THE POSSIBILITY OF USING VACCINE TO CONTROL BOVINE SUBCLINICAL MASTITIS AND HUMAN NEONATAL INFECTION CAUSED BY GROUP B STREPTOCOCCI

PELUANG PENGGUNAAN VAKSIN UNTUK PENGENDALIAN MASTITIS SUBKLINIS PADA SAPI DAN INFEKSI NEONATAL PADA MANUSIA YANG DISEBABKAN OLEH STREPTOCOCCI GRUP B

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ABSTRACT

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Based on the phenotypic expression, group B streptococci (GBS) can be devided into two distinct biovars, the bovine and the human biovar. GBS of bovine mostly grow as sediment with clear supernatant in fluid media, form compact colonies in soft-agar, fail to ferment lactose and have hydrophobic surface character. In contrast, the human GBS grow mostly turbid in fluid media, showed diffuse colonies in soft-agar, ferment lactose and have a hydrophylic surface character. The results of the recent researchs showed that these phenotypic differences were not directly asscociated with the biovar but seemed to be more with the occurrence of polysaccharide capsules. Most of GBS of bovine expressed the protein surface character but not the polysaccharide capsule. This was confirmed with salt aggregation and hexadecane tests. In the pathogenesis of subclinical mastitis, the adhesion process is very important as the initiation step of bacterial colonization on the mammary cell surface. The occurence of haemagglutinin among GBS of bovine is very high. The haemagglutinin and hydrophobic proteins are believed as adhesins in mediating the adherence of this bacteria. The absence of capsule in most of GBS of bovine lead to be phagocytosed easily by the polymorphonuclear (PMN), so that there is a balance condition (homeostatic) between the amount of bacteria and the bacterial elimination by PMN in udder. This might be the explanation about the pathogenesis of the subclinical mastitis. The antiserum against haemagglutinin inhibits the adhesion of this bacteria on the mammary cell surface. The new approach in controlling mastitis by using vaccine should be considered and the vaccine candidates should be selected based on the occurrence of haemagglutinin on the surface of bacterial cells.

ABSTRAK

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Berdasarkan atas ekspresi fenotip streptokokus grup B (SGB) dapat dibedakan menjadi dua biovar, yaitu SGB biovar sapi dan SGB biovar manusia. SGB biovar sapi umumnya tumbuh dalam bentuk sedimen dengan supernatan jernih, membentuk koloni kompak dalam soft-agar, memecah laktose dan memiliki permukaan sel yang bersifat hidrofobik. Sedangkan SGB biovar manusia tumbuh keruh di media cair, membentuk koloni difus dalam soft agar, tidak mampu memecah laktose dan permukaan selnya bersifat hidrofilik. Hasil penelitian akhir-akhir ini menunjukkan bahwa perbedaan fenotip ini ternyata tidak langsung berhubungan dengan biovar tetapi lebih kepada keberadaan kapsul polisakarida di permukaan sel bakteri. SGB biovar sapi lebih banyak mengekspresikan tabiat protein pada permukaan selnya. Hal ini ditegaskan dengan uji salt aggregation dan uji heksadekan. Pada kasus mastitis subklinik, kemampuan adhesi sangat penting sebagai langkah awal kolonisasi bakteri di permukaan sel ambing. Keberadaan hemaglutinin pada SGB biovar sapi sangat tinggi. Hemaglutinin dan protein hidrofobik dipandang sebagai adhesin dalam memperantarai proses adhesi. SGB biovar sapi tidak memiliki kapsul sehingga mudah difagositosis oleh sel radang polimorf (PMN), sehingga terjadi keseimbangan antara jumlah bakteri dengan eliminasi bakteri oleh PMN di dalam ambing. Inilah mungkin penjelasan mengenai patogenesa mastitis subklinik.

Antiserum hemaglutinin menghambat proses adhesi bakteri pada permukaan sel ambing. Pendekatan baru dalam penanggulangan mastitis dapat dipikirkan dan pemilihan kandidat vaksin hendaklah mengacu kepada keberadaan hemaglutinin pada permukaan sel bakteri.

INTRODUCTION

Group B streptococci (*Streptococcus agalactiae*) are well known as causative agent of subclinical mastitis in cows and neonatal septicaemia and meningitis in human. Mastitis has remained the most economically important problem in dairy cattles in Indonesia.

Subclinical mastitis cases cause significant economic loss. These include lost in milk productions (16-38 %), degrading of milk due to poor quality, increase replacement costs, milk discard, drug costs, veterinary fees and labor costs. The decrease of milk production is felt to be more economically important than that caused by clinical mastitis. With respect to mastitis treatment, concern has been focused on its efficacy and economic merit. Greater emphasis has been placed also on antibiotic residue avoidance to ensure a residue-free milk supply for the consuming public. The major change in direction over the past ten years concerning treatment of mastitis is the reduction in the use of antibiotics. According to the results of previous study, the incidence of subclinical mastitis among dairy cattles in Java is very high (85 %) and *S. agalactiae* is the main causative agents (Pasaribu *et al.*, 1994; Wibawan *et al.*, 1995).

In human, this bacteria cause a serious infection in neonatal bacterial pneumonia, septicemia and meningitis. Pneumonia develop as a consequence of aspiration of infected amniotic fluid in utero or from vaginal contents during parturition (Baker, 1990). The cross infection of this bacteria from cows to human and vice versa has not been studied intensively. The *biovar characteristics* can be used as markers to determine the incidence of this cross infections.

Vaccination is thought to be the most important alternative in controlling subclinical mastitis and infection of infant caused by this bacteria. Vaccine candidates should be chosen based on the occurence of virulent factors of the bacteria. The nature, biological activities and the role of these virulent factors in the mechanism of infections should be firstly clearly defined.

BIOVAR MARKERS AND VIRULENT FACTORS

Biovar Markers

Based on the host origins, the GBS could be classified into two biovars, GBS of bovine and of human origins. Each biovar have the specific phenotypic expression and this could be used as *markers* in determining the possibility of cross infection of this bacteria from cows to human or vice versa and for other epidemiological studies. The differences in the phenotypic expression of bacteria seemed to be closely related with the occurence of polysaccharide capsule and protein surface components of bacteria (Wibawan and Lämmler, 1991b). GBS of bovine origin are mostly unencapsulated and express the protein characters on the surface of bacterial cells. In contrast, GBS of human origin demonstrate the formation of capsule and express more polysaccharide characters.

It is of interest, the each biovar develop their interaction mechanism with the respective hosts by creating the valuable surface components which are suitable for the infection process and support the adaptibility of the bacteria to micro environment in the host. GBS of bovine origin are mainly unpigmented in Islam agar, had a long chain formation, grow as sediment with clear supernatant in fluid media and express compact colonies in soft-agar. Contrary to the GBS of bovine origin, GBS of human origin are mostly pigmented, show a short chain formation, grow homogenous turbid in fluid media and form diffuse colonies in soft-agar (Wibawan and Lämmler, 1990a; 1990b; Wibawan *et al.*, 1991a) (Figure 1)

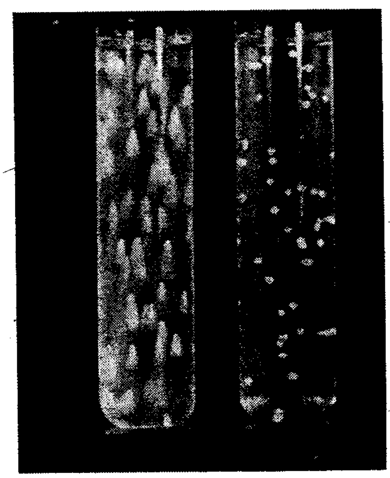

Figure 1. Compact Colonies are Expressed by Most of GBS of Bovine Origin (a) and Diffuse Colonies by Most of GBS of Human Origin (b)

The difference of phenotypic expressions of this bacteria is not directly associated with the bacterial origins but seems to be more with the encapsulation status of the bacteria. The GBS of bovine origin demonstrate aboved characteristic growth because most of them are in the form of nonencapsulated phase. The expression of protein surface components seems to be more pronounced by GBS of bovine origin compared to GBS of human. The GBS of human origin, which are mainly in the encapsulated form, show difference phenotype expression from GBS of bovine origins. The encapsulation of these bacteria had been confirmed by electron microscopic studies. (Wibawan and Lämmler, 1990b; Wibawan *et al.*, 1996).

Virulent Factors

Capsule is believed to be one of virulent factors of the GBS which plays an important role in avoiding the phagocytosis and the killing process of bacteria by polymorphonuclear (PMN) (Wibawan *et al.*, 1994; Valentin-Weigand *et al.*, 1996). The inner phagocytosed life bacteria can use the PMN as vehicles in the spreading in the infected hosts (Figure 2). This indicates the presence of capsule in GBS of human origin plays an important role in the invasion process of bacteria through the host tissue and in the entering the blood sirculation and at last cause bacteriemia, septicemia and meningitis (Rubens *et al.*, 1992; Gibson *et al.*, 1993). In contrast, the presence of capsule by GBS of bovine origin does not always give an advantage, becaused the capsule masks the surface protein components such as hydrophobic surface proteins and haemagglutinin that responsible for the adhesion and the colonization process of bacteria on mammary epithelial cells. Using both proteins, as well as specific antibodies against haemagglutinin and hydrophobic proteins in the inhibition assay lead to the decrease of bacterial adhesion of unencapsulated bacteria on mammary epithelial cells (Wibawan *et al.*, 1993; Wibawan *et al.*, 1997). The invasion of bacteria into the tissue have no or only a little role in the pathogenesis of subclinical mastitis. The adhesion and the colonization of the bacteria are more important than the invasion's ability of bacteria in the subclinical mastitis pathogenesis. This supported by the facts that only small pathological changes can be observed in subclinical infected udder by this bacteria. Further, the elimination of bacteria by phagocytosis activity of PMN in milk is not so effective as in blood circulation because of the presence of lipid components and other alien materials in milk that should be also eliminated by the PMN.

Figure 2. Phagocytosis Activities of PMN to GBS Bacteria.

SEROTYPES

Based on the specific surface antigens *S. agalactiae* can be devided into polysaccharide antigens Ia, Ib, II, III, IV, V and protein antigens c, R and X. The distribution of type antigens for each bacterial biovars have a specific pattern. Most of GBS cultures isolated from bovine have protein type antigen X. This type antigen X seems to be specific for GBS of bovine origin, because upto now, there is no report about the occurence of the antigen X in GBS of human origin. The protein type antigen X could additionally be used as a marker to detect the cross infection of this bacteria. GBS of human origin mainly have polysaccharide type antigen III, Ia, and II alone or in the combination with protein antigen c and R (Wibawan and Lämmler, 1990a; Wibawan *et al.*, 1992a; Pasaribu *et al.*, 1994; Wibawan *et al.*, 1995).

CELL SURFACE CHARACTERISTICS

By using Salt Aggregation Test (SAT) and Hexadecane Addherence Test (HAT) we could determine the surface characteristics of GBS. GBS of bovine origin express hydrophobic characters, have the ability to agglutinate erythrocytes and show adhesive characters on the surface of epithelial cells. The adhesive characters of the bacteria closely related with the occurence of the protein surface components of the bacterial cells. This suggests the role of the protein surface components as the virulent factors of the bacteria which responsible for the adhesion process. The adhesion is the first step of bacterial infections and followed by the colonization. GBS of human origin show hydrophilic characters, express no haemagglutination activities and adhere very poor to epithelial cells. This surface characteristics are thought to be influenced by the presence of polysaccharide capsule by most of human isolates (Wibawan and Lämmler, 1992). The role of polysaccharide capsule and the surface protein components (hydrophobic proteins and haemagglutinin) were studied by comparing the unencapsulated GBS mutant which was produced from encapsulated original strain through transtransposon mutagenesis (Wibawan and Lämmler, 1991; Wibawan *et al.*, 1994) and comparing the positive- and negative haemagglutinin GBS strains (Wibawan *et al.*, 1992b). The same results in adhesion and in phagocytosis process were further observed by using the unencapsulated variant that was separated from encapsulated original strain through percoll gradient centrifugation technique (Wibawan *et al.*, 1996).

GROWTH RATES OF GBS

The growth rates of GBS appear to be closely related with the encapsulation of the bacteria. The encapsulated GBS strains exhibited clearly visible growth after 4 h incubation and change the conductivity of the culture fluid to a point defined as *time to detection* (TTD) within 4 h measured by impedance systems. No compareable results could be observed by unencapsulated GBS cultures.

The TTD values were longer for cultures with protein antigens or for unencapsulated cultures. The influence of polysaccharide capsule to growth rate of GBS could be additionally confirmed by two of GBS of serotype III and their asialo capsular mutants. Encapsulation and differences in growth rate seemed to be also related to other characteristics of the bacteria, such as chain length, growth properties in fluid and soft agar and the surface behaviour of this bacteria (Wibawan and Lämmler, 1993) (Figure 3).

The compareable results about the growth rates and encapsulation of GBS had also been reported by Pincus *et al.* (1992). The growth rates in fluid medium for encapsulated GBS variants lagged behind those for encapsulated GBS.

INTERRELATIONSHIP OF PHENOTYPIC CHARACTERS

As already indicated from our previous results all this properties were significantly related to each other. A hydrophobic surface character might cause a long chain formation of the bacteria and grew as granular sediment and compact colonies. This possibly leads to a depression of growth rate at early logarithmic-phase of the bacteria by steric hinderence. However, the determination of growth rate could help to predict the degree of the encapsulation of GBS cultures and might be importance to understand the differences in virulence of GBS isolates from subclinical and clinical cases.

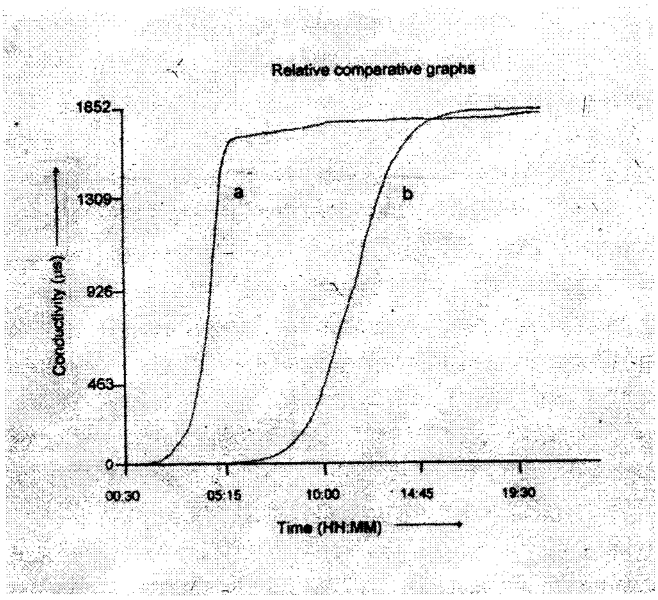

Figure 3. The Growth Rate of Encapsulated (a) and Unencapsulated (b) GBS Detected by Impedance System at Early Logarithmic Phase of Growth

INFLUENCE OF SPECIFIC ANTIBODY TO PHENOTYPIC EXPRESSION

The presence of specific antibody in soft-agar preparation is able to convert the colonies form of GBS from diffuse to compact. Based on our knowledge on this properties, it seems that the specific antibodies inhibited the capsule formation of respective bacteria. Serum-soft agar is a simple method that could be used to demonstrate the influence of specific antibodies in inhibiting the capsule formation. In addition, the serum soft-agar technique can also be used individually to determine the serotype of diffuse growth bacteria (Wibawan and Lämmler, 1990c).

SUBCLINICAL INFECTION

Subclinical infection is an ideal form of interactions between host and bacteria. How the bacteria and the host developed their defense system until no drastic elimination of bacteria and no significant physio-pathological changes in hosts could be occured. The subclinical mastitis case is a good example for the ilustration. The GBS of bovine strains in their normal form create no capsule formation or only a thin capsule formation so that the penetration and the invasion to udder tissue does not optimally occur. In addition, the GBS of bovine strains grow slowly and phagocytosed easily by the PMN and this regulate the certain number or a favourable number of bacteria on the surface of udder epithelial cells.

As the compensation, for the survival this bacteria express more adhesive properties which is very important for the colonization process. Eventhough in vitro condition the unencapsulated bacteria phagocytosed easier than encapsulated bacteria, but in milk the phagocytosis activity of PMN is much lower. This condition will increase the survival of the bacteria.

THE POSSIBILITY OF USING VACCINE

The vaccine candidates of GBS against subclinical mastitis should be chosen based on the occurence haemagglutinin and hydrophobic proteins on bacterial cell surface. Both surface proteins are believed to have an important role in the adhesion and colonization of the bacteria. The specific antibodies of haemagglutinin and hydrophobic proteins decrease the adherence of bacteria on udder epithelial cells.

The specific antibodies against polysaccharide capsule (sialic acid as the main components) could inhibit the capsule formation of GBS. Great amount of sialic acid was found mostly by GBS with type antigen III and 80 % of neonatal septicaemia and meningitis cases due to GBS infections caused by type III GBS (Baker and Barret, 1974; Yeung and Mattingly, 1984).

CONCLUSIONS

Based on the host origin group B streptococci can be devided into two biovars of bovine and human biovars. Each biovar has specific phenotypic characters and can be used as markers in determining the cross infection between both biovar origins. The differences in phenotypic expression between GBS biovars does not directly related to the host origin but seems to be associated with the encapsulation of the bacteria. The GBS biovars develop their characteristic cell surface in response to the need of infection process. Protein surface component of GBS of bovine origin seems to play a significant role in the pathogenesis of subclinical mastitis. In the infection of human, polysaccharide capsule as an antiphagocytic factor played an important role. Vaccination might be as the most prominent alternative in controlling subclinical mastitis and neonatal septicemia and meningitis caused by GBS infections. The vaccine candidate should be chosen based on the occurence of virulent factors of the bacteria which responsible for infection process.

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