Biologic Drugs.

Learning Objectives.

At the end of this course, you should be able to :

1. **briefly describe the role of TNF-a and IL-1 in the inflammatory process**
2. **understand how manipulation of these molecules can affect the progression of joint damage**
3. **appreciate the role of drugs in the management of RA**

Biologic drugs have been developed to help manage disorders such as Rheumatoid Arthritis, where cytokines are produced which damage tissue, and initiate an inflammatory response, i.e. auto-immune disorders. Disorders like this can lead to discomfort, deformity, and loss of function, and may have a major impact on quality of life. Previously, treatment has consisted of managing symptoms such as pain and swelling, as well as minimising further deformity and damage. More recently, management for these disorders has changed with the development of a newer group of drugs which aim to reduce or prevent the auto-inflammatory process, thereby reducing the damage. These drugs are known by the general term of 'biologic agents'.

Biologic agents target a group of proteins known as cytokines, and in particular Tumour Necrosis Factor (TNF) and Interleukin-1 (IL-1). The term 'cytokine' refers to a broad range of structurally diverse molecular families and individual proteins best known for their many roles in immune system function. They are often pleiotropic molecules (the same molecule can produce different effects in different situations), with diverse and cell type-specific activities. Cytokines work by binding to specific receptors, and their activities include regulating cell activation, haematopoiesis, apoptosis, cell migration, and cell proliferation. In effect, they act as chemical messengers. In this capacity, they are involved in virtually all aspects of both innate

and adaptive immune responses. Leukocytes are a primary source of cytokines, although they maybe produced by many other cell types as well.

Overproduction or inappropriate production of certain cytokines by the body can result in disease. For example, it has been found that interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-alpha) are produced in excess in rheumatoid arthritis where they are involved in inflammation and tissue destruction. Biologic drugs have been developed to inhibit IL-1 or TNF-alpha. Kineret (anakinra) was developed as a treatment for rheumatoid arthritis that works by inhibiting IL-1 binding to its receptor. TNF-alpha inhibitors (also called TNF blockers) bind TNF and prevent TNF from attaching to cell surface receptors. Enbrel (etanercept), Remicade (infliximab), and Humira (adalimumab) are TNF blockers.

Tumour Necrosis Factor-alpha

Tumour necrosis factor alpha (TNF-alpha), named for its anti-tumour properties, was isolated almost 30 years ago. It is an important member of the TNF superfamily and has important roles in immunity and cellular remodelling as well as influencing apoptosis and cell survival. Its central role in inflammation has led to the development of TNF-alpha antagonists as effective therapies for rheumatoid arthritis and inflammatory bowel disease. It is a cytokine produced primarily by monocytes and macrophages, and is found in synovial cells and macrophages in the tissues.
It shares many properties with another cytokine, interleukin 1. Its roles include:

- Activation of endothelium
- Stimulation of fibroblast proliferation
- Induces MMP (matrix-metalloprotease) production in synoviocytes
- Stimulates production of IL-1 and IL-6
- Activates osteoclasts via IL-1 stimulation.

Alternative names used for this molecule are lymphotoxin B, and cachectin. Most tissues in the body appear to be affected by TNF-a, and the cytokine serves a variety of functions, many of which are not yet fully understood. The cytokine possesses both growth stimulating properties and growth inhibitory processes, and it appears to have self regulatory properties as well, e.g.TNF-A induces neutrophil proliferation during inflammation, but it can also induce neutrophil apoptosis.

Additional beneficial functions of TNF-a include its role in the immune response to bacterial, and certain fungal, viral, and parasitic invasions as well as its role in the necrosis of specific tumours. Lastly it acts as a key mediator in the local inflammatory immune response. TNF-a is an acute phase protein which initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection. TNF-a secreted by the macrophage causes blood clotting which serves to contain the infection.

The pathological activities of TNF-a have attracted much attention. For instance, although TNF-a causes necrosis of some types of tumours, it promotes the growth of other types of tumour cells, and high levels of TNF-a correlate with increased risk of mortality. TNF-a participates in both inflammatory disorders of inflammatory and non inflammatory origin. Originally sepsis was believed to result directly from the invading bacteria itself, but it was later recognized that host system proteins, such as TNF-a induced sepsis in response. Exogenous and endogenous factors from bacteria, viruses, and parasites stimulate production of TNF-a and other cytokines. Lipopolysaccharide released from bacterial cell walls is a potent stimulus for TNF-a synthesis. When cytokine production increases to such an extent that it escapes the local infection, or when infection enters the bloodstream, sepsis ensues. Systematic oedema results in low blood volume, hypoproteinanemia, neutropenia and then neutrophilia. Body organs fail and death may result. Victims of septic shock experience fever, falling blood pressure, myocardial suppression, dehydration, acute renal failure and then respiratory arrest.

Prolonged overproduction of TNF-a also results in a condition known as cachexia, which is characterised by anorexia, net catabolism, weight loss and anaemia, and occurs in illnesses such as cancer and AIDS. Cachectin and TNF-a were once considered different proteins, but it was realised that the two proteins were actually the identical, and is an example of the pleiotropic nature of cytokines.

Interleukin 1

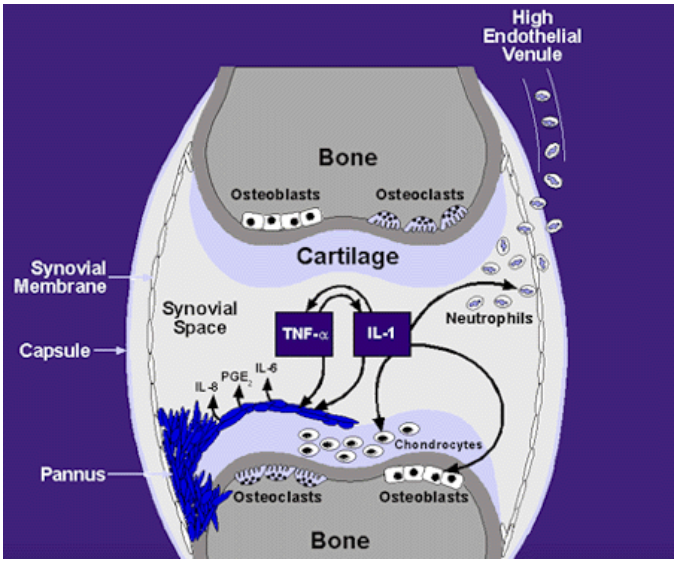
Interleukin 1 (IL-1) is a general name for two distinct proteins, IL-1 alpha and IL-1 beta, that are considered the first of a family of regulatory and inflammatory cytokines,' playing important roles in the regulation of acute inflammation. In the immune system, the production of IL-1 generally results in inflammation. The effects of IL-1 are not limited to inflammation, and have also been associated with bone formation and remodelling, insulin secretion, appetite regulation, and fever induction, IL-1 has also been known by a number of alternative names, including lymphocyte activating factor, endogenous pyrogen, catabolin, hemopoietin-1, melanoma growth inhibition factor, and osteoclast activating factor.

IL-1 is expressed by many cells and has multiple functions including local inflammation. Cells known to express IL-1a include astrocytes, fibroblasts, hepatocytes, keratinocytes, brown fat adipocytes, T cells and eosinophils, dendritic cells, macrophages, monocytes and oligodendrocytes. Both IL-1a and IL-1b form an important part of the inflammatory response of the body against infection. These cytokines increase the expression of adhesion factors on endothelial cells to enable transmigration of leukocytes to sites of infection and re-set the hypothalamus thermoregulatory centre, leading to an increased body temperature which expresses itself as fever. IL-1 is therefore called an endogenous pyrogen. The increased body temperature helps the body's immune system to fight infection. IL-1 is also important in the regulation of hematopoiesis. IL-1b production in peripheral tissue has also been associated with hyperalgesia (increased sensitivity to pain) associated with fever.

IL-1a is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. This cytokine is produced by many cell types but is only secreted by monocytes and macrophages. It is produced as a proprotein, which is proteolytically processed by calpain and released in a mechanism that is still not well understood. Additionally IL-1a is essential for maintenance of skin barrier function, especially with increasing age.

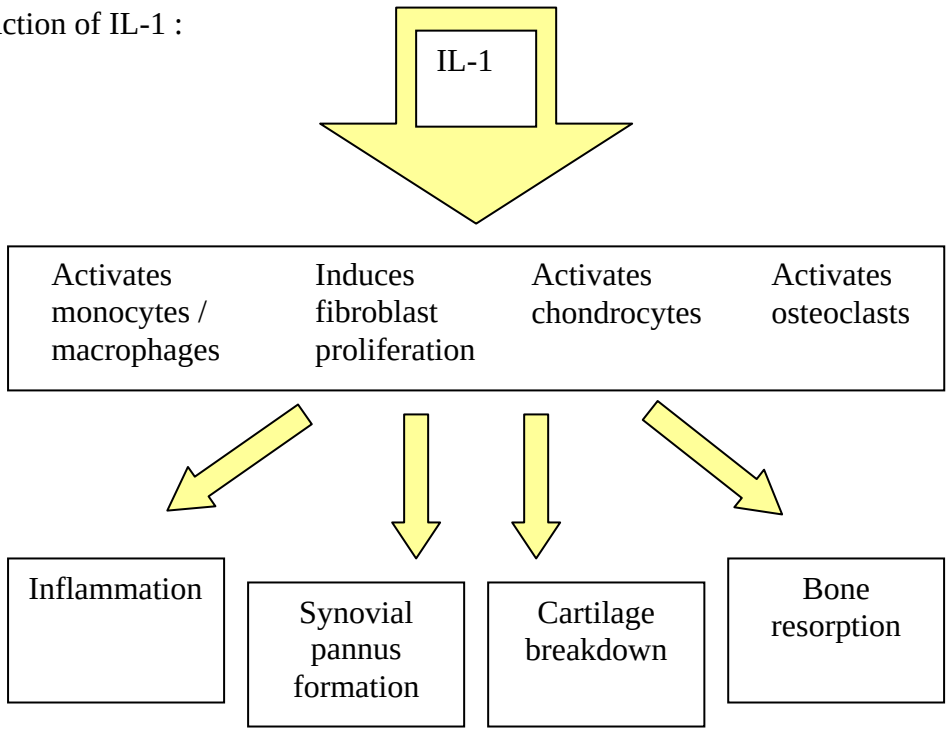
TNF, IL-1, and Rheumatoid arthritis.

Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory systemic disease of unknown aetiology characterised by persistent joint inflammation that results in progressive joint destruction, joint deformity, and physical disability. RA may affect other organs and may also result in an increased risk of premature death. The average life expectancy of RA patients is decreased by 3 to 18 years compared to age and gender matched controls. Damage to the bone and cartilage caused by intense episodic synovitis in RA can be attributed to various potent pro-inflammatory mediators that include IL-1 and TNF, as well as several other cytokines. It is notable that IL-1 and TNF are particularly abundant in the cytokine profile of the synovial lining of the joint.



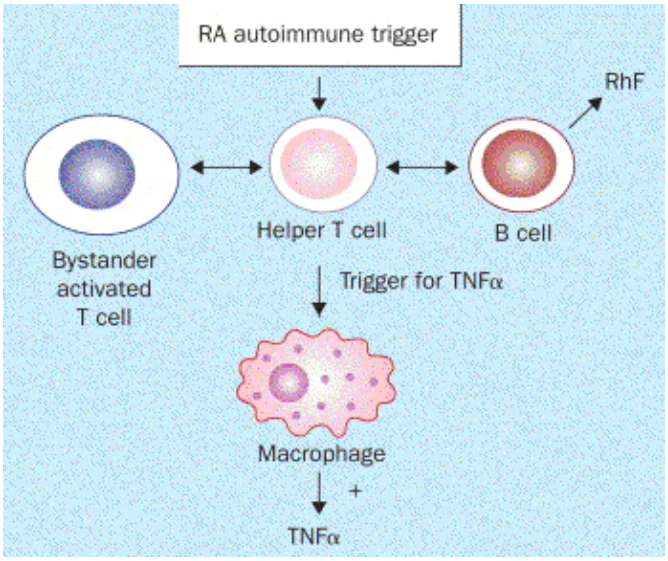
Evidence supports the fact that the level of disease activity in RA, and progression of joint destruction, correlates with plasma and synovial fluid levels of IL-1. IL-1 stimulates production of prostaglandin E2, nitric oxide, and matrix metalloproteases, which promote joint degradation. In addition, IL-1 suppresses joint repair by inhibiting collagen synthesis, regulates the immune system systemically and locally in acute and chronic disease, augments activation of T and B lymphocytes, causes macrophages to release proteolytic enzymes and chemotactic factors, and also stimulates osteoclasts to resorb bone. The expression of the IL-1 gene may be stimulated by various types of cell interactions, by pro-inflammatory cytokines such as TNF-a, or by the autocrine or paracrine action of IL-1 itself. Similar to TNF-a, the principal cell type that produces IL-1 is the activated macrophage.

Action of IL-1 :



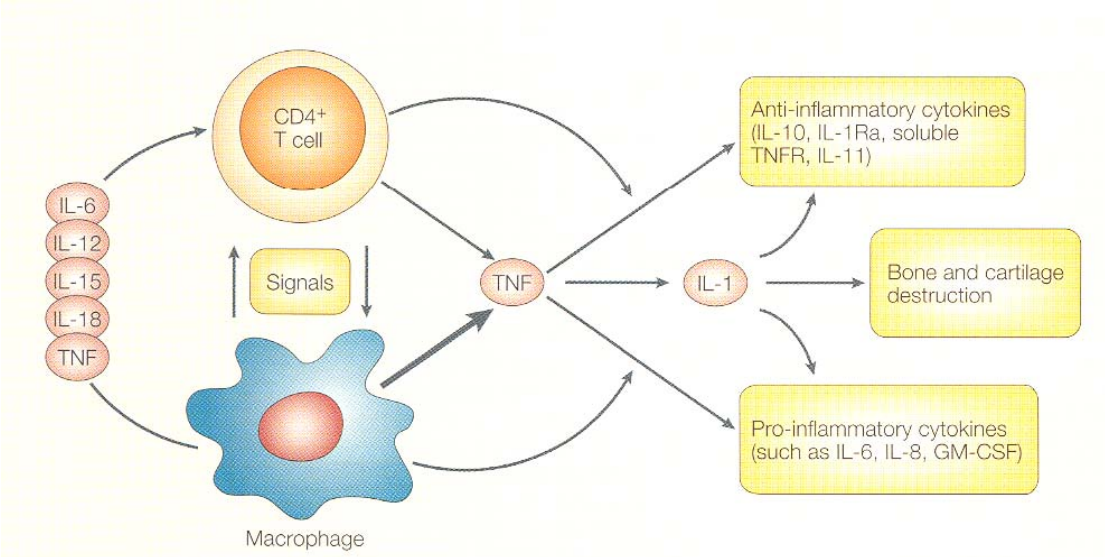
In relation to the production TNF-a in RA, signalling between the T cells and macrophages stimulates the increased release of TNF-a within the joint. The original signalling mechanism is thought to be linked with an autoimmune trigger.

Action of TNF:



Higher levels of TNF-a also induce the expression of adhesion molecules, which enhances the binding of neutrophils and leucocytes within the vascular endothelium, further inducing the inflammatory response. It is also interesting to note that levels of TNF-a and TNF receptors are more abundant at the cartilage-pannus junction on the joint surface, which is the point thought to represent the ‘moving edge’ of destruction within the joint.

In combination, TNF-a and IL 1 act synergistically to induce an inflammatory response, and the beginning of cartilage and joint destruction.



Biologic drugs

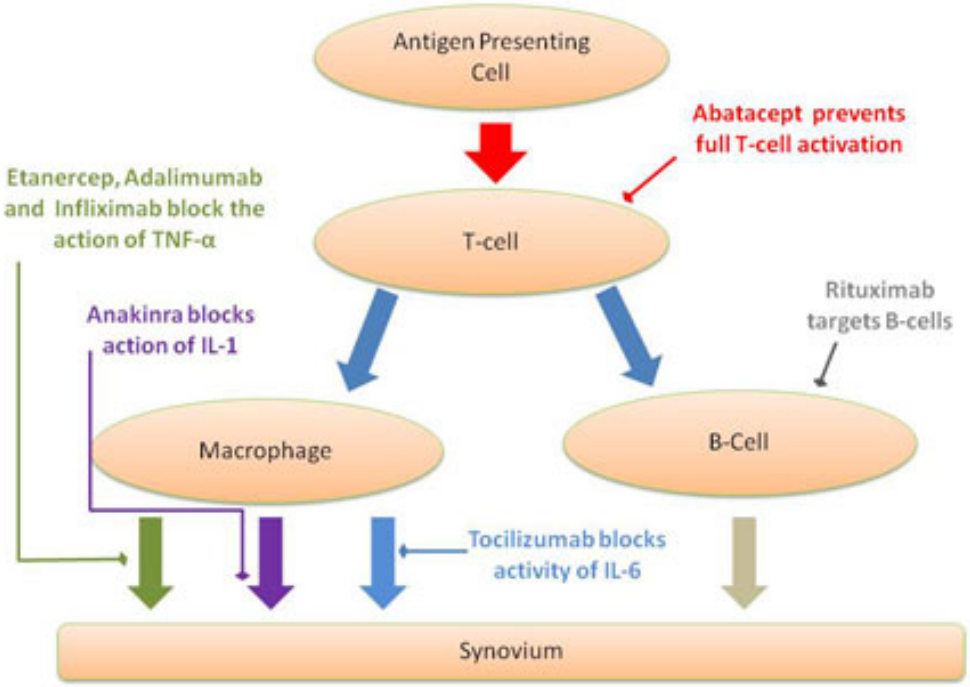
In recent years use of this new class of drugs has become more prominent. Generally these drugs are usually prescribed together with the disease-modifying anti-rheumatic drug, methotrexate. Biological treatments targeting components of the immune system in combination with disease-modifying antirheumatic drugs (DMARDs) are effective in preventing joint damage in some patients, but not all sufferers respond to these treatments and some may relapse despite treatment.

The drugs currently available are :

Tocilizumab (Actemra™)	Abatacept (Orencia®)	Adalimumab (Humera®)
Etanercept (Enbrel®)	Infliximab (Remicade®)	Anakinra (Kineret®)
Rituximab (Mabthera®)		

The newest of these is tocilizumab, which is a humanised monoclonal antibody to the interleukin-6 receptor that blocks the activity of interleukin-6, a cytokine that plays a major role in the RA inflammatory process. This is the first drug to use this approach, and is therefore seen as a new breakthrough in treating RA. It is currently awaiting approval in the United States and Europe, although in Japan, it was approved for the treatment of RA in April 2008.

Other biologics already used to treat RA act at different points in the inflammatory process. Abatacept works by reducing the activation of T-cells, which reduces the activation of other cells in the RA inflammatory process. Adalimumab, etanercept, and infliximab block the action of TNF-a, preventing tissue damage. Rituximab targets B cells, one of the key players in the pathogenesis of RA. Anakinra blocks the actions of the cytokine, IL-1.



Etanercept

Etanercept is effective in reducing the signs and symptoms of RA, as well as in slowing or halting radiographic damage, when used either as monotherapy or in combination with methotrexate. Etanercept is also approved for the treatment of psoriatic arthritis and for ankylosing spondylitis as well as psoriasis. Etanercept is a fusion protein that combines two extracellular binding domains of a type of TNF receptor with a portion of a human IgG1 antibody molecule. The components of the protein are entirely human, and anti-etanercept antibodies are relatively uncommon. Etanercept binds TNF in the circulation and in the joint, preventing interaction with cell surface TNF receptors thereby reducing TNF activity.

The most common dose currently used is 50 mg self-administered once per week by subcutaneous injection. Both prefilled syringes and an autoinjection system (SureClick®) are available. Etanercept is also available in a 25 mg dose which is administered twice per week at this dose. Intermittent or occasional dosing has not been studied. There is limited information on the safety or efficacy at doses beyond 50 mg per week. It will usually begin to have a therapeutic effect within 1 to 4 weeks,

and improving signs and symptoms with additional improvements that can be seen over 3-6 months.

As with all TNF antagonists, there is an increased risk of infection, including serious and opportunistic infections in patients treated with etanercept and other TNF antagonists. Although disseminated tuberculosis due to reactivation of latent disease has been seen more commonly with infliximab and adalimumab than etanercept, screening for latent TB is nonetheless prudent before treatment with any TNF inhibitor. Etanercept and other TNF inhibitors are not recommended in patients with demyelinating disease or with congestive heart failure. Transient neutropenia (lowering of white blood cell counts) or other blood dyscrasias have been reported with etanercept and the other TNF inhibitors. Injection site reactions are sometimes seen with etanercept, but are usually mild.

Infliximab

Infliximab, in combination with methotrexate, is approved for the treatment of RA, and for the treatment of psoriatic arthritis, and ankylosing spondylitis, as well as psoriasis and Crohn's disease. Infliximab is a chimeric monoclonal antibody that binds TNF with high affinity and specificity. The antibody binding site for TNF is of mouse origin, with the remaining 75% of the infliximab antibody derived from a human IgG1 antibody sequence.

Infliximab is effective as monotherapy in reducing the signs and symptoms of RA but anti-infliximab antibodies can develop which can, in turn, reduce the durability of the response. Co-treatment with methotrexate reduces the frequency of these antibodies and is therefore recommended along with infliximab. The combination of infliximab and methotrexate is very effective in reducing clinical manifestations of disease, as well as in slowing or halting radiographic progression of disease in RA.

Infliximab binds TNF in the joint and in the circulation, preventing its interaction with TNF receptors on the surface of inflammatory cells, and eventually clearing TNF from the circulation. Monoclonal antibodies also bind to cell-bound TNF. Through its actions, infliximab inhibits the activity of TNF.

Infliximab is administered via the intravenously, usually over 2-3 hours. The recommended starting dose of infliximab is 3 mg/kg for RA given as an intravenous infusion followed by additional dosing at 2 and 6 weeks, then every 8 weeks thereafter. Infliximab should be given in combination with methotrexate. If the clinical response is inadequate at a starting dose, infliximab can be increased incrementally to a maximum dose of 10 mg/kg and the frequency of infusion increased to every 4-6 weeks.

In clinical practice infections are increased in patients who receive TNF antagonists, both mild and serious. Infliximab is not recommended in patients with congestive heart failure or with demyelinating disease. Patients receiving infused biological agents including infliximab may develop a clinical syndrome of fever, chills, body aches, and headache associated with the infusion of the antibody. Anti-infliximab antibodies occur in 10-30% of patients depending on the dosage and frequency of infusion but are suppressed by concomitant methotrexate therapy.

Adalimumab

Adalimumab is a fully human anti-TNF monoclonal antibody with high specificity for TNF. Like the other TNF antagonists, it is effective as monotherapy and in combination with methotrexate, at reducing signs and symptoms of RA and in slowing or halting radiographic progression of disease. It is administered by subcutaneous injection every two weeks but can be increased to weekly, if needed. Adalimumab is effective in RA, Psoriatic arthritis, and ankylosing spondylitis, and Crohn’s disease.

Adalimumab binds specifically to TNF and blocks its interaction with two types of cell surface TNF receptors, thereby interfering with endogenous TNF activity. Adalimumab binds to both soluble as well as cell bound TNF.

Adalimumab is currently available in a 40 mg dose and is given by self-administered subcutaneous (SC) injection every other week. Both prefilled syringes as well as an autoinjector system (Huimira Pen®) are available. If response to this dose is

inadequate, the frequency of injections can be increased to weekly. The usual time to show an effect is 1 to 4 weeks.

As with other TNF antagonists increased infections, ranging from mild to serious, and including opportunistic infections are seen in clinical practice. Like other TNF antagonists, adalimumab is not recommended for patients with concurrent demyelinating disease or congestive heart failure. Injection site reactions may also be seen with adalimumab, which are typically mild and generally do not result in drug discontinuation.

Abatacept

Abatacept is the first of a class of agents known as T-cell co-stimulatory blockers. These agent interfere with the interactions between antigen-presenting cells and T lymphocytes, and affect early stages in the pathogenic cascade of events in rheumatoid arthritis. T lymphocytes become activated due to an unknown stimulus but likely involving the interaction between an antigen and a cell surface molecule. T cells recognise antigens as foreign and if they receive a second stimulus, will become active, proliferate, move to inflamed sites, and secrete pro-inflammatory cytokines including TNF.

Abatacept works by binding to a surface molecule on the T lymphocyte, preventing the second signal from being delivered, thus turning down the T cell response. Additional effects are decreasing the production of T cell derived cytokines including TNF.

Abatacept is administered via intravenous infusion once per month after initial doses at baseline, 2 weeks, and 4 weeks. The dose is based on body weight, with patients <60 kg receiving 500 mg, 60-100 kg receiving 750 mg, and >100 kg receiving 1000 mg. The medication is administered over a period of approximately 30 minutes to one hour. Responses are typically seen within 3 months.

As with other biologic drugs, infections are increased in patients receiving abatacept. These have ranged from mild to severe. Respiratory infections including pneumonia

are more common in patients with underlying COPD, thus extreme caution is suggested in this population. Malignancies have been seen in clinical trials but it is unclear if the rates are more that expected in patients with rheumatoid arthritis. Opportunistic infections have been seen, though only a few cases of TB have been seen to date. Infusion reactions have been seen in clinical trials that are typically mild.

Rituximab

B cells are an important inflammatory cell with multiple functions in the immune response. They serve as antigen presenting cells, can secrete cytokines, and differentiate into antibody-forming plasma cells. The depletion of B cells has been shown to be effective in reducing signs and symptoms of RA and in slowing radiographic progression. Rituximab is a B cell depleting agent, and is currently available for the treatment of rheumatoid arthritis. Rituximab was originally developed to treat non-Hodgkin's lymphoma and has been used to treat this malignant condition of lymphocytes and lymph nodes for several years. Early studies in patients with rheumatoid arthritis showed rituximab caused a rapid and sustained depletion of circulating B cells in the circulation with clinical improvements in many patients as well. Further clinical studies have now demonstrated that rituximab is effective in decreasing signs and symptoms and in slowing radiographic progression in RA patients who have failed other DMARD therapies.

Rituximab binds to a surface molecule on the B cell, leading to the removal of B cells from the circulation. A single course of ritximab (2 infusions of 1000 mg each given 2 weeks apart) leads to a rapid and sustained depletion of B lymphocytes in the peripheral blood. This effect is sustained for 6 months to 1 year or even longer. The levels of the autoantibody rheumatoid factor decrease, but the levels of other antibodies typically remain within the normal range after the first infusion. The clinical effects are thought to occur from a decrease in B cell cytokines, interactions between B cells and T cells, or due to reductions in autoantibody levels.

Effects from rituximab are not seen for up to 3 months after an infusion. Effects however may last 6 months and up to 2 years following a single infusion course.

The currently approved dose is 1000 mg administered intravenously over 3-4 hours with two doses given 2 weeks apart. Patients typically receive intravenous corticosteroids with each infusion and premedication with diphenhydramine and acetaminophen. The optimal time for readministration is not yet clear. Some have advocated a fixed dosing regimen of every 6 months, while others have advocated waiting until a patient begins to flare before retreating. Studies are ongoing to evaluate redosing schedules. The extent and duration of B cell depletion has not been clearly correlated with efficacy. Nor has the reconstitution of normal levels of B cells been well correlated with loss of efficacy.

Infusion reactions are seen in patients who receive Rituximab infusions. These may include hives, itching, swelling, difficulty breathing, fever, chills, and changes in blood pressure. These are usually mild and respond to slowing the infusion rate or additional medication (such as antihistamines) but may be severe. These are reactions were the most common with the first infusion.

As with other immunomodulatory therapies, infections may be increased in patients who are receiving rituximab, and it may also lead to the reactivation of viral infections that were dormant, including hepatitis B.

Anakinra

Anakinra, a human recombinant IL-1 receptor antagonist, is approved for the treatment of RA. Anakinra can be used alone or in combination with DMARDs other than TNF blocking agents (Etanercept, Infliximab, Adalimumab). Anakinra is not recommended for use in combination with TNF inhibitors because studies have shown increased infections without additive clinical benefit.

Anakinra blocks the biologic activity of IL-1 by binding to IL-1 receptor, thereby preventing binding of IL-1, and activation of the receptor.

The recommended dose of anakinra is 100 mg/day administered daily by subcutaneous injection. The dose should be administered at approximately the same

time each day. An auto-injection system is available for the medication. The usual time for a clinical effect to occur is 2 to 4 weeks.

The most commonly observed side effect with anakinra is injection site reactions, occurring in approximately two-thirds of patients. These reactions are present as erythema, itching, and discomfort and typically resolve over one to two months. In some patients these reactions can be severe leading to drug discontinuation. A modest increase in the risk of serious infection was observed in RA patients in clinical trials treated with anakinra in combination with DMARDS other than TNF inhibitors, compared to placebo with DMARDs (2 % vs 1%). The risk of serious infections of anakinra in combination with a TNF inhibitor is approximately 7% and this combination of biologics is not recommended. Opportunistic infections including tuberculosis are less common with anakinra than with TNF antagonists. Mild to moderate decreases in absolute neutrophil counts were seen more commonly in anakinra treated patients in clinical trials.

Newer areas of interest

Researchers at Imperial College have now identified a new immune trigger that may contribute to the pathology of RA. Tenascin-C is an extracellular matrix glycoprotein specifically expressed at areas of inflammation and tissue damage in inflamed rheumatoid joints. It was found that injection of tenascin-C into the joint cavity in mice caused severe joint inflammation and damage and also that mice lacking tenascin-C were protected from erosive arthritis. In cultures of cells from rheumatoid arthritis patients, tenascin-C induced synthesis of pro-inflammatory cytokines via activation of Toll-like receptor 4 (TLR4). TLR4 is one of a family of receptors that play a key role in pathogen recognition and activation of innate immunity. Stimulation of TLR4 is known to activate macrophages leading to release of TNF-a, one of the targets of existing biological agents used to treat RA patients. Previous studies had shown that mice lacking TLR4 do not show chronic joint inflammation, and blocking the interaction between tenascin-C and TLR4 may provide a new way to combat RA.

KEY LEARNING POINTS.

1. **TNF-a and IL-1 are both cytokines, which are proteins having multiple roles in the immune system, i.e. they are pleiotropic.**
2. **The primary source of cytokines are leukocytes, although they may also be produced by other cells.**
3. **TNF-a is involved in endothelial activation, fibroblast proliferation, induction of matrix metalloproteases, stimulation of interleukin production, and activation of osteoclasts.**
4. **IL-1 is involved with the regulation of acute inflammation, bone formation, insulin secretion, appetite regulation, and fever induction.**
5. **TNF-a and IL-1 are both directly involved in the pathological changes found in rheumatoid arthritis, and have been found to be particularly abundant in the synovial lining of joints.**
6. **The result of increase cytokine action in the joint is inflammation, synovial pannus formation, cartilage breakdown, and bone resorption.**
7. **Biologic drugs act by blocking the action of TNF-a and IL-1, either by directly preventing the increase of the cytokines within the joint, or by blocking the cytokine receptor sites, thereby preventing cytokine activation.**
8. **A common side-effect of all biologic drugs is that by dampening down the immune response, the patient is subject to an increased risk of infection.**