CHAPTER I

INTRODUCTION

Typhoid fever still remains a major public health problem in Thailand and other developing countries . Medical researchers have long been seeking an effective vaccine and find that oral vaccination against typhoid fever has several potential adventages over parenteral vaccines including the ease of administration and lack of adverse side reactions . Moreover , it seems logical that direct stimulation of the intestinal immune system by oral vaccination is the best way to immunize against typhoid infection since it is the portal of entry for this pathogenic organism . Recently , Germanier (1 , 2) has shown that living galactose Epimeraseless (gal E) mutants of S.typhimurium (G_{30}) can provide a high degree of protection against a subsequent challenge with virulent S. typhimurium in mice . Similarly , human volunteers were significantly protected from typhoid fever by an oral vaccine consisting of live gal E mutants of S.typhi (3 , 4) . These gal E mutants of Salmonella have a defective uridine diphosphate (UDP) galactose - 4 - epimerase , which is responsible for synthesis of UDP - galactose and UDP - glucose . Consequently these mutants are " rough " because they are unable to incorporate galactose into their lipopolysaccharide (LPS) and hence have an abbrevieted LPS core . However , in the presence of exogenous galactose the cells produce smooth LPS . The outstanding protective capacity of rough gal E mutants is believed to result from the utilization of galactose present in the host leading to the production of small amounts of smooth O - antigenic LPS in vivo .

Murine infection with the natural mouse pathogen

S. typhimurium is the most widely accepted model for studying immunity to typhoid fever . This model has been adopted in this experiment because S. typhi , the causative agent of typhoid fever , is only virulent for man and chimpanzees .

S.typhimurium is a facultative intracellular pathogen of mice that cause a systemic infection . An essential step in the pathogenesis of typhoid fever in mice or humans is the establishment of a systemic infection which usually develops from loci established in the Peyer' s patches of the small intestine soon after ingestion (5) . After oral feeding of mice with various Salmonella strains (6 , 7) , it was found that the ability of a strain to colinize the Peyer' s patches profoundly influenced the immunogenicity of the oral bacterial vaccine and its ability to protect against oral challenge with S. typhimurium .

The exact mechanisms of protective immunity in typhoid fever are not well characterized . The most important defense mechanism for elimination these ntracellular microorganisms is specific cell - mediated immunity which is the co - function of antigen - specific T lymphocytes and monocytes / macrophages (8) . The spread of S.typhi appears to be due to macrophages in which the pathogen is able to survive and proliferate , nevertheless macrophages are the cells which once activated by lymphokines from S.typhi , specific T lymphocytes exert a bactericidal effect on the engulfed oraganisms . However , specific antibody is required for efficient phagocytosis of Salmonella by either normal or activated macrophages (9, 10) both cell types depending on Fc and/or C3 receptors for this function .

The immune mechanisms which underly an effective protection against enteropathogenic bacteria after oral vaccination are still

far from being understood . Since S.typhimurium is a facultative intracellular parasite . Therefore ,it is expected that S.typhimurium G_{30} , which is oral vaccine against virulent S. typhimurium , should mainly promote a cell - mediated immunity . Macrophage activation and proliferation in the Peyer' s patches following efficient oral vaccination , appears to be a likely immune mechanism which protects against murine typhoid .

In the studies of Moser et al (11) , it was found that S. typhimurium gal E strain G_{30} , following oral feeding to mice , developed a state of immunity to a secondary oral challenge with virulent S. typhimurium . This immunity was concomitant with the development of intestinal and serum antibodies and delayed type hypersensitivity (DTH) to Salmonella antigens infected into the foot-pads of mice . One supposes therefore that a local cell - mediated immunity is also induced in the gut in addition to local antibody production ; thus since the G_{30} vaccine was able to induce a DTH response , it seems likely that G_{30} vaccine can produce an increased macrophage activily within the Peyer' s patches which may be indicative of a local antibacterial CMI against Salmonella .

Research Aims

1. To compare the number of macrophages in the Peyer' s patches of mice after feeding S.typhimurium G_{30} with a control group.

2. To demonstrate the protection afforded by oral vaccination with living cells of S.typhimurium G_{30} , in mice to subsequent oral challenge with S.typhimurium C_5 .

3. To investigate the effect of intracellular killing of

S. typhimurium C_5 in vitro by macrophages in the Peyer' s patches of mice orally immunized with S.typhimurium G_{30} compared with control group .

4. To investigate the requirement of specific antibody for intracellular killing of S. typhimurium C_5 in vitro by macrophages in the Peyer' s patches of mice .

Specific Aims

1. To determine the increase in the number of macrophages in the Peyer' s patches of mice after feeding S.typhimurium G_{30} .

2. To determine the role of macrophages in the Peyer' s patches of mice after feeding S.typhimurium G_{30} in the protection against murine typhoid .