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OLD HERBORN UNIVERSITY SEMINAR ON EFFECTIVE AND INEFFECTIVE DEFENCE MECHANISMS OF THE GASTROINTESTINAL TRACT:

REVIEW OF THE INTERNAL DISCUSSION

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INTRODUCTION

There is a growing interest in the notable interaction between the gastrointestinal immune system tract and the intestinal microflora. Studies dealing with this subject are rare, however, probably due to the complexity of the Gut Associated Lymphoid Tissue (GALT) and the diversity and high numbers of bacterial species colonising the gut. During the internal discussion among the speakers at the fifth Old Herborn University seminar some elements of the gut defence mechanisms were focused. In this overview several aspects have been grouped.

1. The first chapter deals with the immunological defence mechanisms of the gut mucosa, and the interaction between lymphocytes and the gut epithelium. Special attention has been given to the inflammatory bowel diseases ulcerative colitis (UC) and Crohn's disease (CD) and to coeliac disease.
2. In the second and the third chapter attention has been given to the functional role of cytokines e.g. TNF and interleukines, and endotoxin in health and disease.
3. Special notice has been given to monoclonal antibodies, to Lipopolysaccharide (LPS) and their potential use in the treatment of Gram-negative sepsis.
4. the fourth chapter deals with the defence mechanism of the stomach; the protective role of basic fibroblastic growth factor and in *Helicobacter pylori* the pathogenesis of gastric ulcer.
5. Finally some aspects among the neuro-immunological function of TNF and enkephalins are presented in the fifth chapter.

Histo-immunological areas and lymphocytes in the gut

The lymphoid system of the intestinal tract consists of solitary lymphoid follicles (LF), aggregated LF e.g. Peyer's patches (PP), and mesenteric lymph nodes (MLN) (the afferent limb); and isolated T and B lymphocytes and plasma cells in the lamina propria (LP) (the efferent limb).

LF are found along the mucosal lining of the colon up to a number of $2x10^4$. Since it is almost impossible to separate LF cells (afferent limb) from lamina propria cells (efferent limb), information on the cells of the LF cannot be obtained by Fluorescent Activated Cell Sorting (FACS) of single cell suspensions from colon, but can only be drawn from immunohistochemical investigations of cells in tissue sections from colonic mucosa. LF contain mainly B cells (61%). Other cells present in the LF are dentritic reticulum cells, which function as antigen presenting cells, and T cells (30% $CD4^+$, 8% $CD8^+$). The amount of $CD4^+$ and $CD8^+$ T cells in the LP is 56% and 30% respectively. The main proliferative activity is found in the centre of these follicles. The B cells of the LF mainly contain cytoplasmic IgG (78%) and IgM (14%), whereas in the surrounding LP the B cells mainly contain cytoplasmic IgA (92%). No secretory IgA is found in the follicle associated epithelium. This epithelium lacks secretory component (SC) and appears to resemble M cells, which are normally found at the site of PP.

Normally, M cells are responsible for the active uptake of (intestinal) antigens and the primary stimulation of Ig-producing cells takes place in the PP. Studies in chicken showed that the destruction of M cells in the bursa caused severe agammaglobulinaemia.

PP are only found in the small intestine and contain mainly thymus derived T cells. Intestinal antigens are handled primarily in the PP, whereas the secondary immune response is believed to occur in the regional lymph nodes. IgA plasma cells in the LP originate from the PP.

Intra epithelial lymphocytes (IEL) consist of several types of cells which are almost all $CD8^+$ (90%). Some carry the α/β TCR others the γ/δ TCR. The α/β cells are thymic dependent, are derived from the PP and require antigen for stimulation. On the other hand γ/δ cells are derived from the bone marrow and are thymic independent in the mouse; their origin is unknown in man. In man γ/δ T cells are mainly found in the colon, γ/δ IEL are considered to be end-stage cells. To our best knowledge there are no studies on the kinetics of the γ/δ T cells. The repertoire of γ/δ T cells is limited.

Production of IgA

Secretory IgA is the main defence mechanism in the digestive tract. Why IgA is produced in large amounts whereas other intestinal immune mechanisms are suppressed has not been fully elucidated. It might be due to the action of ill-defined contrasuppressor cells. The existence of these cells is still a subject of discussion. In mice intraepithelial γ/δ T cells may have a contrasuppressive function. The isotype switch from IgM to IgA is regulated by a so-called switch cell, originally thought to be a T cell but which in fact may be a macrophage. Switching from IgG directly to IgA is not reported, nor a switch from IgA to IgG. At present there is a lack of information about the actual regulation of the IgA immune response. However, transfer experiments with Ly1-B cells derived from geneti-

cally different donors showed that 50% of the IgA producing plasma cells in the lamina propria originated from Ly1-B cells. At what location in the body the isotype switch occurs is not known. It is unclear whether the IgA^+ B cells in the LP come from PP. Whereas the redistribution process itself is found to be antigen driven, the homing of B cells to lamina propria is regulated by vascular adhesion molecules so-called addressines.

Suppression

Possibly the most important immune mechanism in the digestive tract is suppression which prevents inflammatory reactions to food antigens and indigenous bacteria. Such intestinal hyporesponsiveness may involve clonal anergy, suppressor T lymphocytes, cytotoxic T lymphocytes and/or suppressor macrophages. Antigen handling by an intact gut epithelium seems to be critical. The numerous intraepithelial $CD8^+$ T cells may be involved in induction of hyporesponsiveness (oral tolerance). This type of immune response has been reported to be MHC class II restricted despite the well established MHC class I restriction of $CD8^+$ T cells; the presence of suppressor inducer T cells in the test system, however, was not excluded. MHC class II antigens are constitutively expressed on surface epithelial cells in the human small intestine, but occurs in colon epithelium only in inflammatory conditions.

One recent hypothesis is that down-regulation of immune responses in the gut mainly is caused by an interaction between gut epithelial cells expressing CD1 and T cells expressing CD8. Crosslinking of CD1 and CD8 is probably needed for suppression. Destruction of the gut epithelium, e.g. in inflammatory bowel disease, results in a lack of expression of CD1. This causes abrogation of oral tolerance and preferential stimulation of $CD4^+$ T cells. It has also been shown that $CD8^+$ γ/δ T cells may have helper function in the absence of CD1. The break in oral tolerance will lead to a systemic type of immune response.

There may be three approaches for study of the suppressor function in the gut.

1. One may focus on negative control systems. A lot of different phenomena are gathered under the umbrella of the suppressor cell. It is important to notice that there is not just one type of suppressor cell. Moreover, the function of 'suppressor cells' is very diverse and may depend on the stage at which they are activated during the immune response.
2. Functional studies can be carried out by eliminating parts of the immune system by thymectomy or administration of cytotoxic drugs e.g. cyclophosphamide. It is important to note that in case of athymic animals pre-T cells still occur. Cyclophosphamide has serious side effects including cytotoxicity, bone marrow suppression or possible induction of cytokines.
3. Another approach may be the elimination of single or multiple classes of cells of the immune system e.g. by using monoclonal antibodies directed against T afferent or T efferent cells.

Immunological aspects of Ulcerative Colitis, Crohn's Disease and Coeliac Disease

Examples of diseases in which the oral tolerance is abrogated are ulcerative colitis (UC), Crohn's disease (CD), and coeliac disease. As already mentioned the CD1 molecule is normally present on mucosal epithelial cells, whereas IEL carry the CD8 molecule. Crosslinking of both molecules, CD1 and CD8, is needed for suppression.

In UC there is a lack of epithelial expression of CD1. In mixed lymphocyte cultures with peripheral blood mononuclear cells and intestinal epithelial cells from patients with inflammatory bowel disease, preferential stimulation of $CD4^+$ T cells with helper function was observed. This may explain why polyclonal B cell activation occurs in UC, causing an increase of IgG. The IgG response, which is dominated by the IgG1 class, is strongly complement activating. Formation of IgG1 immune complexes with complement activation is probably the main effector mechanism in UC. High levels of antibodies to aerobic pathogenic bacteria such *E. coli*, *Pseudomonas*, *Yersinia*, *Str. bovis* and anaerobes like *Peptostreptococci* and *Bacteroides* spp. have been found in UC. These antibacterial antibodies are most likely not responsible for the primary lesions in UC, but secondary to the epithelial lesions. There is evidence that the lesions in UC are caused by an autoimmune reaction against a 40 kDa protein (Das-antigen) normally found in the mucus layer in the distal colon. Histological examination of the submucosal area underneath the destroyed epithelium in UC has shown that predominantly granulocytes, IgG plasma cells and macrophages are present but no T cells. In UC the whole colonic mucosa may be affected, whereas CD is characterised by skip lesions and primary lesions at the lymphoid follicle site. The pathogenesis of CD is different from that of UC in several aspects. First, CD appears to be T cell mediated. There is an increase in the total numbers of both $CD4^+$ and $CD8^+$ T cells, however, the CD4/CD8 ratio is unchanged. With respect to the function of T cells, it may be worthwhile to notify that the importance of phenotype assessment can tell us little of the actual functional capacity of these cells.

The aetiology of CD is unknown, however, the recorded immunological changes can be seen as a direct consequence of the destruction of the epithelium. In contrast to UC, this break in tolerance causes a disproportionate increase of IgG2 antibody levels. The target antigens responsible for the increased IgG2 response in CD are still unknown.

It has been shown that T cells and macrophages play an important immunoregulatory role in CD. It is not known whether the normal Ig class switch from IgM to IgA is redirected into a switch from IgM to IgG.

A high antibody response has been found in CD against *Mycobacterium paratuberculosis*. However, PCR studies failed to confirm a correlation between the presence of *M. paratuberculosis* and CD. It is therefore doubtful that *M. paratuberculosis* plays a direct role in the pathogenesis of CD. CD may occur due to an altered self caused by an infectious agent effecting the gut epithelium. Consequently, this may cause an alteration in the epithelial T cell interaction. The latter may functionally change from tolerance induction into activation of cytotoxic or helper-function.

The change in the epithelium has be speculated to be caused by a virus infection e.g. Herpes Simplex Virus (HSV) since lesions found in CD resemble apht-like lesions in the oral cavity, caused by HSV. Moreover such lesions have occasionally been reported in the stomach, in which case HSV was isolated. Until now, HSV has not been isolated from the gut epithelium in CD patients. It might be of interest, however, to search for HSV in the mesenteric lymph nodes in these patients. This may explain why thrombus formation in the small vessels in the submucosa occur. This process is possibly basic to the epithelial lesions in CD. It may be worthwhile to investigate the prevalence of HSV in CD by using PCR tech-

niques. On the other hand genetic predisposition may cause an altered immunoregulatory function of the epithelium.

For future studies on the comparison of UC with CD, it is important to note that one needs to define the degree of inflammation. Influx of granulocytes in inflammatory bowel disease lesions has been found to be regulated by adhesion molecules and upregulated by IFN-γ. Information on the time course and sequential induction of these responses remain subject for future study.

Coeliac disease is a small intestinal disorder characterised by villous atrophy and crypt hyperplasia. Unlike CD and UC, the immune dysregulation in coeliac disease is characterised by a slight break in oral tolerance. This break is reversible probably due to an intact IgA production; the number of IgA-producing cells in the small intestine is strongly increased in coeliac disease.

T independent antigens and superantigens

The categorisation of T independent (Ti) antigens is questionable. There are two classes of Ti antigens, Ti1 and Ti2. In fact the term Ti antigen is simply historic, since most antigens do require some help. This help may come from mast cells secreting IL-4 and IL-5, from stromal cells, or macrophages.

LPS is known to be the strongest stimulator of B cells. Proof is given by studies in germfree mice fed an antigen free diet. These mice only had a small B cell repertoire. In order to get a stimulation by T independent antigens, it is necessary to have crosslinking at the receptor site. Ti1 and Ti2 are both able to induce a primary response in B cells. Only Ti1 antigens also induce a specific secondary response in B cells.

Superantigens are bacterial products which display a special kind of increased immune stimulation compared to (normal) antigens. Examples of superantigens are *S. aureus* toxin and streptococcal M protein. Superantigens are able to bind specifically to the beta chain of the TCR and thereby induce a second signal on T cells. The simultaneous presence of MHC class II antigens increases the stimulatory function of superantigens. In this case superantigens are not presented on the MHC class II molecule after intracellular processing.

Antigen clearance by Kupffer cells

The liver plays an important role in Leishmaniasis. Liver macrophages known as Kupffer cells are not only the host cells which become infected but also play a role as effector cells. IFN-γ activated macrophages have been found to kill intracellular infectious agents up to 90-100% *in vitro*. The defence against *Leishmania* may be augmented *in vivo*, by administration of IFN-γ to the macrophages. Systemic IFN-γ therapy, however, is plagued by a great number of serious side effects, such as fever and increase of MHC class II expression which may result in autoimmune disease. To overcome these side effects, IFN-γ should be delivered directly to the macrophage. Packaging of IFN-γ in liposomes, however, does not serve this purpose properly, because liposomes are directly phagocytised by macrophages. After destruction of the liposomes by cytoplasmic liposomal enzymes, their contents are liberated into the cytoplasm of the macrophage. As a consequence, IFN-γ can not bind to the extracellular receptor on the macrophage and cannot perform its function. It is unknown whether macrophages express

a second messenger upon IFN-γ stimulation. In therapy, focusing on stimulating killing activity of macrophages, more experiments will be needed regarding the receptor signal transducing mechanisms in macrophages.

Kupffer cells are both stimulated by antigen coming from the gut portal vein liver, as well as from the route gut lymphatic vessels thoracic duct arteria subclavia lung blood liver. Uptake of endotoxin takes place via both routes. Because the flow in the thoracic duct is much larger compared to the portal vein, detection of endotoxin in the former correlates well with systemic endotoxaemia. During systemic endotoxaemia in humans, the liver and spleen are the most important sources of TNF. Downregulation of the macrophages in the liver leads to systemic disorders.

Finally, somatostatin is reported to stimulate macrophages in the liver. This may be a tool for future study on the role of macrophages in the immunological defence mechanism.

Mucosal vaccines

In order to get a good immune response by oral vaccines, presentation of the antigen in microspheres may be appropriate. Microspheres are taken up by M cells. Subsequent distribution depends on the size. Microspheres smaller than 3 microns pass the PP and enter the circulation. If their size is between 4 to 7 microns, they are phagocytised by macrophages. Microspheres of 7 to 10 micron stay in the PP and release their contents locally. Repeated doses are required in order to get an immune response. It is unknown whether there is a dose effect. This immune response, however, has only a short type of memory, if any. In order to get a long lasting immunity; i.e. memory, a persistent presence of the antigen is required.

An alternative for the vaccination with microspheres may be the administration of antigen in transgenic bacteria. However, these bacteria may only be present in the gut in limited numbers due to colonisation resistance.

CYTOKINES

Regulation

Cytokines are secreted upon stimulation of promoter genes. These genes all show a great similarity in their DNA sequence. This may be seen as the explanation why many cytokines are secreted by the macrophages, endothelial cells, and smooth muscle cells upon a single stimulus. Stimuli for the release of cytokines are not only endotoxins but also seen as an indirect effect of tissue damage. The type and kinetics of cytokine production and release vary according to stimuli. Upon pancreatectomy for example, IL-6 is produced by smooth muscle cells and endothelial cells. Upon LPS stimulation, first TNF and thereafter IL-1 and IL-6 are secreted by macrophages. This underlines that not all cytokines are released at the same time. In mice it has been found that TNF and IL-6 may act synergistically to modulate physiologic function.

TNF in granulomas

TNF plays an important role in necrotising as well as non-necrotising granulomas. Blocking of TNF by anti-TNF antibodies will inhibit granuloma formation e.g. in CD and in tuberculosis. In tuberculosis, this may result in the unlimited proliferation of micro-organisms. In a similar way cutaneous Leishmaniasis in mice can not be cleared if TNF production or release is blocked. In the lepromatous form of lepra remar-

kably less TNF is produced compared to the tuberculoid form. In addition to its role in granuloma formation, TNF also appears to be a potential stimulator of oxidative killing.

Cytokines in B cell differentiation

Cytokines also have an important function in B cell differentiation and proliferation. B cell maturation factors can be divided into secretion factors i.e. IL-2, IL-5, and IL-6 and switch factors i.e. IL-4, IFN-γ, TGF-β, IL-5, and IL-6. B cell growth factors can be divided into competence factors i.e. IL-1, IL-4, IL-5 and progression factors i.e. IL-2, IL-4, IL-5, IL-6, IL-10, IFN-γ, and C3a.

ENDOTOXIN

Intraluminal endotoxin in the gut

The number of LPS molecules per *E. coli* bacterium is about 10^6. This amount differs per strain and depends primarily on the growth phase of the organism. The concentration of endotoxin in the gut appears to show great variability between individuals. Whether LPS is needed for induction of oral tolerance remains unclear. Another physiological effect of endotoxin is its modulating effect on the gut motility.

Under pathological conditions like irradiation and trauma, intraluminal endotoxin may be responsible for the disruption of the tight junctions of the epithelial and goblet cells. Such alterations result in increased endotoxin in the liver and increase of intra-intestinal bacterial translocation.

Binding and clearance of endotoxin

LPS normally circulates in an aggregated form in blood. De-aggregation is necessary in order to get a response to LPS. Endotoxin interacts with and binds to a number of plasma proteins including LPS binding proteins (LBP) Low Density Lipoproteins (LDL) and High Density Lipoproteins (HDL). Upon stimulation with LPS, hepatocytes release LBP. Alpha 2 macroglobulin is thought to promote the clearance of endotoxin. Binding of LPS by LBP occurs at the Lipid A fragment of the LPS molecule. However, binding does not result in direct clearance of endotoxin. The LBP-LPS complex binds to the CD14 receptor on macrophages. Signal transduction may subsequently take place if crosslinking occurs with an ill-defined CD18 molecule. Possibly, CD18 is a binding protein. It is unknown whether CD18 plays a role in signal transduction in the macrophage.

LBP is an important factor in macrophage stimulation by LPS. In case of depletion of LBP by anti-LBP antibody, more LPS is needed in order to stimulate macrophages to TNF release. Binding of LPS by immunoglobulins and complement has been found *in vitro*. It is unknown whether this binding also takes place *in vivo*. HDL can in part compete with LBP for LPS-binding. When HDL is present, higher threshold of LPS is required for macrophage stimulation.

Clearance of endotoxin is orchestrated by antibodies which bind to the Fc-receptor or via complement binding the C3 receptor on macrophages, erythrocytes and neutrophil granulocytes. Granules of neutrophils contain enzymes that cleave off the fatty acids. This enzymatic cleavage does not result in clearance per se but rather in neutralisation. The clearance of the Lipid A part of the LPS molecule may be regarded as

most important since Lipid A displays high toxicity. LPS clearance occurs predominantly in the liver Kupffer cells. In the Kupffer cell, LPS is first degraded into free Lipid A and its polysaccharide chain. Degradation of Lipid A is a slow process. Biologically active Lipid A may persist in Kupffer cells for periods up to one week or more.

Future experiments should focus on signal transduction mechanisms after LPS stimulation, the intracellular clearance of endotoxin, the distribution of endotoxin clearance mechanisms in the body and the effect of immunoglobulins complexed with LPS.

Toxicity

Long lasting infection with endotoxaemia may result in the accumulation of Lipid A in Kupffer cells. This may act as an endogenous time bomb. This endogenous time bomb is best illustrated by experiments in rabbits which need decreasing doses of endotoxin in order to cause mortality when exposed to slow intoxication with endotoxin.

Anti-LPS antibodies

Most bacteraemias are caused by smooth bacteria instead of rough bacteria. Possibly, rough bacteria are easily lysed upon complement fixation whereas smooth bacteria cannot be lysed because the membrane attack complex cannot reach the membrane due to steric hindrance by the presence polysaccharide chains. Functional phenotype switching may be an important adaptation mechanism to foil the defence mechanism of the host. Phenotype switching of *E. coli* may occur at different levels. During rapid growth, smooth bacteria may switch to the rough phenotype. Bacteria may also switch off plasmid encoded cellular proteins e.g. pili after colonising the mucosa. Cell wall changes may also occur due to antibiotics. Beta lactam antibiotics have been found to increase cell wall permeability upon which fragmentation of bacteria occurs. Aminoglycosides on the other hand do not affect the cell wall permeability. Genetic control of *in vivo* variations of bacteria may be a field of future study.

Rough forms of bacteria e.g. *E. coli* are more susceptible to antibodies. Monoclonal anti-J5 antibodies are known to bind at the core region (Lipid A) of LPS *in vitro*. It is known that anti-J5 sera (HA1A) are protective in patients having endotoxaemia. Therefore, not bacteraemia but endotoxaemia is treated by HA1A. This may implicate that anti-J5 does not work by opsonisation of bacteria but by binding of endotoxin.

The endotoxin concentration in the blood does not always correlate with the number of viable bacteria found in the blood. This would suggest that LPS release either occurs from local sites, is readily bound and/or cleared or originates from dead bacteria. To estimate the severity of septicaemia in patients, TNF levels in serum are more indicative than endotoxin levels. Whether anti-J5 antibodies should be of IgM or IgG isotype remains a matter of controversy.

Use of anti-LPS antibodies for analysis of bacteria by flow cytometry; FACS

FACS not only enables the study of eukaryotic cells, but also of bacteria. Analysis of bacteria by flow cytometry may occur through the use of conjugated probes directed to the bacterial genome and conjugated monoclonal antibodies directed against cell wall determinants. Labelling at the genome level requires cell wall permeability without lysis. Detection of surface components of pure cultures by monoclonal antibodies reveals that within the bacterial population in pure culture not

all antibody binding sites may equally be present. Antigenic variation occurs in time, growth phase, and growth medium components. The antigenic expression should not be regarded as permanent mutations, as it may be due to varying expression of cell wall components. This is contradictory to the adagium that bacteria originating from one single parent cell are strictly homogeneous. This rapid reversible change of bacterial cell wall *in vitro* could perhaps be regarded as a virulence factor to escape the immune system *in vivo*.

Endotoxin in septic shock

It remains questionable whether the organ and physiologic dysfunction seen in septic patients is caused by release of endotoxin and cytokines. *In vitro* macrophages are shown to release a plethora of different cytokines upon endotoxin stimulation. These cytokines were also found in septic patients and showed a good correlation with the APACHE II score; i.e. severity of disease and clinical outcome. Animal model systems have also shown correlations between cytokine levels and physiologic function.

STOMACH

Protective mechanisms

Mucosal damage in the stomach may occur through ischaemia or chemical agents. Direct cellular protection may be provided by intracellular glutathion, intracellular pH, Ca^{2+}, ATP and the plasma membrane. Indirect protection is provided by accelerated gastric emptying, luminal and tissue dilution of damaging agents, and maintenance of blood flow and epithelial restitution.

Basic Fibroblast Growth Factor (b-FGF)

Failure of the protection may cause ulceration. B-FGF is a highly protective agent in experimentally cystamine-induced duodenal ulcer. B-FGF is administered orally and has been found to reduce the size of the ulcer lesions. Unlike cimetidine, b-FGF is not effective by reducing the acid or pepsin output. Instead, its beneficial effect is ascribed to the stimulation of angiogenesis in the normally hypovascular ulcer bed. Some glucocorticosteroids are also reported to have a b-FGF like effect.

Helicobacter pylori

Helicobacter pylori causes gastric ulcers and can be isolated in all patients with acute gastric ulcer. The habitat of *H. pylori* is the antrum. It is often difficult to isolate *H. pylori* from patients with chronic ulcers.

It is important to know that there are different strains of *H. pylori* having different degrees of virulence. For study of the pathogenesis of *H. pylori* induced ulcers an appropriate animal model is required. *H. pylori* does not grow in rodents. In very young pigs, *H. pylori* causes hardly any inflammation. Therefore, primates seem to be the experimental animals of choice. Urea in the gastric mucosa of the host is demolished into highly toxic ammonia by urease released by *H. pylori*. Strains which have lost their urease gene are not pathogenic. Once infected by *H. pylori*, these organisms may persist underneath the gastric epithelium for years. It is not yet clear so far what role is played in this respect by the coccoid dormant forms of *H. pylori*.

The route of transmission of *H. pylori* is unknown. Families being infected with one strain suggest an oral to oral route. Interestingly, there is also a higher incidence of *H. pylori* among gastroenterologists.

The treatment of choice in *H. pylori* gastric ulcer is amoxycillin or tetracycline, metronidazole, or bismuth. Bismuth penetrates and disrupts the *H. pylori* cell wall. Bismuth should not be combined with anti-acids because of disintegration when given simultaneously. Upon successful treatment, serum IgA antibodies against *H. pylori* decrease. The urease breath test also drops after successful treatment. The use of serodiagnosis in *H. pylori* infection is questionable. Commercial tests are not useful in this respect, because of the prevalence of too many serotypes and crossreactions.

It is important to have good parameters available for objective classification of *H. pylori* induced disease. Only histological inflammation markers are found to correlate with *H. pylori* infection.

NEURO-IMMUNOMODULATION

Enkephalins have an immunomodulating activity. There are two kinds of enkephalins (enk), namely Leu-enk and Met-enk; only Met-enk appears to be important. Met-enk injected systemically in high dose, may give suppression of antibody response and also has an anti-inflammatory activity. At low dose Met-enk, however, appears to have a potentiating effect on the immune response, e.g. an increase of $CD4^+$ cells has been described. Met-enk injected intracerebrally or intrathecally show similar effects as when they are injected intravenously. Enkephalins were studied instead of prolactin (pituitary hormones) because enkephalins also appear to be present in the gut. Lymphocytes are supposed to have receptors for enkephalins. Thus in all respects, enkephalins appear to have actions similar to cytokines.

Systemic cytokines e.g. IL1a in the adrenal medulla in rats may have a different effect as compared to cerebral produced cytokines. Similarly, TNF results in cachexia when produced and disseminated systemically, whereas intrathecally TNF only leads to anorexia without protein catabolism. Due to its size, TNF does not cross the blood-brain barrier. A local production of cytokines by neural and glial cells is just another example of the neuro-immune relationship.