Pharmacodynamic Evaluation: Gastroenterology

Petar Nikolov, Georgi Banishki, and Milena Nikolova-Vlahova

Abstract

Pharmacodynamics aims to explain the complex relationship between the medication's dose, physiological or pathological response, and the chemical nature of the drug. The human gastrointestinal tract (GIT) is a strictly hierarchic body system with numerous functions and is often a therapeutic target, crosslink or can even serve as a measurement for drug's physiologic and biochemical effects. The pharmacological effects and the pharmacodynamics evaluation in the GIT would not have been possible without three distinct receptor families that have been known to have an enormous role in the modulation of the GIT functions: serotonergic, cannabinoid, and opioid receptors. In addition, the route of administration can be of great importance for the absorption and pharmacological response of the drug and there are several main routes of administration in Gastroenterology: oral, parenteral, transmucosal, and local. Furthermore, a targeted and individualized approach for drug monitoring was developed that takes into account individual patient variability through careful gathering of pharmacokinetic and pharmacodynamic data: therapeutic drug monitoring, allowing to really individualize patient's dose. Various novel imaging methods are used in Gastroenterology, e.g., PET scan, MRI, and molecular endoscopy, and they all use tracers and contrast agents. They allow for early an accurate detection of various lesions in the GIT. Current understanding of oral tolerance has allowed the development of two groups of medications with a unique pharmacodynamic profile: oral vaccines, inducing immune response and oral tolerogens, initiating immunomodulation with alteration of immune response directed at the development of local and/or systemic immune tolerance.

Contents

P. Nikolov (✉)
Gastroenterology, Ardea Medical Center, Sofia, Bulgaria
e-mail: petar.k.nikolov@gmail.com

G. Banishki
University of Aberdeen, Aberdeen, UK
e-mail: banishki@abv.bg

M. Nikolova-Vlahova
Clinic of Nephrology, University Hospital Alexandrovska, Medical University, Sofia, Bulgaria
e-mail: milena_i_dani@abv.bg

F.J. Hock, M.R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-56637-5_50-1

General Overview

Petar Nikolov

Pharmacodynamics stands for the study of the physiologic, biochemical and molecular effects of medications on the body and involves receptor binding, postreceptor signaling and biochemical interactions. Pharmacodynamics also aims to explain the complex and multifactorial relationship between the medication's dose, physiological or pathological response, and the chemical nature of the drug.

The human gastrointestinal tract (GIT) is a really complex and yet strictly hierarchic body system with numerous functions:

- Provides route and safe passage of food through the body.
- Plays a key role in the food's processing, degradation and utilization.
- Acts as an "opened door" to the outside world and thus has a major role in the life adaptation and preservation of the biological individuality of humans – GIT is extremely important for the proper functioning of the innate and acquired immune response.
- At the same time plays a pivotal role in the maintenance of oral tolerance.
- GIT is a crossroad for all other body systems from a metabolic, regulatory, and signaling standpoint, thus allowing for a therapeutic intervention at many levels.
- Small and large intestine host the intestinal flora, which is sometimes regarded as the "forgotten organ" in the human body as it is biochemical activity is only comparable with the one of the liver.
- Numerous endocrine and exocrine secreting cells adaptively interact with each other, thus keeping an equilibrium with the other body systems, the nutritional habits, and the outside world.
- Oral and rectal drug intake are often the preferred routes of drug administration for many therapies and would not have been possible without the unique and ubiquitous functions of the GIT.
- Liver is the most advanced and sophisticated biochemical laboratory in the human body and plays a primordial and diverse role in the drug's pharmacodynamics.

All the above features of the GIT may be a therapeutic target, crosslink or serve as measurement for drug's physiologic and biochemical effects. With this regard, the human GIT and the science that studies it – Gastroenterology – are an important milestone in the pharmacodynamic evaluation. Moreover, the human GIT hosts a galore of receptors that are of vital importance for the optimal functioning of the human body as it is a crossroad and often the pharmacological effects of the medications aiming at the GIT may biologically go in their effects beyond the GIT due to its ubiquitous function.

Receptors in the GIT

Georgi Banishki

Targeting specific receptors has always been one of the most preferred options in drug development and the GIT has been no exception in that area. There have been three different receptor families that have been identified that have been of the greatest interest to researchers due to their role in modulating GI functions and these are the serotonergic (5-HT), cannabinoid, and opioid receptors.

Serotonergic Receptors

There have been 12 different serotonin receptors identified to date, all of which are G-protein coupled receptors (GPCRs) and for most time

they have been associated with the nervous system and their importance in mood control, depression, anxiety, sleep, etc. However, the greatest store of serotonin in the body is the gut where can be found about 95% of it (Gershon 2013). It was first in the 1950 that was proven that serotonin plays a major role in peristaltic activity as serotonin secreted from enterochromaffin cells in gut mucosa evokes peristaltic activity. Subsequent studies on rats using tryptophan-deficient diet (tryptophan is precursor of serotonin) showed that peristaltic activity was not impaired, but serotonin still has a modulating effect on the gut motility (Gershon 2013). Drugs that target serotonin receptors have been shown to be very effective in patients with IBS. In previously treatment resistant patients, Alosetron, a 5-HT_3 antagonist, was shown to be very effective against IBS with diarrhea, and Tegaserod, a 5-HT_4 agonist, was effective in patient who had IBS with constipation. Both drugs had some severe side effects which have led to their restricted use, but they were a successful proof of concept as it confirmed serotonergic bowel dysfunction to be an important factor in IBS (Gershon 2013).

Another major finding regarding serotonin signaling in the gut is its role in inducing inflammatory response. Animal studies using mice which lacked serotonin reuptake transporter (SERT), the activity of serotonin was enhanced and prolonged, and these mice were susceptible to developing trinitrobenzenesulfonic acid (TNBS)-induced or IL-10 KO-associated colitis (Gershon 2013). The exact pathway for this is yet to be understood although there has been evidence suggesting that by stimulating 5-HT_7 receptors on dendritic cells to launch the innate immune mechanisms, serotonin can cause inflammation of the bowel (Gershon 2013). Studies using KO mice which are missing the synthesizing enzymes for serotonin, tryptophan hydroxylase 1 and 2 (TPH1 and TPH2), have also confirmed the importance of serotonin in gut inflammation. Most interestingly, mice which have TPH1 gene knocked out had a reduction in inflammation while mice where lacked TPH2 inflammation was increased and thus led to the conclusion that serotonin can act as both the sword and shield of the gut (Gershon 2013). Serotonin has also been confirmed as an important factor in liver regeneration with 5-HT_2 receptors found on hepatocytes being involved in promoting DNA synthesis and hepatocytes proliferation (Gershon 2013). Overall, as we understand more about the importance of serotonin in gut function, it is an area that is going to attract even more attention by researchers looking for new therapeutics for different GI disorders.

Cannabinoid Receptors

Cannabis has been used to treat different GI ailments for centuries, but only recently with latest scientific discoveries researchers are beginning to understand the pharmacologic pathway for this phenomenon. Cannabinoids elicit response through two main types of GPCRs called the cannabinoid 1 and cannabinoid 2 receptors (CB1 and CB2). CB1 receptors are found throughout the whole enteric nervous system (ENS) and the colon epithelium, while CB2 receptors are found primarily in the immune system, and thus they are both involved in multiple processes ranging from GI motility to regulating gastric secretion (Izzo and Sharkey 2010). The main active substance in cannabis, THC, has long been associated with craving of food, and there has been significant evidence accumulated which has directly linked CB1 receptors in the brain and gut with increasing food intake and body weight gain (Izzo and Sharkey 2010). Several antagonists have been developed that target specifically CB1s for promoting weight loss and treating obesity, but only one has reached the market (Rimonabant) and it was later withdrawn due to increased risk of depression (Izzo and Sharkey 2010). Nevertheless, this remains an area with very high potential as rat studies have shown that CB1 expression is upregulated in obesity-prone rats thus confirming the therapeutic potential for future CB1 antagonists that are unable to cross the blood-brain barrier (Izzo and Sharkey 2010). On the other hand, activation of CB1 receptors has also been shown to reduce gastric secretions and decrease gastric ulcers in rodents which is another area that presents exciting new opportunities for future drugs (Izzo and Sharkey 2010).

However, probably the greatest interest has been the involvement of CB receptors in controlling inflammation. Cannabis for many years has been used by patients suffering of autoimmune disease including those affecting the GIT such as Crohn's disease (CD) or ulcerative colitis (UC). Preclinical experiments in humans have shown increased expression of CB receptors and/or enhanced endocannabinoid levels in intestinal biopsies of patients suffering from CD, UC, diverticulitis, and celiac disease (Izzo and Sharkey 2010). Both CB1 and CB2 receptors are possibly involved as in vitro studies have shown them to modulate inflammatory responses and CB1s were also shown to promote gut healing (Izzo and Sharkey 2010). In rodent models, both CB1 and CB2 agonists have shown to be effective in reducing inflammation from trinitrobenzene sulfonic acid (TNBS) and oil of mustard induced colitis, and in rodents where CB1 antagonists were applied they were shown to be more susceptible to induced colitis (Izzo and Sharkey 2010). CB2 receptors by being found mainly in immune cells (B cells, killer T cells) has been shown to be involved in suppressing activated macrophages and the secretion of proinflammatory cytokines such as TNFα (Izzo and Sharkey 2010). In addition, as previously stated both CB1 and CB2 play an important role in regulating gut motility and secretion, their activation by an exogenic compound could cause an even greater reduction in gut inflammation through this process as well. All of this accumulated evidence from in vitro and in vivo studies is highly indicative of the huge future potential of CB receptors in the management of diseases such as CD or UC.

Opioid Receptors

Just like cannabis, opium and its many different derivatives have found therapeutic applications long before modern medicine. For centuries, it has been used for treatment of pain and diarrhea in instances such as cholera infections. It has been confirmed that opiates act by targeting specific opioid receptors, but unlike serotonin and cannabinoid receptors their function is much better understood and utilized. Opioid receptors are also all GPCRs and can be subdivided into three classes – μ-opioid receptors (MOR), κ-opioid receptors (KOR), and δ-opioid receptors (DOR). All three types are found in the myenteric and submucosal plexus of the ENS and MORs are also found in immune cells in the lamina propria of the gut. All opioid receptors are directly linked to controlling Cl^- secretions in the gut and thus water movement, delaying transit from the small intestine to the colon, elevating the resting anal sphincter pressure, and regulating intestinal inflammation (Holzer 2009). The most commonly used drug acting on MORs is Loperamide which is the most commonly used drug to treat diarrhea caused by infections of IBS. Loperamide is a MOR agonist; it cannot pass the blood-brain barrier and targets the receptors in the ENS thus causing decreased propulsive motility and intestinal secretion (Holzer 2009). A common side effect as one would expect is constipation, but in overdoses it can also cause fatal arrhythmia and it should be avoided in patients with IBD where it can result in toxic mega colon. There is one other antidiarrheal drug which utilizes the MOR signaling pathway without crossing the blood-brain barrier, but indirectly. Racecadotril or acetorphan inhibits enkephalinases, the enzymes which degrade endogenous opioids, thus increasing their concentration which therefore leads to delayed bowel transit (Holzer 2009).

MOR mediated constipation is also quite a common problem for patient treated with opiate analgesics and which suffer from the so-called opioid-induced bowel dysfunction (OBD). Apart from constipation, OBD also included incomplete evacuation, abdominal distention bloating and discomfort, and gastroesophageal reflux, and it persists throughout the whole treatment of the patient and even though he can develop resistance to the analgesic effects of the opioid, the GI effects remain largely the same (Holzer 2009). This can be somehow managed using naloxone, an inverse MOR agonist which could counteract the undesirable effects without compromising the analgesia. This however is limited by its narrow therapeutic range and ability to cross the blood-brain barrier and at higher doses it can greatly reduce analgesia (Holzer

2009). As a result, one approach that has been attempted is by using peripherally restricted opioid receptor antagonists like n-methylnaltrexone, which has both low oral bioavailability and cannot cross the blood-brain barrier. This concept has subsequently been verified in rat, dog, and human studies using both oral and parenteral formulations, and it has now been approved for human use by both the FDA and EMA (Holzer 2009). Nevertheless, its long-term safety and tolerability are not yet known so the recommendation is not to use it for longer than 4 months. Other such antagonists have been developed (e.g., alvimopan), but they have found only limited clinical use. Nevertheless, peripheral MOR antagonists remain an area of great interest for the future.

Treatment Routes and Drug Delivery

Petar Nikolov

The ubiquitous physiological properties of the human GIT along with the various pathology to be found there predispose for a galore of treatment options to be considered in humans. The development of numerous acid suppressing drugs, antiviral agents for viral hepatitis, biologics for inflammatory bowel disease, live biotherapeutics for intestinal disease, etc. has changed the face of gastroenterology forever. These treatment options come with particular drug delivery techniques. In an attempt to improve the efficacy and safety profile of medications, researchers have developed different methods such as individualizing drug therapy, dose titration, therapeutic drug monitoring, delivering drug at controlled rate, targeted delivery, etc. (Tiwari et al. 2012).

The most commonly used routes of drug administration in Gastroenterology are:

- Oral – delivering the drug into the stomach, small or large intestine
- Parenteral – subcutaneous, intravenous, intraarterial, intramuscular, intralesional
- Transmucosal – transrectal, transnasal, and sublingual
- Local – mostly suppositories or enemas

Oral Drug Administration

The oral drug administration is probably the most commonly used method of drug administration. It is using oral formulations that could open into the stomach, small or large intestine.

There is a great and somewhat unmet need in oral delivery of protein and peptide drugs, suitable devices for delivering the therapeutic agents into the systemic circulation. Numerous gelatin capsules, film tablets, sustained release capsules, etc. have been developed in the last decades so to boost the efficacy and tolerability of numerous medications aiming GIT pathology (Tiwari et al. 2012).

Oral administration in Gastroenterology is only indicated in cases where patients can swallow properly, it is believed that this route of administration would be more beneficial as compared with the others, the drug is not likely to be destroyed or inactivated by stomach acid, pancreatic enzymes, bile acids or colonic bacteria and last but not least the drug would not be inactivated in the intestinal wall and/or the liver (first pass metabolism). Oral medications to be given with a glass of water in an upright position and washed down with a sufficient amount of water. Oral medications should not be given to a recumbent patient due to the risk of aspiration, chocking and also due to the risk of damages to the esophageal mucosa especially by some medications (e.g., tetracyclines, iron salts). To prevent gastric irritation and to achieve the desired concentration, some researchers have developed enteric coated tablets that resist the gastric acid and disintegrate in the intestine alkaline contents. This also helps to achieve the desired concentration of the drug in the small intestine (e.g., in Crohn's disease) and last but not least to retard the absorption of the drug. Furthermore preparations with colonic release have been developed. Oral formulations can be designed so to release the active substance over different period of time so there is a normal and controlled release oral formulations.

The constantly increasing number of peptide and protein drugs being investigated demands the development of novel dosage forms which exhibit also site-specific release. Delivery of drugs into

systemic circulation through colonic absorption represents a novel mode of introducing peptide and protein drug molecules and drugs that are poorly absorbed from the upper GIT (Pinto-Alphandary et al. 2000).

Specific targeting of drugs to the colon is recognized to have numerous therapeutic advantages per se and drugs, which are destroyed by the stomach acid and/or metabolized by pancreatic enzymes or affected by bile acids, are slightly affected into the colon. Colon targeting is of value for the topical treatment colonic pathology such as Crohn's disease, ulcerative colitis, amebiasis, and colorectal cancer. Sustained colonic release of medications can be useful also in the treatment of non-GIT conditions. Peptides, proteins, oligonucleotides, colonic diagnostic agents, and even oral vaccines are potential candidates of interest for colon-specific drug delivery. The diverse microflora and numerous enzymes present in the human colon are being exploited to release drugs in the colon (Tiwari et al. 2012); however, some pharmacodynamic obstacles are also involved in the effective local delivery of drugs to the colon due to the artificial bypass of the stomach and small intestine: unpredictable effect of the gut flora that could vary in its composition from person to person (Lagier et al. 2012), differential pH conditions in the colon, differences in the dietary habits, long transit time during the passage from mouth to colon create difficulties in the safe delivery of drugs to the large intestine (Tiwari et al. 2012).

Recent technological achievements such as drug coating with pH-sensitive and bacterial degradable polymers, embedding in bacterial degradable matrices and designing into prodrugs are aiming to effectively target drugs to the colon. The use of pH changes is similar to the enteric coating and consists of employing a polymer with an appropriate pH solubility profile. The concept of using pH as a trigger to release the drug in the colon is based on the pH conditions that vary significantly down the GIT. Polysaccharide and azopolymer coating, which is refractory in the stomach and small intestine yet degraded by the colonic bacteria, have been used as carriers for colon-specific targeting. Last but not least, the availability of good preclinical models and clinical methods promoted the quick development and evaluation of colon-specific drug delivery systems for clinical practice (Tiwari et al. 2012).

Parenteral Route of Administration

Routes of administration other than the oral are called parenteral. These are used mostly when oral therapy is not possible, not well tolerated (e.g., oral therapy triggers vomiting, diarrhea), patients cannot swallow, drug is not absorbed orally, to avoid drug modification by the GIT and when rapid systemic action and dose accuracy are to be ensured. GIT sometimes limits the bioavailability of certain medications because of its protease enzymes and bacteria-rich environment as well as general pH variability from pH 1–7. These extreme conditions make oral delivery particularly challenging for the some medications, e.g., biologics, insulin.

A common parenteral route of administration in Gastroenterology is the subcutaneous injection. It is used for the application of nonirritant substances (e.g., somatostatin analogues). The drug absorption is slower but the action is sustained and uniform. It often comes with great efficacy and variable tolerability depending on the type of the active substance administered. Overall, the immunogenicity of subcutaneously administered proteins depends upon antigen presentation and processing by lymph nodes and migratory cutaneous dendritic cells in the subcutaneous space (Fathallah et al. 2013). Another parenteral route of administration is the intravenous one. In this case, drugs are given directly into a vein. Normally the drug produces a rapid effect and the target serum concentration can be achieved with lower doses administered. The drug may be given as a bolus, over 5–10 min, or as continuous infusion (e.g., rehydration), over prolonged periods of time. Some medications are considered to have irritant effect when administered intravenously (e.g., iron, cancer chemotherapy, potassium solutions, parental feeding, etc.). Use of intra-arterial administration in Gastroenterology is very limited and is used mostly in cases of angiography and

embolization therapy (e.g., hepatocellular carcinoma). The intramuscular route of administration allows for the administration of soluble substances, mild irritants, colloids, and suspensions. The volume of injection should not exceed 10 ml. The intramuscular administration of vaccines optimizes the immunogenicity of the vaccine and minimizes adverse reactions at the injection site, e.g., HBV vaccine (Zuckerman 2000). Intralesional injections have features of both parenteral and local drug administration. Intralesional injections in Gastroenterology are often given under ultrasound control or via endoscope (e.g., endoscopic intralesional steroid injection in refractory esophageal strictures, endoscopic intralesional injection of diluted epinephrine (1:10,000) in the prevention of recurrent bleeding).

Transmucosal Route of Administration

The transmucosal route of administration is characterized by several main features: it is normally painless; offers greater flexibility in a variety of clinical situations, including patients who cannot swallow oral medications and/or in cases when it is not possible to establish intravenous access. Additionally it is characterized by a rapid onset of pharmacological effect, which is often preferred for drugs, especially in the treatment of the acute disorders. Human mucosa has rich blood and lymph supply and many drugs can cross the rectal mucosal membrane like any other lipid membrane, meaning that unionized and lipophilic substances are readily absorbed. Many drugs are using the so-called transrectal administration route. The rectum has rich blood and lymph supply: the portion of the drug absorbed from the upper rectal mucosa is carried by the superior hemorrhoidal vein into the portal circulation, whereas the portion absorbed by the lower rectum enters directly into the systemic circulation via the middle and inferior hemorrhoidal vein. Because of that absorption pattern approximately 50% of drug absorbed by the rectum bypasses the liver and additionally CYP3A4 is not present in the lower intestinal segments, meaning that the chances for first pass metabolism are significantly lower as compared with oral drug administration (e.g., indomethacin suppositories used after ERCP for the prevention of post ERCP-pancreatitis). The transnasal and sublingual routes are less commonly used in Gastroenterology but again can be really efficient and with a really good safety profile overall (e.g., intranasal fentanyl in procedural and postprocedural pain in children and sublingual nitroglycerin again in the prevention of post ERCP-pancreatitis).

Local Drug Administration

The local drug application in Gastroenterology is mostly given in the form of suppositories (e.g., Mesalazine in distal forms of ulcerative colitis) or enemas. The systemic absorption in local application is negligibly low. Enemas can be divided into retention and evacuant enema. In retention enema the fluid containing the drug (e.g., methylprednisolone in ulcerative colitis, mesalazine foam in ulcerative colitis) is usually 100–120 ml. The evacuant enema (e.g., soap water enema before abdominal surgery, X-ray of GIT) aims to remove the fecal matter and flatus. The liquid stimulates bowel movements by distending the bowel wall, whereas soap acts as a softener. The overall quantity of fluid administered is usually up to 600 mg.

Fecal microbiota transplantation holds a special place in gastroenterology and could be given as a retention enema (but also in the form of oral capsules) for the local treatment of recurrent *Clostridium difficile* infection and also ulcerative colitis (Rossen et al. 2015).

The development of micelles, liposomes, and even nanoparticles are being currently researched and integrated in oral and parenteral GIT medications. The aim of these sophisticated drug delivery systems is to provide enhanced efficacy for existing and novel medications and/or reduced toxicity for patients. These drug delivery systems may be subjected to even further changes such as PEGylation of liposomes and nanoparticles so to boost their efficacy, formation of nanogels, and solid lipid nanoparticles. These novel drug delivery systems are largely experimental but have also

shown some efficacy in some gastrointestinal tumors.

Therapeutic Drug Monitoring

Georgi Banishki

Management of serious progressive diseases of the GIT offers countless challenges to gastroenterologist from lack of therapeutic response, genetic polymorphisms, drug interactions to adverse drug reactions. This requires a targeted approach that takes into account individual patient variability through careful gathering of pharmacokinetic and pharmacodynamic data and this is where therapeutic drug monitoring (TDM) comes into account. TDM has been defined as using laboratory measurements usually a biological matrix of a parameter (e.g., drug metabolites) which after analysis will directly influence patient therapy (Dasgupta 2012). TDM is not applied for all lines of treatment but only in situations when: (1) clinical evidence is deemed insufficient, (2) correlation between serum or whole blood drug concentration and dosage is poor, (3) there is a narrow therapeutic range, (4) drug toxicity may lead to serious adverse events, (5) there is a link between serum or whole blood concentration of the drug and its therapeutic response or toxicity, and (6) there are clinical indications (e.g., toxicity despite no dosage adjustment) which require it (Dasgupta 2012). Naturally, one would expect that chemotherapeutic treatments would be subject to TDM, but that is the case in only a select number of cases. Previously discussed PET imaging has been a huge improvement in monitoring chemotherapy efficacy, but classical pharmacological tests are still rarely used – 5-fluoruracil treatments are one notable exception (Dasgupta 2012). However, in terms of the GIT TDM has started to make a mark in helping choose the best treatment for patients with IBD. In the past, steroids were the preferred choice for IBD management, but nowadays they are primarily used for controlling diseases flares at relapse periods, but for maintaining remission other drugs are now preferred. The main drugs that have been universally accepted for use in IBD maintenance can be subdivided into three classes and these are the aminosalicylates, TNFα inhibitors, and thiopurines. Aminosalicylates (e.g., mesalazine, sulfasalazine) are a class of anti-inflammatories and are the first-line choice for UC, but not so much for CD. These agents have been used for decades without close blood monitoring of metabolites and dosing regimens have been adjusted according to the clinical response and manifestation of symptoms. With anti-TNFs and thiopurines this is not the case due to their narrow therapeutic window and high risk of adverse drug reactions, and close TDM is deemed necessary for all patients. This has allowed for selecting the best therapy for the patient and if needs be altering it in order to account for evolving loss of response (LOR) or patient safety concerns.

Anti-TNFs are all monoclonal antibodies and are the latest line of drugs used to treat IBD that have come during the last decade. They are the primary recommended therapy for advanced CD or UC and the following drugs have been approved for human use – infliximab, adalimumab, certolizumab (only in the USA), and golimumab. The first concern when giving one of these drugs is the occurrence of primary LOR, or lack of effect after the first phase of treatment which can occur in up to one third of all patients (Kopylov et al. 2014). The second concern is secondary LOR which is also quite common and which is much harder to assess and can occur at any point of a treatment regimen. For some drugs like infliximab, it has been reported to appear in up to two-thirds of all patients in the first year, while in others like adalimumab it has reported as every fourth patient. The main factors that can cause primary LOR are disease progression, age of patient, genetic polymorphisms, smoking, and prior exposure to such drugs. For secondary LOR, the main cause has been immunogenicity and the development of antidrug antibodies (ATIs) (Kopylov et al. 2014). ATIs develop for both chimeric and fully humanized anti-TNFs and act by interfering with their binding to TNFα molecules. Consequently, the main tools that have been used for TDM in patients treated with anti-TNFs have been evaluation of serum levels of

drug metabolites and ATIs as well disease activity measurements. The most common method used is double-antigen ELISA in which the drug molecule (e.g., infliximab) is both the capture antigen and the detection antibody. This has the drawback of being unable to detect ATIs in the presence of the drug in the serum which can be fixed by using antihuman λ antigen detection antibody (AHLC) ELISA which has this capacity (Kopylov et al. 2014). Disease activity is measured by regular monitoring of an inflammatory marker (e.g., CRP/FCP) and endoscopy (Fig. 1). In most cases of primary LOR, this is not due to low levels of drug metabolites but due to increased clearance (e.g., fecal loss in UC) and ATI formation which result in low serum levels (Kopylov et al. 2014). Generally, in such cases increasing the dosing regimen has been ineffective, but switching from one medication to another is something that could have positive results (e.g., infliximab replaced with adalimumab). For cases when the patient experiences secondary LOR, a careful assessment of the level of inflammation and drug metabolites is needed. In case that there is active inflammation and high drug levels, the best option would be to switch to another medication. However, if the inflammation is seen, but drug levels are low there would be two options – first would be to increase the dose and the other, but much less common would be to add an immunomodulator in order to suppress ATI formation. Clinical studies where azathioprine or methotrexate was given in addition to infliximab showed reduction of ATIs and the return of clinical response and improvement in disease management (Kopylov et al. 2014).

Thiopurines are immunomodulators or immunosuppressants that act by blocking purine synthesis and thus inhibiting T cell production. The main drug of this class azathioprine was initially developed as an anticancer medication, but was then found out to be extremely effective in transplantations and later became the mainstay in IBD management. In recent years, azathioprine has been gradually replaced as first choice by the monoclonal antibodies as they have been deemed to be more target specific and with less side effects (Kopylov et al. 2014). The most common cause for discontinuation of treatment with thiopurines have been its adverse effects such as myelosuppression due to their interference with DNA synthesis, but there are also about 9% of all patients

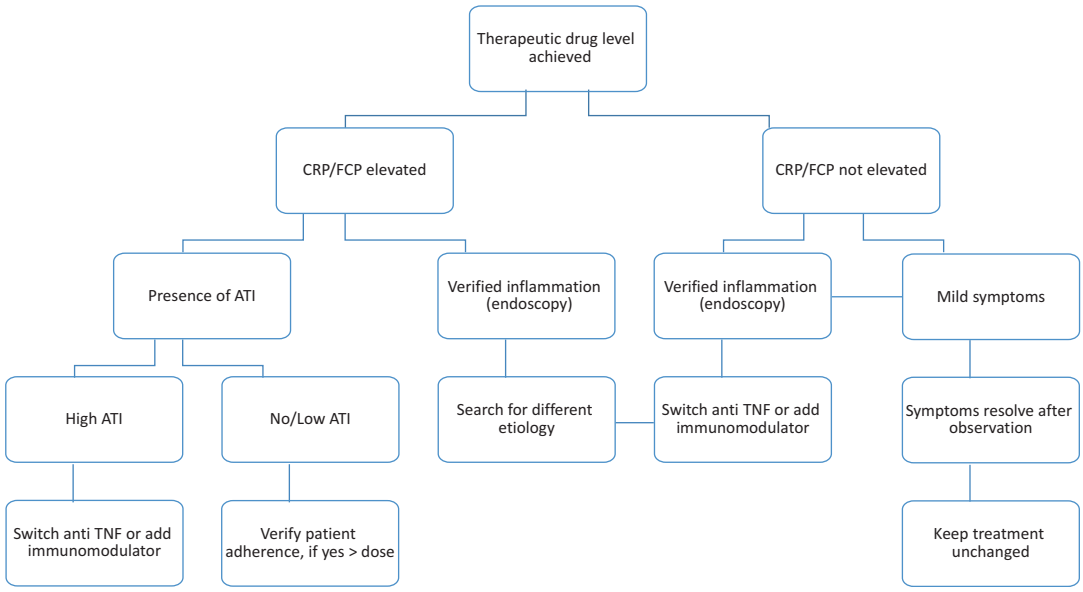

Fig. 1 TDM based algorithm for management of loss of response to TNFα inhibitors (Adapted for use from Kopylov et al. 2014)

who do not respond to this line of treatment (Kopylov et al. 2014). Therefore, therapeutic drug monitoring is done by monitoring thiopurine metabolites, but also by monitoring blood counts and doing routine checks on pancreatic enzymes. One other factor that has also been recommended to be taken into account is genetic polymorphisms. Once administered azathioprine is rapidly converted to active metabolite 6-mercaptopurine (6-MP) by a nonenzymatic reaction and after that there are two competing pathways (Kopylov et al. 2014). The first is mediated by the enzyme thiopurine methyltransferase (TPMT) which converts 6-MP to the inactive 6-MMP which gets excreted or the other pathway which converts it to 6-thianoguanine (6-TGN). 6-TGN is an active metabolite which causes myelosuppression, and in patients who were shown to have low or intermediate acting, TPMT was at much greater risk of myelosuppression due to 6-TGN accumulation (Kopylov et al. 2014). Three such alleles have been confirmed in Caucasian (TPMT*2, TPMT*3A, or TPMT*3C) and one in African-American (TPMT*3C) populations, and the FDA now recommends genotype or phenotype assessment of TPMT prior to initiating azathioprine (Kopylov et al. 2014). In case when patients are found to be homozygous of any of these, azathioprine should be avoided, and in case when they are heterozygous a decrease in recommended dose by 30–70% has been suggested (Kopylov et al. 2014). Nevertheless, even if the patient has been confirmed not to be heterozygous for any of these alleles regular CBCs should be performed as myelosuppression has been seen in patients with normal TPMT after long-term treatment with azathioprine (Kopylov et al. 2014).

Imaging in Gastroenterology

Georgi Banishki

As in other functional areas, imaging plays a major role in diagnosing GI diseases and even though there were times when it relied on radiological studies it has expanded to include novel technologies which utilize tracers and contrast agents. The imaging techniques used play a great role both in diagnosing and assessing treatment efficacy and can be subdivided into two categories: anatomical and functional. The anatomical techniques such as computer tomographic (CT) scans as their name signifies focus on observation of structural changes and identification of anatomical landmarks induced by GI ailments (e.g., solid tumors), while the functional ones rely on detecting functional and metabolic changes. These techniques can include functional magnetic resonance imaging (fMRI), but also the recently developed molecular imaging techniques such as positron emission tomography (PET). It is important to note that no single technique is superior to another which is why they are always used in connection, but this section will focus on the ones that rely on the use of pharmacological agents and their molecular interaction with biological targets.

Positron Emission Tomography (PET) Imaging

Despite of research in the field dating as far as the 1950s, PET scanning has been approved for use in colorectal and esophageal cancer diagnostics and management since 2001 and since then it has revolutionized the field. Several studies have shown that PET results have caused a significant change in cancer management in 25% and in some cases up to 40% of patients (Bailey et al. 2005). PET scanning is a branch of nuclear imaging which uses radioactive isotopes called nucleotides or tracers aiming to particular organs and structures. The radioactive isotope can be swallowed, injected or inhaled depending on the target of interest. Each radiotracer is specifically designed to be taken by a metabolic pathway at the targeted tissues where it will accumulate. All radionucleotides are designed to have short half-lives and as they are decaying they are emitting positrons (e+) which travel in the tissue until they meet an electron (e−) which annihilates both and result in the production of photons which then get detected by the scanning device. Once

Table 1 UK intercollegiate Committee recommended indications for clinical PET studies in the GIT: (A) supported by randomized controlled clinical trials, meta-analyses, and systematic reviews, (B) by experimental or observational studies, and (C) other evidence (Adapted from Bailey et al. 2005)

Oncology applications	Indication	Not indicated routinely	Not indicated
Esophagus	Staging of primary cancer (B) Assessment of disease recurrence in previously treated cancers (C)	Assessment of neoadjuvant chemotherapy (C)	
Stomach	No routine indication (C)	Assessment of gastroesophageal malignancies and local metastases (C)	
Small bowel	No routine indication (C)	Proven small bowel lymphoma to assess extent of disease (C)	
Liver	Equivocal diagnostic imaging (CT, MRI, ultrasound) (C) Assessment pre- and posttherapy intervention (C) Exclude other metastatic disease prior to metastectomy (C)		Routine assessment of hepatoma (C)
Pancreas		Staging a known primary (C) Differentiation of chronic pancreatitis from pancreatic carcinoma (C) Assessment of pancreatic masses to determine benign or malignant status (C)	
Colon and rectum	Assessment of recurrent disease (A) Prior to metastectomy of colorectal cancer (C)	Assessment of tumor response (C) Assessment of a mass that is difficult to biopsy (C)	Assessment of polyps (C) Staging a known primary (C)

all of these emissions undergo computer reconstruction a 3D image is created and in which the targeted areas will be highlighted and thus may indicate that there are metabolic processes associated with a particular disease in this area (Bailey et al. 2005). PET imaging has found extensive application mainly in neurology and oncology, and it is in the diagnosis and treatment monitoring of different cancers that it has found its greatest use in the GIT (Table 1).

Anatomical imaging techniques remain the mainstay in early diagnosing of cancer, but in other aspects such as tumor staging and monitoring treatment outcome and disease progression is where PET being applied to the best benefit. Accurate evaluation of treatment response is critical for optimal treatment decisions in different types of cancer, and Response Evaluation Criteria in Solid Tumors (RECIST) group has come up with criteria on how to evaluate tumor response to treatment (Table 2).

RECIST assesses tumor response by the extent of tumor size reduction and in the past this assessment was done primarily using CT scans or MRI, but as these can be sometimes insufficient in visualizing all tumor infiltration points. This has led to the fusion of PET and CT scan technologies and the development of fluorodeoxyglucose-positron emission tomography (FDG-PET/CT). FDG is a glucose analogue radiotracer which is differentially taken up by malignant cells due to their higher glucose metabolism and this can be used to monitor both short- and long-term metabolic response of tumor after chemotherapy (Van Cutsem et al. 2016). This method utilizes the affinity of tumor cells for FDG, which is strongly linked to tumor grade (aggressiveness) and cellularity. Changes in FDG uptake can be detected after a single course of chemotherapy and as early as 24 h after treatment and thus can help discriminate malignant CRC tumors that are unlikely to respond to treatment. Metastatic CRC is usually highly responsive to FDG, with the exception of mucinous tumors, which may not be detected by a FDG-PET/CT scan (Van Cutsem et al. 2016). Other digestive tumors that can result

Table 2 Response Evaluation Criteria in Solid Tumors (RECIST) (Adapted from Van Cutsem et al. 2016)

Grade	Response criteria
Complete response	Disappearance of all target lesions. Any pathological lymph nodes(whether target or nontarget) must have reduction in short axis to <10 mm
Partial response	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters
Progressive disease	At least a 20% increase in the sum of diameters of target lesions, the appearance of one or more new lesions is also considered progression
Stable disease	Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease

in false-negative results are low grade neuroendocrine tumors, well-differentiated hepatocellular carcinoma, nonmass forming gastric tumors, and mucinous or cystic pancreatic tumors. Nevertheless, FDG-PET/CT has shown to be much more sensitive in tumor detection and especially for evaluating responses to chemotherapy and especially for targeted treatments than CT scans or FDG-PET imaging on their own. For example, in the multicenter SoMore trial, FDG-PET/CT was used to assess early metabolic response following treatment with combination of sorafenib and capecitabine in chemorefractory malignant CRC patients (Van Cutsem et al. 2016). Just after one single treatment cycle (week 3), FDG-PET/CT was able to differentiate responsive from unresponsive lesions (Van Cutsem et al. 2016). These and other such findings support the notion that FDG-PET/CT will be able to greatly improve the management of chemotherapies in the future, but more such studies will be needed in order to verify this. Furthermore, as the RECIST scale was designed for CT scanning assessment a much less ambiguous response scale called the PET response criteria in solid tumors (PERCIST) has been developed which takes metabolic criteria into much greater account (Table 3). Nevertheless, due to its lack of focus on treatment resistance improved FDG-PET response criteria will need to be developed in the future (Van Cutsem et al. 2016).

Table 3 PET response criteria in solid tumors (PERCIST) (Adapted from Poeppel et al. 2002)

Grade	Response criteria
Complete metabolic response	Complete resolution of FDG uptake
Partial metabolic response	≥30% decrease in FDG uptake with absolute drop in standardized uptake value (SUD)
Progressive metabolic disease	≥30% increase in FDG uptake or appearance of new FDG-avid lesions
Stable metabolic disease	No partial metabolic response or disease progression

One other benefit of FDG-PET/CT compared to normal CT scan is that can provide complementary metabolic information that can enable the detection of tumors at unexpected sites or in appearing morphologically normal structures. A common problem associated with colorectal cancer (CRC) is its ability to metastases and most specifically to form metastases in the liver and you cannot achieve disease control without limiting this spread. Once again CT scans and MRI are the preferred method, but FDG-PET/CT is starting to make an impact here as well. FDG-PET/CT can effectively detect extrahepatic disease and has higher sensitivity (64% vs. 89%) and specificity (70% vs. 90%) in this setting (Van Cutsem et al. 2016). Performing FDG-PET/CT in addition to conventional imaging can further support decision-making as one study of 150 patients with metabolic CRC found the addition of FDG-PET to CT resulted in the avoidance of an unnecessary laparotomy in a significant proportion of patients (38%) (Van Cutsem et al. 2016). The expansion of FDG-PET/CT to evaluate the response of metastatic disease remains a growing area of research. FDG remains the golden standard in PET imaging in 90% of all applications, but there are other PET radiotracers that have been tested in oncology and are likely to have greater importance in the future. One such example is ^{15}O-water which can indicate perfusion and hypoxia which are both markers of tumor angiogenesis and increased metastatic activity (Van Cutsem et al. 2016).

There have been attempts to expand PET imaging into other disease areas and one such example

has been lymphocytic gastritis which accounts for about 5% of cases of chronic gastritis and its symptoms are often nonspecific and include abdominal pain, nausea and vomiting, or weight loss (Murphy et al. 2017). Therefore, using endoscopic appearance of the mucosa for diagnosis can be unreliable as it can appear as nodules, erosions, enlarged and prominent rugae, or normal. In a case study by Murphy and colleagues, they were able to diagnose for the first time lymphocytic gastritis using PET scan. A 20-year-old man presented for evaluation of diffuse abdominal pain after he had completed chemotherapy 2 months earlier for stage IV diffuse large B-cell lymphoma (DLBCL). During the examination, abnormal PET findings showed him to have a new area of intense activity within the proximal stomach (Fig. 2a). Subsequent upper endoscopy (Fig. 2b) showed scattered shallow ulcers throughout the stomach and gastric biopsies confirmed the patient to be suffering from lymphocytic gastritis-type pattern with acute inflammation and reactive gastropathy. Further immunostaining showed that these were CD3+ T-cells rather than the B-cells expected in recurrent DLBCL and along with the negative *H. pylori* and Celiac disease serologies confirmed the final diagnosis of lymphocytic gastritis. The patient underwent treatment with several days of daily proton pump inhibitors (PPI) and the repeat upper endoscopy and PET scan demonstrated resolution (Fig. 2c and d). Despite the fact this was a single event, this has led to the speculation that the increase in intraepithelial lymphocytes and associated inflammation is the etiology of hypermetabolic activity seen on the PET scan and could account for other abnormal observations from previous studies (Murphy et al. 2017).

Contrast Enhanced MRI (CE-MRI)

Chronic inflammatory conditions such as Crohn's disease (CD) have always been diagnosed using invasive techniques such as endoscopy and biopsies. However, as like with any other autoimmune disease CD is characterized by alternating remission and relapsing phases which lead to progressive intestinal damage and loss of function this requires a closer examination and a larger view of the gut and endoscopy is insufficient in these aspects. Therefore, cross-sectional imaging techniques like computed tomography enterography (CT) or MRI have been recommended as suitable techniques for assessing both mucosal healing and long-term disease progression and thus can help to establish the best treatment strategy (Savarino et al. 2017). As CD patients have a high risk of developing anal abscesses and fistulas (30–50%), and CT has high radiation hazard, MRI with its high contrast resolution has become the preferred choice for assessing treatment options for CD. MRI has demonstrated high accuracy for the assessment of mucosal lesions and has shown to be a reliable alternative to ileocolonoscopy as it reveals not only the gut mucosa, but all other bowel layers and is thus preferred by the patients and physicians for frequent disease examinations (Savarino et al. 2017).

In order to achieve better contrast of the image, IV injection of Gadolinium-based contrast agents (GBCA) (contrast-enhanced MRI, CE-MRI) has been demonstrated to be of crucial importance for evaluating mucosal inflammation, transmural involvement, and extraintestinal disease (Savarino et al. 2017). GBCAs are small molecular chelates with high stability containing a Gadolinium ion (Gd3+) which causes reduction of the T1 (rate of longitudinal relaxation) and T2 (rate of transverse relaxation) by modifying the relaxation of closer water protons which in turn leads to positive enhancement on T1-weighted images (Savarino et al. 2017). This in turn helps to better distinguish wall thickness and inflammation that are associated with CD. Nine different GBCAs have been approved for human use since 1986 and until recently they were all thought to be quite safe (Savarino et al. 2017). However, there has been accumulating evidence of Gadolinium accumulation in tissues. There have been animal models and clinical findings which have demonstrated Gadolinium accumulation in brain tissue (Savarino et al. 2017). This has been confirmed only for patients who had CE-MRI for neurological conditions, but there is mounting evidence of

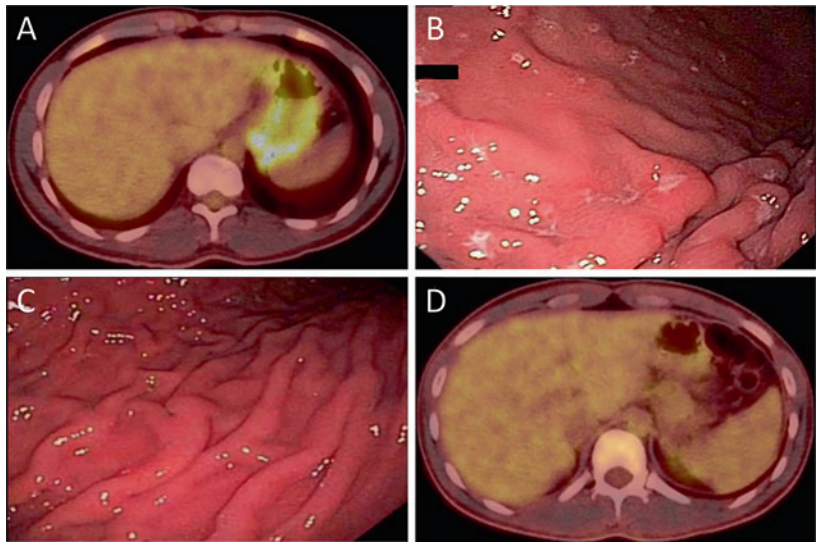

Fig. 2 (**a**) Intense activity in proximal stomach detected by PET; (**b**) shallow gastric ulcers seen by endoscopy; (**c**) full mucosal healing after repeat endoscopy; (**d**) resolution of hypermetabolic activity after repeat PET and PPI therapy (By permission of Murphy et al. 2017)

this also occurring in patients with no brain disorders. GBCAs are also suspected as a possible cause of the life-threatening condition nephrogenic systemic fibrosis (NSF) which could result in potentially fatal renal failure (Savarino et al. 2017). This has led to warning being given to GBCAs being used in patients with estimated glomerular filtration rate <30 mL/min/1.73 m2 and the European Medicines Agency has recommended the suspension of the market authorizations for four linear GBCAs "because of evidence that small amounts of the Gadolinium they contain are deposited in the brain" (gadobenate, gadodiamide, gadopentetate, and gadoversetamide) (Savarino et al. 2017). Nevertheless, CE-MRI remains the most preferred choice for assessing CD treatment response and progression and will likely remain so in the future.

Molecular Endoscopy

Nuclear medicine has its advantages, but in terms of early cancer detection optical imaging such as endoscopy remains the preferred choice due to neoplasms usually occurring in the epithelial and mucosal surfaces of the GIT. However, observation of structural changes and identification of anatomical landmarks can sometimes be insufficient as there are flat genetically heterogeneous precancerous lesions that are not visible to the naked eye and are virtually undetectable when using standard white light endoscopy. This has led to the development of a new emerging technique, molecular endoscopy which has been designed to better visualize genetic and molecular changes typical of cancerous disease by utilizing autofluorescence or fluorescent molecular compounds targeted at disease specific markers. Molecular endoscopy offers the opportunity to substantially improve specificity through detection of targets that are unique to disease. This technique has been demonstrated in preclinical models using genetically engineered animals that replicate the molecular pathogenesis of human disease. Molecular endoscopy has been demonstrated clinically in the colon and esophagus and is being developed for use in the stomach, biliary tract, and pancreatic duct (Table 4) (Lee and Wang 2016).

Molecular endoscopy has been specifically designed to exploit the uniqueness and high expression levels of targets that are specific of GI diseases. Consequently, for molecular imaging have been recognized agents with high affinity

and target specificity as well as rapid clearance and preferably low cost. Fluorophores have been used for quite a while in contract imaging techniques such as flow cytometry and several such as fluorescein isothiocyanate (FITC) have been approved for human use (Lee and Wang 2016). The way that fluorophores work is by emitting light after light excitation and when attached to an antibody with high target specificity they are the perfect for staining and thus identifying objects of interest. Other than antibodies a broad range of platforms for fluorescent dyes have been tried out in both preclinical and clinical studies, including enzyme-activatable probes, peptides, and lectins (Lee and Wang 2016). FITC and other such fluorophore are generally inexpensive, emit light in the visible spectrum and thus provide images with high resolution and reduced depth.

In preclinical studies molecular endoscopies has been demonstrated to detect overexpression of cathepsin B in colonic adenomas and also to monitor tumor shrinkage after treatment with sirolimus in mice animal models (Lee and Wang 2016). In clinical studies, fluorescent antibodies and peptides have been used to demonstrate nonpolypoid lesions in the proximal colon. It had been previously demonstrated that in the proximal colon up to 27% of all lesions can be invisible to white light endoscopy. In a study where 15 patients were injected with Cy5-labeled GE-137 peptide, a peptide that binds to c-Methionine overexpressed in dysplastic crypts, Burggraaf and colleagues successfully demonstrated that flat lesions can be detected with this method (Lee and Wang 2016). Using a modified fiber-optic colonoscope, they showed that all of the 47 tubular adenomas detected had increased uptake of this fluorescent peptide. Furthermore, nine additional adenomas that remained unseen under white light alone were detected (Lee and Wang 2016). These were all small in size and of nonpolypoid nature thus confirming the potential of this imaging technique. Last but not least, molecular endoscopy has been demonstrated also into monitoring therapy effectiveness. Adalimumab is a drug used to treat CD by counteracting Tumor Necrosis Factor α (TNFα) and in up to 50% of patients there is no response to treatment. Atreya and colleagues gave topically 25 patients FITC-labeled anti-TNF antibody in 25 patients and then treated with adalimumab (Lee and Wang 2016). Using confocal laser endomicroscope it was demonstrated that patients with high number of cell with membrane-bound TNF responded much better to the therapy and this was demonstrated over a course of a whole year (Lee and Wang 2016). Molecular endoscopy has also been effectively demonstrated in Barrett's esophagus imaging. In two separate clinical experiments using topically administered FITC-labeled peptide specific for CypA, the fluorescence images collected demonstrated up to 76% specificity for detection of high-grade dysplasia and 97% specificity for esophageal adenocarcinoma and most importantly to distinguish previously unidentifiable flat lesions (Lee and Wang 2016). Overall, this imaging technique has demonstrated huge promise and is currently investigated in other GI areas and overtime with improvements to both endoscopic tools and molecular probes it has the potential one day to become a universally used by gastroenterologists method.

Oral Vaccines and Oral Tolerogens

Milena Nikolova-Vlahova

Oral vaccines and oral tolerogens are only possible because of the unique functions of the human GIT; however, their biological effect goes far beyond the GIT thanks to its ubiquity.

Vaccines are undoubtedly one of the major successes of modern medicine. They are generally composed of killed or attenuated causative organisms, their subunits or nucleic acid. The subunit vaccines are considered to be very selective and specific but are poorly immunogenic. Therefore, in cases when the vaccine has lower immunogenicity, its effect could be promoted with the use of adjuvants that form complexes with the immunogenic parts and ensure slower release and more prolonged exposure to the antigen. These adjuvants are divided in two types: vaccine delivery systems (emulsions, micro- and nanoparticles, immune-stimulating complexes, and liposomes)

Table 4 Summary of key ex vivo and in vivo GI studies using targeted fluorescent labels and the different detection instruments used (Adapted for use from (Lee and Wang 2016)

Indication	Target	Carrier molecule	Fluorescent label	Administration method	Species	Instrument	Year of experiment
Colitis	GGT	Enzyme	Rhodamine green	Topical	Mouse	Wide-field fluorescence endoscopy	2013
Colonic adenoma	Cathepsin B	Enzyme	Cy5.5	Injection	Mouse	Wide-field fluorescence endoscopy	2010
Colonic adenoma	EGFR	Peptide	Cy5.5	Topical	Mouse	Wide-field fluorescence endoscopy	2015
Colonic adenoma	HER2	Peptide	Cy5.5	Topical	Mouse	Wide-field fluorescence endoscopy	2016
Colonic adenoma	Claudin-1	Peptide	Cy5.5	Topical	Mouse	Wide-field fluorescence endoscopy	2016
Colorectal cancer	EGFR	Antibody	FITC	Injection	Mouse	Confocal laser endomicroscopy	2010
Colorectal cancer	VEGF	Antibody	AF488	Injection	Mouse	Confocal laser endomicroscopy	2010
Sessile serrated adenoma	Unknown	Peptide	FITC	Topical	Human	Wide-field fluorescence endoscopy	2016
Colonic adenoma	c-met	Peptide	Cy5	Injection	Human	Wide-field fluorescence endoscopy	2015
Crohn's disease	Membrane-bound TNF	Antibody	FITC	Topical	Human	Confocal laser endomicroscopy	2014
Colonic adenoma	EGFR	Antibody	AF488	Topical	Human	Confocal laser endomicroscopy	2013
Colonic adenoma	Caspr-1	Peptide	FITC	Topical	Human	Confocal laser endomicroscopy	2008
Esophageal squamous cell cancer	Periostin	Antibody	Cy5.5	Injection	Mouse	Wide-field fluorescence endoscopy	2013
Barrett's neoplasia	HER2	Antibody	AF488	Injection	Rat	Confocal laser endomicroscopy	2015
Barrett's neoplasia	Glycans	Lectin	AF488	Topical	Human (ex vivo)	Wide-field fluorescence endoscopy	2012
Barrett's neoplasia	CypA	Peptide	FITC	Topical	Human	Confocal laser endomicroscopy	2016
Barrett's neoplasia	CypA	Peptide	FITC	Topical	Human	Confocal laser endomicroscopy	2013
Gastric cancer	MG7	Antibody	AF488	Injection	Mouse (xenograft)	Confocal laser endomicroscopy	2013

and immunostimulatory adjuvants that are non-immunogenic and nontoxic per se but potentiate the immune response to the antigen (aluminum and its salts, oil emulsions, synthetic polynucleotides, ISCOMs, etc.) (Wang and Coppel 2008; Saroja et al. 2011; Zhu and Berzofsky 2013).

Oral vaccines represent an easy, patient-friendly and needle-free method of protection against mucosal and nonmucosal pathogens. On the other hand, oral tolerogens are antigens that reduce systemic allergic immune response to foreign antigens (Chehade and Mayer 2005). Tolerance is immune phenomenon in which the immune response decreases following repetitive stimulation with high or low grade concentrations of antigen due to alteration of the mucous and systemic immune response.

Oral Mucosal Immune Tolerance, Suppression, and Silencing

When discussing the oral immune system modulation, of both local and systemic immune response, we should elucidate the concept of immune tolerance, suppression, and silencing. Immune tolerance is immune phenomenon associated with decrease or abolishment of immune response during or after administration of low or high doses of antigen (including allergen) due to repetitive contact with the immune system cells and decrease in immune response (Chehade and Mayer 2005; Shnawa 2015). Immune tolerance can be local/mucosal or systemic. Both types are associated with clonal cell deletion and anergy, T-regulatory activity, and clonal negative selection. Mucosal immune tolerance also involves T-cell apoptosis, changes in Th3 and dendritic cell activity and secretory IgA clonal deletion.

Immune suppression is inhibition of immune response due to decreased levels and/or activation of immunocompetent cells, humoral factors, and antibodies. It can be due to inborn defects, contact with myelosuppressive substances (including cytotoxics), radiation, etc., or antagonists to natural immune mechanisms (i.e., monoclonal antibodies against T/B cells, antibodies, cytokines and their receptors) (Shnawa 2015).

Oral mucosal immune silencing (also called oral tolerance) is a mechanism of immune tolerance, related to changes in membrane expression of molecules (i.e., B7-H) on the surface of Langerhans'cells due to contact with microbial antigens and by-products (Shnawa 2015).

Oral vaccines induce immune response and oral tolerogens cause immunomodulation with alteration of immune response directed at the development of local and/or systemic immune tolerance.

Oral Vaccines

Vaccines are an effective strategy aimed of prevention of many infectious and noninfectious disease. Different routes of administration have been developed, including oral, intranasal, intracolorectal, buccal, intravaginal, subcutaneous, intradermal, intramuscular, etc. Oral vaccines ensure durable protection against mucous and nonmucous infections, including polio, rotavirus and adenovirus infection, typhoid fever. Oral vaccines against several nonmucous diseases have been developed, including malaria, Japanese encephalitis, hepatitisB, etc. but their safety, tolerability, and efficacy in humans are to be elucidated.

The immunogenicity of all oral vaccines depends on the stimulation of mucosa-associated lymphatic tissue (MALT), gut-associated lymphatic tissue (GALT), dendridic cells, secretory IgA response, and T-cells. All oral vaccines are composed of killed or attenuated microorganisms or of their immunogenic substances. The *delivery system* is the crucial link, determining the site of liberation of the immunogenic substance and the site of stimulation of mucous immune response (Wang and Coppel 2008; Saroja et al. 2011; Kraan et al. 2014). Moreover, orally administered vaccines can trigger non-gastrointestinal response (urogenital or systemic) depending on the site of liberation of antigens and the delivery system.

Following delivery systems have been developed (Wang and Coppel 2008; Saroja et al. 2011; Kraan et al. 2014):

- Live bacterial vectors expressing recombinant antigens and colonizing the gut (mainly non-pathogenic *Salmonella*).
- Particulate formulations – vaccine particles are covered with substances that protect them from degradation until the antigens reach their target sites (small or large intestine) – microparticles of biodegradable polymers, cochleates, liposomes (in lipopolysaccharides and/or phospholipids), ICOMs (covered with cholesterol + QuilA), virus-like particles (VLPs). Moreover, the antigens can be incapsulated in a structure along with targeting substances. The encapsulated antigens induce stronger and site-specific immune response. Some of these techniques have been proven to be effective in animal models but their efficacy, safety, and tolerability on humans need to be further elucidated.
- Nanoparticles – these small particles of encapsulated antigens ensure safe passage of the vaccine to the site of action (usually the small intestine) and effective stimulation of the immune response – both humoral and cellular. As in microparticles, the vaccine antigens are encapsulated in polymers, starch, lipid-containing vesicles, VLPs and ISCOMs, that are dissolved and subsequently absorbed in the small intestine and exercise both their local and systemic immunogenic effect with subsequent immune response.
- Transgenic plants – genetically modified plants that express antigenic proteins expressed on the surface or within the plant cell – a cost-effective alternative of particulate formulations.
- Mucosal adjuvants – cholera toxin and heat-labile *E.coli* toxin – toxic and unsafe in humans.
- Several oral malaria vaccines have been developed. The main principles of their action are: naked bacterial antigens, oral vectors expressing plasmodium antigens (*Salmonella, Lactococcus*), encapsulated plasmodium antigens (i.e., PLGA-encapsulated SPf66).
- Oral vaccines against hepatitis B – live vectors of HBs antigen, encapsulated HBs antigen (in biodegradable capsula in the form of microparticles), expression of HBs antigen by genetically modified potatoes and lettuce.
- Oral vaccines against Japanese viral encephalitis – viral protein with CpG adjuvant and/or expressed by genetically modified *E.coli*.
- Several new vaccine delivery systems that ensure needle-free administrations of vaccines have been developed, including the MucoJet system (Miller and Greenberg 2017). The MucoJet has been tested in laboratory animals (rabbits). It uses a gas-generating system that produces a high-pressure jet of vaccine that penetrates the buccal mucosa and is capable of inducing both local and systemic immune response (Miller and Greenberg 2017).

The immune potentiators (adjuvants) that are added to orally administered vaccines in order to increase their immunogenicity are two biologically similar enterotoxins – cholera toxin and heat-labile *E.coli* toxin, and the Toll-like receptor (TLR) ligands – Poly(I:C), FSL-1, MPLA, Pam3CSK4, cytosine-phosphate guanosine, etc. (Wang and Coppel 2008; Saroja et al. 2011; Zhu and Berzofsky 2013). These immunogens activate the NK cells, NKT, B cells, CD4+ T cells in the spleen and induce stronger Th1 response after sublingual vaccine delivery. Aluminum and its salts, oil emulsions, and synthetic polynucleotides are nontoxic and nonimmunogenic but non-specifically enhance the vaccine-triggered immune response.

Oral Tolerogens

Oral tolerogens are antigens that after repeated oral administration lead to a decrease in allergic (IgE-mediated), secretory (IgA-mediated), or systemic (IgG-mediated) immune response (Chehade and Mayer 2005).

The oral administration of antigens change both Th2 (IL-4/IL-10) and Th3 (TGF-beta) response, dendritic cells and antigen-presenting cells in general, along with CD4 + CD25+ and LAP + T cells, cytokine levels and affect other metabolic and immune pathways (retinoic acid,

Tres expression transcription factor FoxP3) (Chehade and Mayer 2005; Wang et al. 2013). Moreover, the development of oral tolerance is augmented by multiple humoral factors, i.e., IL-4, IL-10, anti-IL-12, TGF-beta, cholera toxin B subunit, Flt-3 ligand, anti-CD40 ligand, and repetitive oral contact with the allergen/antigen.

Oral tolerogens are used both in allergic (i.e., allergy to cow's milk, contact allergy) and in autoimmune diseases (systemic lupus, encephalomyelitis, myasthenia, arthritis, uveitis (Chehade and Mayer 2005; Wang et al. 2013). Several researches have been performed in chronic diseases, such as atherosclerosis, asthma, stroke and colitis. In immune disease all oral tolerogens have been tested mainly in experimental animals and in humans several studies in lupus, multiple sclerosis, uveitis, myasthenia, and arthritis have been conducted.

Effect of oral tolerogens depends mainly on three factors: antigen dose and form, host factors (age, genetics, and normal flora of the host) (Chehade and Mayer 2005).

- The effect is dose-dependent - frequent administration of low doses leads mainly to suppression of the immune response (allergic or inflammatory), whereas high doses lead mainly to clonal anergy and deletion.
- The form of the antigen – soluble antigens have more pronounced tolerogenic effect than particulate. Moreover, the possibility for cross-reaction and antigen mimicry should not be forgotten.
- Host genetics – in mice, the development of tolerance to ovalbumin is dependent on MHC genes, IL-4 and IL-10 secretion, interferon-gamma secretion, etc. In humans, the presence of DRB1*08, DRB1*08/12tyr16, and DQB1*04 is associated with higher prevalence of peanut allergy.
- Host age – the allergic and immune reactions are known to be more severe in younger subjects and especially in neonates, probably due to increased intestinal permeability and lymphocytic response in this age. Therefore, the tolerogenesis is expected to have different efficacy in different age groups.
- Normal flora of the host – The development of oral tolerance is highly dependent on the bacterial colonization of the host's GIT. The concomitant administration of *Lactobacillus spp.* along with oral desensitization to cow's milk leads to more effective tolerogenesis. Therefore, the normal bacterial flora promotes the development of normal oral tolerance.

Oral tolerance has been tested in humans in autoimmune diseases, such as multiple sclerosis, lupus, arthritis, uveitis, contact sensitivity. The studies in systemic lupus failed to demonstrate long-term efficacy of abetimus (LJP 394) for the activity of systemic lupus and lupus nephritis. Studies in humans are ongoing for other immune disease.

References

Bailey DL, Townsend DW, Valk PE, Maisey MN (2005) Positron emission tomography in clinical medicine. Positron emission tomography, 1st edn. Springer, New York, pp 1–12

Chehade M, Mayer L (2005) Oral tolerance and its relation to food hypersensitivity. J Allergy Clin Immunol 115 (1):3–12

Dasgupta A (2012) Chapter 1 – introduction to therapeutic drug monitoring: frequently and less frequently monitored drugs. In: Dasgupta A (ed) Therapeutic drug monitoring, 1st edn. Elsevier, London, pp 1–29

Fathallah AM, Bankert RB, Balu-Iyer SV (2013) Immunogenicity of subcutaneously administered therapeutic proteins - a mechanistic perspective. AAPS J 15 (4):897–900

Gershon MD (2013) 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obesity 20(1):14–21

Holzer P (2009) Opioid receptors in the gastrointestinal tract. Regul Pept 155:11–17

Izzo AA, Sharkey KA (2010) Cannabinoids and the gut: new developments and emerging concepts. Pharmacol Ther 126:21–38

Kopylov U, Ben-Hurin S, Seidman E (2014) Therapeutic drug monitoring in inflammatory bowel disease. Ann Gastroenterol 27:304–312

Kraan H, Vrieling H, Czerkinsky C et al (2014) Buccal and sublingual vaccine delivery. J Control Release 190:580–592

Lagier J-C, Million M, Hugon P et al (2012) Human gut microbiota: repertoire and variations. Front Cell Infect Microbiol 2(136):1–19

Lee JH, Wang TD (2016) Molecular endoscopy for targeted imaging in the digestive tract. Lancet Gastroenterol Hepatol 1:147–155

Miller CS, Greenberg RN (2017) MucoJet: a novel oral microjet vaccination system. Oral Dis. https://doi.org/10.1111/odi.12697

Murphy CJ, Swanson B, Markow M et al (2017) Lymphocytic gastritis identified by abnormal PET scan. ACG Case Rep J 4:e62

Pinto-Alphandary H, Andremont A, Couvreur P (2000) Targeted delivery of antibiotics using liposomes and nanoparticles: research and applications. Int J Antimicrob Agents 13:155–168

Poeppel TD, Krause BJ, Heusner TA et al (2002) PET/CT for the staging and follow-up of patients with malignancies. Eur J Radiol 70:382–392

Rossen NG, MacDonald JK, de Vries EM et al (2015) Fecal microbiota transplantation as novel therapy in gastroenterology: a systematic review. World J Gastroenterol 21(17):5359–5371

Saroja CH, Lakshmi PK, Bhaskaran S (2011) Recent trends in vaccine delivery systems: a review. Int J Pharm Investig 1(2):64–74

Savarino E, Chianca V, Bodini G et al (2017) Gadolinium accumulation after contrast-enhanced magnetic resonance imaging: which implications in patients with Crohn's disease? Dig Liver Dis 49:728–730

Shnawa IMS (2015) Oral mucosal immune tolerance versus oral immune silencing: mini review. Am J Biomed. Life Sci 3(4–1):7–9

Tiwari G, Tiwari R, Sriwastawa B et al (2012) Drug delivery systems: an updated review. Int J Pharm Investig 2 (1):2–11

Van Cutsem E, Verheul HMW, Flamen P et al (2016) Imaging in colorectal cancer: progress and challenges for clinicians. Cancers 8:1–14

Wang L, Coppel R (2008) Oral vaccines delivery: can it protect against non-mucosal pathogens? Expert Rev Vaccines 7(6):729–738

Wang X, Sherman A, Liao G (2013) Mechanisms of oral tolerance induction to therapeutic proteins. Adv Drug Deliv Rev 65(6):759–773

Zhu Q, Berzofsky JA (2013) Oral vaccines. Gut Microbes 4(3):246–252

Zuckerman JN (2000) The importance of injecting vaccines into muscle: Different patients need different needle sizes. Br Med J 321(7271):1237–1238