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# Neuroimmune mechanisms in postoperative ileus

G E Boeckxstaens,<sup>1,2</sup> W J de Jonge<sup>2</sup>

<sup>1</sup> Department of Gastroenterology, University Hospitals Leuven, Catholic University of Leuven, Leuven, Belgium; <sup>2</sup> Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands

Correspondence to: Professor G E Boeckxstaens, Department of Gastroenterology, University Hospitals Leuven, Catholic University of Leuven, Herestraat 49, 3000 Leuven, Belgium; [guy.boeckxstaens@med.kuleuven.be](mailto:guy.boeckxstaens@med.kuleuven.be)

## ABSTRACT

Postoperative ileus (POI) is a common clinical condition arising after almost every abdominal surgical procedure, leading to increased patient morbidity and prolonged hospitalisation. Recent advances in insight into the underlying pathophysiology have identified intestinal inflammation triggered by handling of the intestine as the main mechanism. Not only does the local inflammatory process compromise the contractile activity of the handled intestine, but it also activates inhibitory neural pathways and possibly triggers inflammation at distant untouched areas, leading to a generalised impairment of gastrointestinal motility. Macrophages residing in the muscularis externa and mast cells are the key players in this inflammatory cascade. Pharmacological interventions preventing the activation of these immune cells reduce the influx of leucocytes into the intestine, an effect associated with a reduction of the duration of POI. New potential therapeutic strategies to shorten POI based on these new insights will undoubtedly enter the clinical arena soon.

In the past century, the introduction of antibiotics and sterile surgical conditions has contributed to a revolutionary improvement in postoperative morbidity and survival. Yet, each patient undergoing an abdominal surgical procedure, even if minimally invasive techniques are applied, will develop a transient episode of impaired gastrointestinal motility or postoperative ileus (POI). Although some argue that uncomplicated POI should be considered as a “normal” or “physiological” response of the intestine to a traumatic event and thus should be disregarded, it clearly has a significant impact on patient morbidity, with prolonged hospitalisation and thus increased costs. The annual costs related to POI have been estimated to be as much as US\$1.47 billion annually in the USA, illustrating its large socio-economic impact.<sup>1</sup> Over the past decade, our insight into its pathophysiology has increased exponentially. The role of inflammation triggered by handling of the intestine is now generally accepted as the key event in POI. In particular, insight into the bidirectional interaction between the immune system (mast cells, macrophages and other leucocytes) and the autonomic nervous system (afferents and efferents), also referred to as neuroimmune interaction, has significantly contributed to a better understanding of the pathophysiology of POI. Moreover, it has become clear that inflammatory mediators released by leucocytes within the gut wall also directly impair smooth muscle contractility. The present

manuscript will mainly describe these new developments with the emphasis on their potential clinical relevance.

## DEFINITION—CLINICAL ASPECTS

Although POI may occur following extra-abdominal operations, it is most pronounced and inevitable after every abdominal surgical procedure. It presents clinically as the inability to tolerate food, with abdominal distension, absence of bowel sounds and lack of flatus and defecation. Nausea and vomiting, pain and postoperative fatigue further contribute to the morbidity and prolonged hospitalisation of patients. On average, this period lasts 2–4 days for conventional abdominal procedures, but decreases to as little as  $\leq 2$  days in the case of laparoscopic surgery.<sup>2</sup> Some surgeons consider the inability to tolerate food and absence of bowel sounds during the first few postoperative days as a normal phenomenon, and only consider “prolonged” or “pathological paralytic ileus”, which lasts  $>3$  days after surgery, as clinically relevant.<sup>3</sup> Others propose to prolong this period to  $>6$  days.<sup>4</sup> The incorrect usage of prolonged or paralytic ileus to define POI has introduced some confusion in the literature regarding the exact definition of POI. During a recent consensus meeting, however, POI was defined as the time from surgery until passage of flatus or stool together with the time to adequate oral intake maintained during 24 h. Secondary POI was defined by the same symptoms but precipitated by a complication of surgery, such as an anastomotic leak, abscess or peritonitis.<sup>5,6</sup> Such prolonged or complicated ileus develops in  $<10\%$  of abdominal surgeries, but may increase to up to 25% of patients undergoing a hemicolectomy<sup>1</sup> (table 1). Obviously, the pathophysiological mechanisms involved and the treatment of the latter condition are completely different and will not be discussed here.

Transient inhibition of gastrointestinal motility is well documented as the underlying mechanism and involves the entire gastrointestinal tract. It was first described at the turn of the century by Bayliss and Starling.<sup>7</sup> By now, we know that not all segments are equally affected; small intestinal motility is on average disturbed for approximately 24 h, gastric motility for between 24 and 48 h, whereas colonic motility is impaired for between 48 and 72 h (reviewed in Benson and Wingate<sup>8</sup>). It should be emphasised, however, that normalisation of motility, for example the return of the migrating motor complex in the small intestine,

**Table 1** Differences between postoperative ileus and prolonged/paralytic ileus

	Definition	Duration	Frequency
Postoperative ileus	Time until first flatus or stool + adequate oral intake during 24 h	2–4 days	Almost every abdominal surgical procedure
Prolonged or paralytic ileus	Precipitated by complication of surgery	>6 days	10–25%

does not necessarily imply that normal function and transit have returned. Nevertheless, these studies underscore that colonic motility is the main determinant of clinical recovery. This explains why first defecation and flatus are often used as primary outcome parameters in clinical trials. These parameters are, however, non-specific. Passage of flatus strongly depends on patient reporting, whereas passage of stool may simply reflect rectal emptying and not necessarily therefore inform of recovery of purposeful gastrointestinal contractile activity. With the possibility that new treatments for POI may arise in the near future, there will be a definite need for better outcome variables or other biological markers in order to evaluate new treatments better and objectively.

### PATHOPHYSIOLOGY

Although it is now clear that opening of the peritoneal cavity and manipulation of the intestine is the main mechanism underlying POI, other factors impairing neuromuscular function, such as anaesthetics and postoperative pain medication, certainly contribute to the postponement of recovery of normal transit. The effect of anaesthetics, especially that of volatile drugs, is, however, short lasting and therefore of only marginal importance. The use of postoperative opioids to control postoperative pain on the other hand has a much greater impact on postoperative motility. It should be acknowledged that the introduction of postoperative opioids has significantly improved the patient's comfort in the very early postoperative phase. Nevertheless, these drugs potentially inhibit gastrointestinal transit, even when patient-controlled epidural morphine is administered. Efforts to reduce the dose of opioids or to antagonise their effects by peripherally acting opioid  $\mu$ -antagonists such as methylalantrexone<sup>1</sup> or alvimopan<sup>9</sup> are therefore important to minimise the detrimental effect of opioids on gastrointestinal motility.

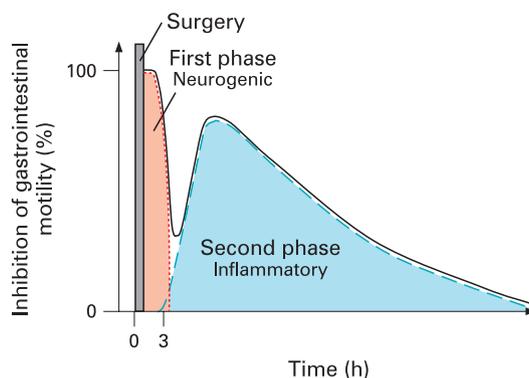
The main cause of POI, however, clearly relates to the surgical procedure itself. In the last decade, evidence has accumulated that abdominal surgery triggers two different phases, each with its own dynamics and underlying pathophysiological mechanism (fig 1). The first or early phase is neurally mediated and involves neural reflexes activated during and immediately following surgery. In the late 1990s, the concept was introduced that the manipulation of the intestine triggers the influx of leucocytes in manipulated intestinal segments, impairing the contractile properties of the inflamed intestine.<sup>11–15</sup> This second phase starts 3–4 h after surgery and is responsible for the

sustained and thus clinically more relevant inhibition of gastrointestinal motility. From a clinical perspective, interference with or prevention of this second phase is clearly expected to be most relevant and most effective in the treatment of POI. It should be emphasised, however, that data obtained in rodents may not necessarily translate to the human situation for obvious reasons of "species" differences, for example due to different expression of receptors, different mediators released or absence of vomiting in rodents. Moreover, mainly for simplicity and to increase reproducibility, most animal models only involve manipulation of the intestine/colon but do not include intestinal resection. The latter could not only be important from a pathophysiological point of view, but may also be extremely relevant to exclude potential side effects on healing of anastomoses. Therefore, animal models dealing with these shortcomings are certainly warranted.

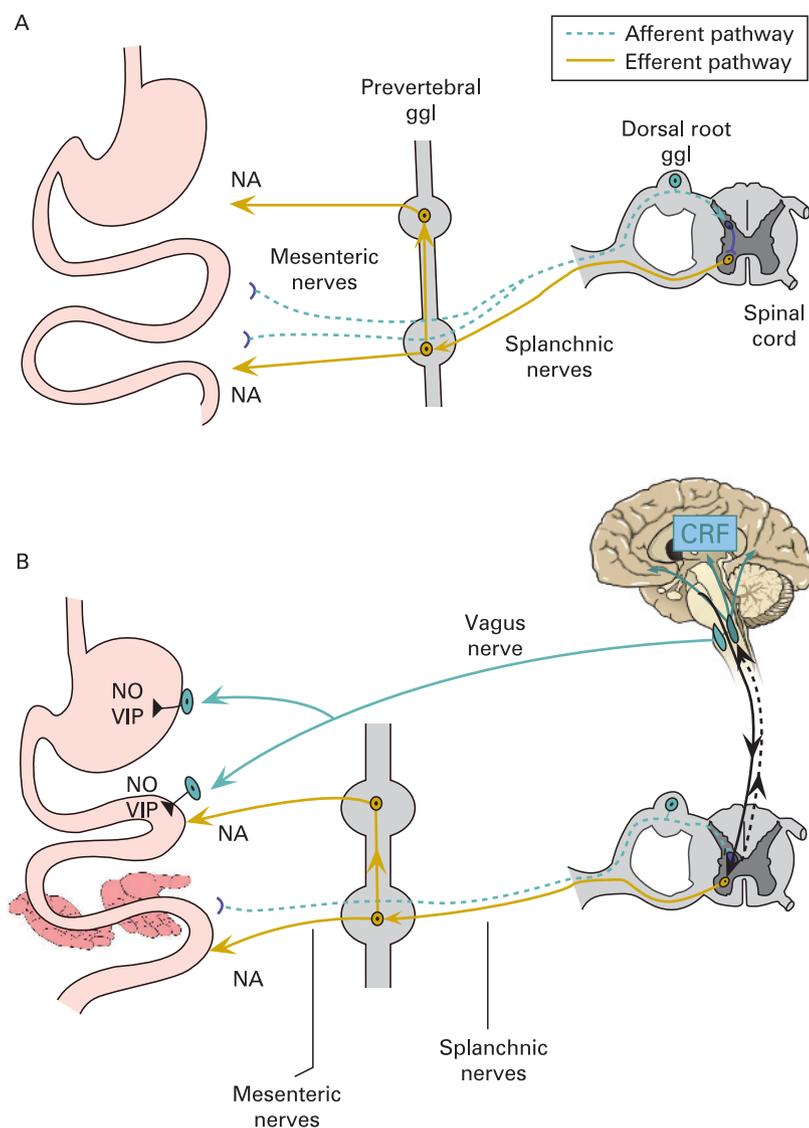
Abdominal surgery triggers two different phases—that is an early neurogenic and a late inflammatory phase—each with its own dynamics and underlying pathophysiological mechanism.

### The early neurogenic phase of POI

Early physiological studies revealed that the intensity and the nature of the nociceptive stimuli applied strongly determine the duration of the period of ileus. Incision of the skin or a simple laparotomy briefly interrupts gastrointestinal motility. This brief inhibition is neurally mediated and is adrenergic in nature since depletion of adrenergic nerves prevents interruption of normal motility.<sup>14–17</sup> Most probably, the activated neural pathway involves a spinal loop with afferent splanchnic nerves synapsing in the spinal cord and efferents travelling back to the gut (fig 2A).<sup>3</sup> In contrast, more intense activation by intestinal



**Figure 1** Schematic representation of the two phases involved in postoperative ileus. The first phase starts during abdominal surgery and ends soon after it. The second inflammatory phase starts approximately 3–4 h after surgery, lasts much longer and is therefore clinically more relevant.



**Figure 2** Schematic representation of the neural pathways involved in the inhibition of gastrointestinal motility induced by laparotomy (A) and more intense nociceptive stimulation during intestinal manipulation. After laparotomy, spinal afferents are activated, synapsing in the spinal cord where they activate an inhibitory pathway involving prevertebral adrenergic neurons, abolishing the motility of the entire gastrointestinal tract (A). During intestinal manipulation, additional pathways are activated mediated by the brainstem. Afferent signals are transmitted to the brainstem where they trigger an increased autonomic output to the neurons of the intermediolateral column of the thoracic cord, where sympathetic preganglionic neurons (releasing noradrenaline (NA)) are located. In addition, the motor nucleus of the vagus nerve is activated, synapsing to inhibitory nitergic (NO) and vipergic (VIP) neurons. CRF, corticotrophin-releasing factor; ggl, ganglion.

manipulation results in prolonged inhibition of motility which can only be partially blocked by adrenergic antagonists. Acute studies focusing on the first 30–90 min after surgery identified the involvement of high-threshold supraspinal pathways activating specific hypothalamic and pontine–medullary nuclei such as the nucleus tractus solitarius and the paraventricular and supraoptic nucleus of the hypothalamus<sup>18–20</sup> (fig 2B). Within this pathway, corticotrophin-releasing factor (CRF) seems to play a central role. It is hypothesised that

CRF release stimulates neurons in the supraoptic nucleus of the hypothalamus, which send projections to the spinal cord, including the intermediolateral column of the thoracic cord, where sympathetic preganglionic neurons are located.<sup>18–21</sup> Activation of these nerves subsequently inhibits motility of the entire gastrointestinal tract. In addition to this adrenergic inhibitory pathway, intense stimulation of splanchnic afferents triggers an inhibitory non-adrenergic, vagally mediated pathway<sup>22</sup> (fig 2B).

Although insight into the neural pathways and neurotransmitters involved in this early short-lasting phase is important, it should be emphasised that activation of nociceptors and/or mechanoreceptors by mechanical stimuli per se during abdominal surgery will cease once the abdomen is closed. Other factors such as mediators released by tissue damage or subsequent inflammation therefore must come into play, explaining the more prolonged nature of POI.

### The late inflammatory phase of POI

In 1978, Bueno *et al* observed two phases of inhibition after abdominal surgery in dogs implanted with intestinal electrodes.<sup>23</sup> The first or “primary” phase consisted of complete inhibition of electrical spiking activity which transiently ceased at the end of surgery. After 3–4 h, a second period of inhibition was observed, its duration being dependent on the nature of surgery. In particular, resection of the small bowel resulted in a long-lasting reduction of spiking activity with recovery of the first myoelectric complex after 94 h.<sup>23</sup> Although the authors demonstrated that the first phase was mediated by an inhibitory neural pathway, the exact origin of the second phase remained unclear.

Almost 20 years later, Kalff *et al*<sup>11–13</sup> demonstrated that this second, long-lasting phase of POI was mainly due to inflammation of the intestinal muscularis (fig 1). It was hypothesised that intestinal manipulation activates resident macrophages present in the intestinal muscularis externa. These normally quiescent macrophages are organised into a layer or “network” at the level of the myenteric plexus and at the serosal side of the intestine.<sup>24–26</sup> Activation of these phagocytes subsequently resulted in cytokine and chemokine release, followed by an influx of leucocytes starting approximately 3–4 h after surgery. Most interestingly, the spontaneous and stimulated contractile activity of muscle strips obtained from the inflamed intestine was significantly impaired, consistent with observations in POI. Moreover, pretreatment of animals with antibodies or antisense oligonucleotides against intercellular adhesion molecule-1 (ICAM-1) not only prevented the influx of leucocytes, but also preserved normal neuromuscular function of muscle strips, providing the proof of concept that inflammation induced by manipulation indeed largely contributes to POI.<sup>13 27 28</sup>

### Mechanisms triggering the local inflammatory response

In general, the innate immune system plays a key role in host defence and in initiating an inflammatory response. The latter is due to recognition of a variety of macromolecules through pattern recognition rather than by reacting to specific antigens. The innate immune system recognises two large classes of macromolecules: first, those related to pathogens or pathogen-associated molecular patterns (PAMPs), and secondly, molecules released in response to cell damage or damage-associated molecular patterns (DAMPs).<sup>29–30</sup> The prototype of PAMPs is lipopolysaccharide (LPS), a constituent of the Gram-negative bacterial cell wall, but many other types exist such as peptidoglycan, bacterial flagellin, or lipoteichoic acid from Gram-positive bacteria. Intracellular molecules such as ATP, uric acid, heat-shock proteins or S100 proteins are examples of DAMPs, also referred to as “alarmins” or danger signals. Both DAMPs and PAMPs are recognised by pattern recognition receptors such as Toll-like receptors (TLRs) and RAGE (receptor for advanced glycation end-products). For example, LPS is recognised by TLR4 and its binding results in activation of the immune cells expressing the TLR4 receptor. DAMPs activate a variety of (intracellular) receptors and lead, for instance, to the activation of a multiprotein complex, the “inflammasome”, a structure required for the synthesis of biologically active interleukin 1 (IL1) and IL18.<sup>31</sup>

In POI, most evidence so far identifies mast cells, most probably peritoneal mast cells, and resident macrophages as the main players of the innate immune system involved in the inflammatory response to intestinal handling. As shown in fig 3, one of the earliest observations in rodent models and even in human is the activation of peritoneal mast cells and the subsequent release of mediators such as histamine and mMCP-1 (murine monocyte chemoattractant protein) in the peritoneal cavity.<sup>32–33</sup> In patients undergoing surgery, even gentle inspection of the intestine at the very beginning of the surgical procedure triggers the release of mast cell mediators. This may be an important step in the inflammatory cascade as it will lead to a

transient increase in intestinal permeability with translocation of intraluminal bacteria and bacterial products (see below). Subsequently, resident intestinal macrophages will be activated, followed by phosphorylation of transcription factors, upregulation of inflammatory genes and secretion of cytokines and chemokines. The latter induce the upregulation of endothelial adhesion molecules and the subsequent influx of leucocytes at a later stage (fig 3). In addition, the release of DAMPs in response to tissue damage evoked by handling can activate the resident macrophages. In the following paragraphs, this inflammatory cascade will be discussed in more detail.

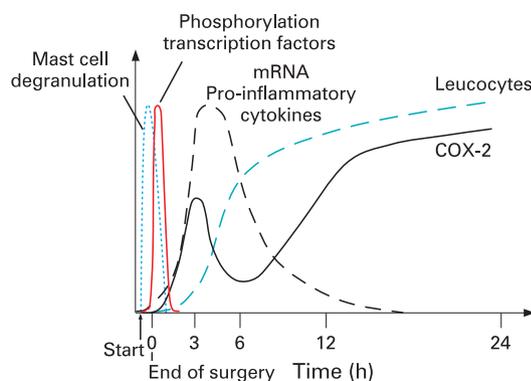
**Mast cells and macrophages are the key players in the pathophysiology of postoperative ileus.**

### Activation of peritoneal mast cells

Within the serosa and mesentery, mast cells are found close to blood vessels before entering the gut wall, often in twos or threes, and particularly closely associated with afferent nerve fibres (<25 µm).<sup>34</sup> Mast cells are not only involved in adaptive immunity, they are also vital for the recruitment of neutrophils and the elimination of bacteria from the peritoneal cavity. Mast cell-deficient mice indeed show a significantly increased mortality and impaired bacterial clearance in a model of acute septic peritonitis.<sup>35</sup> Mast cells therefore should be considered as sentinels of the peritoneal cavity providing protection against potential threats.

The importance of mast cells in the inflammatory cascade triggered by intestinal manipulation was demonstrated in experiments using mast cell stabilisers.<sup>32</sup> Both ketotifen and doxantrazole reduced the inflammatory response and delayed gastric emptying 24 h after abdominal surgery. Conversely, incubation of intestinal loops in solution containing the mast cell activator 48/80 induces an inflammatory response and POI. Finally, W/W<sup>V</sup> mutant mice that lack mast cells fail to develop an intestinal infiltrate following intestinal manipulation. Reconstitution with wild-type mast cells on the other hand restores the capacity of mutant animals to recruit leucocytes to the intestine after surgery.

To date, the exact trigger(s) activating the peritoneal mast cells are still unclear, but neuropeptides such as substance P or calcitonin gene-related peptide (CGRP) released from activated afferent nerves could be involved.<sup>36</sup> Mast cells in the mesentery are indeed in close proximity to afferent nerves.<sup>34</sup> Once activated, vasoactive and proinflammatory substances such as histamine and proteases are released in the peritoneal cavity. Both in rodents and in human, these agents can indeed be detected in the peritoneal fluid immediately after intestinal manipulation.<sup>32–33</sup> Given the anatomical location of mesenteric mast cells—that is,



**Figure 3** Schematic representation of the timing of the inflammatory events triggered by abdominal surgery. COX-2, cyclooxygenase 2.

adjacent to the mesenteric blood vessels where these enter the intestinal wall<sup>34</sup>—mast cell mediators will easily diffuse into the mesenteric blood vessels (fig 4). We hypothesise that this could explain the diffuse increase in mucosal permeability observed after intestinal manipulation.<sup>37,38</sup> When fluorescent LPS and fluorescent microbeads are introduced into the intestine prior to surgery, intestinal handling results in translocation of fluorescent material through the mucosa into the intestinal wall. This period of increased permeability occurs only during a short time window within 3–4 h after manipulation. Once the beads enter the intestinal wall, they are phagocytosed by the resident macrophages or transported to the lymph nodes via the lymphatics<sup>37</sup> (fig 4). As such, mast cell activation could represent a key event that triggers the next stage of the inflammatory cascade—that is, activation of the resident macrophages.

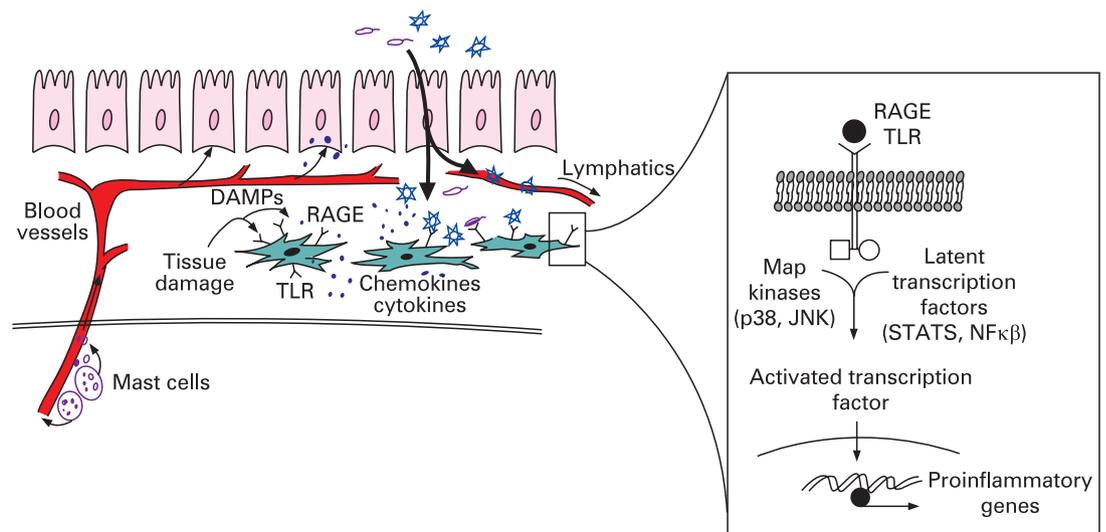
#### Activation of resident macrophages

The intestinal mucosa, submucosa and muscularis externa are densely populated with several subsets of resident phagocytes and antigen-presenting cells (APCs) of haematopoietic origin.<sup>39</sup> Under healthy conditions, such resident macrophages are organised into a layer at the level of the myenteric plexus (between the longitudinal and circular muscle layer) and in the intestinal serosa.<sup>24–26</sup> Most of these cells possess phagocytic properties, express LPS-binding receptor CD14<sup>39</sup> and are activated by LPS.<sup>26,40,41</sup> Moreover, muscularis macrophages stain for macrophage scavenger receptor CD163, which has been shown to possess

bacteria binding and sensing capacities.<sup>42</sup> This phagocyte population in the muscularis externa has an interesting nature and most probably consists of different subsets of APCs, including macrophage-like cells expressing F4/80, and dendritic cell (DC)-like cells expressing most common DC markers such as CD11c and DEC205.<sup>39</sup> However, in mouse bowel wall, major histocompatibility (MHC) II<sup>+</sup> cells outnumber F4/80<sup>+</sup> cells indicating that the majority of these resident muscularis macrophages function as phagocytes rather than APCs.

Hence, the exact cellular constituents of the phagocyte population are yet to be defined, but their importance in the development of intestinal inflammation following intestinal manipulation was first demonstrated by Kalff *et al*<sup>12,15</sup> (fig 4). Surgical manipulation caused an increase in resident phagocytes that stained for the activation marker lymphocyte function-associated antigen-1 (LFA-1). Moreover, pharmacological or genetic depletion (*op/op* mice) of resident macrophages resulted in a decrease of inflammatory mediators and diminished the recruitment of leucocytes into the muscularis.<sup>43</sup> Moreover, macrophage-altered animals had near normal *in vitro* jejunal circular muscle function and gastrointestinal transit despite surgical manipulation, clearly illustrating the importance of these phagocytes in POI.

Several potential mechanisms may trigger the activation of these resident macrophages (fig 4). First, damage of the intestine due to manipulation will release DAMPs,<sup>44</sup> such as ATP, which has been shown to be a potent activator of muscular phagocytes.<sup>45</sup> Secondly, cytokines or LPS entering



**Figure 4** Proposed mechanism involved in the inflammatory response following intestinal handling. Mast cells residing around the mesenteric vessels are activated by intestinal handling and release vasoactive substances diffusing into the blood vessels. These substances increase the mucosal permeability, allowing the entrance of luminal bacteria or bacterial products (lipopolysaccharides) to enter the lymphatics or to interact with the Toll-like receptor (TLR) on the resident macrophages. Another route of resident macrophage activation is by binding of damage-associated molecular patterns (DAMPs) released by damaged tissue. Binding of the TLRs or RAGE (receptor for advanced glycation end-products) activates intracellular signalling pathways (see inset) with transcription of proinflammatory genes in the nucleus. JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-κB; STATS, signal transducers and activators of transcription.

the systemic circulation may activate the network of resident macrophages.<sup>40 46 47</sup> For example, injection of LPS results in nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation and subsequent upregulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) in resident macrophages. Finally, evidence points towards translocation of bacteria or bacterial products such as LPS (see above). Pre-treatment with antibiotics indeed improved the inflammatory response to intestinal handling and, moreover, postoperative small intestinal smooth muscle contractility was significantly less impaired 2 h after surgery in TLR4 knock-out animals.<sup>38</sup>

Phagocytosis of the incoming bacteria and/or activation of TLRs and RAGE by PAMPs and DAMPs stimulates macrophages to secrete proinflammatory cytokines and chemokines. Regulation of this process is largely determined and controlled by intracellular signalling pathways involving the activation of a series of kinases that finally lead to phosphorylation and activation of transcription factors.<sup>48</sup> The latter migrate to the nucleus to start the transcription of proinflammatory genes (fig 4). Activation of the intracellular signalling pathways p38, JNK/SAP (stress-activated protein) and extracellular signal-regulated kinase (ERK)1/2 has been demonstrated within 1 h after intestinal manipulation.<sup>49 50</sup> After translocation of the phosphorylated transcription factors (such as NF- $\kappa$ B) to the nucleus, proinflammatory cytokines (tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), IL1 $\beta$  and IL6) and chemokines (MCP-1 and macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ )) are secreted by the resident macrophages leading to the upregulation of adhesion molecules (ICAM-1) in the endothelium and the progressive influx of leucocytes.<sup>12 13 28 33 38 51</sup> The influx of leucocytes into the muscularis starts approximately 3 h after manipulation, gradually increasing until 24 h post-operatively (fig 5), with monocytes, neutrophils and mast cells as predominantly infiltrating leucocytes.<sup>11</sup> Finally, intestinal manipulation induces the synthesis of enzymes such as iNOS and COX-2 in the resident macrophages,<sup>52-55</sup> a phenomenon that greatly contributes to the impaired gastrointestinal motility which characterises POI.

Inflammation of the intestinal muscularis is the key pathophysiological mechanism underlying the second long-lasting phase of postoperative ileus.

#### How does surgery-induced local intestinal inflammation lead to generalised POI?

##### Local inflammation and impaired neuromuscular function in POI

Impaired neuromuscular function due to inflammation has been extensively demonstrated in a variety of inflammatory models.<sup>56 57</sup> In POI, evidence has been reported that upregulation of iNOS

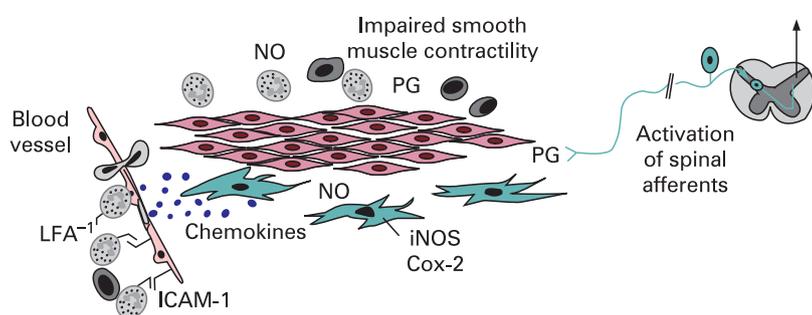
and COX-2 in the resident macrophages and infiltrating leucocytes to a large extent mediates the blunted contractile response of inflamed tissue<sup>52-54 58 59</sup> (fig 5). Blockade of iNOS and COX-2 indeed normalises spontaneous contractility of muscle strips and restores transit 24 h after surgery. Similar findings were obtained in mice genetically deficient in iNOS and COX-2, clearly demonstrating that increased production of NO and prostaglandins (PGs), including PGE<sub>2</sub>, is largely responsible for the compromised neuromuscular function of the manipulated and inflamed intestinal segments.

#### Mechanisms leading to generalised hypomotility in POI

Impaired contractility of the manipulated areas due to local inflammation does not, however, explain the complete clinical picture. POI is mainly a condition of generalised hypomotility, which includes areas that have been left untouched by the surgeon. We therefore proposed that other mechanisms must be involved and anticipated that the inflammatory process triggers neural pathways inhibiting gastrointestinal motility of distant untouched areas. Manipulation of the mouse small intestine indeed delayed gastric emptying even though no gastric inflammation was observed. Neural blockade with the ganglion blockers guanethidine and hexamethonium to prevent the inhibitory input to the stomach normalised gastric emptying, illustrating that the local inflammatory response activates an adrenergic inhibitory pathway impairing motility of distant areas.<sup>27</sup> This was further corroborated by increased c-fos expression (a marker of neural activation) in the spinal cord and brainstem, and increased nerve activity of spinal afferent nerves triggered by the intestinal infiltrate 24 h after surgery.<sup>27 53 55</sup> These findings thus indicate that neural pathways activated by the local infiltrate indeed play a role in the generalised paralysis of the gastrointestinal tract (fig 5). Interference with this mechanism by, for example, epidural blockade with local anaesthetics such as bupivacaine may explain the beneficial effect observed in clinical trials, provided the blockade is positioned at the level of visceral input to the spinal cord—that is, at the thoracic level. Lumbar or low thoracic epidural administration of local anaesthetics on the other hand will be clinically ineffective.<sup>60</sup>

Finally, the Bauer group demonstrated that especially colonic manipulation leads to pan-enteric molecular expression of proinflammatory mediators such as IL6, MCP-1, iNOS and COX-2. This is most probably due to bacterial translocation into the circulation and systemic circulation of cytokines.<sup>37 38 44</sup> As discussed earlier, mast cell mediators diffused throughout the peritoneal cavity may contribute to promote translocation of intestinal bacteria and/or bacterial products along the largest part of the intestine and trigger a more general inflammatory response.

Taken together, it is becoming increasingly clear that manipulation of the intestine during surgery



**Figure 5** Mechanisms underlying the impaired contractility of the intestine following abdominal surgery. Activated resident macrophages release inflammatory cytokines and chemokines. This leads to upregulation of endothelial adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), attracting leucocytes to invade into the intestinal muscularis externa. These leucocytes and the resident macrophages produce large amounts of nitric oxide (NO) and prostaglandins (PGs) (by upregulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (Cox-2)), impairing the contractile activity of the smooth muscle cells. In addition, PGs activate and increase the sensitivity of spinal afferents contributing to the generalised picture of postoperative ileus. LFA-1, lymphocyte function-associated antigen-1 (LFA-1).

triggers a cascade of events orchestrated by the immune system compromising neuromuscular function of the entire gastrointestinal tract by recruiting both neural and inflammatory mechanisms.

In summary, intestinal handling during surgery triggers a cascade of events leading to intestinal inflammation. In addition to local damage of the intestine, mast cell activation triggers a brief period of increased mucosal permeability allowing passage of intraluminal bacteria or bacterial products which will activate resident macrophages in the muscularis externa. The subsequent release of cytokines and chemokines will initiate the recruitment of leucocytes into the intestine, impairing the contractility of the inflamed intestine by increased production of NO and COX-2 metabolites. Moreover, activation of inhibitory adrenergic neural pathways by the infiltrate will impair neuromuscular function of distant areas, explaining the generalised character of POI. Impaired transit at distant areas can also result from activation of the resident macrophages by circulating cytokines and bacterial products or even bacteraemia induced by manipulation of the intestine further contributing to the generalised impairment of gastrointestinal motility.

#### Intestinal inflammation and POI: evidence in humans

Comparably with the rodent models, several lines of evidence support that the pathophysiological mechanisms described above also apply to the human situation. Mast cell mediators are detected in peritoneal lavage fluid very early during surgery. Even very gentle inspection of the intestines at the beginning of the abdominal procedure increased the level of peritoneal trypsinase.<sup>33</sup> In contrast, patients undergoing a laparoscopic or a vaginal hysterectomy hardly showed an increase in trypsinase. Inflammation induced by handling of the intestine is also demonstrated in human tissue. Intestinal tissue removed during surgery shows

activation of resident macrophages and time-dependent induction of IL6, IL1 $\beta$ , TNF $\alpha$ , iNOS, COX-2, ICAM-1 and LFA-1.<sup>33-61</sup> In line with this, increased levels of the cytokines TNF $\alpha$ , IL6, IL8 and IL10 have been documented in the peritoneal fluid and blood of patients undergoing abdominal surgery.<sup>33-62</sup> Next, influx of leucocytes was clearly demonstrated in intestinal tissue removed at the end of the surgical procedure and in tissue obtained from re-operated patients.<sup>33-61</sup> Finally, in vivo recruitment of radiolabelled leucocytes to the intestine was demonstrated in patients undergoing conventional abdominal surgery, but not in patients undergoing a laparoscopic procedure.<sup>33</sup>

As in rodents, inflammation significantly impairs the neuromuscular function of the intestine. Spontaneous contractile activity and the response to bethanechol of muscle strips from patients re-operated on 24 or 48 h after an initial abdominal procedure was significantly impaired, and was restored by incubation with specific pharmacological blockers of iNOS and COX-2.<sup>61</sup> Moreover, we found that delay of clinical recovery was correlated with the influx of radiolabelled leucocytes into the intestine.<sup>33</sup> Although certainly more evidence confirming the role of intestinal inflammation in man is awaited, the data are very suggestive that drugs targeting the inflammatory cascade described here may be effective in reducing POI.

Also in humans, intestinal handling induces influx of leucocytes underlying postoperative ileus.

#### IMPLICATIONS FOR THE TREATMENT OF POI

For an extensive review on the preventive techniques and treatment of POI, the reader is referred to excellent reviews specifically dealing with this issue.<sup>5-60</sup> It is important to stress, however, the fact that for new drugs to enter the clinical arena, they will have to prove their clinical benefit against or in combination with the current new and exciting initiatives in perioperative patient care. In particular the fast track programme, a multimodal approach for patients undergoing colonic surgery, has proven to reduce significantly the rate of perioperative morbidity, hospital stay and costs.<sup>5</sup> In this programme, several perioperative measures—that is, restricted fluid management, optimised analgesia, forced patient mobilisation and early oral feeding—are introduced into patient management with impressive results. Most probably fluid restriction and an effective epidural analgesia are the key factors determining the outcome.<sup>63</sup> To what extent a similar improvement is achieved in other types of surgery and whether the fast track programme can easily be implemented in a general surgical ward remains to be determined. Nevertheless, these studies clearly

illustrate that postsurgical recovery can be significantly improved with relatively simple and cheap measures. The focus of the following paragraphs will be on new therapeutic approaches based on new insights into the pathophysiology of POI.

Accepting that the “inflammatory” or “second prolonged” phase of POI is clinically most relevant, treatment should preferentially aim to prevent or reduce the inflammatory response to intestinal handling. It is important to stress, however, that interference with the immune response may have devastating effects on the first defence against bacterial infection and perhaps even more importantly on wound healing. The latter is of great clinical importance as increased risk of anastomotic leak is the most feared consequence of any immune-modulating therapy. Even if drugs prove to be safe, ideally handling of the intestine should be prevented or minimised, most probably explaining the shortened POI reported after minimally invasive or laparoscopic procedures. Moreover, one would prefer to prevent rather than to treat inflammation, again provided that treatment does not interfere with the healing process or does not lead to an increased risk of infectious complications. Interference early in the inflammatory cascade may also be more effective compared with drugs administered at a later stage when inflammation is well established and a variety of inflammatory mediators are released. Therefore, given the fact that mast cells and macrophages initiate and to a large extent orchestrate the cascade of events, these immune cells seem to be the most interesting targets for treatment.

#### **Mast cells as a target for treatment**

Stabilisation of mast cells has been proven successful in our mouse model of POI.<sup>32</sup> Pretreatment with the mast cell stabilisers ketotifen and doxantrazole significantly reduced the release of mast cell mediators in the peritoneal cavity, impaired the inflammatory response to intestinal handling and prevented POI. Based on these findings, we conducted a dose-finding pilot study on 60 patients undergoing major abdominal surgery for gynaecological malignancy with standardised anaesthesia, randomised to oral treatment with ketotifen (4 or 12 mg) or placebo.<sup>64</sup> Gastric retention 1 h after liquid intake was significantly reduced by the highest dose compared with placebo. Abdominal cramps improved significantly in patients treated with 12 mg of ketotifen, whereas other clinical parameters were unaffected. Although this study is promising, there is much room for improvement. As peritoneal mast cells should preferentially be targeted, peritoneal lavage with mast cell stabilisers may be a more effective approach. These experiments are currently underway.

#### **Resident macrophages as a target for treatment**

In rodents, pharmacological depletion and inactivation of the resident macrophages by chlodronate liposomes reduces the upregulation of inflammatory

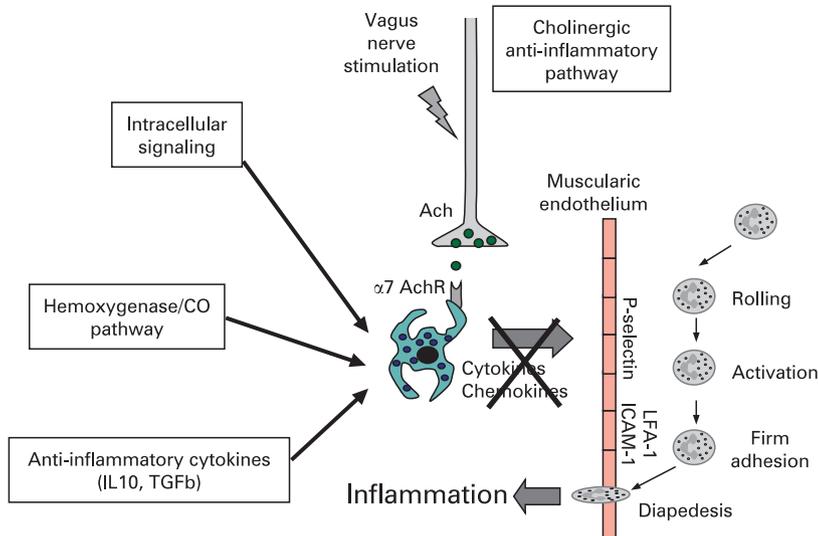
mediators and adhesion molecules, restoring normal transit in the postoperative period.<sup>43</sup> These data underscore that reducing the activity of the resident macrophages may indeed represent an interesting new approach to treat POI. This can be achieved by several different strategies (fig 6).

#### **The haem oxygenase/CO pathway**

The Bauer group has reported abundant evidence that activation of the haem oxygenase-1 (HO-1) pathway or carbon monoxide (CO) potently prevents POI. HO-1 is the rate-limiting enzyme in the degradation of haem, converting haem into iron ions, biliverdin and CO. It is highly inducible under conditions of oxidative stress, as in ischaemia/reperfusion injury and inflammatory conditions, and is one of the cell's first lines of defence against oxidative stress.<sup>65</sup> Inhalation<sup>66 67</sup> or intraperitoneal lavage with CO<sup>68</sup> or intraperitoneal injection of the water-soluble CO-releasing molecule CORM-3<sup>49</sup> are all effective in reducing intestinal inflammation and preventing POI. CORM-3 is most probably active via reduction of oxidative stress induced by intestinal manipulation and further induction of HO-1 in a p38-dependent manner.<sup>49</sup> As CORM-3 did not result in increased levels of carboxyhaemoglobin and can easily be administered prior to or during surgery, this type of drug is very attractive to prevent POI.

#### **The cholinergic anti-inflammatory pathway**

Another interesting approach is to downregulate resident macrophages and thereby prevent the onset of the inflammatory cascade via activation of the cholinergic anti-inflammatory pathway (CAIP) (fig 6). The latter was recently described by Tracey and co-workers and proposed as a powerful endogenous anti-inflammatory system.<sup>69 70</sup> In sepsis models, electrical stimulation of the vagus nerve potently prevented LPS-induced sepsis, reduced splenic TNF production and increased survival.<sup>71 72</sup> This effect is mediated by acetylcholine interacting with  $\alpha 7$  nicotinic acetylcholine receptors (nAChRs) located on macrophages and can be mimicked by specific receptor agonists.<sup>73 74</sup> Recently, we extended the concept of the CAIP to the gastrointestinal tract.<sup>51 75</sup> Vagus nerve stimulation dampened the levels of the macrophage inflammatory mediators TNF, IL6, MIP-2 and MIP-1 $\alpha$  in the peritoneal fluid 3 h after abdominal surgery. In addition, the inflammatory response of the muscularis in the manipulated intestine was reduced and gastric emptying 24 h after surgery was normalised. This beneficial effect of vagus nerve stimulation was mediated by activation of nicotinic receptors on macrophages present in the intestinal muscularis.<sup>51</sup> Most importantly, we showed that these macrophages are in close contact with cholinergic nerve fibres, providing anatomical evidence supporting the concept of cholinergic innervation of the resident macrophages in the intestinal muscularis. Given its anti-inflammatory potency, activation of the CAIP or mimicry of its



**Figure 6** Potential treatment routes to inhibit the activation of resident macrophages (see text). Ach, acetylcholine; AchR, acetylcholine receptor; ICAM-1, intercellular adhesion molecule-1; IL10, interleukin 10; LFA-1, lymphocyte function-associated antigen; TGF $\beta$ , transforming growth factor  $\beta$ .

effect by  $\alpha 7$ nAChR agonists may represent a new approach for the treatment of POI.

Activation of the CAIP resulting in dampening of inflammation can be achieved by activation of the motor neurons of the vagus nerve in the brainstem by intracerebroventricular administration of agents such as acetylcholinesterase inhibitors,<sup>76</sup> muscarinic agonists,<sup>77</sup> ghrelin<sup>78</sup> or the p38 inhibitor CNI-1493.<sup>69–79</sup> Intracerebroventricular injection of CNI-1493 reduced POI and intestinal inflammation in our POI model, an effect abolished by vagotomy, illustrating that the anti-inflammatory effect of CNI-1493 is mediated via the vagus nerve.<sup>80</sup> Interestingly, peripheral administration of these agents results in intracerebral concentrations high enough to activate the CAIP,<sup>79</sup> making these drugs very interesting tools to treat inflammatory conditions. Most probably, although the prokinetic effect of ghrelin will certainly contribute, the beneficial effect of ghrelin in a recent phase II trial for POI could also be explained by this mechanism.

An alternative approach to activate the CAIP is to stimulate the vagus nerve via a more physiological approach, for example via enteral feeding. High fat enteral nutrition was reported to exert an anti-inflammatory effect in a model of haemorrhagic shock mediated via CCK-mediated activation of the vagus nerve.<sup>81</sup> To what extent the beneficial effect of gum chewing<sup>82</sup> on POI is mediated via activation of the vagus nerve is speculative, but certainly deserves further study. Similarly, early introduction of feeding after surgery, an important aspect of fast track surgery,<sup>5</sup> may contribute to the faster clinical recovery of POI after fast track surgery.

Finally, specific agonists of the  $\alpha 7$ nAChR, mimicking the effect of CAIP activation, could be an interesting new class of anti-inflammatory

drugs.<sup>74</sup> Recently, we demonstrated that the specific  $\alpha 7$ nAChR agonist AR-R1779 indeed reduced POI in mice via a peripheral effect.<sup>83</sup> It should be emphasised though that nicotine more potently dampened cytokine production in vitro, as compared with  $\alpha 7$  agonists, suggesting that that other nAChRs or cells may mediate the in vivo effects of AR-R1779.<sup>83</sup> The latter is supported by recent experiments failing to demonstrate  $\alpha 7$ nAChR transcripts in mouse peritoneal macrophages.<sup>84</sup>

Activation or mimicry of the cholinergic anti-inflammatory pathway may be an interesting new approach to treat postoperative ileus.

#### Intracellular signalling pathways

Given the fact that intestinal handling activates macrophages with concomitant activation of mitogen-activated protein kinase (MAPK) signalling p38, ERK1/2 and JNK MAPK<sup>49–50</sup> and NF- $\kappa$ B signalling,<sup>50</sup> interference with these pathways may be an interesting alternative approach to treat POI (fig 6). Semapimod, a p38 MAPK inhibitor indeed reduced POI by dampening the expression of the proinflammatory genes MIP-1 $\alpha$ , IL6, MCP-1 and ICAM-1. Moreover, it inhibited NO production in the muscularis externa by its NOS-inhibiting properties,<sup>50</sup> contributing to a further improvement of gastrointestinal transit. Lastly, we and others showed that semapimod also activates the cholinergic anti-inflammatory pathway,<sup>79–80</sup> providing a third mechanism of action, making this drug a very attractive tool to prevent POI.

Activation of cytosolic transcription factors by phosphorylation is a crucial step in the initiation of the synthesis of proinflammatory mediators. Interference at this level therefore will also dampen the release of a variety of mediators and will theoretically be more efficient than blockade of one cytokine or chemokine. A single bolus injection of tyrphostin AG 126, a protein tyrosine kinase inhibitor, before surgery reduced the transcription of genes encoding the proinflammatory mediators IL1 $\beta$ , MCP-1, iNOS and COX-2, and significantly inhibited the activation of the transcription factor NF- $\kappa$ B.<sup>85</sup> In line with these findings, mice lacking the early growth response gene-1 (Egr-1) had a downregulation in the release of NO, prostanoids, MCP-1, MIP-1 $\alpha$ , IL6, IL1 and granulocyte colony-stimulating factor, as well as a decrease in the recruitment of leucocytes into the manipulated muscle wall of the intestine compared with wild-type mice.<sup>86</sup> To what extent interference with the activation of transcription factors carries a risk of seriously compromising the immune response in general, leading to serious side effects, certainly requires further study. Nevertheless, these data contribute further to the insight that prevention of

the inflammatory response to intestinal handling is indeed an effective strategy to treat POI.

#### Other new treatments for POI

Once the inflammatory cascade is initiated, adhesion molecules such ICAM-1 will be upregulated to attract leucocytes from the circulation. Antibodies or antisense molecules to, for example, ICAM-1 may therefore be an elegant approach to down-regulate leucocyte trafficking and prevent the influx of leucocytes into the intestine. In rodents, this approach indeed improved smooth muscle contractility *in vitro* and normalized gastric emptying and intestinal transit *in vivo*.<sup>13 27 28</sup> Antisense inhibition of ICAM-1 expression has proven safe and well tolerated in several trials performed in patients with inflammatory bowel disease, unfortunately with varying success.<sup>87</sup>

Given the impact of the metabolites of COX-2 on smooth muscle function and their stimulatory effect on intestinal afferents, selective inhibitors administered prior to surgery should be clinically effective. In rodents, the COX-2 inhibitor DFU and the genetic absence of COX-2 indeed prevented POI and diminished the leucocytic infiltrate by 40–50%.<sup>53</sup> In current clinical practice, non-steroidal anti-inflammatory drugs are usually given in the postoperative phase to reduce the use of opioids and thereby contribute to shortening of POI,<sup>5</sup> but based on the current knowledge earlier administration of COX-2 inhibitors (before surgery) may perhaps be even more efficient.

It remains questionable whether prokinetics can overcome the “brake” on intestinal motility exerted by the intestinal infiltrate.

Prokinetics have been advocated as potential therapy for several decades; however, no clinical studies are available providing solid evidence underscoring this.<sup>60</sup> In fact, one can seriously question whether prokinetics can overcome the “brake” exerted by the intestinal infiltrate. Drugs with combined prokinetic and anti-inflammatory properties, such as ghrelin, on the other hand, will theoretically have much greater potency to shorten POI effectively. Most studies with ghrelin or the ghrelin agonist RC-1139<sup>88 89</sup> in rodents, however, have studied the effect of these agents on the acute phase of POI, which is clinically less important. Very recently, another ghrelin agonist TZP-101 was shown to enhance recovery of transit up to 48 h after surgery,<sup>90</sup> but unfortunately no data on inflammation were provided. A phase IIb clinical trial with TZP-101 has recently been completed. Over 200 patients were randomised in a multinational double blind fashion receiving either placebo or TZP-101 within the first hour after surgery for up to 7 days. The median time to first bowel movement was reduced from 89.6 h to 68 h

for the highest dose tested (480 µg/kg) (<http://www.drugs.com>). Although confirmation is certainly awaited, these data look very promising.

Finally, peripherally acting µ-opioid receptor antagonists such as methylnaltrexone<sup>1</sup> and alvimopan<sup>9 10</sup> are extensively studied as treatment of POI, of which alvimopan has been approved in the USA to accelerate the time to upper and lower gastrointestinal recovery after bowel resection. It appears though that these agents only block the detrimental effects of opioids on motility but do not interact with the underlying mechanisms described above.<sup>91</sup> The fact that alvimopan shortened the time to discharge compared with placebo in several phase III trials<sup>10</sup> therefore most probably results from antagonism of the opioid-induced delay in recovery.

#### SUMMARY

POI is an inevitable consequence of abdominal surgery caused by a combination of several factors such as the use of pharmacological agents (anaesthetics, opioids) in the perioperative period, neural mechanisms and intestinal inflammation. From a clinical and therapeutic point of view, the inflammatory response of the intestine following manipulation during surgery is without any doubt the most important pathophysiological mechanism. During the past decade, insight into the underlying mechanisms has grown exponentially and will lead to new pharmacological treatments in the near future. It should be emphasised though that the mechanisms described here mainly if not solely apply to uncomplicated POI following elective abdominal surgery. In prolonged POI due to leakage or other complications, additional mechanisms will be activated, requiring a different therapeutic approach. The same applies for surgical procedures performed under acute emergency conditions on, for example, polytrauma patients or patients entering the operating room in hypovolaemic shock. In these cases, the inflammatory cascade of events has already started and other strategies such as administration of anti-inflammatory cytokines such as IL10<sup>44 92</sup> may perhaps offer better results.

As discussed earlier, drugs interfering with the inflammatory response carry great potency to shorten POI and thus hospitalisation. Future clinical trials will have to prove this concept further and will have to evaluate the effect of administration of these agents before and shortly after surgery on clinical recovery. If the fast track programme, however, proves to be effective for any type of abdominal surgery and can be introduced in any clinical ward, clearly these new drugs will have to prove their superiority against this new approach of perioperative patient care. Nevertheless, the near future will be bright with several potential new drugs and targets in the pipeline.

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National Cancer Institute, in the USA, with a minimum of 12 nodes, the required number. However, while the association between survival and node number is evident, the theory of stage migration is lacking in support.<sup>8</sup> Furthermore, statistical analysis of the actual number of nodes needed to accurately determine nodal status (85% probability) puts the figure closer to 30.<sup>9</sup> This data coupled with large-scale figures from the US showing that less than 50% of institutions adhere to the guidelines brings the radical lymph node dissection theory into doubt<sup>10</sup> Therefore, it would appear that once more we have provided a flawed explanation for a very real finding. The question remains: What is it that is causing increasing survival?

### LYMPH NODES: NUMBER VERSUS FUNCTION

Rather than scrutinise surgical techniques it is possible that the answer lays in the pathologist's laboratory and the processes behind lymph node retrieval. The search for lymph nodes is primarily performed by vision alone. Fat clearing techniques are much vaunted but in reality are rarely used. Accordingly, larger nodes are easier to find whether they are infiltrated or not and patients with bigger nodes will have higher lymph node counts. It is possible that it is the ease by which nodes are found rather than their absolute number that has a bearing on prognosis.<sup>11</sup> This concept is neither new nor controversial yet is consistently overlooked in the consideration of the role of lymphatics in cancer control. It is counter-intuitive to think that cancer causes no immune response and that lymphatics act only as vehicles of malignant spread, yet this is the role to which they are most commonly assigned. Innate immune response to colorectal cancer has been shown to be an independent prognostic indicator,<sup>12</sup> possibly superior to our current tumour/node/metastasis staging system. Genes associated with surveillance and immune response are differentially downregulated in more advanced rectal tumours, indicating prediction of tumour invasion based on genetic profiling of the primary cancer.<sup>13</sup>

Faced with these exciting advances, the search for lymph nodes purely to register their absolute number represents a missed opportunity to gain real insight into prognosis.

### NEOADJUVANT THERAPY AND LYMPH NODES: AN UNHAPPY ALLIANCE

A further note of caution, regarding the use of lymph node number as a measure of quality, must be raised in the era of neo-adjuvant therapies. Combination radiation and chemotherapeutics cause tumour regression in a significant proportion of rectal cancers.<sup>14</sup> This can result in tumour shrinkage allowing for sphincter-saving surgery or, in a small cohort, complete clinical

and histological response which may negate the need for surgery altogether.<sup>15</sup> The anti-tumoural effects are not confined to the rectum, however, and have been shown to cause inversion of regional lymph nodes. Not surprisingly, TME specimens from patients post neo-adjuvant therapy consistently contain fewer nodes.<sup>16</sup> Clearly, the surgical procedure is not the dependent variable; however, the current guidelines do not allow for this ever-growing patient cohort. Indeed we have no data to confirm whether lymph node number post neo-adjuvant therapy still impacts on survival, the premise on which the guidelines are based. While the need for long-term prospective data is implicit the unfortunate drive toward surgeon assessment based on lymph node retrieval rates behoves us to address the issue now. Are we creating an environment where the need to produce consistently high nodal harvests may impact on our operative timing or compromise the use of chemo/radiotherapy? Sphincter preservation has untold positive impact on patient quality of life and it is essential that any questioning of this intervention is performed in an evidence-based manner. Regardless of the uncertain validity of an isolated quality measure in the age of multidisciplinary patient management, the application of guidelines based on an incomparable patient cohort must raise concern.

### CONCLUSION

There is an over-riding drive to find parameters of quality in surgery for rectal cancer. The need for standardised care motivates the search and wide variance in survival data validates it. When faced with such an imperative, solutions are required; however, it may now be the time to hasten slowly. The high standards we seek for our patients must also be applied in the ongoing search for performance benchmarks and quality control.

#### R Kennelly, D C Winter

Institute for Clinical Outcomes Research and Education (ICORE), St Vincent's University Hospital, Dublin, Ireland

**Correspondence to:** Dr R Kennelly, Institute for Clinical Outcomes Research and Education (ICORE), St Vincent's University Hospital, Dublin 4, Ireland; kennellyrory@yahoo.ie

**Competing interests:** None.

#### Study background and organisation

RK is a registrar in general surgery with a special interest in colorectal disease. DCW is a consultant laparoscopic colorectal surgeon in a university teaching hospital. This article originated from a discussion following a multidisciplinary meeting in our hospital. DCW noted that international guidelines were quoted by all interest groups and we wanted to satisfy ourselves that the literature supported this uniform acceptance. Analysis of the literature was performed by RK and both authors contributed to drafting and editing. DCW is guarantor.

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### CORRECTION

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G E Boeckstaens, W J de Jonge. Neuroimmune mechanisms in postoperative ileus. *Gut* 2009;**58**:1300–11. On page 1303, “mMCP-1 (murine monocyte chemoattractant protein)” should read “mMCP-1 (murine mast cell protease 1)”.