New Frontiers in the Pharmacological Prevention of Post-ERCP Pancreatitis: The Cytokines

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Summary

Acute pancreatitis is a major complication of endoscopic retrograde cholangiopancreatography (ERCP), its incidence varying with the indications for the procedure (<5% for the management of common bile duct stones and up to 20% in the case of sphincter of Oddi dysfunction) and also with events occurring during the ERCP such as acinarization and pancreatic sphincterotomy.

If the triggering event of premature intracellular activation of trypsinogen is unknown, the acinar cell injury leads to oxidative stress, nuclear translocation of nuclear factor kappa B and subsequent transcription of chemo- and pro-inflammatory cytokines. These events are followed by chemoattraction and activation of mononuclear cells, T lymphocytes and neutrophils which are responsible for acinar necrosis and amplification of the pro-inflammatory cascade. Finally, after amplification by Kupffer cells, systemic inflammatory response syndrome and multiple organ failure occur. All these events take place within a very short period of time, thus offering a very short therapeutic window during which it is theoretically possible to modulate the severity of human pancreatitis.

The use of immunomodulators in the prevention of human post-ERCP pancreatitis is actually restricted to recombinant interleukin-10. Three of four randomized clinical trials, confirmed by a meta-analysis, have shown that prophylactic injection of recombinant interleukin-10 can significantly reduce the incidence of acute pancreatitis and may decrease the length of the hospital stay. The use of recombinant interleukin-10 in this indication has to be established in a multicenter prospective trial and we need to investigate the safety and efficacy of other immunomodulatory drugs and develop new specific targets.

Cytokines and Acute Pancreatitis

Acute pancreatitis (AP) is a major complication of endoscopic retrograde cholangiopancreatography (ERCP), its incidence varying among indications for the procedure (<5% for the management of common bile duct stones and up to 20% in the case of sphincter of Oddi dysfunction [1, 2, 3]). If the triggering event of premature trypsinogen activation is still unknown in this particular case, all intra-cellular and intra-pancreatic pro-inflammatory cascades of events are the same as for other causes of AP.

Physiopathogeny of Acute Pancreatitis

Regardless of the etiology of acute pancreatitis, the first pancreatic events occur at the level of acinar cells. The primary injury of the acinar cell leads to intra-pancreatic...
activation of trypsinogen and blockade of enzymes secretion. This is very quickly followed by the release of reactive oxygen intermediates (ROI) (within minutes) [4, 5] and oxidative stress responsible for the lesions of cells membranes and cytoskeleton, lipidic peroxidation, intra-cellular depletion of anti-oxidants such as reduced glutathione (GSH) and vitamins E, A, C and the translocation of the nuclear factor kappa B (NF-Kappa B) into the nucleus [6].

Nuclear translocation of NF-Kappa B has been well-demonstrated in several models of experimental AP induced by cerulein [7], taurocholate [8] or bile duct ligation [9] for example. Within the first 15 minutes following AP induction by cerulein injections, phosphorylation of inhibitory kappa B alpha (IKappa Bα) occurs, followed by that of IKappa Bβ, both leading to the detachment of NF-Kappa B units. These events induce the release and the nuclear translocation of NF-Kappa B1, followed by those of RelAp65 and finally NF-Kappa B2 [7, 10]. Within the nucleus, NF-Kappa B units will induce the transcription of several target genes. This results in intra-acinar transcription of chemokines - monocyte chemoattractant protein 1 (MCP-1), MOB-1, interleukin 8 (IL-8), interferon inducible protein 10 (IP-10), etc. [8, 11, 12] - which happens within the first 30 minutes. Transcription of pro-inflammatory cytokines such as IL-1, tumor necrosis factor alpha (TNF-α), IL-6 or those of adhesion molecules (i.e., intercellular adhesion molecule: ICAM-1) [13] occurs within the following hour. These expression and release of chemokines, adhesion molecules and pro-inflammatory cytokines is responsible for the pancreatic invasion by monomacrophages, T lymphocytes and polymorphonuclear neutrophils (PMN), but without any trypsinogen activation. We also know from other experimental studies that translocation of NF-Kappa B alone is not able to induce trypsinogen activation [19]. Therefore, the primary trigger of intra-acinar activation of trypsinogen is still not identified in post-ERCP pancreatitis. Nevertheless, intra-pancreatic transcription of pro-inflammatory cytokines is probably multifactorial in this case: activation of trypsinogen, intraductal hyperpression, oxidative stress, ischemia, etc..

Acinarization and intra-ductal hyperpression may probably increase the ischemia of the pancreatic tissue which occurs during AP. During tissue hypoperfusion, cells become ischemic and their reperfusion leads to oxidative stress, release of ROI, lipids peroxidation, transcription of pro-

**Physiopathology of Post-ERCP Pancreatitis**

Currently, little is known about the physiopathology of post-ERCP pancreatitis. Several factors are mentioned such as chemical, mechanical or microbiological factors which can trigger the premature intra-acinar activation of trypsinogen into trypsin, and which have recently been reviewed by Pezzilli et al. [16]. But at this time, we have still not identified the cause of this activation. As we know from human data, acinarization, due to high volume contrast (or air) injection and hyperpression in the pancreatic duct, is associated with an increased incidence of post-ERCP pancreatitis [16, 17, 18]. In their experimental study, Vaquero et al. [8] demonstrated that saline injection and secondary hyperpression within the rat pancreatic duct leads to nuclear translocation of NF-Kappa B and to the subsequent intra-acinar transcription of IL-6, MCP-1, KC, etc., but without any trypsinogen activation. We also know from other experimental studies that translocation of NF-Kappa B alone is not able to induce trypsinogen activation [19]. Therefore, the primary trigger of intra-acinar activation of trypsinogen is still not identified in post-ERCP pancreatitis. Nevertheless, intra-pancreatic transcription of pro-inflammatory cytokines is probably multifactorial in this case: activation of trypsinogen, intraductal hyperpression, oxidative stress, ischemia, etc.
inflammatory cytokines, and finally chemoattraction of monomacrophages and PMN which, in turn, increase the pro-inflammatory cascade and induce tissue necrosis.

Furthermore, as in every AP (whatever its etiology), nuclear translocation of NF-Kappa B and subsequent chemo- and pro-inflammatory cytokine transcription occur in post-ERCP pancreatitis [20, 21], therefore representing ideal targets for an immunomodulation.

**Experimental Immunomodulation of Acute Pancreatitis**

Immune mechanisms and their modulation have become a major topic of interest in acute pancreatitis; there are many experimental studies which have shown that early modulation of pancreatitis is feasible (Table 1). However, it has also become clear that all these modalities are effective when the drugs are given before or shortly after the appearance of the disease. This observation correlates in clinical practice with the fact that only a narrow therapeutic window exists during which it is still possible to modulate the severity of acute pancreatitis. Therefore, there are two potential clinical settings in which immunomodulation could be attempted: the early modulation of predicted severe cases and the modulation of the unique model of AP that exists in human, namely post-ERCP, acute pancreatitis. Indeed, we know that in high risk cases, we will induce pancreatitis, by endoscopic manipulation, in 5-20% of the cases [22, 23, 24].

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**Table 1. Experimental modulation of acute pancreatitis.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Modulator scavenger</th>
<th>Experimental model</th>
<th>Pancreatic lesions</th>
<th>Systemic complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guice [42]</td>
<td>SOD and catalase</td>
<td>Cerulein/Rat</td>
<td>Decrease</td>
<td>ND</td>
</tr>
<tr>
<td>Wisner [43]</td>
<td>Cerulein/Rat</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Koichiro [44]</td>
<td>Ischemia-reperfusion/Rat</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Schoenberg [45, 46]</td>
<td>Cerulein/Rat</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Rutledge [47]</td>
<td>CDE/Mouse</td>
<td>No effect</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Schoenberg [48]</td>
<td>Taurocholate/Rat</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Lützen [49]</td>
<td>OCT</td>
<td>Cerulein/Mouse</td>
<td>Decrease</td>
<td>ND</td>
</tr>
<tr>
<td>Neuschwander-Tétri [50]</td>
<td>Cerulein/Mouse</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Wisner [51]</td>
<td>Allopurinol</td>
<td>Cerulein/Rat</td>
<td>Decrease</td>
<td>ND</td>
</tr>
<tr>
<td>Niederau [52]</td>
<td>Cerulein/Mouse</td>
<td>Decrease</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Lankisch [53]</td>
<td>Cerulein/Mouse</td>
<td>No effect</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Norman [28]</td>
<td>mAb anti-TNF</td>
<td>CDE/Mouse</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Hughes [29]</td>
<td>mAb anti-TNF</td>
<td>Bile duct infusion/Rat</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Tanaka [31]</td>
<td>IL-1RA</td>
<td>Deoxycholate/Rat</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Norman [30]</td>
<td>Cerulein/Mouse</td>
<td>Decrease</td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td>Gukovsky [7]</td>
<td>PDTC</td>
<td>Cerulein/Rat</td>
<td>Decrease</td>
<td>ND</td>
</tr>
<tr>
<td>Fujimura [32]</td>
<td>Anti-PAF</td>
<td>Cerulein/Rat</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Formela [33]</td>
<td>IL-1 RA</td>
<td>Microvascular ischemia/Rat</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Rau [54]</td>
<td>mAb anti-ICAM</td>
<td>Taurocholate/Rat</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

CDE: cholin deficient ethionin supplemented diet  
ICAM: intercellular adhesion molecule  
IL-1 RA: interleukin-1 receptor antagonist  
mAb: monoclonal antibody  
ND: not determined  
OCT: L-2-oxothiazolidine-4-carboxylate  
PAF: platelet activating factor  
PDTC: pyrrolidine dithiocarbamate  
SOD: superoxide dismutase
Modulation of oxidative stress is a matter of study and different anti-oxidative compounds have been already tested, with varying success only when administered before AP induction. Even if this theory is interesting, it is not the purpose of this review.

Trying to reduce or to block the synthesis and the release of pro-inflammatory cytokines is a unique method for the immunomodulation of AP. Modulation of synthesis, release and bioactivity of pro-inflammatory cytokines can theoretically be performed by:

- general inhibition of cytokine transcription using drugs or cytokines which block or reduce nuclear translocation of NF-Kappa B, and thus subsequent target gene transcription: IL-10, N-acetylcystein (NAC), catalase, corticoids, etc.;
- post-transcriptional specific inhibition: IL-10, IL-11;
- specific inhibition of bioactivity: monoclonal antibodies, receptor antagonists.

Experimentally, in mice and rats, pyrolidine dithiocarbamate (PDTC) [7], recombinant IL-10 [25, 26], recombinant IL-11 [27], monoclonal antibodies and soluble receptors to TNF-α [28, 29] and interleukin-1 receptor antagonist (IL-1 RA) [30, 31], platelet activating factor (PAF) antagonist [32, 33] administration have already been tested and allow the reduction and control of the release of pro-inflammatory cytokines, and the severity of AP lesions and their systemic complications.

Up to now, in clinical practice, only IL-10 has been used for this indication.

**Interleukin 10 and Acute Pancreatitis**

**IL-10**

Briefly, IL-10 is a pleiotrophic cytokine expressed by almost all cells but principally by activated monomacrophages and Th2 CD4+ T lymphocytes [34]. This cytokine discloses major anti-inflammatory properties acting through 1) an inhibition of nuclear translocation of NF-Kappa B and subsequent transcription of pro-inflammatory cytokines such as IL-1, TNF-α or adhesion molecules such as ICAM-1 [35]; 2) the post-transcriptional control of cytokines by enhancing their mRNA instability (i.e. TNF-α) [36]; 3) the induction of natural agonists of

<table>
<thead>
<tr>
<th>Authors</th>
<th>Design</th>
<th>Timing of drug administration</th>
<th>Experimental model</th>
<th>Pancreatic lesions</th>
<th>ARDS</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kusske [25]</td>
<td>rIL-10</td>
<td>At induction then every 8 h</td>
<td>Mice CDE</td>
<td>Decrease</td>
<td>ND</td>
<td>Decrease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After 33 h then every 8 h</td>
<td></td>
<td>Decrease</td>
<td>ND</td>
<td>Decrease</td>
</tr>
<tr>
<td>Rongione [55]</td>
<td>rIL-10</td>
<td>1 h before then every 3 h</td>
<td>Rat Cerulein</td>
<td>Decrease</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 h after then every 3 h</td>
<td></td>
<td>Decrease</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>van Laethem [26]</td>
<td>rIL-10</td>
<td>30 min before, then every 4 h</td>
<td>Mice Cerulein</td>
<td>Decrease</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Osman [56]</td>
<td>IT 9302 (IL-10 agonist)</td>
<td>30 min before</td>
<td>Rabbits Bile injection</td>
<td>Unchanged</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Gloor [57]</td>
<td>IL-10 KO</td>
<td></td>
<td>Mice CDE</td>
<td>Increase</td>
<td>Increase</td>
<td>ND</td>
</tr>
<tr>
<td>van Laethem [58]</td>
<td>Anti IL-10 mAb</td>
<td>2 h before</td>
<td>Mice Cerulein</td>
<td>Increase</td>
<td>Increase</td>
<td>ND</td>
</tr>
</tbody>
</table>

CDE: cholin deficient ethionin supplemented diet
KO: knock out
mAb: monoclonal antibody
ND: not determined
rIL: recombinant interleukin
cytokines (IL-1 or TNF-α [37]); 4) the capability of deactivating macrophages [34] and decreasing macrophage antigen-presenting cell (APC) function through the inhibition of major histocompatibility complex (MCH) class II molecules, B7-1 and ICAM-1 expression [34, 38]; 5) by inducing Th2 phenotype of CD4+ T lymphocytes [39]. It must be noted however that, regardless of its anti-inflammatory properties, IL-10 induces recruitment, growth and differentiation of CD8+ T lymphocytes, natural killer and B lymphocytes. Its also enhances the cytotoxic properties of natural killer cells and CD8+ T lymphocytes [34]. Therefore, its clinical use by systemic administration seems to be better adapted to modulating an acute inflammatory condition.

**IL-10 and Experimental Acute Pancreatitis**

Prophylactic or early therapeutic injection of recombinant interleukin 10 (rIL-10) reduces AP severity and its systemic complications in various experimental models (Table 2). On the contrary, injection of specific antagonists (i.e., IT 9302) or genetic defects of IL-10 (IL-10 knock out (KO) animals) increase the severity of pancreatic and associated systemic lesions (Table 2).

**IL-10 and Post-ERCP Acute Pancreatitis**

Based on previously described anti-inflammatory properties of IL-10 and on experimental animal data, trials have been designed with the aim of reducing the incidence of post-ERCP pancreatitis by IL-10 prophylactic administration. In 2001, our group reported on a single-center, double blind, placebo-controlled trial performed on 144 patients comparing a single injection of recombinant human IL-10 (at 2 different doses: 4 and 20 µg/kg, respectively) given 30 minutes before an ERCP procedure, to a placebo [18]. If primarily designed to evaluate the safety of rIL-10 treatment and its effects on hyper-hydrolasemia, this trial demonstrated that a single injection of rIL-10 given 30 minutes before ERCP was able to decrease the incidence of post-ERCP pancreatitis independently of other risk factors, with an odds ratio (OR) of 0.46. Two other independant risk factors were identified: pancreatic sphincterotomy (OR: 5.04) and acinarization (OR: 8.19) stressing the fact that IL-10 could also be effective in these very high risk cases. Moreover, rIL-10 treatment tended to decrease serum TNF release in patients presenting hyperhydrolasemia, and to decrease the length of the hospital stay. Another important double-blind placebo-controlled study was published in 2001 but was not conclusive [40]. Two hundred patients were included: 101 of them received rIL-10 at a dose of 8 µg/kg and 99 received a placebo i.v. injection 15 minutes before ERCP. If rIL-10 treatment tended to decrease the incidence of pancreatitis and to reduce the length of hospitalization, it did not reach statistical significance, probably because it focused on lower risk patients, including those undergoing diagnostic ERCP.

To date, four randomized clinical trials have been performed and were the subject of a meta-analysis [Singh et al., DDW2002, abstract T1726] concluding that IL-10 is effective in the prevention of post-ERCP acute pancreatitis. Pooling all patients, 294 patients received IL-10 before ERCP and 259 patients received a placebo. The incidence of post-ERCP pancreatitis was 7.1% in the IL-10 groups, and 13.9% in the placebo groups. Statistical analysis concluded that IL-10 significantly reduces the risk of AP (relative risk: 0.46, P=0.003; absolute risk reduction: 6.8%).

Even if the use of rIL-10 in the form of a prophylactic intravenous injection seems to be effective in preventing post-ERCP pancreatitis, this cytokine has advantages and disadvantages, and there are still questions about the standards of its administration. rIL-10 is easy to use and only needs one i.v. bolus injection before ERCP. Moreover, it remains active for 24 hours [41] and can be administered to patients treated on an ambulatory basis. Unfortunately, the cost is still unknown and could be a limiting factor for general administration. It is also necessary
to define the lowest effective dose. Moreover, if we can select patients at higher risk of AP (acinarization, sphincterotomy), we will be able to restrict rIL-10 administration to these cases only. As a parallel with animal studies (Table 2), effectiveness of a delayed injection (during or immediately after the ERCP procedure) might be preserved, but this needs to be confirmed by future studies. A multicenter study is now ongoing in the US and Europe.

**Conclusions: Future of Immunomodulation**

Acute pancreatitis is a frequent and major complication of ERCP. Its incidence varies greatly according to the indications and also with events occurring during the ERCP such as acinarization, pancreatic sphincterotomy and the need for precutting. If the use of rIL-10 in the prevention of post-ERCP pancreatitis is relatively well-established, it needs to be refined and definitively proven and, to this end, there is currently a large ongoing multicenter study. The use of other immunomodulatory drugs also deserves attention. Three specific targets should be investigated: decreasing or blocking the nuclear translocation of NF-Kappa B, development and use of other anti-inflammatory cytokines and specific neutralization of pro-inflammatory cytokines. In experimental animal models, all these targets have been studied. Even if such animal models are very important and helpful, clinical application is generally limited due to the toxicity of some compounds or to their secondary effects. Therefore, we need to test the efficacy and safety of existing molecules, but also to develop and discover new specific targets and drugs which are safe for human administration.

**Keywords** Acute Disease; Cytokines; Interleukin-10; NF-kappa B; Pancreatitis

**Abbreviations** AP: acute pancreatitis; APC: antigen-presenting cell; ARDS: acute respiratory distress syndrome; CDE: cholin deficient ethionin supplemented diet; ERCP: endoscopic retrograde cholangiopancreatography; GSH: reduced glutathione; ICAM: intercellular adhesion molecule; IKappa B: inhibitory kappa B; IL: interleukin; IL-1 RA: interleukin-1 receptor antagonist; IP-10: interferon inducible protein 10; KO: knock out; mAb: monoclonal antibody; MCH: major histocompatibility complex; MCP: monocyte chemoattractant protein; NAC: N-acetylcystein; ND: not determined; NF-Kappa B: nuclear factor kappa B; OCT: L-2-oxothiazolidine-4-carboxylate; OR: odds ratio; PAF: platelet activating factor; PDTC: pyrrolidine dithiocarbamate; PMN: polymorphonuclear neutrophils; rIL: recombinant interleukin; ROI: reactive oxygen intermediates; SIRS: systemic inflammatory response syndrome; SOD: superoxide dismutase; TNF: tumor necrosis factor

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