Surgical Research – General Subjects

European Surgical Research

Eur Surg Res 2002;34:18–21

Surgical Research and *Helicobacter pylori* Infection – A Contradiction?

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Key Words

Helicobacter pylori · Gastritis · Peyer's patches · ELAM-1 · ICAM-1 · TNF-α

Introduction

Historically surgical research has dealt mostly with techniques to improve surgery on e.g. operation on the open heart, organ transplantation, minimal invasive surgery and peri- and postoperative pathophysiology. In this regard there were fruitful research activities in the field of sepsis, microcirculation, neurotrauma or the development of lithotripsy of kidney and gallstones [1–6]. This research has mostly been performed in model systems including animal experiments.

The availability of new techniques in molecular biology has boosted the research in a way that now samples routinely taken from patients for diagnositic purposes can be used to be analysed by these methods and bring further insights into the pathology/pathophysiology of the diseases. This can be illustrated by the analysis of relevant expressed genes within the course of *H. pylori* related gastritis where data from basic immunology of the GI-tract can be illustrated in a relevant human disease.

The understanding of the regulation of an immune response in the gut is of interest for many different diseases. The hyperreactivity to food components like in coeliac disease and food allergy or the autoimmune reaction in chronic inflammatory bowel disease may illustrate this. On the other hand the induction of oral tolerance or the understanding why bacteria can not only colonize the intestine but also can induce severe infections and eventually transmigrate and end in a septic syndrome in e.g. immunocompromized patients are all interesting questions which could be answered by a better understanding of the mucosal immune system. In the investigation of GI immunology surgical models gave new insights into the function of Peyer'patches for the organisation of the local immune system in the intestine. Using traditional techniques of removing the patches it became clear that effector cells in the Peyer's patches like M-cells (fig. 1) are important players for initializing the immune response. After reaching contact with luminally presented antigens, they transfer this information to immune cells in the patches which migrate to the mesenteric lymph nodes where the local immune response becomes organized ending with the spread of effector cells into the lamina propria and the intraepithelial space of the small intestine [7–9]. This theory is now well appreciated and finds further support with the notion that the transfer of the prion protein form BSE might find its way by this pathway [10]. The organisation of the immune system of the stomach on the other hand is not as well defined. There are usually no lymph follicles resembling the Peyer's patches and by morphology no antigen presenting cells like M-cells in the epithelial layer despite reports on the function of epithe-

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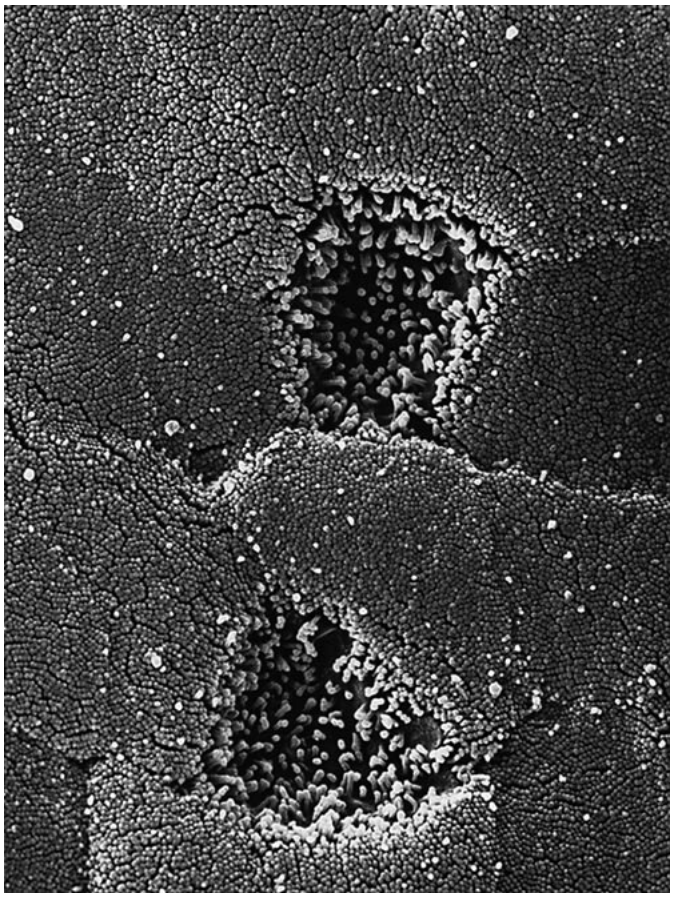

Fig. 1. Scanning electron microscopy of the epithelium covering a Peyer' patch of the rat. Two M-cells are clearly visible in the middle characterized by the microfolds (× 3,600).

lial cells as antigen presenting cells in other parts of the intestine [11–13]. There are early reports on the induction of an immune response in the stomach resulting in a hormone response as well as in hypersensitivity [14–16]. A more challenging view on the organization of the immune system in the stomach came with the observation that a bacterium is the causative agent in the induction of type B gastritis, ulcer disease and eventually cancer. *Helicobacter pylori* has been recognized to be responsible for these disease [17, 18]. So the question arises on the colonization and the regulation of the local immune response with special reference to how a protective immune response might be inducible.

There is little known about how *H. pylori* initially overcomes host defence and persists in a hostile environment. The fact that many patients who harbour the bacterium remain asymptomatic without developing manifest dis-

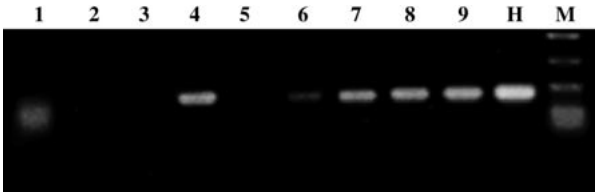

Fig. 2. Analysis of IL-8 expression in biopsy specimen by RT-PCR. Lane 1–4 are from controls, lanes 5–9 from *H. pylori* associated gastritis. H = Stimulated HUVEC as positive control, M = molecular weight marker. Fragment size on a 1% agarose gel is 255 bp. Amplification was for 35 cycles.

ease made it a model for investigating the time course and severity of the immune response in the stomach as well as for studying strain differences of the bacterium. The power of molecular biologic methods made it possible to gain insight on sequential gene expression by means of analysing the amount of specific RNA expressed in biopsies taken from patients by e.g. RT-PCR. One or two biopsy specimens are usually enough for the analysis of up to 10 different genes including standardization for a housekeeping gene. Using such methods genes activated during the development of an inflammatory response as well as immune regulation can be easily analysed during the course of a disease.

Materials and Methods

Biopsies were taken from patients during diagnostic endoscopy. Usually the equivalent of one biopsy was used to isolate total RNA. After homogenization of the biopsy in lysis puffer the total RNA was isolated by its selective binding to a silica-matrix in spin columns (RNAeasy; Qiagen, Hilden, Germany). After elution RNA was precipitated and either stored at –70°C or used for reverse transcription. The cDNA was prepared according to a standard protocol using MMLV-reverse transcriptase (Gibco BRL; Paisley, Scotland).

The amplification of a particular target gene by PCR was performed as described [19]. Specific primers were selected from known sequences and extensively tested against a data bank (GenBank, BLAST). PCR was quantified after different cycle numbers on ethidium bromide stained agarose gels using a densitometer with a sophisticated software and normalized for housekeeping genes.

Results and Discussion

Using this method it became clear that in the early phase of the infection the contact of the bacterium with the epithelial layer of the stomach induces severe chemokine production which is able to attract granulocytes into the

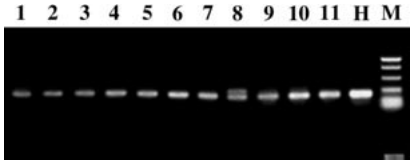

Fig. 3. ICAM-1 (CD54) expression on control (lanes 1–5) and *H. pylori* associated gastritis (lanes 6–11). H = Stimulated HUVEC as positive control, M = molecular weight marker. Fragment size is 238 bp. Amplification was for 30 cycles.

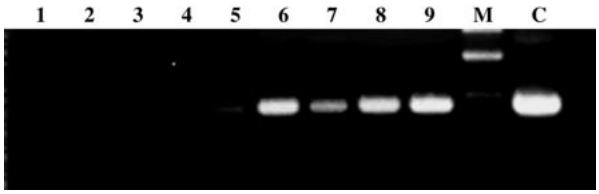

Fig. 4. TNF-α expression in biopsy specimen from control (lanes 1–4) and *H. pylori* associated gastritis (lanes 5–9). M is the molecular weight marker, C are stimulated macrophages as positive control. Fragment size is 444 bp, amplification for 35 cycles.

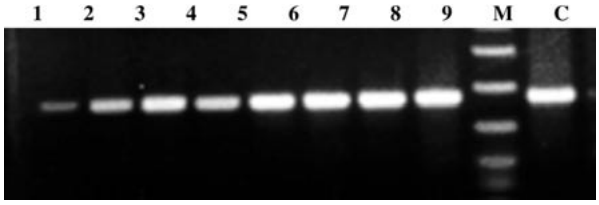

Fig. 5. IL-1β expression in biopsy specimen from control (lanes 1–4) and *H. pylori* associated gastritis (lanes 5–9). M is the molecular weight marker, C are LPS stimulated PBL as positive control. Fragment size is 447 bp, amplification for 35 cycles.

site of inflammation. As shown in figure 2 abundant IL8 message could be detected in the biopsy of patients with gastritis. So the presence of the bacterium in the stomach seems to be a trigger for local chemokine expression. This observation could also be extended to the more epithelial cell specific chemokine ENA-78 as shown previously [20]. Attraction and activation of granulocytes can be induced by these cytokines. This induction was dependent on the adherence of the bacterium to epithelial cells as shown by in vitro stimulation experiments using gastric cell lines [21]. Now chemokines are able to attract granulocytes as well as lymphocytes. For granulocytes to leave the vessel it is important that surface receptors on the granulocytes interact with adhesion molecules on the local endothelial cells. This interaction has been shown to either induce rolling of cells along the vessel wall or stick before emigrating out of the blood vessel. The former is mediated by the interaction of CD62P on the vessel and its ligand PSGL-1 or CD62P on the blood cells. Solid adherence on the other hand is mediated by the interaction of integrins on blood cells and CD54 (ICAM-1) on the endothelial wall. ICAM 1 (fig. 3) was found expressed in all biopsies with *H. pylori* associated gastritis and controls. The expression could also be shown by immunohistochemistry where higher expression was found in gastritis. As counter receptor on migrating cells the β-2 integrin CD11/18 heterodimer is effective. In this regard we and others were able to show that soluble *H. pylori* products had the ability to induce the upregulation of CD11b on granulocytes [22].

Inducible adhesion molecules might be more effective in the attraction of activated blood cells. Candidates are CD62E and CD 106 (VCAM) as well as the early marker CD62P are candidates. With the exception of CD62E all of these molecules could be found upregulated on endothelial cells in gastritis specimens [19]. Apart from the other molecules studied VCAM was not only detected on some endothelial cells but also on follicular dendritic cells in lymph follicles which appear in the lamina propria after *H. pylori* infection [23]. As CD62 E (ELAM-1) is found in chronic inflammatory lesions of the bowel it was surprising that it could not be detected in *H. pylori* associated gastritis since potent inducers for CD62E like IL1-β and TNF-α were detected in the same biopsies. Interestingly, whereas TNF-α was induced (fig. 4) IL1-β was constitutively expressed in all samples (fig. 5). IL1-β has been shown to have dramatic effects on the acid output of the stomach [24, 25]. This seems to suggest that acid production could be regulated by a different pathway in health and disease. On the other hand TNF-α is a potent inducer of adhesion molecules on endothelial cells. So it may well be that in the stomach adhesion molecule regulation differs from other parts of the intestine.

With the help of molecular biological methods it is now possible to get data from routine biopsies which expand the knowledge on the pathogenesis of a particular disease. In this respect *H. pylori* induced gastritis serves as a paradigm for an immune response in the stomach. Not only can the local reaction of the patient be analysed and followed after eradication but also reasons for recurrence of the disease studied. In addition basic data on the immune regulation in the stomach can be obtained. However, why patients who are *Helicobacter pylori* infected vary in their clinical presentation of manifest disease from being

asymptomatic to developing gastric carcinoma remains a baffling question. A broad variation in the genetic repertoire of the patient as well as of the bacterium may be the answer. With the disclosure of the complete *H. pylori* genome every variation and gene shift should be detectable [26]. Even the publication of the human genome offers additional chances to evaluate differences in the reaction of the immune system in the stomach wall.

So is this the end of *H. pylori* research in the stomach with the description of the pathogenesis and the possibility to eradicate the bug? Eradication studies have shown that some changes in the pathology persist even after longer periods of time [27]. For example a possible increase in Barrett carcinomas after eradication has been observed [28–30]. So *H. pylori* infection may even have some beneficial effects on some unknown defence mechanism as for example acting protective on the gastro-oesophageal junction. These new aspects are open for investigations on the genetic repertoire expressed by the bacterium as well as by the particular patient. So the combination of a surgeon having access to human tissues and a powerful lab using molecular biological methods allows the investigation of the pathophysiological events and offers new insights into clinically relevant disease.

Acknowledgement

This work has been supported by grants from the LMU (Weigand Stiftung) and the Chiles foundation, Portland, Oregon.

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