184 "Respiratory Burst" in Macrophages Isolated from Inflamed Human Intestinal Mucosa

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The macrophage plays an important role in local defence. An important bacterial mechanism is oxygen radicals (OR) which, however, may produce tissue damage, perhaps as an early step in inflammatory bowel disease (IBD). We wanted to study: (i) the production of OR ("respiratory burst") by macroscopical macrophages in patients with or without IBD, (ii) whether these macrophages respond to inflammatory mediators; and (iii) whether a difference exists between resident macrophages and newly arrived CD14+ monocyte-like cells.

Methods: Monocytes from healthy donors were separated by Lymphoprep and by adherence to plastic. Cultures of lamina propria-mononuclear cells (LPMNC) were isolated from IBD and CD patient biopsies as well as patients with IBD by EDTA/collegenase/dishase technique. CD14+ cells were depleted by anti-CD14 immunomagnetic beads (Dynal). Adherent LPMNC cultures were then established by incubation for 2 h on gelatin- and plasma-coated microtiter plates, followed by repeated washings. Relative number of colonies was determined by nuclear staining (crystal violet). The remaining macrophages was determined by butyrate esterase staining. PMN (phorbol 12-myristate 13-acetate) stimulated the production of OR (measured as the amount of reduced cytochrome C, after addition of cytochrome C and PMMA for 2.5 h). Except for the control wells, the cultures were stimulated with IFN-y (200 U/ml) and without LPS (1 mg/ml) for 48 h. Results: The fraction of adherent macrophages from non-inflamed and inflamed cultures was similar, but OR-production was increased in the latter (p < 0.01). OR production in cultures from inflamed mucosa depleted of CD14+ cells was reduced (p < 0.001) to 31% (range 21–78%), similar to the value for fairly non-inflamed macrophages from the same patient. The OR production triggered by PMN did not significantly increase after stimulation with IFN-γ in the presence or absence of LPS, contrasting the significant increase seen in vitro-matured monocytes after similar stimulation. Conclusions: Increased OR production was observed in macrophages from inflamed IBD mucosa. This increase was mainly a result of recently recruited CD14+ monocyte-like cells and did not reflect upregulation in resident macrophages.

185 Function of HLA-DQ2 and HLA-DQ8 as Susceptibility Molecules in Coeliac Disease

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Coeliac disease (CD) is an immunological disorder of the small intestine, precipitated in susceptible individuals by ingestion of gluten. Approximately 90% or more of CD patients carry the cis or trans encoded HLA-DQA1*0501, *010201 heterodimer, i.e. HLA-DQ2. Most of the remaining CD patients carry the HLA-DQA1*0101, *010101 heterodimer, i.e. HLA-DQ8. To investigate the functional role of DQ2 and DQ8 in CD, we challenged biopsy from the small intestine of various CD patients with peptic-tryptic digest of gluten and positively selected the cells which became activated; i.e. expressed the IL-2 receptor. These T cells were then expanded in vitro and T cell lines and T cell clones were established. HLA-DQ2 and HLA-DQ8 are the CD4+ gluten-reactive T cells from DQ2 patients recognized gluten antigen when presented by DQ2, and not when instead presented by any other HLA class II molecule of the patient. In a DQ8 patient DQ8 was similarly found to be the preferential restriction element. In contrast, when gluten-reactive T cells from the peripheral blood were investigated, both DR, DQ2 or DQ8 restricted T cells were found. Thus a major proportion of gluten-specific T cells in the intestinal mucosa of CD patients recognize gluten-derived peptides when presented by the disease associated DQ2 or DQ8 molecules, suggesting preferential antigen presentation by these DQ molecules in the intestinal mucosa as a possible immunological mechanism behind the HLA association in CD.

186 Cathepsin E: A Novel Marker of Lymphoepithelium. Its Expression by Normal and Inflamed G.I. Mucosa

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Cathepsin E (CE) is a non-lysosomal aspartic peptidase which seems to be involved in antigen processing for presentation by class II major histocompatibility complex. By means of specific antibodies we localized histochemically CE in folic acid associated epithelium (FAE) of human and rat intestines as well as in palatine, pharyngeal and lingual tonsils. Outside the gut, CE was found in the epithelium overlying bronchial/bronchiolar associated lymphoid tissue and in some professional antigen-presenting cells (Langerhans cells, interdigitating reticulum cells). As a rule, CE-reactive epithelial cells co-expressed HLA-DR. Ultrastructural immunocytochemistry showed CE, in endosomal vesicles and endoplasmatic reticulum of intestinal M cells and M-like cells of tonsils. In biopsies or resection specimens from 70 pts with ulcerative colitis (UC) and 63 pts with Crohn disease (CD), CE reactivity was spread also outside the follicle associated epithelium. CE was overexpressed in all groups of UC and CD, with diffuse staining of epithelial cells in active UC and patchy distribution in CD. Coexisting HLA-DR overexpression was found in 66% of UC (limited to active forms) and 75% of CD cases, respectively. CE and HLA-DR were also studied in 85 gastrectomy specimens either histologically normal or affected with chronic gastritis of different severity. While HLA-DR was absent in patients with gastric epithelium, weak CE immunostaining was found at the base of normal superficial/foveolar cells. De novo expression of HLA-DR and enhanced production of CE characterized H. pylori gastritis. These findings were mostly evident in active gastritis, where heavy staining was found in foveolar and neck epithelium by both CE and HLA-DR antibod- ies. Ultrastucturally these cells showed a striking expansion of the endotyloc-endothelial compartment, at times containing H. pylori antigenic material. Conclusions: (1) CE is a specific marker of lymphoepithelium, where it is usually coexpressed with HLA-DR. (2) CE is widely overexpressed (together with HLA-DR) in IBD as well as in H. pylori-associated chronic gastritis. (3) the development and/or expansion of an epithelial compartment with the capacity to process CE (+) and present (HLA-DR) antigens to T cells may be relevant in maintaining long-standing inflammation typical of such diseases.

187 Thoracoscopic Vagotomy without Drainage: A New Technique for Treatment of Chronic Duodenal Ulcer

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A total of 40 patients were considered for thoracoscopic vagotomy: 37 males and 3 females. The age of the patients ranged from 21–60 years with a mean of 35 years. All the patients had a long history of chronic duodenal ulcer disease, lasting for a mean duration of 6.5 years. Out of the 40 patients 29 were presented with intractability, 6 with haemorrhage, 1 with perforation, 2 with anastomotic ulcer and 2 patients with recurrent ulcer after highly selective vagotomy. The median operating time was 35 minutes with a range of 15–80 minutes. There were no deaths, but major complication occurred in one patient. He developed gastric stasis 48 hours postoperatively due to rupture of emphysematous bullae during the process of lung inflation at the end of the procedure, managed by a chest tube with a low pressure continuous suction. The mean post operative hospital stay was 2.4 days. Follow-up upper endoscopy 2 months post operative showed ulcer healing and ulcer acid study done 3 months post operative indicated complete vagotomy in all patients. Post operative assessment of gastric stasis included clinical fullness, or vomiting, barium meal and upper endoscopic studies. One patient developed signs of gastric stasis 48 hours post operative, managed by gastrojejunostomy through minilaparotomy. Another patient developed stasis 3 weeks later and the other a patient 3 months later. Thoracoscopic vagotomy without drainage is an effective and elegant method in treating chronic duodenal ulcer.

188 Laparoscopic Vertical Banded Gastroplasty


With the development of staple instruments for laparoscopic use a new op- tion became available for the treatment of morbid obesity. In the period October-December 1993 eleven patients underwent vertical banded gastroplasty through laparoscopic approach. Patient selection was the same as for open bariatric surgery. The operation was performed mainly according to Mason with a gastric window made by a 25 mm circular stapler, and the vertical staple line with a 60 mm four-line row linear stapler. The stoma was reinforced by a Gore-tex band. Calibration of the stoma was made with a flexible 9 mm tube or a 10 mm gastroscope. In one patient had to be converted to open surgery during the initial performance, but 2 patients were reopened: One laparoscopically for reinforcement of a vertical staple line defect caused by a gastric tube. The other patient was reopened openly on the first postoperative day because of a rupture in the vertical staple line. Compared to a reference group of 35 obese patients operated with open laparoscopic surgery, the laparoscopic procedure was less painful and were more rapidly mobilized. Several patients were capable of returning to full activity within two weeks after the operation. It is concluded that a vertical banded gastroplasty can be performed with a laparoscopic approach using staple instruments now available. Postoperative results indicate that the patients will have a shorter postoperative recovery period.
Complications After Open and Laparoscopic Cholecystectomy in Norway

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The benefit of routine intraoperative cholangiography is debated in Norway. The main argument for preoperative cholangiography has been to visualize bile duct anatomy, and hence avoid CBD injuries. If this is important, the Norwegian national registry would be expected to reveal a frequency of CBD injuries above other countries.

Methods: A national registry was established in April 1993, including all patients undergoing cholecystectomy. Most Norwegian hospitals had by then practiced the laparoscopic technique for some time, and the period does not cover the first part of the learning curve. Also patients operated with the open technique were included. Indications, preoperative investigation and health condition together with per- and postoperative complications were recorded.

Results: During the first nine months 906 patients were registered, 705 operated laparoscopically, 201 openly (22%). Only in nine of the laparoscopic patients (1.2%) peroperative cholangiography was performed. 75 patients in the laparoscopic group (11%) were converted to open technique. Altogether 135 patients underwent an emergency operation due to acute cholecystitis, 58 laparoscopically, 77 openly. Serious complications in the laparoscopic group were two full CBD transections (0.3%), one partial CBD injury (side-hole), five perforations of visceral organs with Verres needle and one sepsis. One patient died from myocardial infarction after laparoscopic cholecystectomy (mortality 0.1%). In the open group, two patients died from myocardial infarction and one from septic shock due to cholangitis (mortality 1.5%). Other complications to open cholecystectomy was one partial CBD injury (sidehole) and four sepsis.

Conclusion: Our main quality problem in surgical treatment of gallstones is the high mortality after open cholecystectomy. The frequency of CBD injuries is similar to other countries.

Comparison of Sequential and Fixed-Sample Designs in a Controlled Clinical Trial with Laparoscopic Versus Conventional Cholecystectomy


The aim of the study was to compare a fixed-sample and a sequential design with regard to study duration, sample size and medical results in a real-life situation. A randomized study comparing laparoscopic and conventional cholecystectomy was carried out with a fixed sample design parallel to a sequential design. The main variable was duration of postoperative convalescence.

In the fixed-sample trial the necessary number of patients was calculated to be 72. The sequential trial was conclusive after inclusion of 24 patients and reduced the study duration from 43 to 18 weeks. The mean difference in duration of postoperative convalescence between the two surgical methods was 25.8 days in the fixed sample trial and 27.5 days in the sequential trial in favour of laparoscopic cholecystectomy (p < 0.01). Additionally the sequential trial reached the same conclusions as the fixed-sample trial for all the observed variables except for one.

The study indicates that sequential designs should be used more frequently in clinical trials in order to involve the smallest possible number of patients necessary to reach a conclusion.


Prospective Case Registration of Laparoscopic Cholecystectomy in Denmark

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When laparoscopic cholecystectomy was introduced in Denmark in 1991, the Danish National Registry of Laparoscopic Cholecystectomy was established by the Danish Surgical Society. The primary aim was to monitor complications. All patients for laparoscopic cholecystectomy are reported prospectively to the registry. All 53 departments in Denmark currently performing laparoscopic cholecystectomy have agreed to report their cases, and the registry is probably complete. Primarily conventional open procedures are not included.

Data include age, sex, indication for cholecystectomy, previous abdominal surgery, preoperative investigations, duration of surgery, preoperative cholangiography, preoperative complications, reason for conversion, blood transfusion, postoperative course and complications, duration of hospital stay and time to return to work.

By the end of 1993, the registry included data on 3897 patients. 933 (10%) were converted to an open procedure. In 106 (3.7%) the conversion was forced due to a complication. Preoperative cholangiography was used in 21%.

Postoperatively, the course was without complications in 86%. The most frequent complications were cardio pulmonary (3%), abdominal complications not requiring laparotomy or endoscopy (4%) (mainly abdominal discomfort), and wound infection (2%).

21/8987 (0.54%) sustained a bile duct injury. Six had a lesion of the right hepatic duct, and seven a transection of the common bile duct. Five had a minor bile duct lesion and two required a clip-injuries of the duct. Two patients experienced duct obstruction due to tenting occurred. One patient developed a stricture, probably due to thermal injury. There were no fatalities among these patients.

Mortality was 0.28% (11/8987), all were 72 years or older. Seven died from causes unrelated to the operation, while four had procedure related complications.

Median postoperative stay was two days (interquartile range 1-3, range 0-67), while median time to return to work was 10 days (4-14, 1-165).

HLA-DQ Restricted T-Cell Clones From the Small Intestinal Mucosa of Coeliac Disease Patients Recognize Several Different Gliadin Epitopes

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Coeliac disease is precipitated by wheat gliadin. Each wheat cultivar carries approx. 40 different gliadins, classified as α-, β- and γ-gliadins. In most patients, HLA-DQ2 confers the disease susceptibility whereas in those who are DQ2 negative, HLA-DQB is the probable disease susceptibility determinant. We recently found that most gliadin-specific T-cell clones (TCC) from the small intestinal mucosa of coeliac disease patients recognize gliadin when presented by DQ2 or DQ8. We now study the gliadin recognition by the TCC with one purified α/β-gliadin and two purified γ-gliadins from the wheat cultivar Kadett and with synthetic peptides from the N-terminal region of α-gliadin.

One TCC recognizes both the α/β-gliadin and the two γ-gliadins from Kadett, another TCC recognizes the α/β-gliadin only, whereas three other TCC only recognize the two γ-gliadins. Further TCC recognize other gliadin fractions which are heterogeneous with respect to α/β-, γ- or α-ω-gliadins. Some TCC only recognize wheat gliadin, others also proteins from rye. None of the TCC recognize synthetic α-gliadin peptides from the cultivar Scout 66, Kolibri and Cheyenne. Since there are many α/β-gliadins with minor sequence variations, the epitopes may for some of the TCC still be found in this region.

The results suggest that the T-cell response towards gliadins in coeliac disease is diverse. Thus, the existence of large numbers of different, gliadin-specific T-cells in the small intestinal mucosa may take place and hence be an important feature of the disease immunopathogenesis.

Beneficial Effect of Dietary Pectin on the Recovery of Mice with Griseofulvin-Induced Porphyria

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The study was aimed to establish whether dietary pectin could exert a beneficial effect in already induced experimental hepatic porphyria, as an interference of pectin with enterohemepatic circulation of protoporphyrin could be expected. Thirty-two male Balb C mice were fed with standard diet, containing 1% griseofulvin for 7 days. In a group of 8 animals killed immediately after the last feeding of the griseofulvin the excessive amounts of protoporphyrin in the liver (a 450-fold increase) and in the stools (a 34-fold increase) were found. The other animals were divided by 8 into three groups, which were fed for another 7 days with following diets: standard food; standard food, containing 4% high methylic esterification pectin; standard food, containing 4% low methylic esterification pectin.

A beneficial effect of pectin enriched diet was observed. The withdrawal of griseofulvin for 7 days led to a 2.3-fold increase of hepatic protoporphyrin in mice fed standard diet, only but a 4.5-fold reduction was established in the animals fed pectin diet. Parallel changes in faecal protoporphyrin were registered, which was inconsistent with the assumption for interference of dietary