CHAPTER I

INTRODUCTION

In developing countries, in which 75% of the world's population lives, infectious diseases are responsible for nearly one – third of the total of 52 million annual estimated deaths. Acute lower respiratory infections from various bacterial pathogens cause 3.7 million deaths per year. Another 2.9 million deaths are caused by tuberculosis and 2.5 million deaths are caused by diarrheal bacteria (Madigan et al., 2000).

Bacteria are prokaryotic cells. They have a single chromosome that is not enclosed in a nuclear membrane. Depending upon the type of the cell wall structure, bacteria can be further differentiated on the basis of the interaction between the cell wall and Gram stain. Using this staining technique, most bacteria can be classified as gram-positive bacteria, such as *Staphylococcus aureus*, or gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.*

Staphylococcus aureus is one of the most important and widespread hospital pathogens. It is the most common cause of surgical wound infections and the second most common cause of blood infections. *Escherichia coli* is the common cause of urinary tract infection in the hospital. Infections from *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are also very common (Madigan et al., 2000). Bacteria cause diseases by the release of toxins. Toxins of bacteria fall into two groups : exotoxins and endotoxins. Bacterial exotoxins are produced mostly by gram-positive bacteria. They are proteins released extracellularly as the organism grows. Endotoxins are produced only by gram-negative bacteria and consist primarily of lipopolysaccharide (LPS). LPS is a structural component of the outer membrane of gram-negative bacteria, which

is released upon cell lysis. The LPS molecule is glycolipids composed of a complex lipid portion called lipid A, a core polysaccharide region and O-specific side chains. Lipid A appears to be the principal component responsible for the endotoxin activity (Koneman et al.; 1997, Madigan et al., 2000).

Lipoproteins are particles composed of lipids and proteins circulating in the vascular system. Distinct families of lipoproteins have been described, each of which plays defined roles in lipid transport. These families are classified in terms of their densities, determined by ultracentrifugation. The standard lipoprotein classification includes, in an increasing order of density : chylomicron, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).

HDL is a group of lipoprotein particles which have the highest density in the circulation. The main lipids of HDL are cholesterol and phospholipid, whereas the major protein is apolipoprotein A-I (apo A-I). Plasma HDL cholesterol levels inversely correlate with the risk of coronary artery disease, suggesting that HDL plays an important role in protecting against atherosclerosis. HDL has been shown to mediate the removal of cholesterol from cells and return it to the liver for elimination in a pathway known as "reverse cholesterol transport" (RCT). Moreover, HDL can protect LDL, the most atherogenic lipoprotein, against oxidative modification, the process necessary for the development of atherosclerosis.

Several lines of evidence also show that HDL plays an important role in innate immunity. By using a radioactive technique, Braude et al. (1955) studied the distribution of endotoxin *in vivo*. After intravenous injection of lethal doses, more than 80% of radiolabelled endotoxin was present in plasma, but not in erythrocytes (Braude et al., 1955; Herring et al., 1962, Ulevitch and Johnston 1978) described a reaction of LPS in plasma or serum which resulted in a marked reduction in the buoyant density of the LPS. In addition, the reduction

in buoyant density of LPS was associated with the abrogation of the biologic responses of the host to LPS and could represent a major pathway of LPS detoxification. Subsequent *in vivo* and *in vitro* studies demonstrated that HDL could bind LPS resulting in detoxification of LPS (Weinstein and Ulevitch, 1979; Ulevitch et al., 1979; Ulevitch et al., 1981; Munford et al., 1981; Emancipator et al.,1992; Levine et al., 1993; Parker et al., 1995; Levels et al., 2001). *In vitro*, LPS bound to lipoprotein was 20- to 1,000-fold less active than the unbound form in inducing monocytes and macrophages to release cytokines (Cavaillon et al., 1990). A study of LPS-LDL complex demonstrated that LPS could insert itself into LDL and form an LPS-LDL complex (Victorov et al., 1989). Therefore, it has been proposed that the lipid-A domain of LPS can be masked by insertion into the phospholipid bilayer resulting in decreasing the biological activity of LPS (Levine et al., 1993). Brandenburg et al. (2002) studied the interaction of HDL with LPS by a variety of physical techniques and biological assays and found that the functional groups of LPS interacting with HDL were the phosphates and the diglucosamine backbone (Brandenburg et al., 2002). HDL could also inhibit the toxic effects of lipoteichoic acid (LTA), an active cell wall component of gram-positive bacteria (Grunfeld et al., 1999).

In vitro, human HDL inhibits the growth of *Staphylococcus epidermidis* (Tada et al., 1993). It has been proposed that inhibition of bacterial growth may be due to the insertion of particular HDL components, lipids or proteins, into bacterial cell membrane resulting in cell lysis (Tada et al., 1993), although the exact mechanism is not yet known. Furthermore, human HDL can inhibit the growth of *Trypanosome* protozoa by inducing cell lysis. Phospholipid composition of HDL might be important for this activity (Rifkin, 1991). Recently, one HDL-associated protein, apo L-I, was able to lyse *Trypanosomes* by forming anion channels in the lipid bilayer of lysosomes which caused

membrane depolarization, continuous influx of chloride, and subsequent osmotic swelling (Perez-Morga et al.,2005).

HDL also demonstrates anti-inflammatory properties *in vivo*. Intravenous infusion of reconstituted HDL or HDL apoprotein protected normal mice from the toxic effects of LPS (Levine et al., 1993). When transgenic mice with a 2-fold elevation of plasma HDL levels were injected with LPS, they had more LPS bound to HDL, lower plasma cytokine levels, and improved survival rates compared with control mice (Levine et al., 1993). Beside the effects on monocytes and macrophages, LPS also activates endothelial cells. Infusion of reconstituted HDL inhibited infiltration of neutrophils and the expression of adhesion molecules on endothelial cells induced by a periarterial collar in rabbits (Nicholls et al., 2005). However, whether HDL could inhibit the effects of LPS on endothelial cells in vivo has not been studied.

During bacterial infection, a wide range of alterations in metabolism occur. These are part of the body's reaction known as **the acute-phase response (APR)**, which helps to protect the host from further injury and facilitates the repair process (Gabay and Kushner, 1999). The APR also induces a variety of alterations in lipid and lipoprotein metabolism (Khovidhunkit et al., 2004). HDL circulating during the APR, which is called acute-phase HDL (AP-HDL), has been shown to contain different in lipid and protein composition compared to that of normal HDL, which leads to alterations in various functions of HDL (Khovidhunkit et al., 2004).

Although HDL is well known in its role in protecting against atherosclerosis, the role of HDL in innate immunity is lesser known. Accumulating evidence found that HDL could inhibit LPS-induced cytokine release, toxic of LTA and growth of gram-positive bacteria, *S. epidermidis, in vitro* (Parker et al., 1995; Levels et al., 2001, Cavaillon et al., 1990, Grunfeld et al., 1999, Tada et al., 1993). Moreover, HDL could also attenuate LPS-induced

cytokine release and improve survival rates *in vivo* (Levine et al., 1993, Nicholls et al., 2005). However, whether AP-HDL could inhibit the growth of gram-negative and gram-positive bacteria *in vitro* and attenuate LPS-induced leukocyte adhesion on endothelial cells *in vivo* has not yet been studied.

Therefore, the purposes of present study are to determine

1. whether normal HDL and AP-HDL inhibit both gram-negative and gram-positive bacterial growth *in vitro*.
2. whether normal and AP-HDL can inhibit LPS-induced leukocyte adhesion on endothelial cells *in vivo*.
3. what's components of HDL responsible for its inhibiting effects.