REVIEW OF LITERATURES

Pathogenesis of Salmonella Infection

S.typhi , the causative agent of typhoid fever , is avirulent for mice . The parenteral 50 % lethal dose (LD_{50}) of S. typhi for mice of all inbred strains examined to date is $> 10^{8}$ bacteria (12 – 14) . By contrast , S. typhimurium which evokes gastroenteritis in man , causes a typhoid fever – like disease in mice (call murine typhoid) . Both human typhoid and murine typhoid are systemic illnesses . In mice infected orally with S. typhimurium or in humans who ingest food or water contaminated with S. typhi , the bacteria either multiply in the small bowel or directly penetrate the intestinal mucosa without apparent enteric colonization (12 , 15 , 16) . Studies in mice have established that the foci from which Salmonella disseminate are the Peyer's patches of the small intestine (16,17) . The bacteria apparently gain access to the circulation via the lymphatics , seed the reticuloendothelial cell system (RES) and replicate within splenic and hepatic tissues . A secondary bactermia ensues following growth of Salmonella in these RES organs which , in turn , furthers systemic dissemination of the organisms . The pathogenesis of murine typhoid is illustrated in Figure 1 (18) . It should be emphasized that the sequence of events that occurs once S. typhimurium becomes systemic is the same whether animals are inoculated orally or parenterally .
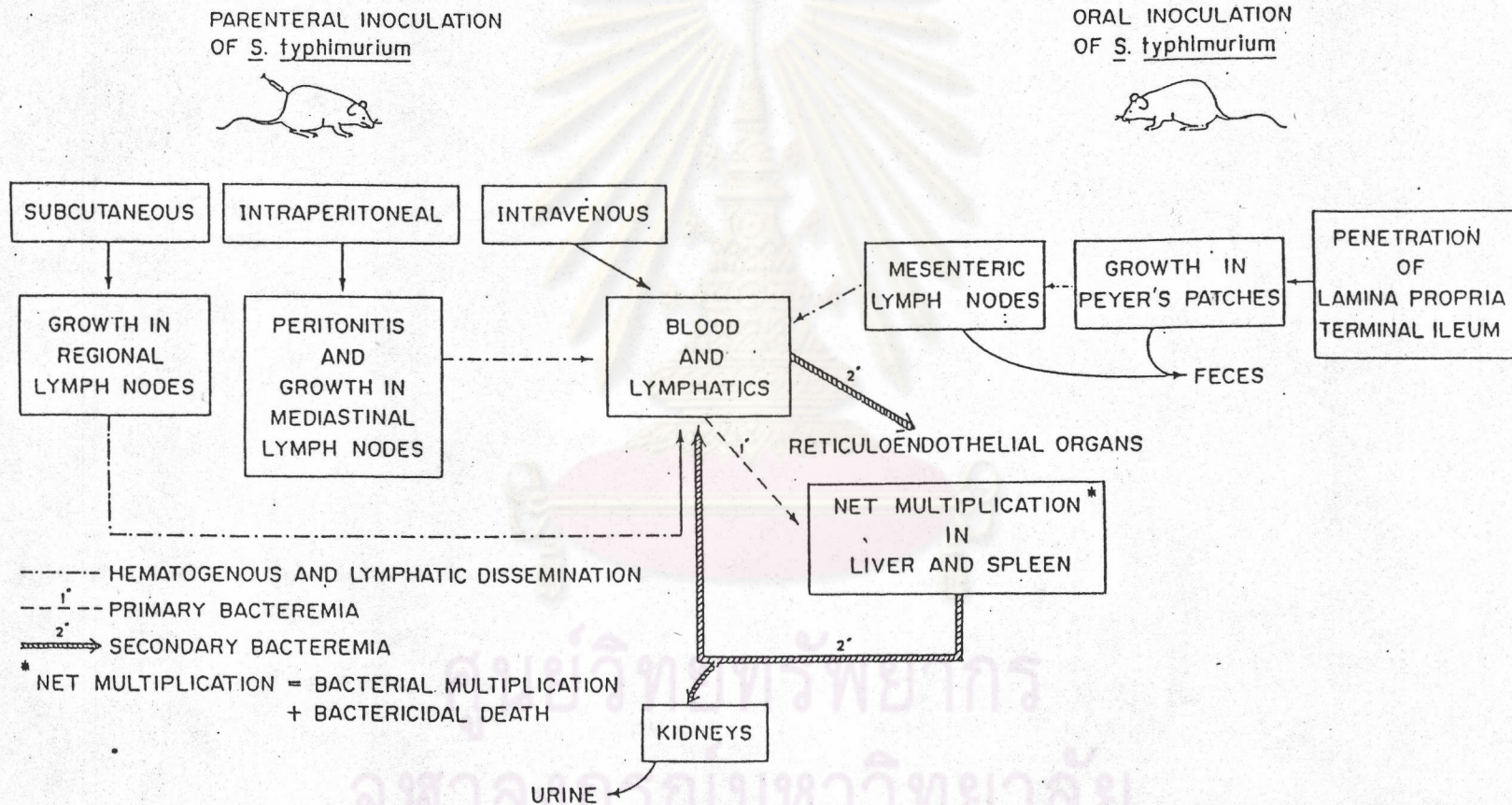
Figure 1   Pathogenesis of Murine Typhoid

Virulence Factors in Salmonella

As in other organisms , several surface components such as the Vi antigen or smooth LPS increase the virulence of this pathogen by hindering or preventing phagocytosis or complement mediated killing . Further , the presence of pili or other appendages that enable the organism to adhere to mucosal surfaces will promote colonization of the intestinal tract and incraese its virulence .

Oral Typhoid Vaccines

Typhoid fever is a disease for which medical reserchers have long been seeking an effective vaccine . Although currently available parenteral vaccines are effective (19 , 20) , they are not widely used because undesirable side - effects are frequently reported ; the development of a safe and effective oral vaccine has generally been considered the ultimate goal . Furthermore , it has been an axiom of local immunity that responses are best stimulated by the local application of antigen and clearly an understanding of the route by which orally administered material is taken up and processed by the intestinal immune system is essential to an appreciation of the process of oral immunization .

1. Oral Killed Vaccines

Killed whole - cell vaccines for oral application have been commercially available for at least 50 years . They were introduced for practical reasons , such as ease of administration , lack of side - reactions , simplicity of production and absense of any hazards . The efficiency of these oral vaccines has , however , never been demonstrated in clinical challenge studies or properly

controlled field trials . In a challenge study , volunteers received six tablets containing 10^{10} killed S. typhi . No protection was demonstrated . When the dosage was doubled to to 12 tablets , an efficacy of 30 % became evident (21) .

In the first field trial performed in a large population of children in India , the same vaccine administered in three doses of one tablet showed an effectiveness of 24 % (22) . Taking into account the results obtained in the volunteer studies in the second and third trials , the concentration of killed bacteria per tablet was increased to 3×10^{11} and 4×10^{11}, respectively . The marginally encouraging results of the first trial could not be confirmed (23).

2. Oral Live Vaccines

There is as yet no reliable serological test available that could predict vaccine efficacy in man . Experimental work on immunity to Salmonella infection has to be carried out in an animal model . From studies with the S. typhimurium mouse model , it appears that optimal protection comparable to that induced by a sublethal infection with virulent bacteria can only be achieved by vaccination with live attenuated bacteria . This is particularly evident when the oral route of administration is used . Multiple of killed vaccines can delay the proliferation of virulent challenge bacteria somewhat in the macrophages ; they are , however , unable to prevent subsequent multiplication to lethal concentration . Only live vaccines provide the mice with protection comparable to that induced by a sublethal first infection with virulent bacteria .

The first oral live vaccine tested in man consisted of streptomycin - dependent S. typhi bacteria . This vaccine proved to

be safe in volunteer studies but failed in its final lyophilized form
to induce clinical protection (24) . Based on the results obtained in
the S. typhimurium mouse model , S.typhi mutant Ty 21a , which lacks
the enzyme UDP -4 - galactose - epimerase, was developed as a
candidate vaccine strain (3) . The main characteristic of this
attenuated strain is that cell - wall lipopolysaccharides , which are
responsible for virulence and which appeared to be essential for the
protective capacity of attenuated strains , are only synthesized
under conditions which induce autolysis of the bacteria . This
mechanism renders the S. typhi Ty 21 a bacteria avirulent in spite of
the presence on the bacterial cell wall of the single known virulence
factor of Salmonella .

The attenuated S.typhi strain Ty 21 a was tested for its
stabilily , safety and efficacy as a live oral vaccine in 155
American Volunteers (4) . These individuals ingested five to eight
doses of up to 3 - 10 x 10^{10} of freshly prepared live bacteria
without significant adverse reaction . Despite this high dosage ,
vaccine bacteria were seldom secreted on the day after vaccination
and never for longer than two to three days . Of the 958 S.typhi
isolates from coproculture , none showed any signs of reversion to
the wild type . Efficacy of the vaccine was evaluated in an
experimental challenge study and was shown to be 87 % (4) .   This
result was obtained from a challenge dose that caused typhoid fever
in 53 % of the non - immunized control group . In earlier studies ,
parenteral vaccines demonstrated no protection against such a high
challenge dose . In addition to protection against clinical disease ,
the Ty 21 a vaccine also stimulated local immunity : the vaccinees
excreted the virulent challenge bacteria for a shorter period of time
than the controls .

In 1978 , a controlled field trial was eventually started

in Alexandria , Egypt , to evaluate the efficacy of oral typhoid vaccine Ty 21 a in an area endemic for typhoid fever (25) . More than 32,000 six to seven - year - old school children were involved in this trial ; half of them received three doses of vaccine containing on average 3×10^9 live Ty 21 a organisms , and the remainder three doses of placebo . To neutralize gastric acidity , 1 g of bicarbonate was given a few minutes before the vaccine . The vaccine was well tolerated . During a pilot study on 884 children , the vaccine strain was not isolated from any of the stool specimens which were collected two and seven days after vaccination . These children , as well as a third group of 25,628 school children of the same age who received neither vaccine nor placebo , were submitted to the same surveillance . One single case of typhoid fever , as confirmed by positive blood culture , occurred in the vaccinated group as compared to 22 cases in the placebo and 39 cases in the non - vaccinated group . Three probable typhoid cases , detected by positive stool culture and (or) sero - diagnosis , were reported in the vaccine group as compared to 41 cases in the placebo and 60 cases in the non - vaccinated group . These figures represent a 95 % vaccine efficacy . These results indicate that in the form and dosage used , the live oral vaccine Ty 21 a is safe , stable and effective against typhoid fever for at least three years . Subsequent test of this vaccine incoporated into enteric coated gelatin capsules , carried out in schoolchildren in Santiago , Chile provided 67 % efficacy for at least 3 years . (26) Following the development of the Ty 21a mutant , Stocker et al (27) described the production of aromatic auxotrophic strains of S.typhi which were made avirulent by their nutritional requirements ; such strains , since they are of wild - type antigenic character , have proved to be effective live vaccines. Indeed these authors report that a single intraperitoneal dose of one of these aromatic - dependent strains (S. typhimurium SL 3235) to

hypersusceptible mice , proved safe and protected well against a later challenge with a virulent S.typhimurium strain .

3. Gal E Mutant of Salmonella typhimurium

S. typhimurium G_{30} is a gal E mutant strain of S. typhimurium C_5 which is the agent of murine typhoid .

Gal E mutant of S .typhimurium have a defective uridine diphosphate (UDP) galactose – 4 – epimerase , the enzyme is responsible for the synthesis of UDP – galactose , thus these mutants are unable to incorporate the sugar into their lipopolysaccharide and the organisms are " rough " . However , unlike most rough Salmonella, gal E mutants are as efficient at conferring immunity to mice as a sublethal infection by a smooth organism (1) . From Figure 2 , in the presence of exogenous galactose , UDP – galactose is formed via galactose – 1 – phosphate , thereby by – passing the defective epimerase and smooth lipopolysaccharide is synthesized (28) . The lack of an effective epimerase prevents the normal metabolism of exogenous galactose and its accumulation in the cell as galactose – 1 – phosphate iniates the lysis of the growing bacterium (29) . Germanier and Furer (2) have suggested that the protective capacity of the mutant in mice may be the net effect of these 2 properties . Thus , the lack of virulence together with their immunogenicity could be explained by an initial period of growth followed ultimately by lysis induced through utilization of host galactose .
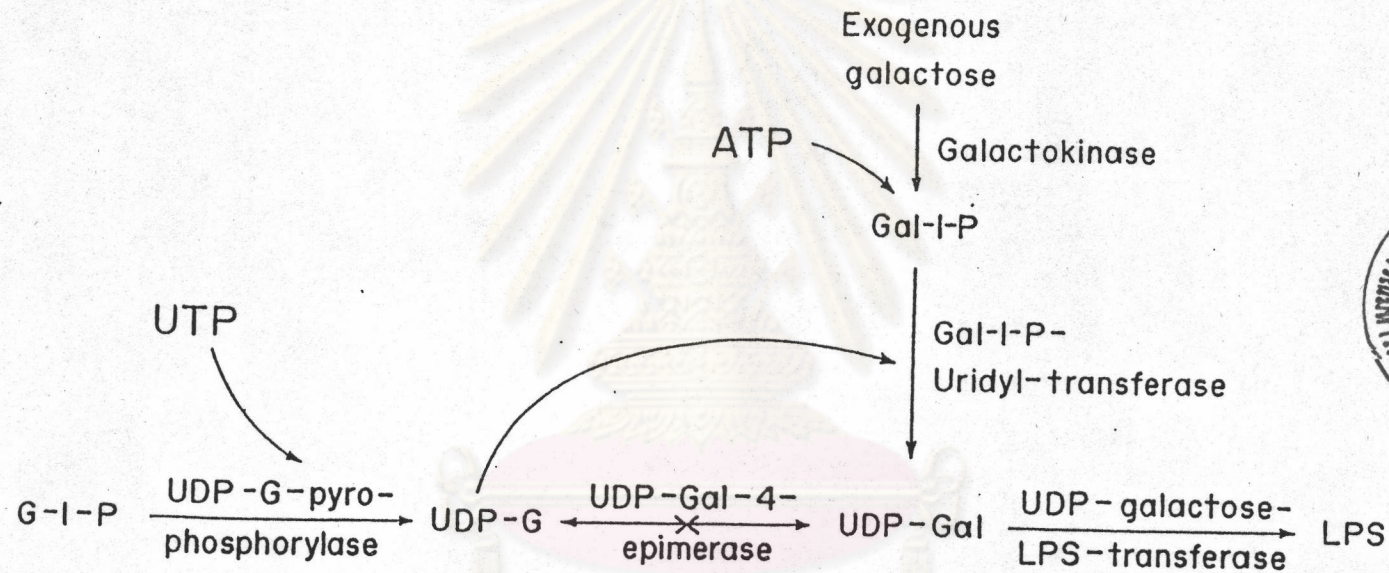
Figure 2   Schematic Representation of the Incorporation of Exogenous

Galactose into the Lipopolysaccharides of gal E Mutants

Salmonella typhimurium Resistance Genes of Inbred Mice

Several experimental parameters influence whether mice survive challenge with S.typhimurium , and these include : the strain of S.typhimurium , the dose and route of infection , and the particular strain of mice (30 ,31) . When mice of different inbred strains are inoculated via the same route , they exhibit dose - dependent variable susceptibility to S.typhimurium (30 ,32 , 33) ;the parenteral LD_{50} of highly virulent S.typhimurium for some strains (e.g. , BALB/c , C57BL/6) is <90 bacteria , whereas the LD_{50} for other strains (e.g. ,CBA .A/J) is >10^{4} (34) . The differential response of mice to S.typhimurium may therefore serve as a model for the genetic control of resistance of an infections organism and as a probe to evaluate mechanisms of immunity to typhoid fever . Furthermore , recent studies indicate that expression of several distinct host genes determines whether S.typhimurium - inoculated mice survive murine typhoid (31) , and genes appear to act at different phases of the infectious process . Thus , a careful dissection of how specific host genes affect the response of mice to S. typhimurium infection will facilitate a better understanding of the importance of immunologically specific and nonspecific resistance mechanisms during each stage of murine typhoid .

The response of mice to S.typhimurium infection (murine typhoid) is controlled by the expression of at least three distinct host genes . One of these genes , Ity , is located on chromosome 1 (35 ,36) , and inbred strains of mice carry either its dominant resistance (Ity^{r}) or recessive susceptibility (Ity^{s}) allele (33) . A 2 nd salmonella - response gene , Lps^{d} , is a mutant allele of the chromosome 4 LPS - response gene (37) . Mice that express this allele are refractory to the biologic effects of endotoxin (37 ,38) and are

salmonella susceptible (39) . The 3 rd gene , xid , is x — linked (4Ø) and confers both salmonella sensitivity (41) and a B cell functional defect (42 , 43) on CBA/N mice and F_1 male progeny derived from CBA/N female parents .

Although the mechanisms whereby these genes regulate the course of S.typhimurium infection have not been delineated , most investigators agree that macrophages play a critical role in the early phase of the infections process . Several lines of evidence support this hypothesis . First , in vivo treatment of innately resistant Ity^r mice with silica , an agent that is selectively toxic for macrophages (44) , converts such animals to a salmonella — susceptible phenotype (45) . Second , Ity^s mice are unable to control the initial net growth of the bacteria in the liver or spleen , whereas Ity^r mice can control the replication of Salmonella in these reticuloendothelial cell organs (30 , 32 , 33 , 35 , 36) Presumably , the early deaths of Ity^s mice reflect this unrestricted bacterial multiplication . Third , Maier and Oels (46) demonstrated that macrophages from Ity^r mice kill salmonella better than do Ity^s macrophages .

1. Ity : Genetics

The host gene which regulates how well mice control the early replication of S.typhimurium in splenic and hepatic tissues after intravenous (i.v.) or subcutaneous (s.c.) challenge with S.typhimurium was designated Ity (for immunity to S. typhimurium by Plant and Glynn (47) . These investigators recogniqed the existence of Ity by the response to s.c. challenge of F_1 , F_2 , and backcross generations derived from matings of Salmonella — resistant (LD_{50} of S.typhimurium C_5 by s.c. route for BALB/C mice < 1Ø) parental strains

(33) . They observed that resistance among these hybrid mice was controlled by a single , autosomal , non - H - 2 - linked gene , and they called the susceptibility allele Ity^s and the resistance allele Ity^r Plant and Glynn subsequently mapped Ity to mouse chromosome 1 by a series of linkage studies with known chromosomal markers (35) . They confirmed that the Ity locus was located on mouse chromosome 1 by evaluation of the pattern of Salmonella susceptibility among 48 recombinant inbred strains of mice (36) .

Protective Factors Operating in the Gut

The local immune system within the intestine provides a number of mechanisms whereby pathogenic microorganisms are expelled from the gut , but in addition the intestinal tract possesses features of innate resistance which , since they act in synergy with those of the immune system , must be considered in immune mediated protection in the gut . However a fundamentally important factor in the expression of immunity at intestinal tract is as follow :

1. Innate Resistance

1.1 Gastric Acidity

The stomach with its low pH acts as an efficient bactericidal trap limiting the number of viable bacteria and viruses which can enter the intestine . At pH 3.0 the bactericidal effect is complete (48) , although the susceptibility of bacteria and viruses to low pH varies . The importance of gastric acidity in protecting the intestine can be seen from the increased infection rates in individuals with a neutral pH (49) . In addition to bactericidal effects gastric pH can also reduce the antigenicity of protein (50)

and this is further affected by proteolytic enzymes within the small intestine (51) .

1.2 Peristalsis

Peristalsis is probably the major factor reducing bacterial numbers within the small intestine . Organisms introduced into the small intestine are rapidly cleared by peristalsis (52) . So effective is peristalsis that it is probably essential for microorganisms that colonise the small intestine to adhere to the epithelial surface via specific receptor mechanisms (53) and inhibition of this adherence is a powerful immune strategy .

1.3 Mucus

Mucus has a number of functions within the intestinal tract . It lubricates the epithelial surface , protects it from the low pH of the stomach , and act as a medium in which digestion and absorption can occur . In addition , it has direct antimicrobial effects by cleansing the epithelial surface . It can entrap bacteria (54) and so facilitate their removal . The mucus molecule has been shown to possess regions mimicing the receptor sites for bacteria present on epithelial cells and this may facilitate their trapping (55) .

1.4 Normal Microflora

The normal microflora of the small intestine represents a stable ecosystem which appears to be able to resist colonization by pathogenic microorganisms . The most dramatic demonstration of this comes from the observation that while normal

mice require an oral dose of $\underline{S.\ enteriditis}$ organisms of 1×10^5 to cause infection , a single dose of oral streptomycin will reduce the number required to as few as 10 (56) . The normal bacteria do this either by competing for space , or by occupying specific receptor sites , or by successfully competing for essential nutrients . In addition , they may render the environment toxic to the newcomers by altering the pH or producing toxic agents , such as colicins or volatile fatty acids . In addition to these direct effects the intense competition that the indigenous microflora provides will amplify any small antibacterial effect and so lead to the rapid displacement of any bacterial species which is selectively disadventaged ,even though the degree to which it is discriminated against may be quite small .

2. Immune Resistance

2.1 Immunoglobulin A (IgA)

IgA is the major immunoglobulin in the intestinal secretions of most species and as such is presumed to play an important role in immune defence of mucosal surfaces . It is secreted through the epithelial cell and appears to become associated with the mucus layer (57) . Its effects within the intestinal lumen appear to be essentially passive . It probably does not activate complement (58) and can not opsonize . It may participate in or facilitate antibody - dependent cell mediated cytotoxicity (ADCC) (59) . Of greater significance is the effect of IgA in interfering with the binding of microorganisms and their products to the epithelium . As noted above , Such binding is assential to ensure the colonization of microorganisms within the small intestine and it has been shown that secretory antibody which prevents this adhesion results in the

elimination of the bacteria . Thus , IgA antibody against <u>Vibrio cholera</u> will prevent disease by interfering with the attachment of the bacteria to the intestinal wall , even - though the number of organisms within the loop are not reduced (60) .

2.2 Other Immunoglobuins

Although IgA is quantitatively the major immunoglobulin in most species except ruminants (61) selective IgA deficiency is the commonest immunodeficiency (62) and is often asymptomatic (63) , probably because the functions of IgA are taken over by IgG and IgM (64) . In normal individuals IgG is only a minor component of secretions and appears as a result of passive transduction although it may effect , albeit inefficiently , events within the intestinal lumen (65) . It has been suggested that IgG may in these circumstances be harmful by virture of its more active Fc region and in particular complement activation may cause damage to the epithelial barrier . In vivo and in vitro evidence suggests that although IgG will effectively block absorption of its specific antigen across an epithelial surface , absorption of non - cross reading by - stander antigen is increased (66 , 67) . Other effects of IgG may be more useful to the host . In particular IgG antigen - antibody complexes can bring about the release of mucus from goblet cells and increase its cleansing activity (68) .

IgM is the major secretory immunoglobulin of young animals (69) . IgA deficient individuals (70) and during the early secretory immune response (71) . Direct evidence for its efficacy within the small intestine is limited but can be inferred from experiments in which the in vivo effects of IgA , IgG and IgM were compared , where all three immunoglobulins were found to be equally

effective against cholera using the infant mouse gut as an experimental model (72) .

2.3 Cell Mediated Immunity (CMI)

Little is known of the functions of cell mediated immunity (CMI) in the intestinal tract . Delayed type hypersensitivity (DTH) responses have been observed in the intestine (73 , 74) and evidence exists that lymphokine activated macrophages may contribute to local resistance to salmonellosis and Listeriosis (75) . Cytotoxic responses may also be of importance in the intestine . Cytotoxic T lymphocytes (CTL) , natural killer (NK) and killer (K) cells have all been identified among the intraepithelial lymphocyte and lymphocytes of the lamina propria (76) and preliminary evidence suggests that cytotoxic reactions may be implicated in defense against several pathogens , including S.typhimurium in mice (77) . While it is too early to assess the significance of these results , it is probable that CMI response contribute significantly to mucosal protection in a number of diseases .

The Immune Response to Oral Immunization

Oral immunization has been proposed as a method of administering vaccines for a large number of years and several different reasons have been advanced to justify this approach . Besredka (78) suggested that only by copying a disease will the disease be overcome and this approach not only inspired the early studied but also led to the sucessful development of the oral polio vaccine . The discovery of the mucosal immune system and an apperciation of its importance in enteric disease , however , laid a more rational framework which extended this earlier intuitive

approach and it is now clear that only by the efficient stimulation of mucosal immunity will enteric disease controlled through vaccination .

Oral immunization is not new . Indeed according to Raetting (79) it was practised by the ancient Egyptians , but its history really started with the work of Besredka . In experiments and field trials involving both Shigella and Salmonella organisms , Besredka (78) showed that a significant degree of protection could be achieved through the feeding of inactivated bacteria .

1. Gut Associated Lymphoid Tissue

Gut associated lymphoid tissue (GALT) consists of discrete lymphoid aggregates which line the intestinal and are known Peyer' s patches , as well as a variety of lymphoid cells dispersed throughout the lamina propria and epithelial layer . Purely physical considerations would suggest that the induction of an immune response is most likely to occur . In the Peyer' s patch because the physical separation of cells from one another in the lamina propria and epithelium would make the various cellular interactions that are necessary for induction much less likely to occur than in Peyer' s patches where the cells are in close contact . Support for the concept of the Peyer' s patch as the major site in GALT for immune induction comes from two rather special properties of Peyer' s patches . The first of these is the dome epithelium (Figure 3) which covers the luminal surface of the Peyer' s patch . Specialised cells present in this dome epithelium , called M Cells , have been shown to pinocytose luminal materials and transport them to the lymphoid cells of the Peyer' s patch (80) . Peyer' s patches can thus be viewed as specialised antigen sampling sites along the intestine . A second

special feature of the Payer's patch is that it appears to represent a major site for the induction of IgA responses (81) , and it is well known that the immunization which follows an antigen exposure via the intestine is proferential for the IgA class .

The induction of an immune response in GALT presumably involves a series of cellular interactions . There is a potential regulatory step which precedes all of these cellular events in the Peyer's patch , namely the uptake of antigen into the patch via M cells . (Figure 4)

1.1 Histology of Peyer's patches

The gastrointestinal tract is replete with lymphoid tissue capable of mounting an immunologic response to prevent the penetration of the epithelial barrier by antigens . Lymphocytes and macrophages are present in abundance as aggregates in the Peyer's patches of the small intestine or as a diffuse population of cells in the lamina propria of the small and large intestine , where they co-exist with immunoglobulin secreting plasma cells . The Peyer's patches which are formed by groups of lymphoid follicled are also distributed throughout the ileum and appendix . Their distribution in humans increases distally from a few follicles to more than 900 in the terminal ileum (82) ; there is a similar increase in bacterial concentration . In mice or rats , patches of roughly uniform size occur throughout the small intestine and usually consist of 3 to 9 follicles (83) . In mice , Peyer's patches appear as white bulges on the serosal surface . The active follicles form mounds , pushing aside the ville and projecting in to the lumen (Figure 3) . In Balb/c mice , follicles are large and round with rapidly replicating lymphocytes . Some mouse strains , such as C57 , have smaller ,

flatter follicles with a less active lymphocyte population .

Owen (84) gives the following description of Peyer's patches in mice . The surface of the Peyer's patch follicle consists of columnar cells , covered by closely knit microvilli and intervening M cells whose surfaces are roughened and irregular . The M cell provides a reduced barrier to particles , and large molecules and the membrane - like attenuation of M cell cytoplasm facilitates the approach of underlying lymphocytes (mainly T - cells) and macrophages to the intestinal lumen (Figure 4) .

Macromolecules are transported by M cells from the lumen , by vesicles , into the space between cells surrounding the migrating lymphocytes (80) . Beneath the dome epithelium is an area populated largely by macrophages and lymphocytes (85) which seem to be in constant transit in and out of the epithelium and into the lymphatics .

Beneath this traffic area lie the germinal centers consisting largely of B cells and macrophages containing cellular and non cellular debris (86) . Germinal centers lie deeper in the patch tissues and these develop only when the surfaces of the patches are exposed to luminal antigens (84) .

1.2 Peyer's patch Macrophages

Because macrophages play such a central role in the induction of the immune response , one would presume that Peyer's patch macrophages must play an important role in enteric immunization . This has made particularly puzzling the observations in vitro experiments that murine Peyer's patch macrophages are

functionally deficient (88 ,89) although they are evident in histology sections of Peyer's patches (90 ,91) . Some recent work may provide an explanation . Functional macrophages can be demonstrated in murine Peyer's patch in vitro but they are not recovered by the conventional technique of teasing the Peyer's patches apart . When murine Peyer's patches were dissociated with a neutral protease instead , 5 - 10 % of the cells were macrophages on the basis of non specific esterase staining and phagocytosis of latex beads (92) . Furthermore , macrophage - dependent functions , such as non - specific mitogen - induced T cell proliferation and mitogen induced immunoglobulin synthesis , could be demonstrated in Peyer's patch cells recovered using the enzyme treatment . Peyer's patch macrophages are also capable of presenting specific antigen to antigen primed T cell (93) . Peyer's patches macrophages released by collagenase treatment could present antigen after being pulsed in vitro or alternatively after being pulsed in vivo by intragastric feeding of the antigen some hours before sacrifice . Functional macrophages were not demonstrable in any of these experiments if Peyer's patches were simply teased apart , as had been done in previous work . These results indicate that murine Peyer's patch macrophages are fully capable of presenting antigen but that they are more firmly situated on a supporting stroma then macrophage in other lymphoid tissue such as the spleen .

The importance of macrophages in Peyer's patches in phagocytosis was demonstrated by Owen (94) . His studies in mice with Giardia infection (as a model of gut immunity) showed that macrophages apparently function within and below the intestinal epithelium by phagocytizing dying cells , particles and microorganisms especially in the lymphoid follicles where the uptake of luminal organisms is enhanced . Macrophages entering as extending

pseudopods into follicle epithelium supplement lymphocytes in taking
up macromolecules transported from the lumen by M cells .

1.3 Antigen Uptake by GALT

Particulate transport appears to result from the
functioning of a system which provides controlled contact between
luminal Ags and immunologically responsive cells . The process ,
which has been described as the " sampling " of intestinal contents
(80 ,95 - 97) , apparently takes place by the routing of material
from the intestinal lumen through the cytoplasm of special epithelial
cells . Owen (80) has described the uptake of horseradish peroxidase
by " membranous " (M) cells in the Peyer' s patch epithelium of mice.
M cells had irregular , stubby microvilli , attenuated cytoplasm and
prominent apical vesicles which contained horseradish peroxidase a
few minutes after the imtroduction of this substance into the lumen
of the mouse intestine . The M cells enfolded lymphocytes which were
often seperated from the intestinal lumen by only a thin margin of M
cell cytoplasm . An hour after its introduction , horseradish
peroxidase was present in lateral intercellular spaces and was being
pinocytosed by enfolded lymphocytes . Besides , Joel et al (98) have
described carbon particles within large vacuoles in the Peyer's patch
epithelium of mice given particulate carbon by gavage .
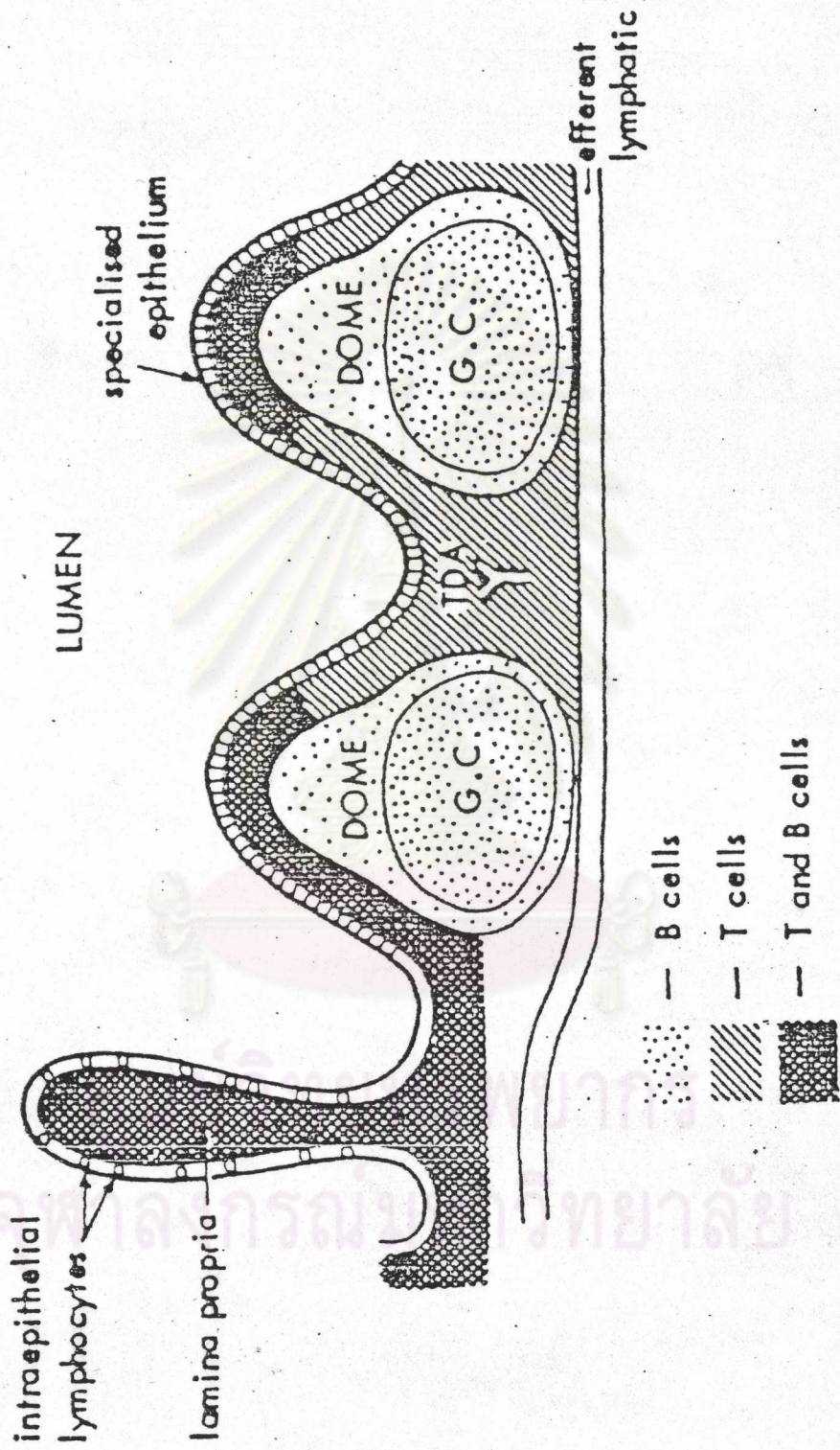
Figure 3   Diagram of Peyer's patches structure in the mouse.

Gc = germinal center , TDA = Thymus – dependent area (37) .
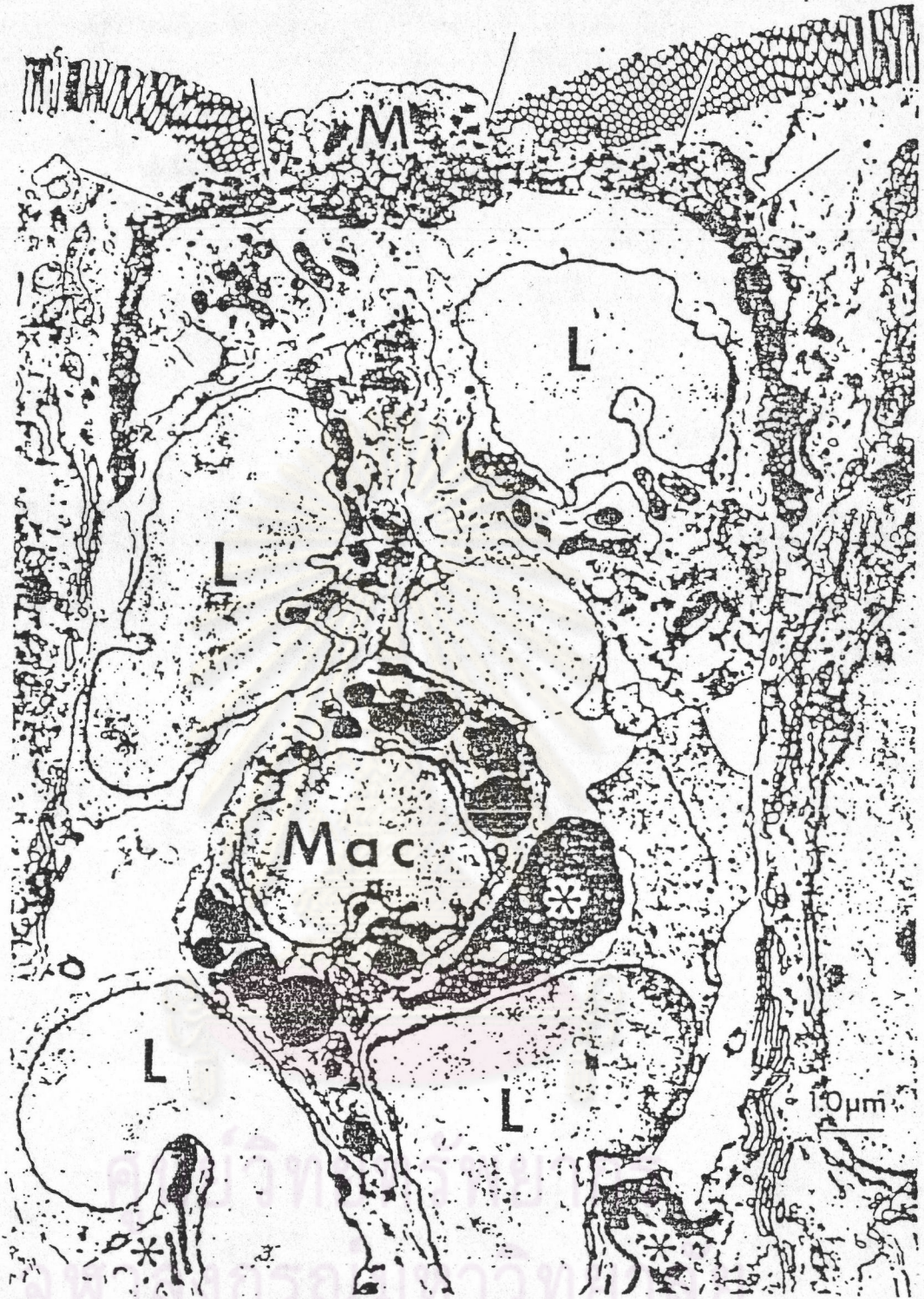
Figure 4 Darkly stained cytoplasm of a Mouse M cell (M) streched between two microvillus - covered columnar cells (c) like a membrane seperating wandering lymphocyte (L) and a macrophage (MAC) from the lumen above . Vesicles (arrows) are transporting exogenously administered horse - radish peroxidase from lumen to enfolded lymphoid cells .

2. Antibody Responses Following Oral Immunization

Studies in which mice have been orally immunized with S.typhimurium (99) or Escherichia coli (100) have all shown there is a close relationship between the amount of antigen fed and the magnitude of antibody elicited , with the greatest response requiring very large closes of antigen . Similarly , following the injection of bacterial suspensions into the Peyer's patches of rats the amount of biliary IgA antibody stimulated is dependent upon dose (101) . However , whilst it is clearly possible to stimulate the production of IgA antibodies in the gut secretions of pigs (102) and calves (103) by feeding dead bacteria , it has been possible to compare , by making use of the immunological link that exists between the gut and mammary gland (104) , the efficacy of live and dead E.coli antigens . Such investigations have shown that the IgA response in both milk and gut secretions to E.coli is both reduced and delayed in sows fed heat killed organism (105) compared to those fed live bacteria . Further , it has been shown that in order to stimulate a secretory detectable by serological means with the live organism , a dose sufficiently large to promote multiplication within the tract is required (106) . Interestingly , whilst rabbits immunized by feeding 10^{10} live Shigella on three - weekly occasions , elicit a good secretory IgA antibody response when challenged 60 days later (107) those fed with the dead organism show no evedence of memory .

3. Cell Mediated Immune Response Following Oral Immunization

Much of the work of characterizing the mucosal immune system and developing oral vaccines has focussed on the humoral immune system . However , it is clear that cell mediated immune

mechanisms may also be activated and a variety of parameters have been measured following oral presentation of parasites ; e.g. Schistosoma mansoni in rats (108) and Giardia in mice (109) . Besides A direct in vitro test measuring the antibacterial activity of lymphocytes from GALT has previously been employed to test successfully cell - mediated immunity in mice against S.typhimurium (77 , 110 - 112) .

Macrophage Functions in Antimicrobial Defense

1. Mechanisms of phagocytosis

Phagocytosis is the principal host mechanism for the elimination of most microorganisms and is a function of monocytes , which in the tissues differentiate into macrophages . The monocytes/ macrophages lineage is generated in hemopoietic tissue , in adult mammalian organisms mainly in the bone marrow (113) . Mechanisms of phagocytosis are illustrated in Figure 5 (114) Attachment of microorganisms to the phagocyte is facilitated by Fc and c3b receptors on the phagocyte surface . IgG may bind first to the microorganism and then to the phagocyte , or the antibody may bind first to the phagocyte , as so - called cytophilic antibody , and then to the microorgamism . Cytophilic antibody may be IgG or IgM , and its Fc region thus binds to one of several different Fc receptors on the phagocyte . C3b may be generated on the microorganism surface by the classes or alternate pathways . Such C3b - coated microorganisms are then bound to the phagocyte at the C3b receptor . The attached microorganism is then surrounded by pseudopodia of the phagocyte and ingested . The resultant vesicle or phagosome fuses with a lysosomal vesicle to form a phagolysoome . Within this structure , the microorganism is destroyed by oxygen - independent

and oxygen - dependent mechanisms . The later depend very heavily on
the generation of intracellular peroxide .

PHAGOCYTOSIS

O_2 –INDEPENDENT MECHANISMS	O_2 –DEPENDENT MECHANISMS
Low pH	
Lactoferrin	$H_2 O_2$
Lysozyme	
Glycosidases	$[O^-]$
Proteases	$[OH^-]$
Lipases	
Granular cationic proteins (PMN's only)	$[O_2^-]$

ATTACHMENT   INGESTION   PHAGOSOME FORMATION

DESTRUCTION OF THE MICROORGANISM   PHAGOLYSOSOME FORMATION
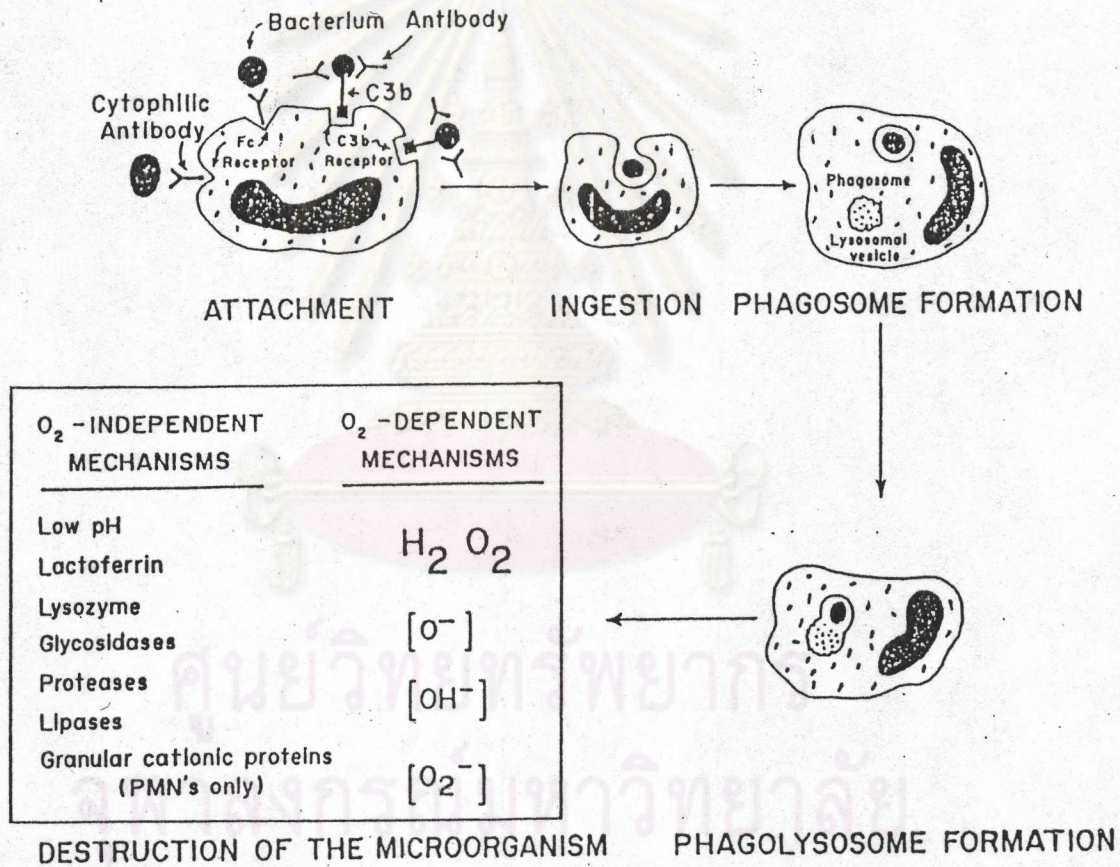
Figure 5   Mechanism of Phagocytosis

2. Activation of Macrophages

The term " activated macrophage " was first introdnced by Mackaness in the 1960' s to describe the enhanced microbicidal activity of macrophages from animals undergoing a cell – mediated immune response to intracellular pathogens . The investigations of Lurie (115) concerning tuberculosis provided the first direct demonstration that macrophages harvested from vaccinated animals infected with tubercle bacilli in vitro display an enhanced capacity for inhibiting the intracellular growth of bacteria . This type of immunity has been shown to be nonspecific in its expression (116) , since macrophages from animals immunized against a particular bacterium display increased microbicidal activity against a variety of unrelated bacteria . However , the acquisition of these mechanisms depends on the generation of a specific T cell – mediated immune response (117 , 118) . Numerous reports have further established that macrophages are activated by antigen – stimulated lymphocytes through the action of their secretion products or lymphokines (119 , 120) .

2.1 Activating Agents of Macrophages

As already mentioned above , activated macrophages have been originally abtained from animals infected with bacteria such as Brucella abortus (121) , Salmonella typhi (122) , Listeria monocytogenes (123) , Corynebacterium parvum (124) or BCG (125) . The same agents activate normal macrophages in vitro , provided sensitized lymphocytes are present . Indeed , the effector function of activated macrophages is nonspecific , though greater resistance is obtained with the original infecting agent (126) . However , its generation requires a specific contact between antigen and T cell ,

resulting in the secretion of soluble factors , or lymphokines . They modulate the physilolgy of macrophages , as first demonstrated by the inhibition of macrophage migration from capillary tubes (127) . The factor inducing this macrophage activation : MAF (Macrophage Activating Factor) has been partially purified (128) and although its identity with MIF (Migration Inhibitory Factor) has been claimed for a long time , two groups have successfully siperated these two activities (129 , 130) .

Several agents induce macrophage activation without any requirement for sensitized lymphocytes . They include bacterial endotoxin (131) and polyanions , such as double stranded RNA (132) as well as pyran copolymer (133) . It has been proposed that the action of these molecules might be mediated by interferon (132) or components of complement (134) . Macrophage cytotoxicity can also be induced by immunoglobulins or immune complexes (131) .

3. Attachment of Microbes to the Macrophage Surface

In contrast to phagocytosis , attachment of particulate mather , especially microorganisms ,to the macrophage surface does not require energy and is relatively independent of temperature . The two processes are ,therefore , not necessarily linked to each other (135) . The binding of bacteria and other microbes to the cell surface is commonly thought to be mediated by surface receptors . Fibronectin has been termed a " molecular glue " and may , as well as heparin , be instrumental in attaching small particles to the phagocyte surface (136) . Lectins also exhibit opsonizing properties (137) . Probably much more important are C3b - receptors since C3 is produced by macrophages and can be activated directly via the alternative pathway by a variety of microorganisms (138) .

Theoretically , this should enable macrophages to attach C3b - coated bacteria to their surface in the absence of a specific immune response . Of even greater importance than C3b - receptors are Fc - receptors which seem to be distinct for different Ig classes and subclasses (139) . Particles covered by immune complexes also attach to Fc - receptors of macrophages (140) .

4. Phagocytosis of Microorganisms by Macrophages

As mentioned before , phagocytosis of a particle attached to the phagocyte surface is a temperature - and energy - dependent process . Binding to one type of receptor is thought to require displacement of identical receptors to the site of particle attachment before endocytosis can occur . In addition , a reserve of membrane components must be made available , be it (with the help of the Ca^{2+} - dependent phospholipase A_2 ?) via new synthesis of phospholipids , be it by " stretching " membrane folds (141) . Phagocytosis is made possible by changes in the sol/gel properties of contractile proteins which allow for membrane protrusions to " engulf " the particle (142) .

5. Microbial Activities of Macrophages

One ability of the professional phagocytes is to kill other cells , including microorganisms . It is generally accepted that O_2 - dependent bactericidal mechanisms are more efficient than those that occur in the absence of oxygen . In fact , the killing capacity of phagocytes correlates in many model systems with the magnitude of the respiratory burst that is elicited . It involves an enhanced catabolism of glucose via the pentose shunt , and an activation of oxidases , in particular NADPH - oxidase , and - to a

lesser extent - glutathione peroxidase and reductase . Intermediate products of oxygen reduction comprise superoxide anions (O_2^-) , hydrogen peroxide $(H_2 O_2)$, hydroxyl radicals $(OH.)$ and singlet oxygen (o_2) . These highly reactive agents are toxic to all cells (142) . Macrophages can be primed for an enhanced respiratory burst by prior exposure to proteolytic enzyme (143) . It remains to be examined if macrophages , though the release of such enzymes , may be able to improve each other's killing capacity . In view of the highly toxic oxygen derivatives produced during a respiratory burst , the question arses as to how macrophages protect themselves against these offending agents . Oxygen radicals are probably generated in high concentrations only in minute compartments of the cytoplasm , e.g. the phagocytic vacuole , or they are released into the extracellular space . It should be remembered in this context that the NADPH - oxidase appears to be a membrane - bound enzyme . In addition , $H_2 O_2$ is counteracted by catalase which is present in most cells . Antioxidants such as ascorbic acid , vitamin E and the glutathione redox system may protect the cell from damaging effects exerted by other O_2 - derivatives .