CHAPTER III

REVIEW OF THE LITERATURES

1. History of *Streptococcus*

The *Streptococcus* was discovered by Billroth and Ehrlich in 1874 as globular microorganisms growing in chain-forming coccus from erysipelas lesions and infected wounds (McCarty, 1990). In 1884, Rosenbach gave the name *S. pyogenes* to cocci that grew in chains and had been isolated from suppurative lesions in man. In 1887, Nocard and Mollereau reported the production of mastitis in the cow and goat by inoculation the *Streptococcus* into the udder of the milk cow. In 1887 and 1888, Schutz described streptococci isolated from the lesions of equine pneumonia and strangles (Parker, 1984). Similar organisms, eventually named streptococci (Gr. Streptos, winding, twisted), were isolated from the blood in puerperal fever and from the throat in scarlet fever. However, a number of different kinds of streptococci may be cultured from human patients and animals, and the first classifications were based on their capacities to hemolyze red blood cell. In 1919, Brown introduced the term's alpha (α), beta (β), and gamma (γ) to describe the three types of hemolytic reactions observed on blood agar plates (McCarty, 1990).

The genus of *Streptococcus* is one of five genera belonging to the family Streptococcaceae and the other genera are *Aerococcus*, *Gemella*, *Leuconostoc* and *Pediococcus* (Ginsburg, 1982). Primarily through the efforts of Lancefield in the early 1930s, the β-hemolytic streptococci were further differentiated into a number of immunogenic groups designed by the letters A through U. Most strains causing human infections were found to belong to group A. The group specific antigens were identified as carbohydrates and the type-specific antigens as proteins (McCarty, 1990).

The focus of this thesis is on GCS and GGS streptococci. However due to the closed similarity between GAS, GCS, and GGS and since the data of GCS and GGS is much less comprehensive than GAS, thus, these reviews were discussed by including GAS information as well.

2. The organisms

2.1 Morphology and cultural characteristics

The streptococci are a large group of gram positive cocci that were grow in pairs or in chains of up to 50 cells or more. Individual cells are typically 0.5-1.0 μm x 1.0-2.0 μm (Kilian, 1998). Streptococci grow as pairs in extremely rich media, but they grow in progressively longer chains, as they are cultured in simpler media. Intercellular bridges through which the cytoplasm of adjacent bacteria can pass connect the streptococci in the chains (Walker, 1998). Capsulation is not a regular feature of streptococci but some form a capsule of hyaluronic acid or of other distinct polysaccharides (Kilian, 1998). Most group A, B, and C strains produce capsules composed of hyaluronic acid and the capsules are most noticeable in very young cultures and they resist to phagocytosis (Jawetz, et al., 1987, 2001). Streptococci are facultative anaerobes but some strains will grow better under anaerobic conditions. An atmosphere containing increased CO_2 (5% - 7%) also stimulates many isolates. Medically important streptococci, enterococci, and aerococci are homofermentative, meaning that the sole product of glucose fermentation is lactic acid (Koneman, et al., 1992, 1994). The temperature range for the growth of most pathogenic streptococci is 22-42 ^{0}C with an optimum of 35-37 ^{0}C. On blood agar, colonies are usually less than 1 mm in diameter, gray-white or colorless, dry or shiny and usually irregular in outline. Most of the pathogenic streptococci produce hemolysins (Chesbrough, 1984). In general, colonies of the streptococci on blood agar are non-pigmented (Kilian, 1998). The ability of certain clinically important streptococci to induce zones of complete hemolysis (β-hemolysis) around colonies on blood containing agar media was one of the first characters to be recognized and used for distinguishing between isolates.

Other isolates of streptococci induce a zone of greenish discoloration (α-hemolysis) on blood agar, and yet others cause no detectable changes to the red blood cells (γ-hemolysis) (Kilian, 1998). In this study, we are interested in β-hemolysis streptococci, which are GCS and GGS only.

2.2 Structure and antigenic compositions of GAS, GCS, and GGS

The structures have shown that the compositions of particular importance for the virulence of the *Streptococcus* and its classification are located in the surface layers of the bacterium. The cellular compositions of GCS and GGS are most similar to GAS (Rotta, 1986) which is a major pathogen and the information is more comprehensive than GCS and GGS, therefore, we will describes instead of the information of GAS. The cellular compositions, the outer part of the cell surface is the capsule and beneath the capsule of GAS is the cell wall, which in a simplified way, can be visualized as having a three-layer structure (Figure 1). The outer most cell wall layer contains lipoteichoic acid and a number of protein components, namely, M, T, R antigens, MAP (M-associated protein, or so-called non-type specific protein), SOF (serum opacity factor) and the Fc-binding factor. The second layer consists of polysaccharide, and the third layer is peptidoglycan. It should be pointed out that the outer most layers usually carries fimbriae, which is important in virulence factor and can cause various diseases and the peptidoglycan layer has an intercalating mosaic structure (Rotta and Facklam, 1983)

The cell wall of streptococci consists of the shape-forming peptidoglycan, various carbohydrate structures including teichoic acids, and a number of proteins (Kilian, 1998). Cell wall peptidoglycan accounts for about 40 to 80 percent of the cell wall (Ginsburg, 1982). The streptococcal cell wall may contain several forms of polysaccharides, which is one of some species have constitutes the Lancefield group antigen (Kilian, 1998). The work of Rebecca Lancefield laid the ground for the serologic classification of the cell wall of the streptococci and hemolytic streptococci can be divided into serologic groups (A-H, K-U) (Kilian, 1998; Jawetz, et al., 2001) and certain

groups can be subdividing into types. The polysaccharides GAS and GCS contain an identical backbone consisting of α-1,2- and α-1,3-glycosidically linked rhamnose molecules, but differ in their limited side branches. GAS carries N-acetylglucosamine residues, GCS disaccharides of N-acetylgalactosamine, and GGS carries galactose, galactosamine, and rhamnose (Ginsburg, 1982), which are the immunodominant substituents (McCarty, 1959; Coligan, Kindt, and Krause, 1978; Kilian, 1998). Cell walls always contain the amino sugar glucosamine and muramic acid, while galactosamine is a variable component (Hardie, 1986). Streptococci produce an array of proteins associated with the cell wall (Kehoe, 1994; Kilian, 1998). The cytoplasmic membrane encloses the cytoplasm and is situated within the cell wall. The cytoplasm contains a complex of nucleoproteins and proteins, some with enzymatic activity (Rotta and Facklam, 1983).

2.2.1 Structures on the surface of GAS

- Lipoteichoic acid (LTA), which are structure that enables the microorganisms to adhere to the epithelial cells of the human oral mucosa by means of the fatty acid moiety of its molecule. There are also receptor for LTA on the surface of human polymorphonuclear leucocytes which are involved in the recognition of GAS and enable attachment in the process of phagocytosis (Rotta and Facklam, 1983).

- M protein is a major virulence factors that responsible for resistance to phagocytosis, and is the type specific substance in GAS. The information about M protein was discussed in the part of M protein typing.

- The M-associated protein (MAP) is a non-type specific substance, but closely associated with the M protein (Rotta and Facklam, 1983). MAP is found in all M protein-containing GAS and some strains of GCS and GGS but not in M-negative strains. It is antigenically related to sarcolemmal components of the myocardium. Antibody responses to MAPs are usually highest in-patients with acute rheumatic fever (Joklik, et al., 1992).

- T protein type antigens, which are trypsin resistant that occur in several antigenic patterns. Most of them shared with a number of M types and some T antigens restricted to a single M type, whereas others may be shared by several M types (Joklik, et al., 1992). T antigen has also been found in some GCS and GGS strains (Rotta, 1986).

- R protein antigens are also trypsin resistant and occur in some strains of some types only. They have also been found in strains of groups other than A and have been identified in four antigenic forms, the most common being R28 and R3. They have no relation to virulence factor (Rotta, 1986).

- The serum opacity factor (SOF) is a trypsin sensitive protein antigen causing opacity of serum (Rotta, 1986) and was found to be a second type specific substance in some types of GAS that are nontypeable by M antigen (Rotta and Facklam, 1983; Rotta, 1986).

- The Fc reacting factor binding the Fc fragment of the heavy chain of immunoglobulin and thus factor is also present in some strain of GCS and GGS (Rotta, 1986).

- Nucleoprotein were extracted by weak alkali yields mixtures of protein and other substance of little serologic specificity, called P substance, which probably make up most of the streptococcal cell body (Jawetz, et al., 2001).

2.2.2 Extracellular products of GAS

- Hemolysins found in many streptococci, is able to hemolyze red blood cells in vitro in varying degrees (Jawetz, et al., 2001). They have two hemolysins one that is hemolytic in the reduced form and the other is oxygen stable and releasing in serum containing media. They have been termed streptolysin O (oxygen labile) and streptolysin S (serum soluble) (Kilian, 1998). Streptolysin O (SLO) is a protein (MW

60,000) that is formed by most GAS and by many strains of GCS and GGS, but not by member of other groups (Parker, 1984) which is reversibly activated from the reduced form. It is antigenic and the antigen and its antibody can be quantified in a standard hemolytic system using a reference serum (Rotta, 1986). An antistreptolysin O (ASO), and antibody that appears in humans following infection with any streptococci that produce streptolysin O and this antibody blocks hemolysis by streptolysin O. An ASO serum titer in excess of 160-200 units is considered abnormally high and suggests either recent infection with streptococci or persistently high antibody level due to an exaggerated immune response to an earlier exposure in a hypersensitive person (Jawetz, et al., 2001). Streptolysin S (SLS) is an oxygen stable nonantigenic peptide (MW 2,800) (Ginsburg, 1982) that is responsible for the β-hemolysis around colonies of many streptococci on aerobically incubated blood agar plates. Oxygen stable streptolysins of the S type are formed by most streptococci of GAS, GCS, and GGS and also by members of a number of other groups of pyogenic streptococci (Parker, 1984).

- Erythrogenic toxin (pyrogenic exotoxins) is responsible for the rash in scarlet fever (McCarty, 1990). They are formed only by GAS (Parker, 1984). There are three antigenically distinct streptococcal pyrogenic exotoxin A, B, and C (Jawetz, et al., 2001). For its production, the *Streptococcus* must be in a lysogenic state and the majority of strains produce toxins of types B and C simultaneously (Rotta, 1986).

- Streptokinase (fibrinolysin) was produced by most GAS and some strains of GCS and GGS (Huang, et al., 1989). It is an activator of the fibrinolytic system of human blood. Streptokinase is a protein with a molecular weight of approximately 47,000 (Ginsburg, 1982; Parker, 1984). It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins. This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody (antistreptokinase) (Jawetz, et al., 2001).

- Streptodornase (streptococcal DNAase) was produced by strains of GAS, GCS, and GGS (Parker, 1984). All strains of GAS form at least one

DNase. Of the 4 enzymes (A-D) that have been detected, the B enzyme is the predominant nuclease in GAS (Kilian, 1998). Antibody titers to DNAase B are helpful in diagnosing streptococcal pyoderma and poststreptococcal glomerulonephritis (which most often follows pyoderma), because these diseases are often not accompanied by elevated ASO titers (Walker, 1998).

- Nicotinamide adenine dinucleotidase (NADase) was produced by strains of GAS, GCS, and GGS (Rotta, 1986). Streptococcal strains that cause glomerulonephritis (the so-called nephritogenic strains) are also good producers of NADase and anti-NADase titers can be used to establish these strains as etiologic agents in suspected cases of streptococcal pyoderma and glomerulonephritis (Walker, 1998).

- Hyaluronidase production is common in GAS and this enzyme is also produced by strains of GBS, GCS, and GGS (Rotta, 1986). Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source. Following infection with hyaluronidase producing organisms, species antibodies are found in the serum (Jawetz, et al., 2001).
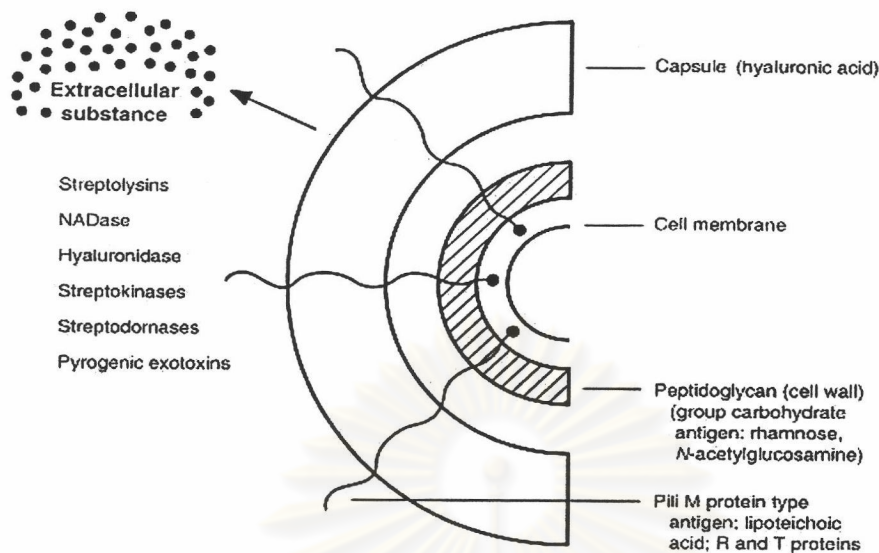
Figure 1: Structure and cellular compositions of GAS

3. The classification of *Streptococcus*

The classification of streptococci into three major categories based on a series of observations including colony morphology and hemolytic reactions on blood agar, serologic specific of the cell wall group specific substance (Lancefield classification), and biochemical reactions and resistance to physical and chemical factors (Jawetz, et al., 2001).

3.1 Colony morphology and hemolytic reactions on blood agar

The classification of hemolytic patterns is used primarily with the streptococci and not with other bacteria that cause disease and which typically produce a variety of hemolysins (Jawetz, et al., 2001). When cultured on blood agar, aerobic *Streptococcus* species can be classified as follows:

- α-Hemolysis is partial lysis of the erythrocytes surrounding a colony that causing a gray-green or brownish discoloration in the media

- β-Hemolysis is complete lysis of the red blood cells surrounding a colony that causing a clearing of blood from the medium

- γ-Hemolysis is no hemolysis and consequently, which is no color change of the medium surrounding a colony. Organisms showing no hemolysis are generally termed nonhemolytic rather than γ-hemolytic

- α-Prime or wide-zone α-hemolysis is a small zone of intact erythrocytes immediately adjacent to the colony, with a zone of complete red blood cell hemolysis surrounding the zone of partial lysis. This type of hemolysis may be confused with β-hemolysis (Koneman, et al., 1992, 1994; Larsen, 1995)

In some classification systems, β-hemolytic strains include strains that show α-hemolysis after overnight incubation on 5% sheep blood agar. In other classifications, only strains that show β-hemolysis are considered to be hemolytic, and the α-hemolytic strains are included with the nonhemolytic strains (Jawetz, et al., 2001). In addition, β-hemolytic isolates of GAS, GCS, and GGS can be subdivided into two groups: large-colony (>0.5 mm in diameter) and small colony (<0.5 mm in diameter) formers (Ruoff, 1995).

3.2 Serologic specific of the cell wall group specific substance (Lancefield classification)

Rebecca Lancefield developed the most commonly used classification scheme in the 1930's. She found that the C carbohydrate could be extracted from the streptococcal cell wall by placing the organisms in dilute acid and heating for 10 minutes (Larsen, 1995). β-hemolytic streptococci and some other streptococci produce group specific carbohydrates (C carbohydrates) that can be identified using group specific antiserum (Chesbrough, 1984). These C carbohydrates, many of which are teichoic acids, could be identified using type-specific antibody. Twenty-one groups of C

carbohydrates were identified and assigned letter designations from A to U. Based on the Lancefield classification system, most human streptococcal disease is caused by group A β-hemolytic streptococci (*S. pyogenes*), while group B streptococci (*S. agalactiae*) and group D organisms (such as *E. faecalis*) are also important pathogens. *S. pneumoniae* does not exhibit Lancefield antigens (Walker, 1998). In addition, streptococcal species other than those that produce β-hemolytic are found possess C carbohydrate. Some are found as normal flora in animals or as animal pathogens, and other may be found in both humans and animals. The Lancefield groups most commonly seen in human infections are A, B, C, D, F, and G, although not all Lancefield groups commonly cause human infection (Larsen, 1995).

3.3 Biochemical reactions and resistance to physical and chemical factors

Biochemical tests include sugar fermentation reactions, tests for the presence of enzyme, and tests for susceptibility or resistance to certain chemical agents. Biochemical tests are most often used to classify streptococci after the colony growth and hemolytic characteristics have been observed. Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group specific substances, group A, B, C, F, and G (Jawetz, et al., 2001). Test for fermentation of carbohydrates may be carried out in meat-extract broth or in serum sugar. All the streptococci ferment glucose and maltose and most of them ferment lactose and sucrose. Other sugar reactions may be of value in distinguishing between closely related streptococci, but departures from the modal fermentation pattern are not infrequent (Kilian, 1998). Traditionally, large colony forming group C isolates have been differentiated into species based on their abilities to ferment various carbohydrates (Ruoff, 1995). Briefly, the carbohydrate broth is inoculated with a loopful of broth or several colonies from an agar plate. The carbohydrate broth is incubated at 35 ^{0}C for up to 7 days. A positive reaction is recorded when the indicator turns yellow (if bromcresol purple is the indicator). Acid formation in broth containing trehalose or sorbital is used to differentiate the β-hemolytic group C and G streptococci (Table 1) (Kilian, 1998).

In addition, streptococci have also been classified according to physiologic characteristics. This classification divides the species into four groups that include pyogenic streptococci, lactic acid streptococci, enterococci, and viridans streptococci (Parker, 1984; Larsen, 1995). The pyogenic streptococci are those that produce pus, which these organisms are mostly β-hemolytic and constitute the majority of the Lancefield groups. The lactic acid streptococci are non-hemolytic organisms often found in dairy products that they are part of Lancefield group N. The enterococci comprise those species found as part of the flora of the human intestine that this group of organisms is now part of the genus *Enterococcus*. The viridans streptococci are not parting of Lancefield's classification because they do not have a C carbohydrate that they are widely found as normal flora in the upper respiratory tract of humans. The viridans streptococci are α-hemolytic or non-hemolytic and are often seen as opportunistic pathogens (Larsen, 1995).

Table 1: Identification of GCS and GGS (Joklik et al., 1992; Koneman et al., 1994; Kilian, 1998)

Species	Lancefield serogroup	Hemolysis	Presence of β-glucuronidase	Fermentation of			
				Ribose	Sorbitol	Lactose	Trehalose
S. equi	C	β	+	-	-	-	-
S. zooepidemicus	C	β	+	*V	+	+	-
S. dysgalactiae	C	α, β	+	+	+	+	+
S. equisimilis	C, G	β	+	+	-	V	+
S. canis	G	β	-	+	-	V	+
S. anginosus	A, C, F, G	α, β, γ	-	NA**	-	-	+
S. constellatus	C, G	α, β, γ	-	NA	-	v	V
S. intermedius	C, G	α, β, γ	-	NA	-	+	+

*V = variable

**NA = not available

4. Streptococcal species in Lancefield serogroup C and G (Table 1)

Streptococcus equisimilis is the most common GCS and can be found in GGS (Vandamme, et al., 1996) to colonize and cause infection in humans. It ferments trehalose but not sorbitol, and it produces streptokinase and streptolysin O but not streptolysin S. The streptokinase used for human thrombolytic therapy is derived from *S. equisimilis*. Because *S. equisimilis* produces streptolysin O, infections caused by this organism may result in elevated antistreptolysin O (ASO) antibody titers, which are classically used to screen patients for previous group A β-hemolytic streptococcal infection. *S. equisimilis* has been isolated from the throat, nose, and genital tract of asymtomatic carriers (Christensen, et al., 1974; Goldman and Breton, 1978) and from the umbilicus of up to two thirds of asymtomatic newborns (Drusin, et al., 1973). Domestic animals (e.g., horses, cattle, pigs, and chickens) may also be infected (Johnson and Tunkel, 2000).

Streptococcus dysgalactiae is uncommon in humans but causes mastitis in cows and suppurative polyarhtritis in lambs. It generally produces α-hemolysis or no hemolysis on blood agar, ferment trehalose, and produces a single hemolysin that is not streptolysin O or S (Johnson and Tunkel, 2000).

Streptococcus zooepidemicus causes significant, often epidemic, infections in domestic animals (horses, cattle, sheep, and pigs). Most cases of human infection can be traced to an animal source (Bradley, et al., 1991). *S. zooepidemicus* has been isolated as the etiologic agent in cases of bovine mastitis, equine respiratory tract infections and infertility, and severe infection in poultry (Wilson, et al., 1978). It ferments sorbitol but not trehalose, produces a novel hemolysin but not streptolysin O or S, dose not produce streptokinase, and is not considered part of the normal human flora (Deibel and Seeley, 1974). Human infection is uncommon and has been associated with consumption of homemade cheese and unpasteurized cow' s milk (Duca, et al., 1969; Barnham, et al., 1983).

Streptococcus equi is primarily a pathogen of young horses that causes strangles, which is a serious and highly contagious respiratory disease (Bryans and Moore, 1972; Wilson, et al., 1978). It ferments neither trehalose nor sorbitol that produces a soluble hemolysin but not streptolysin O or S, and dose not produce streptokinase (Johnson and Tunkel, 2000).

Streptococcus canis is a species originally proposed in 1986 (Deviere, et al., 1986; Whatmore, et al., 2001) for streptococci isolated from dogs and cows possessing the Lancefield group G antigen. The species has since been isolated from a variety of other animals including cats, rats, mink, mice, rabbits, and foxes. Although *S. canis* isolates may often represent commensal flora of the canine skin and mucosa that they have been implicated in a variety of canine diseases associated with urinary tract infections, abortion, vaginitis, mastitis, and skin infections. In addition, severe invasive *S. canis* infections in dogs, analogous to human streptococcal toxic shock syndrome and necrotizing fasciitis associated predominantly with GAS that have been reported (Miller, et al., 1996; DeWinter, et al., 1999; Whatmore, et al., 2001).

Streptococcus anginosus, *Streptococcus constellatus*, and *Streptococcus intermedius* are small colony forming β-hemolytic streptococcal strains (the minute hemolytic streptococci) that the genetically different from large colony forming pyogenic group A, C, and G streptococci and included in the species comprising the *S. milleri* group. These organisms may have variable hemolytic reactions, β-hemolytic strains are found principally in the species *S. anginosus* and *S. constellatus*. The small colony forming β-hemolytic streptococci constitute part of the normal flora of the pharynx and upper respiratory tract and can also be isolated from faeces. Pathogenic factors of small colony forming β-hemolytic streptococci include fibronectin-binding ability and production of hydrolytic enzymes. These organisms have been isolated from purulent infections of the oral cavity, upper respiratory tract, central nervous system, gastrointestinal and genitourinary tracts, abdominal cavity, skin, soft tissues and bone (Ruoff, 1995).

5. Pathogenesis and clinical findings

The clinical significance of these organisms is similar to GAS, which is a major pathogen. The streptococci are usually found as parasites of humans and other animals. Some strains are normal flora of the alimentary, respiratory and genital tracts by colonizing the skin and mucous membrane (Ruoff, 1995). A variety of distinct disease processes are associated with streptococcal infections. The biologic properties of the infecting organisms, the nature of the host response, and the portal of entry of the infection all greatly influence the pathogenic picture (Jawetz, et al., 2001). They can cause a wide range of infection varying from clinically mild infections to severe infections and can also cause post-streptococcal sequelae (Ruoff, 1995).

5.1 Disease caused by GAS

GAS was transmitted from person to person through the respiratory route that cause pharyngitis, tonsilitis, sinusitis, otitis media, cervical adenitis, pyoderma, lymphadenitis, impetigo, erysipelas, cellulitis, bacteremia, osteomyelitis, arthritis, and endocarditis. This infection is characterized by a severe sore throat, with malaise, fever, and headache. Rare cases of meningitis and pneumonia have also been reported (Bisno, 1990). Severe GAS infections associated with a toxic shock-like syndrome, with presentations similar to those found in patients with classic staphylococcal toxic shock syndrome that have also been described (Cone, et al., 1987; Stevens, et al., 1989). These clinical characteristics include hypotension, renal dysfunction, hypoalbuminuria, thrombocytopenia, hypocalcemia, and respiratory failure (Koneman, et al., 1994). When primary acute GAS infections are not treated with antibiotics, some patients develop late sequelae that are immunologically mediated. The two most important late sequelae are acute rheumatic fever (ARF) and acute glomerulonephritis (AGN). In each case, disease is associated with a specific subset of GAS strain, as identified by their M protein profiles (Walker, 1998). Rheumatic fever, which can occur 1 to 5 weeks after acute pharyngiitis that is a multisystemic collagen vascular disease characterized by carditis, polyarthritis, subcutaneous nodules, and erythema marginatum (rash). ARF

occurs in about 3% of patients who have suffered from streptococcal pharyngitis, but ARF can also follow an asymtomatic pharyngeal infection. ARF occurs in-patients infected with rheumatogenic M serotypes 1, 3, 5, 6, 14, 18, 19, 24, 27, and 19 (Walker, 1998). Although the course is self-limited, chronic or progressive damage to the heart valves may occur. Another poststreptococcal sequlae is glomerulonephritis, which is an inflammatory disease of the renal glomerulus that may occur as soon as 10 days after pharyngitis or 21 days after pyoderma caused by a nephritogenic strain of GAS. AGN most often follows pharyngitis caused by M serotype 1, 4, or 12 or pyoderma caused by M serotype 49, 55, 57, or 60 (Walker, 1998). It is associated with diffuse glomerular lesions, hypertension, hematuria, and proteinuria (Koneman, et al., 1994).

5.2 Disease caused by GCS

GCS have been implicated in variety of infections including pharyngitis, epiglottitis, sinusitis, meningitis, soft tissue and bone infections, intraabdominal abscesses, pericarditis, and endocarditis (Murray, et al., 1994). In man, GCS can be associated with respiratory tract, skin and wound infections. It has repeatedly been demonstrated as an etiological agent in endocarditis, meningitis, urinary tract infection and other clinical conditions (Stamm and Cobbs, 1980). Sometimes GCS can cause post-streptococcal complications such as acute glomerulonephritis (Barnham, et al., 1983). One study was reported that GCS pharyngeal carriage might be capable of causing rheumatic fever, which is GCS, might have acquired rheumatogenic factors from GAS by horizontal transfer of genetic material (Haidan, et al., 2000). One study was reported that GCS bacteremia in-patients infected with the human immunodeficiency virus (HIV) (Chalasani, et al., 1995). In animals, GCS strains produce septicemia in cows, rabbits and swine. Frequently GCS were isolated from wound infections of horses. Sometimes GCS were associated with various avian diseases (Peckham, 1966). GCS were isolated from milk and udders of cows with acute or sometimes mild mastitis. Also GCS were isolated from blood and tissues of lambs suffering from polyarthrits (Rotta, 1986). *Streptococcus equisimilis*, the most common human group C isolates, has been recovered from the pharynges of carries and from

those with exudative pharyngitis and tonsillitis. It has also caused several other human infections, including sepsis in neutropenic hosts, puerperal sepsis, cellulitis, necrotizing fasciitis, pneumonia, epiglottitis, empyema, bacteremia, meningitis, brain abscess, osteomyelitis, septic arthritis, and endocarditis (Stamm and Cobbs, 1980; Kuskie, 1987; Arditi, et al., 1989; Salata, et al., 1989; Ortel, et al., 1990; Koneman, et al., 1992, 1994). *S. zooepidemicus* causes various types of diseases in animals, include bovine mastitis, respiratory infections in horses, and genital tract infections in poultry. This species that has also been found in human infections, include pneumonia, endocarditis, meningitis, and cervical lymphadenitis (Barnham, et al., 1983; Barnham, et al., 1987; Koneman, et al., 1992, 1994). *S. equi* is the cause of a respiratory tract infection in horses called " strangles". Strangles is characterized by a high fever, a mucopurulent nasal discharge, and abscessed in the submandibular and retropharyngeal lymph nodes that eventually rupture and drain into the respiratory tract of the infected animals (Bannister, et al., 1985). This organism is rare in humans. *S. dysgalactiae* causes bovine mastitis and purulent arthritis in lambs and goats and they have a single case report of meningitis in a premature infant (Kuskie, 1987; Koneman, et al., 1992).

5.3. Disease caused by GGS

GGS constitute a part of the normal human gastrointestinal, vaginal, oropharyngeal, and skin flora. Infections caused by this organism include pharyngitis, otitis media, pleuropulmonary infection, cellulitis, septic arthritis, septic thrombophlebitis, bacteremia, endocarditis, and meningitis (Auckenthaler, et al., 1983; Vartian, et al., 1985; Watsky, et al., 1985; Yanelli, et al., 1987; Koneman, et al., 1992, 1994; Murray, 1994). GGS can possibly be complicated by acute glomerulonephritis (Reid, et al., 1985; Gnann, et al., 1987). One study was reported that GGS pharyngeal carriage might be capable of causing rheumatic fever, which is GGS, might have acquired rheumatogenic factors from GAS by horizontal transfer of genetic material (Haidan, et al., 2000). GGS can cause severe infections of bone and joint prostheses. GGS cellulitis at sites of parenteral injection and bacteremia with subsequent hematogeneous complications have been reported frequently in intraveneous drug users (Golshlack and

Blackburn, 1984; Craven, et al., 1986; Lebar, et al., 1988; Koneman, et al., 1992). Bacteremia with this organism may occur in the clinical settings of underlying malignancies, puerperal sepsis, septic abortion, chronic pulmonary disease, and congestive heart failure. GGS meningitis and sepsis has also been reported in a patient with acquired immunodeficiency syndrome (Raviglione, et al., 1990; Koneman, et al., 1992). *S. canis* have been implicated in a variety of canine diseases associated with urinary tract infections, abortion, vaginitis, mastitis, and skin infections. In addition, severe invasive *S. canis* infections in dogs, analogous to human streptococcal toxic shock syndrome and necrotizing fasciitis associated predominantly with GAS that have been reported (Miller, et al., 1996; DeWinter and Prescott, 1999; Whatmore, et al., 2001).

6. Laboratory diagnosis

6.1 Direct gram-stained smears

Gram-stained smears of clinical specimens that yield streptococci on culture generally show gram positive or gram-variable cocci arranged in pair and chains. Chains of cells in both specimens and broth cultures tend to appear as chains of pairs of cells rather than as chains of individual cells. Individual cell shapes range from those that resemble diplococci to those that are coccobacillary or coryneform, and this morphology is often observed on smears from broth cultures and form solid media as well (Koneman, et al., 1992, 1994). GCS and GGS have ovoid or spherical cell 0.6-1.0 μm in diameter that sometimes in pus the long axis of the cells is transverse to the long axis of the chain and at other times parallel with the long axis of the chain. In the latter case resembling streptobacilli that bacillary forms may occur, which is grow in pairs, short or long chains, very long chains being common in broth (Rotta, 1986).

6.2 Culture media

Most streptococci grow aerobically and anaerobically. The temperature range for the growth of most pathogenic streptococci is 22-42 ^{0}C with an optimum of 35-37 ^{0}C. On blood agar, colonies are usually less than 1 mm in diameter, gray-white or colorless, dry or shiny and usually irregular in outline. Most of the pathogenic streptococci produce hemolysins (Cheesbrough, 1984). The appearance of surface or subsurface β-hemolysis GCS or GGS colonies does not differ sufficiently from that of GAS colonies to be of any value in identification (Facklam and Washington, 1991). GCS have wide zone of β-hemolysis observed on blood agar, which is the small, watery colonies dry out rapidly and ultimately leave flat, glistening colonies. A soluble hemolysin, distinct from streptolysin O and S that is produced in serum-fortified broth culture (Rotta, 1986). GGS have matte-type colonies and have the board zones of β-hemolysis on blood agar may be larger than GAS and streptolysin O is produced (Rotta, 1986).

6.3 Serological methods

6.3.1 Antigen detection methods

Base on the exploring work of Rebecca Lancefield, which is the serological classification of human streptococci, is based on the detection of the group specific carbohydrate antigen from cell wall of the organism. The various techniques by which serologic identification of streptococci may be accomplished include the following:

- The capillary precipitin test, which from the method used by Lancefield, in which the extracted antigen is layered over group-specific antisera in a capillary tube and the information of a precipitin reaction at the extract-antiserum interface that provides the group designation of the organism (Koneman et al., 1994).

- Coagglutination, in which visible agglutination of the staphylococcal cells coated with a specific antiserum, provides the group designation of the organism (Koneman et al., 1994).

- Latex agglutination, in which polystyrene latex beads are used as the carriers for the group specific antisera that are reacted with the organism extract, with visible agglutination of the latex particles serving as a positive test (Facklam et al., 1979; Daly and Seskin, 1988).

Several commercial kits were based on coagglutination and latex techniques, which are available (Koneman, et al., 1994). Lancefield antigens of group A, C, and G, however, are not specific to a single type of *Streptococcus*. When nongroupable β-hemolytic isolates that fail to react with Lancefield group A, B, C, F, and G are encountered, physiological method may aid in identification (Ruoff, 1995).

6.3.2 Antibody detection methods

The streptococcal antibody tests are useful in establishing previous streptococcal infection in cases of suspected rheumatic fever or glomerulonephritis. By the time these diseases appear suppurative infection has resolved and streptococcal culture is often negative. In general, anti-streptococcal antibody levels rise above normal limits within 2-3 weeks of the acute infection that can be estimated such antibodies include antistreptolysin O (ASO) that is particularly in respiratory disease, anti-DNAase and antihyaluronidase that is particularly in skin infections, anti-streptokinase, anti-M types-specific antibodies, and other. Of these, the anti-ASO titer is most widely used (Jawetz, et al., 2001). Table 2 were summarized the principle streptococcal antibody test. It is difficult to demonstrate a rising antibody titer by the time the patient presents with symptoms of non-suppurative sequelae. Therefore, sera are evaluated for titers that are evaluated above the " upper limit of normal " a value that is variable in populations of different ages, in different locations and with different frequencies of streptococcal infection was summarized in table 3 (Ruoff, 1998). Anti-

streptolysin O and other anti-streptococcal antibodies may also be produced in response to infection with large colony forming GCS and GGS (Efstratiou, 1989; Ruoff, 1998).

Table 2: Principle of streptococcal antibody tests (Ruoff, 1998)

Test	Antigen	Method
ASO	Streptolysin O	Antistreptolysin O antibodies in patient serum inhibit streptolysin O-mediated red blood cell lysis in reaction mixture. End point is last serum dilution that shows no hemolysis. Titers may be expressed in Todd units or international units depending on how streptolysin O was standardized.
Anti-DNAase B	DNAase B	Anti-DNAase B antibodies in patient serum inhibit DNAase B from hydrolysing a DNA-methyl green colored conjugate. End point is last serum dilution with and arbitrary (2+ to 4+) degree of color.
Anti-A-CHO	Group A carbohydrate	ELISA assay measuring amount of antibody in patient serum binding to purified group A carbohydrate.

Table 3: Normal limits of streptococcal antibody titers (Wannamaker and Ayoub, 1960; Ruoff, 1998)

Antibody	Upper limits of normal		Lower limits for
	5-12 years	Young adults	rheumatic fever
Antistreptolysin O	333	200	250
Antihyaluronidase	110	80	300
Anti-DNAase B		80	320
Anti-NADase		130	175

7. Typing system of β-hemolytic streptococci

β-hemolytic streptococci is very important, especially GAS is the most common group found in human pathogenic. The typing system of β-hemolytic streptococci is considerable important in particular cases of streptococcal disease and in special epidemiological situations. The application of *Streptococcus* typing is in microbiological and epidemiological studies of streptococcal infections and their sequelaes (Rotta and Facklam, 1983). The *Streptococcus* strains (GAS, GCS, and GGS) can be typed in different way, e.g., M typing, T typing, R typing, OF typing (Hardie, 1986). Detail about T protein, R protein, and SOF factors were previously described in section " structure and antigenic compositions of GAS, GCS, and GGS ". M typing is discussed in detail later in section " M typing ". The T typing system is based on the agglutination reaction. The T typing of GAS has been important in the investigation of epidemiology of GAS infections and has identified strains associated with outbreaks when the M type was not identifiable (Cunningham, 2000). Some T antigens are restricted to a single M type, whereas several M types may share other T antigens (Table 4). T antigens are not associated with virulence. Antibodies to T antigens are not protective (Joklik, et al., 1992). R protein has been identified, either pepsin or trypsin destroys one (designated R3) the other (R28) is destroyed only by pepsin (McCarty, 1990). They have no relation to virulence factor (Hardie, 1986). The SOF test detects the opacity factor (OF) of β-hemolytic streptococci that the test is useful in epidemiological tool for that association with specific M types (Widdowson, et al., 1971; Rotta and Facklam, 1983).

Table 4: Relation of T patterns to M types (Joklik, et al., 1992)

T complex	M types bearing T complex
1	1
2	2
3/13/B3264	3, 13, 33, 39, 41, 43, 52, 53, 56
8/25/Imp.19	2, 8, 25, 55, 57, 58
5/11/12/27/44	5, 11, 12, 27, 44, 59, 61
14/49	14, 49
15/17/19/23/47	15, 17, 19, 23, 30, 47, 54

7.1 M typing

M protein is a major virulence factor that found in group A, C, and G streptococci (Bisno, et al., 1996). The M typing system, which detects the variation in the M protein molecule between different strains is the basis of the Lancefield M serotyping scheme for *S. pyogenes* (Lancefield, 1962). GAS can be further differentiated into 100 serogroups by their M proteins (Walker, 1998). M antigens, which are resistant to heat and acid, are precipitated by ethanol and destroyed by trypsin. M protein structure and function in GGS and GCS were similar to GAS (Jones and Fischetti, 1987; Bisno, et al., 1987, 1996; Collins, et al., 1992). The GGS and GAS appear to be able to share their M protein genes by horizontal transfer (Simpson, et al., 1992; Sriprakash and Hartas, 1996). M protein serotype identification is not done routinely, but it serves as an important epidemiological tool. M typing is useful in certain streptococcal diseases (such as rheumatic fever, scarlet fever, glomerulonephrtis, toxic streptococcal syndrome, and necrotizing fasciitis) that associated with a restricted number of streptococcal strains (Walker, 1998). For example, acute rheumatic fever associates with rheumatogenic strain which are M type 1, 6 and 12 and acute glomerulonephritis associates with nephritogenic strain which are M type 1, 5, 6 and 19 (Robinson and Kehoe, 1992).

The structure of M protein may be detected by electron microscopy on the surface cells as fibrils protruding up to 200 ηm outwards from the cell (Swanson, Hsu and Gotschlich, 1969; Robinson and Kehoe, 1992; Kilian, 1998) (Figure 2). The amino terminal region extends from the surface of the streptococcal cell wall, while the carboxy terminal region is within the membrane. The M protein is anchored in the cell membrane by the LPSTGE motif identified by Fishchetti and colleagues (Fischetti, et al., 1990). Each fibre consists of a dimeric M protein molecule, which, with few exceptional domains, has a α-helical coiled-coil structure with a 7-residue periodicity (Fischetti, 1989). The age of molecular biology brought cloning technology to the study of streptococcal antigens, the cloning and sequencing of the *emm* genes revealed repeating sequences motifs within (I) the N-terminal region, (II) the mid molecule region and pepsin sensitive region, and (III) the conserved carboxy-terminal region. The N terminal region, called the A repeat region, confers serotype specificity on the GAS and was found to be highly variable among M protein serotypes. The mid-region was also variable and was called the B repeat region (Fischetti, 1989). The carboxy-terminal region also contained amino acid sequence repeats, which extend throughout the carboxy-terminal one-third of the molecule. Figure 3 shows a diagram of the M protein molecule, illustrating the repeating regions and pepsin cleavage site (Cunningham, 2000).

M proteins have been divided into class I and class II molecules (Bessen, et al., 1989, 1997). The division of the M proteins into two classes is based on their reaction with antibodies against the C repeat region of M protein. Class I M proteins are reported to contain a surface-exposed epitope on whole GAS that reacts with the antibodies against the C repeat region. Streptococcal strains containing the class II M proteins do not react with these antibodies and do not contain the class I epitope (Bessen, et al., 1989, 1997). In addition, the class I M protein serotypes were opacity factor negative, while the class II serotypes were opacity factor positive (Cunningham, 2000).
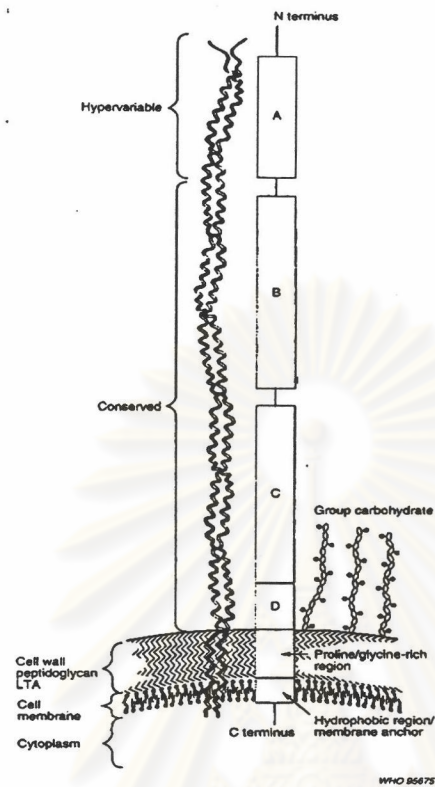
Figure 2: The structure of M protein (Johnson and Kaplan, 1996)

Figure 3: The A, B, C, and D repeat region of M protein, with the protein anchor and pepsin cleavage site shown. The A repeat region varies between serotypes and contains the highly serotype-specific amino acid sequence of M protein at the N-terminus. The B repeat region varies from serotype to serotype, while the C repeat region contains a conserved sequence shared among all of the serotypes. The anchor region contains the LPXTGX motif required to anchor gram-positive proteins in cell membrane (Cunningham, 2000).

As mention above, M protein is very important due to its the function as a major virulence factor that has highly resistant to phagocytosis. The antiphagocytic activity of M protein is due to two mechanisms that it binding of complement regulatory protein factor H and fibrinogen. One mechanism is binding of factor H, which inhibits the activation of the complement pathway (Horstmann, et al., 1988). Factor H is a regulatory component of the complement pathway, which inhibits the deposition of soluble C3b. Factor H binds to the C repeat region of the M proteins, and deletion of the C1 and C2 repeat regions reduces factor H binding (Peres-Casal, et al., 1996). The antiphagocytic behavior of GAS is also mediated by the binding of fibrinogen to the surface of M protein (Whitnack and Beachey, 1985). Fibrinogen binding to the surface of GAS blocks the activation of complement via the alternative pathway and greatly reduces the amount of C3b bound to streptococci, which therefore reduces phagocytosis by polymorphonuclear leukocytes (Horstmann, et al., 1992). Figure 4 illustrates opsonization of GAS by M type specific antibody and complement.

Figure 4: Opsoniztion of GAS by M type specific antibody and complement. How the immune system recognizes GAS and uses opsonization by complement and type-specific antibody against M protein or any other surface molecule capable of generating opsonic antibody. Fc receptors shown on macropharges bind to the antibody Fc region, inducing phagocytosis and killing of the streptococi (Cunningham, 2000).

N-terminal of M protein has hypervariable part that is highly heterogeneous for typing that is called M serotyping or Lancefield M serotyping. Serological M typing has identified more than 100 M types (Facklam, et al, 1999).

Antibodies to hypervariable part of M protein have been proved to be protective (Robinson and Kehoe, 1992). Protective immunity has two major mechanisms. First, organisms entering the host can be blocked from attachment to mucosal surfaces by IgA specific for the C repeat region of M proteins (Figure 5). Second, the GAS has entered the host tissue that is effectively eliminated by opsonization with type specific antibody and complement, with subsequent phagocytosis and killing (Figure 4). One mechanism prevents colonization, while the other mechanism prevents multiplication in the host tissues or blood (Cunningham, 2000). Most recent vaccine strategies have targeted either the type specific N-terminal region of the M protein or highly conserved carboxy-terminal region of the M protein molecule. Vaccination against the N-terminal type specific region induced protective bactericidal and opsonic antibody against the specific M protein serotype, while vaccination against the conserved carboxy-terminal region of the M protein protected against multiple serotypes and prevented colonization at mucosal surfaces. The development of vaccine has always been met with enthusiasm, but certain problems must be overcome. First, it should not intensify the rheumatic disease that the vaccine would be designed to prevent. Second, the immune response should provide lasting protection. Third, because more than 100 different M protein serotypes cause infections, only a limited number of M protein serotype are practical for type specific vaccine (Hauser, et al., 1991; Kaplan, 1991; Cunningham, 2000). Therefore, it is believed that effective vaccine should compose of M protein from multiple types that is important in causing diseases.
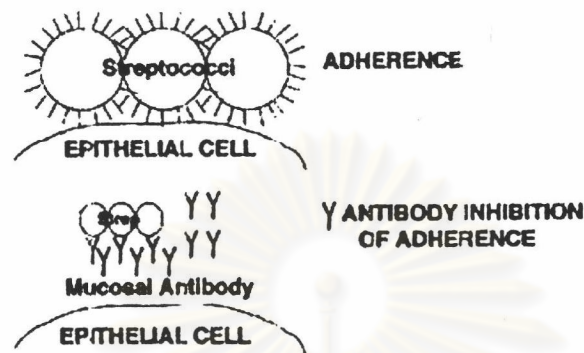
MUCOSAL IMMUNITY AGAINST
GROUP A STREPTOCOCCI

Figure 5: Streptococcal adherence and inhibition of adherence to the mucosa by specific antibody. Mucosal antibody against surface adhesins or eptopes in the C repeat region of M proteins protects against colonization with GAS (Cunningham, 2000).

M serotyping is very useful in epidemiology study that has different type in different part of the world. In 1998, Zurawski and colleges reported that all 94 isolates have M1 (16%), M12 (12%), and M3 (11%) were most common found in invasive GAS disease in Metropolitan Atlanta (Zurawski, et al., 1998). In 2001, Brandt and colleges reported that all 216 isolates have M1 (18.5%) was most common, followed by M12 (15.7%), M3 (14.4%), and M28 (13.9%) that isolated from throat infections in the region of Aachen, Germany (Brandt, et al., 2001). M serotyping can also be used to identify source of outbreaks and to monitor streptococcal carriage within region of endemicity (Gardiner and Sriprakash, 1996). GAS are endemic in Thailand, and ARF is a severe health problem in that area (Phornphutkul and Markowitz, 1981). In 2000, Pruksakorn and her colleges reported that M25 is endemic to Northern Thailand (Pruksakorn, et al., 2000). M serotyping is useful for monitor and follows up of diseases (Gardiner and Sriprakash, 1996).

However, M serotyping has several limitations. First, the producing of type specific M typing antisera are difficult and specialized (Beall, et al., 1997; Facklam, et al., 1999). Second, the antibodies are cross-reactive (Robinson and Kehoe, 1992). In 1997, Beall and his colleague examined the production of M typing sera that were collected from M typing reference strains. They found that Lancefield extracts of many strains no longer consistently gave positive results with their respective M type specific serum, and positive reactions were often very weak. Thus, its appears that many of the stocks of M typing sera may have lost their activity. Unfortunately, time and labor constraints prevent the refill of these stocks, and many of the typing sera will no longer be used (Beall, et al., 1997). Many GAS isolates are currently not M protein typeable because of either a loss of M antigen expression under cultivation or a lack of availability of appropriate M antisera (Kaufhold, et al., 1992). Since production of M type precipitating antisera is very expensive and labor intensive, the potential usefulness of a nonserologic typing system for sequencing the 5' end of the M protein (*emm*) gene toward a molecular based typing system was examined (Facklam, et al., 1999).

7.2 *Emm* gene

Emm gene is a virulent gene that encoded the M protein. *emm* gene family are located in a cluster occupying 3-6 kb between the regulatory gene *mga* (previouly *mry/virR*) and the coregulated *scpA* gene encoding C5a peptidase within the vir regulon on the chromosome (Caparon and Scott, 1987; Simpson, et al., 1990; Perez-Casal, et al., 1991; Kilian, 1998). Most strains contain 1-3 tandemly arranged genes that have evolved through gene duplication followed by sequence and functional divergence (Hollingshead, et al., 1994; Kehoe, 1994; Kilian, 1998). The majority of GAS strains harbor vir regulons, the first that contain *emm*-related genes (Hollingshead, et al., 1993), consists of *virR-fcrA* (-like)-*emm-ennX* (-like)-*scpA* that termed large vir regulons (LVR). The second, regulons without *emm*-related genes are known as small vir regulons (SVR) that contains *virR-emm-scpA*. The last, designated unusual vir regulon (UVR), resembles SVR but contains additional heterogeneous sequences between *emm* and *scpA* (Podbielski, 1993). M protein family (*emm*) genes were divided into two groups

also. M type have been identified into two classes (OF+ and OF-), based on the ability of OF+ M types to express a poorly characterized serum opacity factor (Figure 6) (Widdowson, et al., 1970). The structure of *emm* gene, at 5 ' end or amino terminus is highly heterogeneous that were used to identify the strain of the *Streptococcus* and this system, called *emm* typing. *Emm* typing focuses on the 5 ' ends that are highly heterogeneous (Figure 7). To consider as a given *emm* type by Centers for Disease Control and Prevention (CDC, USA), if it has greater than or equal 95% identity over the first 160 bases of sequences is classified to be the same type and if it has less than 95% identity is classified to be the different type. Single frame-shifts of up to 7 codons, or single in frame deletions/insertions of up to 7 codons are disregarded for these *emm* gene designations, only if flanking these altered sequences a total of 95% identity over 160 bp is obtained. The sequences starting with ' *emm*' indicate that several laboratories besides the CDC, USA streptococcal laboratory have validated this type and sequences starting with '*st*' (sequence type) have not yet been validated by all of the reference laboratories (http://www.cdc.gov/ncidod/biotech/strep/strepindex.html). The *emm* genes of GGC and GCS display sequence heterogeneity at their 5' ends, giving rise to at least 30 distinct *emm* sequence types (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html).

The *emm* typing system has the potential to classify isolates that cannot be typed by serological methods, unnecessary for producing of antibody and high level sensitivity and specificity (Beall, et al., 1996). In 1996, Beall and his colleague reported one advantage of using *emm* typing for M-nontypeable strains by the analysis of seven *emm53* strains, which were obtained within a very short time from the same hospital outbreak of GAS infection (Beall, et al., 1996). Therefore, *emm* typing is useful and reliable epidemiological tool for subdividing especially GAS, GCS and GGS (Beall, et al., 1997; Facklam, et al., 1999).
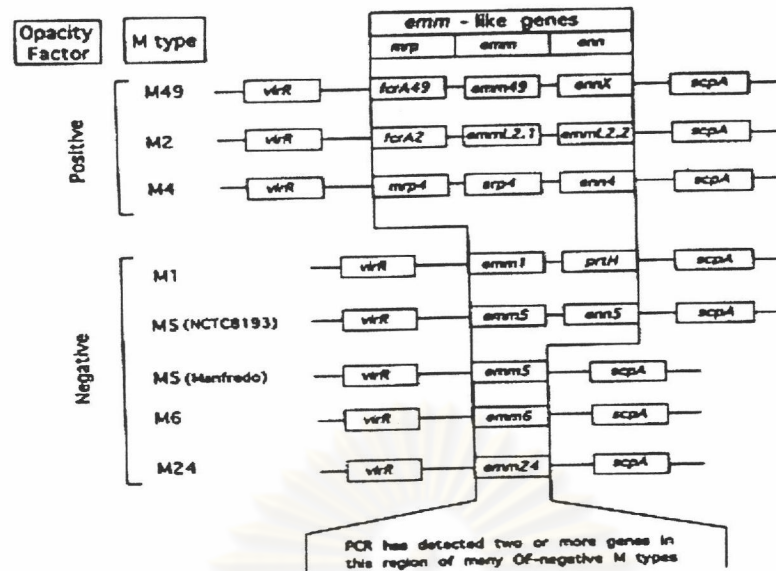
Figure 6: Complication of vir regulon structures. Subdivisions of *emm*-like genes are described by the generic terms *mrp, emm*, and *enn* as indicated at the top of the diagram. The relative positions of genes are well characterized OF+ vir regulons correspond to these subdivisions and that the *emmL* subdivision can be further divided into OF+ and OF- *emmL* genes on the basis of characteristic sequence difference between their conserved regions (Kehoe, 1994; Whatmore and Kehoe, 1994).
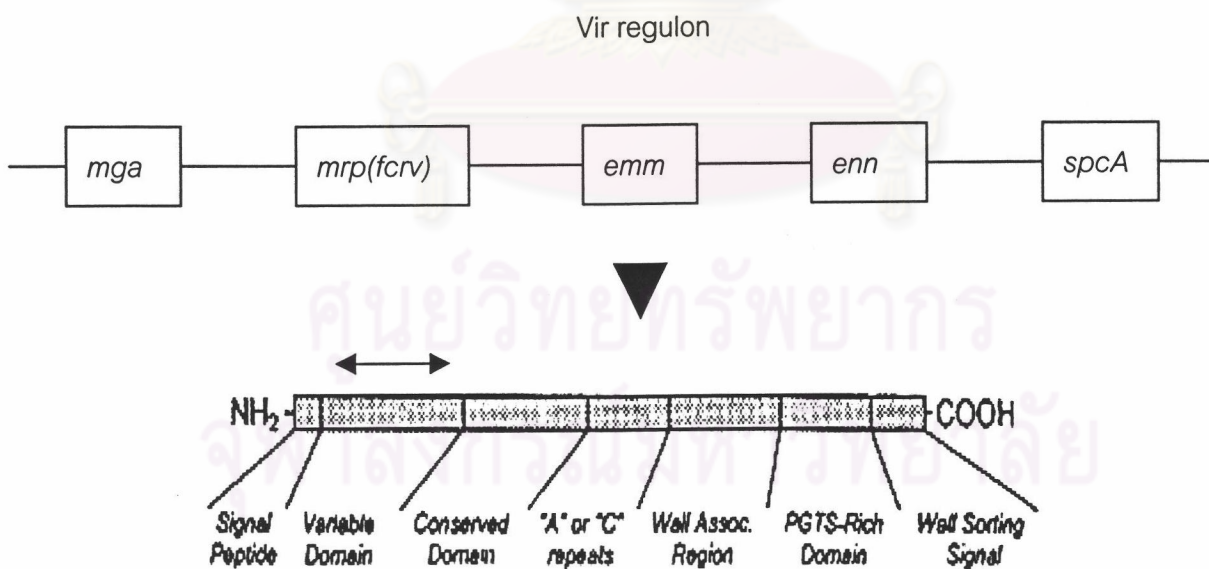

Figure 7: The structure and location of *emm* gene (Navarre and Schneewind, 1999)

8. The reason for this study

GCS and GGS are the focus of this study. These organisms have higher incidence of infection in human and other animals (Bisno et al., 1987, 1996) that associated with severe infection and complications, which are similar to GAS infections (Bessen, et al., 1989, 1990). In addition, there is evidence of horizontal gene transfer of *emm* gene between 3 group of *Streptococcus* (A, C, and G). Therefore, *emm* gene of GCS and GGS can be the source for GAS (Schnitzler, et al., 1995). At present the study of *emm* typing in GCS and GGS are less than GAS infections.

There is only one report of GGS *emm* study by Schnitzler and his colleges study in 1995. The main objective of Schnitzler and his colleges study, they analyzed a number of human and animal associated GGS strains. The samples of Schnitzler study include 38 GGS strains from human specimens and 12 GGS strain from animal specimens. Thirty-eight GGS *emmL* genes could be associated to 14 GGS *emmL* gene type arbitrarily designated types a to n. They also found the horizontal gene transfer between GAS and GGS. For example, when the GGS *emmL* gene sequences were compare with published GAS *emm* or *emmL* sequences, gene portions of *emmLG480* and *emmLG656* were found to exhibit >85% homology to the GAS *emm57* and *emm12* sequences.

There is no publication of GCS *emm* study. However, there are some reports of *emm* sequences from GCS and GGS in the CDC, USA database. There are 12 *emm* types of GCS and 23 *emm* types of GGS reported in CDC, USA database (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.htm)

In this study, the *emm* gene sequences of GCS and GGS from Thai patients was examined. *Emm* typing were used to identify these organisms, relies upon the use of the two highly conserved primers to amplify a large portion of the *emm* gene. The hypervariable sequence encoding M serospecificity lies adjacent to one of the amplifying primer sequences, allowing for direct sequencing. In addition, we study *emm* pattern from both invasive and non-invasive specimens from each GCS and GGS in order to compare the different type of *emm* gene. These data will provide information of *emm* type from GCS and GGS in Thai patients and the pattern in invasive and non-invasive groups.