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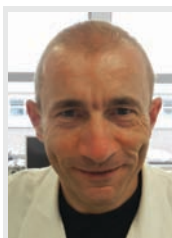
Clinical development of lactocepin: a novel bacterial biologic?

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“This totally unanticipated, potent anti-inflammatory effect of *prtP*-encoded lactocepin suggests a therapeutic potential in chemokine-mediated inflammatory disease such as inflammatory bowel disease.”

Inflammatory bowel disease (IBD) is a group of chronic intestinal inflammatory conditions, with the major types being Crohn's disease and ulcerative colitis (UC). Both are complicated diseases that mainly affect young adults. Complexity lies both in the unclear, multifactorial origin and the implications thereof on treatment. Better understanding of the mechanism of action has led to more rational approaches for medical intervention. Molecular immunology and genetic analysis have tailored the current array of IBD therapeutics. The expansion on the concept of IBD being an immune disease, which stems from the observation of massive lymphocyte infiltration and therapeutic effect of corticosteroids, has been the major lead for drug design. Since the description of *Nod2* [1], a large array of genetic variants, many of which map within immune pathways, have been associated with disease development. Genetic understanding has strengthened our immunologic insight into IBD and along the way led to better clinical target selection. The clinical development of novel anti-inflammatory therapeutics, small molecules, neutralizing monoclonal antibodies and cytokines has become a bastion of modern biopharmaceutical industry.

Since not all carriers of risk genotypes develop IBD, it is obvious that genetics is not the only factor determining the development of IBD. Environmental factors such as food composition and pathogen invasion have long been suspected to drive at least parts of IBD onset and

maintenance. The seminal finding that intestinal bacteria are recognized by mucosal antibodies has, however, greatly shaped the state of mind in this field [2]. Although clear-cut evidence remains scarce, most experts now agree that antigens derived from the – omnipresent and personally unique – intestinal microbiota drive IBD development. Multinational consortia such as the human microbiome project [3] and MetaHit [4] were established to portray the human microbiome composition. Adequate methodology and a broadly available species atlas enables crystallization of the relevant differences between microbiota composition of the healthy and the diseased [5]. Using this knowledge to treat IBD – excluding the malign or (re)introducing benign microflora – is obviously a tempting idea. There are many questions that arise regarding potential uses of this knowledge. Will it be possible and useful to re-tailor IBD-associated microbiota towards a 'healthy' state? [6] Will this result in a long-lasting cure of IBD? Will microbiota composition sway back or will once 'healthy' species now become the driver of the immune reaction? Core to these considerations is the as-of-now unanswered question of whether the stratification of species causes IBD development (i.e., a combination of species that trigger the immune system) or a result of selection by the diseased immune system. How can the above principles be translated into broadly applicable clinical therapy? Development of any drug requires a clear description in terms of

identity, purity, potency and stability. A mechanism of action and good laboratory practice toxicology are essential elements of such description. It is difficult to imagine re-introduction of healthy microflora according good clinical practice experimentation, as required for drug development.

The use of selected type strains – probiotics – represents a more readily applicable approach to design microbial therapeutics for IBD. However, only relatively modest successes have been reported (recently reviewed [7]). This may relate to difficulties in current good manufacturing practice production (identity, purity) but also unclear mechanisms of action (potency). Molecular research can point out the active components in probiotic microflora; the identification thereof consequently unveils useful tools that altogether better fit the paradigms of drug development. Indeed, bacterial proteins are successfully being developed as intravenous drugs. As examples, streptococcal streptokinase, the thrombolytic tissue-type plasminogen activator, is currently a marketed product, and for *Staphylococcus aureus*-derived staphylokinase (THR-100, ThromboGenics, Belgium), Phase III clinical trials have been conducted. Thus, selected active components from (beneficial) microbes may well represent the next – non-self – class of ‘bacterial’ biologicals to combat IBD. Examples of potential candidates include anti-inflammatory proteins from *Yersinia enterocolitica* [8] and *Faecalibacterium prausnitzii* [9]. In fact, in a recent issue of *Cell Host and Microbe*, von Schillde and co-workers [10] have described the anti-inflammatory activity of lactocepin, a protease produced by the *Lactobacillus paracasei* (L.p) constituent of the probiotic mixture VSL#3 [11].

Lactocepins comprise a broad group of cell envelope-associated *prtP*, *prtB* and/or *prtH* encoded proteinases. PrtP are mainly expressed by lactococci and lactobacilli. They are best known for their caseinolytic properties, which are important during cheese making. In addition to its role in milk casein degradation, PrtP was reported to mediate lactococcal adhesion to different types of solid surfaces and solvents [12]. Also, PrtP of *Lactococcus lactis* was shown to degrade autolysin AcmA, which is required for cell separation and autolysis during the stationary phase of growth [13]. Recently, it was demonstrated that VSL#3-derived L.p expresses a *prtP*-encoded lactocepin that both *in vitro* and *in vivo* selectively degrades pro-inflammatory chemokines, notably interferon gamma-induced protein 10 (IP-10). Treatment of inflamed TNF^{AARE/+} mice with intraperitoneal injections of VSL#3-derived L.p-conditioned supernatants resulted in selective loss of IP-10 protein in the ileal tissue, correlating with reduced monocyte infiltration. Feeding with VSL#3, in contrast, had no effect on the development of ileal inflammation in the same model of ileitis [14]. *Lactobacillus casei* (L.c) BFLM 218, a fecal isolate from human volunteers, was tested positive for IP-10 degradation. A *prtP* disruption mutant of this strain (L.c prtP^{dis}) failed to degrade IP-10, proving that PrtP is the active component. This hypothesis was also confirmed *in vivo* as ingestion of L.c, compared with L.c prtP^{dis}, reduced cecal inflammation, cecal T-cell infiltration and IP-10 tissue levels in a T-cell transfer model. This totally unanticipated, potent anti-inflammatory effect of *prtP*-encoded

lactocepin suggests a therapeutic potential in chemokine-mediated inflammatory disease such as IBD.

The clinical development of lactocepin or lactocepin-expressing isolates holds specific challenges. Although other lactobacilli are known to encode *prtP*, they did not exert IP-10 degrading activity despite high (> 98%) sequence homology. Sequence analysis revealed that L.c BL23 encodes a *prtP* that is identical to L.c BFLM 218 *prtP* and is 99% homologous to L.p *prtP*. Also, *prtM* (involved in PrtP maturation) was identical for both L.c strains. L.c BL23 does express PrtP in milk, but apparently not in conditioned media, so environmentally driven differences in expression of lactocepin are expected. Therefore, development of PrtP-producing bacteria as oral treatment may not be straightforward.

Conventional protein purification and formulation as injections for intestinal delivery can obviously be tried, but von Schillde and co-workers observed positive effects of bolus injection of conditioned supernatants in the cecum only [10]. We have earlier laid the groundwork for exploiting a completely innocuous bacterium, *Lactococcus lactis*, for the topical and active (TopAct™) delivery of therapeutic proteins. *L. lactis* is a non-colonizing dairy bacterium that has been used in the food industry over centuries. A meticulous development lies between its initial report [15] and early use in a physical contained clinical setting [16] to arrive at a technology that has now been shown to be amenable for good clinical practice experimentation. Multicenter, multinational clinical trials have been conducted with well-described, reliable and stable products derived from good manufacturing practice. This now makes TopAct a technology that easily allows the insertion of any therapeutic protein. The fact that wild-type dairy *L. lactis* produces prtP probably makes TopAct ideal to deliver lactocepin. The paper by von Schillde and co-workers [10] disclosed the value of lactocepin. The use of a suited delivery vehicle may possibly overcome the lack of effect in the colon.

Questioning the potential to develop a novel therapeutic implies benchmarking against conventional medication. Will the novel therapy be capable of penetrating in between the existing, in a competitive or complementary way? Again, IBD is a complex multifactorial disease that holds both acute as well as chronic phases, periods of remission and unexpected relapses. Despite the fact that many successes have been booked, there still exists an unmet medical need. The appropriate response to such heterogeneity may well be found in a more diverse repertoire of tools. Current IBD treatment uses corticosteroids for remission induction. 5-Aminosalicylic acid is used for the induction of remission in mild to moderate UC and remission maintenance in UC and possibly Crohn's disease. The most powerful biological pharmaceuticals are selective monoclonal antibodies that target the ongoing immune cascade. Most prominent are TNF-neutralizing monoclonal antibodies, such as Remicade, Humira and Cimzia [17], which are as yet unsurpassed in their effectiveness for remission induction and mucosal healing. However, they are not indicated in asymptomatic patients, anti-idiotypic antibodies will arise,

care must be taken to monitor and avoid adverse events and cost of treatment may be prohibitive. The very nature of the activity of lactocepin makes it suited to prevent early, acute inflammation, characterized by the infiltration of many types of lymphocytes. Provided its delivery is adequately addressed, a suitably inexpensive formulation of lactocepin certainly merits further evaluation against the standard-of-care, probably as an agent for remission maintenance.

Will it be possible to develop a lactocepin-based clinical approach for IBD? It is important to note that the available current medication gives a broad answer to treat IBD. Still, subpopulations of patients remain unresponsive and loss of response to (biologic) therapy is a common problem in clinical practice. Differences in inflammatory mechanisms, development of neutralizing antibodies and pharmacokinetic alterations in the patient are known to form the basis of (acquired) unresponsiveness to

therapy. So indeed, ample opportunity exists for novel therapeutics to penetrate the market.

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