ASCA: genetic marker, predictor of disease, or marker of a response to an environmental antigen?

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Anti-Saccharomyces cerevisiae antibodies (ASCA) may be a marker of an immune response to an environmental antigen that occurs in the context of early stage Crohn’s disease.
Osteopontin

Osteopontin: a new addition to the constellation of cytokines which drive T helper cell type 1 responses in Crohn’s disease

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Osteopontin, a cytokine which promotes Th1 immune responses, is overexpressed in the gut of patients with Crohn’s disease or ulcerative colitis. The main cellular source of this cytokine appears to be gut plasma cells.

Crohn’s disease appears to be caused by an excessive CD4+ T helper cell type 1 response directed against undefined antigens of the commensal bacterial flora.1 T cells from affected areas of Crohn’s disease mucosa produce enhanced amounts of interferon γ (IFN-γ) and tumour necrosis factor α. Other markers of Th1 cells, such as expression of the transcription factor T-bet, the high affinity β2 chain of the interleukin 12 (IL-12) receptor, and activated STAT4, all indicate that the mucosal environment in Crohn’s disease favours Th1 polarisation.1

It is important to emphasise that CD4 T cells in normal bowel are also Th1 skewed and express T-bet, so that the differences seen in Crohn’s disease are quantitative rather than qualitative.1 Normal mucosa T cells are however susceptible to apoptosis, whereas this is not the case in Crohn’s disease, suggesting that it is the persistence and accumulation of Th1 cells which drives tissue injury.

Factors which commit virgin T cells to the Th1 or Th2 pathway are still under investigation. T-bet appears to be of primary importance.2 It is induced by IFN-γ itself and is capable of promoting IFN-γ production, not only in Th1 cells, but also in Th2 cells. This results in increased expression of the IL-12Rβ2 chain. Macrophage and dendritic cell derived IL-12 is crucial in Th1 immune responses.3 The IL-12 receptor is made of two chains, β1 and β2, but only the β2 chain can signal and phosphorylate STAT4 which then migrates to the nucleus and further boosts IFN-γ production. Other factors also augment Th1 polarisation. IL-18 activates the transcription factors AP-1 and nuclear factor κB in T cells, and acts synergistically with IL-12 to boost IFN production. Type 1 interferons can also activate STAT4. IL-7, IL-15, and IL-21 also act in synergy with IL-12 to boost IFN-γ production and all, including IL-18, are overexpressed in Crohn’s disease.4 The IL-12 p40 chain can form a heterodimer with p19 protein to form a recently described cytokine, IL-23, which appears to be important in the activation of memory Th1 cells.5 There are no publications on IL-23 in Crohn’s disease.

In this issue of Gut, Sato and colleagues6 identify osteopontin (also known as early T lymphocyte activation gene 1 (Eta-1) as yet another cytokine involved in the Th1 response of Crohn’s disease (see page 1254). Osteopontin is a 60 kDa phosphoprotein constitutively secreted by epithelial cells and bone.6 It contains the characteristic RGD sequence seen in extracellular matrix proteins and shares receptor binding on cells with
extracellular matrix proteins, including αv and β1 integrins. It also binds to CD44. Osteopontin is expressed in many distinct forms depending on differential splicing and the glycosylation/phosphorylation status of the core protein. Proteolytic cleavage by thrombin and matrix metalloproteinases also changes its function. Osteopontin is important in many different tissues. In the bone it regulates calcium deposition and it is also a chemoattractant for cancer cells. However, it is also important in the immune system as it is made by activated T cells, macrophages, and dendritic cells. These cells also have osteopontin receptors.9

Osteopontin increases the adhesion of activated T cells and is a T cell chemoattractant, but importantly it supports Th1 responses and inhibits Th2 responses9; thus its identification in Crohn’s disease adds another member to the increasing number of cytokines which drive Th1 immune responses (fig 1). Osteopontin deficient mice show impaired Th1 immune responses and fail to make granulomas.9 There was therefore a strong rationale for looking at osteopontin in Crohn’s disease. Sato and colleagues’ now show that osteopontin transcripts and protein are elevated in Crohn’s disease and ulcerative colitis. Osteopontin also increases IL-12 production specifically in Crohn’s disease lamina propria mononuclear cells but has little effect on IL-10 production. Arguably, the most important finding of the paper is the source of the osteopontin. There was a predictable increase in immunoreactivity in epithelial cells, macrophages, and around granulomas in Crohn’s disease. However, the most striking feature in inflammatory bowel disease (IBD) was coexpression of osteopontin with mucosal IgM, IgG, and IgA plasma cells. Especially in Crohn’s disease, osteopontin was particularly associated with IgA plasma cells. This result has some similarities with a previous study on osteopontin in Crohn’s disease and normal ileum where production in plasma cells was also noted, although overall no differences were seen between controls and Crohn’s samples.10 However, gene array analysis has shown markedly increased osteopontin transcripts in ulcerative colitis colon compared with normal colon.11 The role of plasma cells in IBD has been somewhat overlooked in recent years, with most emphasis on cellular immunity. However, quantitatively, plasma cells are as abundant as T cells in normal and inflamed gut. By far the largest population of plasma cells in normal and inflamed gut secrete IgA, generally considered to be a beneficial non-phlogistic antibody. In IBD, the largest quantitative increase is in IgA plasma cells, but the biggest proportional increase is in IgG plasma cells as these are uncommon in the normal gut. In Crohn’s disease, IgG plasma cells tend to be found around ulcers whereas in ulcerative colitis they are present along the length of the diseased mucosa.12 It has been suggested that some of this IgG has specificity for gut autoantigens, such as epithelial tropomyosin, indicating that at least part of the pathogenesis of ulcerative colitis may involve antibody mediated autoimmunity.12 The results of Sato et al suggest that IgA and IgG plasma cells may be far more important than previously considered, with a role greater than simply being antibody secreting factories.

The work of Sato and colleagues’ also poses some interesting questions. Is osteopontin always made by plasma cells and is its presence in the gut merely a feature of the fact that the gut contains more plasma cells than the rest of the body combined? Do plasma cells secrete bioactive osteopontin? If osteopontin is present in plasma cells in healthy gut, why does it not deliver a survival signal to CD4 cells? Is osteopontin involved in the migration of T cells into normal and inflamed gut? It would also be of interest to examine trinitrobenzene sulphonate acid colitis in osteopontin deficient mice.

The importance of the therapeutic benefit of neutralising Th1 inducing cytokines in Crohn’s disease is well demonstrated by the clinical success of anti-IL-12 antibodies.13 However, the antibody used in this study is against the p40 subunit of IL-12 and theoretically can also neutralise IL-23. However, there may be considerable heterogeneity between patients in the relative importance of other Th1 inducing cytokines, such as IL-18 and osteopontin, which deserve further investigation.
Silencing RNA: a novel treatment for pancreatic cancer?

N R Lemoine

The high sequence specificity of RNA interference may make it suitable to treat diseases that are linked to selective or elevated expression of particular identified genes, such as in pancreatic cancer

The antiapoptotic gene Bcl-2 has been a target for downregulation by nucleic acid based strategies for more than a decade but the recent failure of the synthetic antisense oligonucleotide agent Genasense in phase III clinical trial caused many to think again about the worth of this approach. However, a new optimism about this and other targets is spreading through the community following several encouraging applications of the recently discovered technology of RNA interference, which is essentially a new biological version of the antisense system.

RNA interference is considered to have begun as an evolutionarily ancient mechanism for protecting organisms from viruses. Many viruses have RNA, rather than DNA, as their genetic material and go through at least one stage in their life cycle in which they make double stranded RNA. Perhaps not surprisingly, all multicellular organisms have evolved a well conserved protein apparatus that destroys double stranded RNA but this has also been found to play a role in maintenance of the organism’s own genome stability by suppressing the movement of mobile genetic elements, such as transposons and repetitive sequences.

The gene silencing process of RNA interference (RNAi) involves the manufacture of short double stranded RNA molecules by an enzyme called DICER, which cleaves RNA duplexes into 21–26 base pair oligomers. These small interfering RNAs (siRNA) cause sequence specific, post-transcriptional gene silencing by guiding an endonuclease, the RNAi induced silencing complex (RISC), to mRNA. This process has been seen in a wide range of organisms such as Neurospora fangius (in which it is known as quelling), plants (post-transcriptional gene silencing), and mammalian cells (RNAi). Downregulation of target gene expression has been found to involve interactions at multiple levels. Where there is complete or near complete sequence complementarity between the small RNA and the target, the Argonaute 2 component of RISC mediates cleavage of the target transcript.

In contrast, where there is sequence mismatch between the miRNA and the target transcript, the mechanism appears to involve repression of translation predominantly. More recently, it has been recognised that siRNA molecules can induce transcriptional silencing through promoter methylation.

In principle, the high sequence specificity of RNA interference might make it suitable to treat disease that is linked to selective or elevated expression of particular identified genes. This may make it particularly appropriate for combating cancers associated with mutated endogenous gene sequences. An early example of the potential power of this approach came in a study of pancreatic cancer. RAS genes are frequently mutated in human cancers, particularly in pancreatic and colon carcinomas. Mutant RAS oncogenes often contain point mutations that alter only a single amino acid, which locks the oncogenic RAS proteins in a persistently activated GTP bound state. A complication in using RAS oncogenes as targets in anticancer therapy is that at present it is not possible specifically to inhibit the biochemical function of only the oncogenic RAS alleles. This may be essential as the wild-type R-KAS gene appears to be required for viability, as evidenced by the embryonic lethal phenotype of mice nullizygous for K-ras.

Retroviral delivery of siRNAs can specifically inhibit the mutant K-RASV12 allele in human pancreatic carcinoma cells, while leaving the wild-type K-RAS allele untouched.

In spite of the fact that pancreatic carcinoma cells have many genetic alterations, loss of K-RASV12 expression leads to loss of tumorigenicity in experimental animal models.

In this issue of Gut, Ocker and colleagues explore the use of siRNAs against another gene that is aberrantly expressed at high frequency in pancreatic cancer, the antiapoptotic gene bcl-2 (see page 1298). Their results suggest that the target can be selectively downregulated in tumour cells in vitro and that intraperitoneal administration of the naked nucleic acid agent can produce variable antitumour effects against malignant deposits growing subcutaneously in vivo. In this study—as in most experiments using RNAi to target particular genes in mammalian cells—the results are interpreted as indicating induction of sequence specific transcript cleavage. However, at this early stage in our understanding of RNAi, it is important not to rule out the possibility that interference mediated through protein translational repression or genomic modification (DNA methylation or histone modification) may also be playing a role in mediating gene specific silencing and any derived RNAi phenotype.

The great attraction of therapeutic epi-genetic gene specific silencing lies in its inheritable nature, meaning that, unlike post-transcriptional gene silencing that requires the continued presence of an siRNA molecule targeting a coding sequence, long lasting suppression of gene expression could be achieved from a single exposure to a specific methylation inducing RNAi agent targeting a promoter sequence.

The major challenge in turning RNA interference into an effective therapeutic strategy is the delivery of RNA
interference agents, whether they are synthetic, short double stranded RNAs (as in the paper by Ocker and colleagues) or viral vectors directing production of double stranded RNA, to the target cells within the body. While siRNA technology has proven extremely powerful and robust for cell culture work, translating this success reliably to animals or humans is proving very difficult, due to insufficient bioavailability of the compounds. However, an important step in the right direction is that Jürgen Soutschek and colleagues have recently been able to demonstrate siRNA mediated downregulation of apolipoprotein B in the liver (and jejunum) of mice using cholesterol conjugates delivered systemically. The effects were preferentially seen in the liver, which is a relatively easy organ to target, and relatively high dosages (three injections each of 50 mg/kg) were required for the effect. In view of the extremely high potency of the siRNA in vitro cell cultures, one must conclude that only a very small fraction of the injected siRNA actually reaches its molecular mRNA target in liver cells. Thus it is unfortunately not likely that simple cholesterol conjugation will solve the general delivery problem of siRNA. Other cationic cell penetrating peptides such as penetratin, Tat, and more recently transportan and oligo arginine have been proposed as general transmembrane carriers for a variety of cargoes, including oligonucleotides and PNA. However, it appears that the main uptake route for most, if not all, of these peptides is endosomal, and thus the reagents have to escape the endosomal compartment in order to enter the cellular compartments of action: the cytoplasm and/or nucleus. The problem of cellular delivery is yet more complex for clinical application where the real challenge for an adjuvant therapy agent is delivery and maintenance of the compound in cancer cells in multiple organs in humans. However, the biotechnology industry has readily recognised the potential silencing properties of RNA mediated interference, with at least 15 companies active in the field, and two companies (Ribopharma and Benitec) have patents for RNAi based clinical applications. Significant progress in delivery technology is required before the concept of RNAi can realistically benefit cancer patients, but exploitation of the decade of clinical experience of antisense and viral gene therapy agents gives investigators a head start.


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Conflict of interest: None declared.

REFERENCES