The early stages of rheumatoid arthritis: New targets for the development of combinational drug therapies

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Abstract

Introduction

The current methods of treating rheumatoid arthritis (RA) do not address the early phases of the disease progression and instead merely treat the symptoms. Whilst this approach may help to alleviate much of the pain and discomfort associated with RA, it does not stop the disease progression and the destruction of joint tissue still occurs. Furthermore, a number of serious side effects are associated with the current treatment modalities. A better strategy may be to eradicate the cause of the disease, thereby bypassing the later phase events. Until recently, this has not been possible due to a lack of understanding of the etiology of RA.

However, recent advances have identified Proteus mirabilis as a trigger of the disease and have provided some understanding of the disease progression. An understanding of the early phases of the disease has identified a variety of new targets for anti-RA drug development which may allow for the development of new treatment regimes with increased efficacy and/or less serious side effects.

Conclusion

The current report examines the P. mirabilis bacterial trigger of RA and how it results in self-reactive antibody production. In so doing, the report highlights some targets for drug treatments aimed at blocking the early phases of RA before the more serious later phase events occur. Whilst it is unlikely that any single drug will be able to treat all aspects of this disease, resveratrol and other stilbenes are highlighted as a class of compounds which may have beneficial effects on several phases of RA disease progression.

Introduction

There is currently no cure for rheumatoid arthritis (RA). Instead, the current treatment modalities for the control and treatment of the disease are generally targeted at alleviating the symptoms (particularly inflammation, pain and swelling) via the use of anti-inflammatory drugs and analgesics. Non-steroidal anti-inflammatory drugs (NSAIDs) are most frequently used for this purpose. However, NSAIDs merely target the symptoms of RA and do not affect the course of the disease. Thus, whilst NSAID use may alleviate suffering, the RA associated joint/tissue degradation still occurs. Furthermore, NSAIDs should be used cautiously for individuals with gastrointestinal, cardiovascular or kidney problems\textsuperscript{1}. For example, cyclooxygenase-2 (COX-2) inhibitors are a class of NSAIDs that directly target the COX-2 enzyme which is responsible for inflammation and pain. However, treatment with COX-2 inhibitors is often associated with an increase in myocardial infarction\textsuperscript{2}.

Other treatment modalities aim to halt or modify RA disease progression through the usage of disease modifying anti-rheumatic drugs (DMARDs). These are a diverse grouping of drugs with no common mechanism of action. Instead, they are grouped together based on their common usage to improve the symptoms of RA, decrease joint damage and improve function and mobility. The cancer chemotherapeutic drug methotrexate is the most commonly used DMARD, although sulfasalazine, lefunomide, sodium aurothiomalate and cyclosporine are also regularly used. Multiple mechanisms appear to be involved in methotrexate's inhibition of RA:

- Inhibition of purine nucleotide metabolism, resulting in adenosine accumulation\textsuperscript{3}.
- Inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells\textsuperscript{4}.
- Increased CD95 sensitivity of activated T cells\textsuperscript{5}.
- Inhibition of methyltransferase activity, resulting in inhibition of enzyme(s) involved in the immune systems function\textsuperscript{6}.

However, methotrexate also has numerous side effects including ulcerative stomatitis, decreased white cell counts (and thus a higher level of other infections), fatigue and dizziness\textsuperscript{7}. Its usage may also result in hair loss, nausea, headaches and skin problems. Furthermore, not all individuals treated with methotrexate are responsive to treatment, even with prolonged treatment periods. Biological DMARDs may be used when other drugs are not effective. These include tumour necrosis factor \( \alpha \) (TNF\( \alpha \)) blockers (e.g. infliximab), interleukin 1 blockers (e.g. anakinra) and T cell costimulation blockers (e.g. abatacept). However, this class of drugs are generally only used when more conventional treatments are ineffective due to their prohibitive high costs and their side effects (lymphoma, serious infections including tuberculosis, heart failure). Furthermore, TNF\( \alpha \) inhibitors may induce the production of further self-directed antibodies, thereby
increasing the joint damage associated with RA. This may also result in the induction of other auto-immune disorders such as lupus, demyelinating disease and ankylosing spondylitis.

There is a need to develop safer, more effective drugs for the treatment of inflammatory diseases. Targeting earlier events prior to the induction of the immune response would not only alleviate the symptoms and discomfort of RA, but would also lessen/prevent the joint damage associated with chronic inflammation. Thus, targeting the trigger(s) of RA is an attractive option for safer, more effective treatments.

**Discussion**

**Evidence of the Involvement of a Microbial Trigger in RA**

Whilst the causes of RA are not comprehensively understood, it is generally accepted that it is an autoimmune disorder which is triggered by specific microbial infections in genetically susceptible individuals (individuals with the MHC class 2 allele HLA-DR4). Since the establishment of the MHC in the autoimmune mechanism, there have been several bacteria and viruses proposed as potential microbial triggers of RA. Most of these have subsequently been ruled out as causative agents because of contradictory evidence and now only a single bacterial species (Proteus mirabilis) is considered a likely trigger of RA.

P. mirabilis infections have been proposed to trigger RA based on several lines of evidence:

- Elevated serum levels of P. mirabilis specific cross-reactive antibodies have frequently been reported in individuals suffering from RA.
- P. mirabilis antibodies from RA patients have cytopathic effects on joint tissue possessing P. mirabilis cross-reactive antibodies.
- P. mirabilis infections have been frequently reported in urine samples from patients with RA.
- Sera from rabbits immunised with HLA-DR4 positive lymphocytes bind specifically to P. mirabilis.
- Amino acid sequence homologies have been identified between the 'EQ/KRRA' motif present in RA HLA-susceptibility antigens and the 'ESRRA' amino acid sequence present in P. mirabilis haemolysins.
- A further sequence homology between the 'LRREI' sequence of type XI collagen (present in joint cartilage) and the 'IRRET' motif present in P. mirabilis urease enzyme has also been reported.

Based on the evidence linking Proteus bacterial infections with pathogenesis, a mechanism of RA disease progression has been proposed (Figure 1). From this proposed mechanism, several broad phases of RA disease progression can be recognised as targets for drug design and/or discovery:

1. Gastrointestinal P. mirabilis acts as a trigger for RA. Thus, limiting the levels of gastrointestinal P. mirabilis may prevent RA initiation and minimize its downstream effects.
2. Gastrointestinal P. mirabilis will not initiate the autoimmune events associated with RA unless it is able to interact with the immune system. This may occur when epithelial lesions (or other epithelial interruptions) allow for the production of anti-P. mirabilis antibodies. Inhibition of the causative agents of gut lesion forming conditions (e.g. Crohn's disease) would also be expected to decrease RA initiation events.
3. The prevention and early detection of urinary tract infections (the major pathway for interaction of P. mirabilis with the immune system) and/or the colonization of the bladder may block the onset of RA and lessen downstream effects.
4. In the urinary tract, P. mirabilis undergoes a conversion to a 'swarming' form of the bacteria to allow colonization. It appears that the swarming form of P. mirabilis is the form most often associated with cross reactive antibodies. Thus, inhibition of urinary tract bacterial conversion to a swarming form would not only block colonization (UTIs), but would also decrease the possibility of antibody production.
5. Blocking the immune response by blocking the interaction of P. mirabilis with immunological cells or by immunomodulation also prevents the production of self-reactive antibodies or the cross-reactivity with self-tissue, thereby diminishing the later phase events of RA and thus the disease symptoms. However, immunomodulatory therapy should be used with caution as inhibiting the patient's immune capability would also expose the patient to a variety of other infections.
6. Most current RA therapies target the later phase events by blocking the inflammatory cascades or by decreasing the symptoms of RA (e.g. pain, swelling, heat). Whilst drugs targeting the late events are effective in easing patient discomfort, they still allow tissue damage (which is associated with the self-reactive antibody action) to occur.

**Proteus mirabilis**

Whilst Proteus mirabilis has been linked with the induction of RA (as well as cystitis and kidney stones), it is a normal part of the microbial flora of soil and water, and also is part of the normal flora of the human gastrointestinal tract. It is generally not pathogenic in most individuals. It is only when it enters the urinary tract or interacts with the bloodstream that it may become pathogenic and stimulate an immune response. P. mirabilis produces high levels of haemolysins and urease and these enzymes contribute to several diseases linked to this bacterium. High levels of urease in the urinary tract (in P. mirabilis UTIs) results in hydrolysis of urea to ammonia,
significantly increasing the urine pH, resulting in crystal formation and subsequently, the formation of kidney stones\textsuperscript{28}. It is believed that the \textit{P. mirabilis} urease and haemolysin enzymes are also targeted by the immune system and may be responsible for the induction of RA in genetically susceptible individuals\textsuperscript{11}. It has been postulated that Proteus infections (most commonly in the urinary tract) induce the production of anti-ESRAAL and anti-IRRET antibodies and these antibodies are cross-reactive with tissues containing EQ/KRRA or LRREI peptide sequences, particularly hyaline cartilage in the joints of the hands and feet\textsuperscript{11} (Figure 1). This antibody binding activates a number of downstream processes including inflammatory cascades involving acute phase proteins, components of the complement cascade, vasoactive amines, as well as cytokines, tumour necrosis factor-\textalpha (TNF-\textalpha) and various other cytotoxic components\textsuperscript{29,30}. Furthermore, this antibody’s cross-reactivity activates further cellular processes, including those involving the action of natural killer cells. These events subsequently result in the development of Proteus reactive RA. Initial infections often result in mild symptoms. Recurrent infections result in enhanced production of cross-reactive antibodies and intensification of the immunological reactions with resulting tissue damage.

**Targeting Gastrointestinal Proteus mirabilis to Block the Onset of RA**

Eradication of the cause of an inflammatory disease is an attractive target for drug design as this would not only block/decrease the late phase inflammatory symptoms, but would also block the immune response and subsequent tissue damage associated with auto-immune inflammatory disorders. Based on the evidence linking Proteus bacterial infections with pathogenesis, several lines of research are aimed at the treatment and management of RA by targeting the bacteria itself. One strategy is the development of anti-Proteus vaccines. Whilst the production of a vaccine may block proliferation of the Proteus species, it is also a
problematic approach as anti-Proteus antibodies could also cross-react with the host’s connective tissue in susceptible individuals, thus exacerbating the symptoms of RA. If antibodies lacking cross-reactive epitopes are developed in the future, this approach may be effective as it would enable susceptible individuals to resist some consequences of a Proteus infection. However, the development and usage of Proteus-sensitive antibiotics may prove a more effective means of blocking RA initiation/progression. Destroying the Proteus bacteria would be expected to greatly reduce the impact of the bacteria on inflammation, diminish the production of anti-Proteus antibodies and thus decrease disease progression. Many antibiotics are already known to inhibit Proteus growth and/or have bactericidal effects towards Proteus spp. However, the development of super resistant bacterial strains has resulted in currently used antibiotic agents failing to end many bacterial infections31,32. For this reason, the development of new anti-P. mirabilis chemotherapeutic agents for the prevention and treatment of RA has received recent attention. Recent studies have examined the anti-P. mirabilis activity of conventional antimicrobials such as carbapenems33 and of complementary and alternative therapies including nanomaterial preparations34 and traditional South African medicinal plants35. A re-examination of traditional medicines for the treatment of inflammation and rheumatic conditions is an attractive prospect as the antiseptic qualities of medicinal plants have been long recognised and recorded. Furthermore, there has recently been a revival of interest in herbal medications due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals.

Unfortunately, whilst prophylactic targeting of P. mirabilis with antibiotics may initially appear as a viable method of controlling RA in susceptible individuals, it is also problematic for several reasons:

1. Prolonged antibiotic treatment/exposure would result in the production of antibiotic resistant bacterial strains:

   Whilst ongoing prophylactic treatment with a single antibiotic would certainly result in resistant bacterial strains, combinational therapies would be less likely to allow for the development of resistant strains. Several studies have reported that combinational therapies significantly inhibit the generation of resistant strains and some severe infections (e.g. Mycobacterium tuberculosis) are successfully treated this way36,37. In this respect, the use of plant extracts with potent antibacterial activity would be particularly attractive option as the extracts would be expected to contain several antibiotic compounds which would be likely to function via several different mechanisms. There is a growing trend in the animal husbandry industry to switch to the usage of crude plant extracts rather than using pure antibiotic compounds for this reason. A literature search has been unable to find any reports of any bacterial species developing resistance to crude plant extracts.

2. Antibiotic treatment may also inhibit beneficial gastrointestinal bacteria, disrupting the balance of beneficial/pathogenic bacteria:

Numerous studies have focused on the use of probiotics in conjunction with antibiotics to return the gastrointestinal microflora to a beneficial balance38,39. Thus, co-administration of a probiotic with prophylactic antibiotic treatment may be required for long term usage to be a viable treatment option.

**Targeting UTIs to Control RA**

The usual route for the establishment of UTIs is via the urethra. In most cases, the bacteria infecting the urethra originate from the bowel. Women have a much greater incidence of UTIs than men as the urethra is shorter and closer to the anus in females than males. The higher incidence of UTIs in women may subsequently be responsible for the higher incidence of RA in women than in men. Epidemiological studies have reported that approximately three times as many women are affected by RA as men40. Furthermore, onset generally occurs in women when they reach middle age or older, as their estrogen levels decrease. The loss of estrogen correlates with a loss of protective vaginal flora, subsequently resulting in an increased incidence of UTI41. The incidence of UTI is also significantly increased during hospitalization, especially when urinary catheterization is involved42. Whilst E. coli accounts for approximately 80-85% of UTIs, Proteus infections also account for a significant number43.

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Following entry to the urinary tract, P. mirabilis (and/or the other pathogenic bacteria) need to attach to the bladder wall and form a biofilm to colonise and to resist the host’s immune system\textsuperscript{44}. For Proteus bacteria, this involves the conversion to a ‘swarming’ form of the bacteria. Interaction between individual bacterial cells (quorum sensing) results in biosurfactant production which is accompanied by a number of morphological changes. The cells become hyper elongated, hyper flagellated and grouped into multicellular raft structures\textsuperscript{45,46,47}. Whilst the fundamental role of swarming in P. mirabilis is unknown, it is believed that it increases the bacteria’s resistance to antibiotics and thus may be a survival mechanism.

A number of measures have been proposed to lessen the chances of a UTI and the subsequent triggering of RA in susceptible individuals. The majority of these involve behavioral modification techniques (e.g. personal hygiene methods after urinating or defecating). None of these has been definitively confirmed to affect the incidence of UTIs. However, several medical interventions have proved successful. A prolonged course of antibiotics is often advised, especially for recurrent infections. Commonly used medicines include nitrofurantoin and sulfamethoxazole. Alternative medicines have also been reported to assist with UTIs. In particular, cranberry juice or extract decreases the frequency of UTIs in individuals prone to recurrent infections\textsuperscript{39,40}. However, it is noteworthy that this type of treatment is not always possible for the treatment of RA as the symptoms of RA often manifest long after a UTI has cleared up. Antibiotic treatment at this time may well block further bacterial colonisations of the urethra, but will be unlikely to affect the later phase events of RA. If a safe effective medicine is ultimately developed that is effective as an UTI preventive, this may ultimately block the induction of RA in many individuals.

**Blocking Proteus mirabilis Interaction with the Blood**

Whilst UTIs appear to be the main route for P. mirabilis to interact with the immune system, the bacterium may also interact directly with the immune system via gastrointestinal interruptions\textsuperscript{21}. Inhibition of the causative agents of lesion forming conditions (e.g. Crohn’s disease) would also be expected to block RA initiation events. Acute treatment of Crohn’s disease is often quite harsh and may involve the use of antibiotics as well as salicylates (e.g. 5-aminosalicylic acid) and corticosteroids (e.g. prednisone) to reduce the inflammation, as well as the use of immunosuppressive drugs (e.g. azathioprine). Methotrexate is often also used. Some of these treatments may also assist with the symptoms and maintenance of inflammation and thus may have pleuripotent effects in the treatment of RA.

Similarly, Helicobacter pylori co-infections also affect the integrity of the gastrointestinal epithelium. To avoid the acidic environment of the gut, H. pylori burrow through the mucus lining of the stomach to harbor in the less acidic environment of the epithelial cell layer\textsuperscript{49}. The bacterium further modifies its pH environment within the stomach through the action of its urease enzyme, which breaks down urea to release ammonia. This subsequently neutralizes the gastric acids and increases the pH of the bacterial local environment. The resultant epithelial damage disrupts the tight junctions between gastric epithelial cells, allowing interaction between the immune system and gastric bacteria (including P. mirabilis). When the resultant inflammation is severe, the protective mechanisms of the stomach and duodenum become overwhelmed and gastric ulcers are formed. However, even less severe infections without obvious ulcers still result in interaction between the immune system and gut bacteria due to a disruption of the tight junctions between epithelial cells. Thus, control of H. pylori (even when gastritis is not evident) is an important consideration in blocking the onset of RA. Treatments are already available for H. pylori induced peptic ulcers. These usually involve a combinational therapy consisting of proton pump inhibitors (e.g. omeprazole) and the antibiotics clarithromycin and amoxicillin (or metronidazole). However, such treatments are best considered short term treatments (usually 1 week) and are not useful in the prevention of H. pylori infections. Much current research is directed towards vaccination against H. pylori, and in the future this approach may help lessen a number of diseases associated with H. pylori infections (e.g. RA, stomach cancers, asthma, inflammatory bowel disease, gastroesophageal reflux disease).

Other regions of the gastrointestinal tract may also be affected by other microbes, allowing interaction between the immune system and the gastrointestinal microflora. For example, gastrointestinal protozoal parasites of the genus Giardia (especially G. lamblia and G. duodenalis) may infect the upper and middle regions of the small intestine (duodenum and jejunum). They attach to the brush border of the epithelial cells, flattening the villi and opening the tight junctions between the cells, allowing interaction between the immune system and the gut microflora\textsuperscript{30}. When an acute infection occurs, it may be treated with metronidazole. However, prevention through hygienic food handling practices and the consumption of sterilized water is generally a better option.

**Immunosuppressants in Controlling RA**

Blocking the immune response by blocking the interaction of P. mirabilis with immunological cells (as outlined in sections 5 and 6) or by immune-modulation may also prevent the production of self-reactive antibodies and thus would also diminish the later phase events of RA and the disease symptoms. However, immune-modulatory therapy should be used with caution as inhibiting the patient’s immune capability would also expose the patient to a variety of other infections. Indeed, several reports have noted an increased incidence in other autoimmune inflammatory disorders, including ankylosing spondylitis\textsuperscript{50}. Furthermore, immune-suppression also results in a decreased cancer immune-surveillance and may result in
an increased incidence of some cancers. Cortisone was the first immune-suppressant to gain widespread medicinal usage. However, its usage has remained limited due to its wide (and often serious) range of side effects. Today, cyclosporine, which suppresses the immune system by inhibiting the proliferation and function of T cells, has gained much wider acceptance due to its less serious side effects.

**Natural Products as Anti-inflammatory Agents**

Herbal remedies have long been used to alleviate rheumatic complaints in all traditional medicine systems. In some cases, the major anti-inflammatory components of these medicines have been determined in the laboratory and are already available in a pure form as anti-inflammatory agents. These compounds include a variety of polyphenolic compounds, of which 3,5,4’-trihydroxy-trans-stilbene (resveratrol; Figure 2a) has received much recent attention due to its presence in many plants used in the treatment of inflammation, and due to its potent anti-inflammatory activity51,52 (Figure 3). Most studies examining the role of resveratrol in the treatment of inflammatory conditions focus on its ability to act as a potent specific inhibitor of NF-κB activation which may act as an inducer for two major inflammatory cytokines, TFN-α and IL-1β51. Thus, resveratrol treatment is known to block cytokine production and inflammation via its inhibition of NF-κB activation. Resveratrol also has direct effects on immune cell function. In vitro exposure of CD8+ and CD4+ T cells to high doses of resveratrol suppresses their proliferation and inhibits IFN-γ, IL2 and IL4 production53. Interestingly, a biphasic effect was reported, with the opposite effect seen on exposure to low resveratrol concentrations. The same study noted a similar biphasic effect on natural killer cell (NK) cytotoxic activity, with an enhancement at low concentrations, and suppression at higher doses.

Resveratrol also has been reported to directly inhibit the growth of several bacterial species54. Interestingly, it was unable to inhibit the growth of P. mirabilis in studies in our laboratory, even at high concentrations55. Furthermore, we were unable to find any reports of in vitro anti-Proteus growth inhibition in the published literature. Several studies have reported on the ability of resveratrol to inhibit P. mirabilis swarming and virulence factor expression in vivo, so it is likely that resveratrol does affect P. mirabilis colonisation and infection of the urinary tract52, albeit possibly by mechanisms other than...
bactericidal or growth inhibition mechanisms. Any combinational therapy or extract that is capable of inhibiting Proteus spp. growth and that also contains resveratrol would be likely to have anti-RA activity via several mechanisms (growth inhibition, colonisation blocking, cytokine production inhibition) and therefore would be particularly effective in treating RA.

Other stilbenes and stilbene glycosides (including resveratrol glycosides) may also have anti-RA activities. The resveratrol glycoside piceid [2-[(3-Hydroxy-5[(E)-2-[(4-hydroxyphenyl) ethenyl] phenoxy]-6-(hydroxymethyl) oxane-3,4,5-triol] (Figure 2b) may be hydrolysed in vivo to remove the glucose moiety, thus releasing resveratrol. Piceid, and other glycosylated stilbenes, also have direct anti-inflammatory potential. Several glycosylated stilbenes (including piceid) have both been shown to block inflammation by decreasing IL-17 production in stimulated human mononuclear cells. However, another study determined that the antibacterial activity of resveratrol is due to a protein tyrosine kinase activity. This same study also reported that resveratrol glycosides do not have the same bacterial inhibitory activity that has been reported for resveratrol, indicating that free hydroxyl groups on both phenyl groups are required for antibacterial activity. Similarly, the stilbene 2,3,4,5-tetrahydroxystilbene-2-0-beta-D-glucoside (TSG) inhibits inflammation by suppressing the induction of pro-inflammatory mediators by reducing NF-kB binding to DNA. The same study detected TSG in numerous herbs used to treat inflammation in Chinese traditional medicine. Furthermore, nine stilbene and stilbene derivatives isolated from the roots of Cicer spp. (chickpeas) were shown to inhibit bacterial and fungal growth. Combretastatins A-1 (Figure 2c) and A-4 (Figure 2d) are well known for their potent ability to block cancer cell progression and induce apoptosis by binding intracellular tubulin, thereby disrupting microtubule formation. Accounts of direct anti-inflammatory activity of combretastatins are lacking. However, it is believed that they act by a similar mechanism to that of colchicine (N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl] acetamide) by binding the colchicine binding site on the tubulin peptide and inhibiting polymerisation. It is thus likely that they may have a similar anti-inflammatory activity and mechanism to colchicine.

Conclusions

RA is a complex disease which may occur in genetically susceptible individuals when the production of self-reactive antibodies is triggered. This manuscript reviews some of the early events in RA disease progression and highlights several targets for the development of drugs to prevent the onset of the disease. It is unlikely that any single drug will be completely effective at preventing the onset of RA due to the complexity of the triggering events. Instead, the use of combinational therapies may be a preferred treatment modality, especially combinations that inhibit the onset of the disease as well as treating the later symptoms. Resveratrol (and other stilbenes) have been highlighted as having pleuripotent effects on inflammation and may be particularly useful, especially when used in combination with drugs targeting other phases of the disease progression. Crude plant extracts (especially those containing stilbenes) have appeal in the treatment of chronic inflammatory diseases for a number of reasons:

- There is less likelihood of developing resistant strains.
- Multiple phases of the disease progression may be targeted simultaneously.
- They are generally cost effective and easy to produce.

However, to be a viable therapeutic option, herbal medicines need to be held to the same standards as conventional medicines. They must be standardised for their bioactive components. Furthermore, rigorous toxicity testing and examination of side effects needs to occur. The same is true if conventional medicines are used together as combinational therapies. These combinations need to be evaluated for potential synergistic or inhibitory effects.

References


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