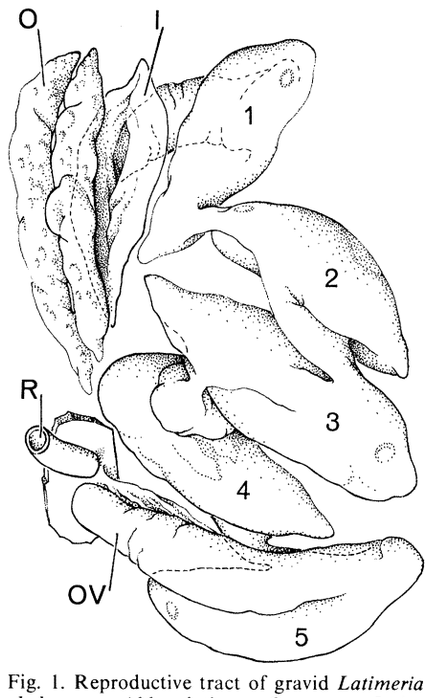

Fig. 1. Reproductive tract of gravid *Latimeria chalumnae*. Abbreviations: *O*, ovary; *I*, infundibulum of the oviduct; *OV*, distal part of the oviduct; *R*, rectum; *1–5*, yolk-sac young in expanded portions of the oviduct.

Despite the number of specimens available for study, the mode of reproduction of *Latimeria* has remained unknown. The anatomy of the urogenital system and orifices of adult males and females was described by Millot and Anthony (*1–4*), but the scarcity of mature females and the absence of any obvious copulatory organ in males left unanswered the key question of whether *Latimeria* lays eggs or gives birth to living young. On the basis of a female found with eggs in her oviduct, Millot and Anthony (*2*) concluded that *Latimeria* is oviparous. Another female containing 19 apparently ripe eggs (8.5 to 9.0 cm in diameter) confirmed these authors' point of view (*3, 5*). Griffith and Thomson (*6*), however, believed that osmoregulatory requirements would make it impossible for such a shell-less egg to survive outside the body of the female and they concluded that *Latimeria* must be ovoviviparous.

With regard to the paleontological evidence, Watson (*7*) described two small skeletons of the Jurassic coelacanth *Undina* (= *Holophagus*) found inside the body cavity of a much larger specimen of the same taxon. He suggested that *Holophagus* was viviparous. More recently, Schultze (*8*) described several isolated larvae of the Pennsylvanian coelacanth *Rhabdoderma* with preserved yolk sacs and, noting that coelacanths lack any fins in the form of intromittent organs, he postulated that these fishes were oviparous. Schultze interpreted Watson's specimen as a case of cannibalism.