Understanding the Immune System

Introduction
The immune system defends us from infection. Infections are illnesses caused by the presence and multiplication of tiny organisms (micro-organisms) in our bodies. These micro-organisms come in many different forms and the threat that they pose to our survival thus demands many different defence mechanisms. Without any of the mechanisms of immune defence the best medical care cannot prevent death from infection for more than a week or two. This rarely occurs because there are so many parts to the immune system and although each of these is important its loss can be compensated for by the other parts. Our knowledge of the immune system has grown greatly over the last 30 years and is relevant to myeloma because:

1) Myeloma arises from bone marrow plasma cells and these are part of the immune system. Understanding normal bone marrow plasma cells can help us understand myeloma and develop new treatments.

2) Both myeloma and some of the therapies for it impair immune function and thus increase risk of infection – a significant cause of illness and death in myeloma. Knowledge of the mechanisms and consequences of this impaired immunity can help us prevent and/or manage infection which occurs in up to a quarter of individuals in the first few months from diagnosis.

3) The immune system has the potential to recognise and eliminate malignant cells; exploitation of this potential is one of the exciting new approaches to the treatment of myeloma

The aim of this article is to describe the immune system as a whole in health and disease with focus on the three areas stated above.

Threats and external defences
Our immune systems defend us against the threats of infection and cancer of our own cells. These threats come in many different forms requiring a variety of mechanisms of defence.

Viruses are about one millionth the size of a human cell; a human cell is one hundredth of a millimetre in diameter. Viruses cannot reproduce until they have infected one of our cells. For example a flu virus infects a cell lining the upper airways and uses that host cell's manufacturing capacity to make thousands more viruses that will then leave the host cell to infect others. Viruses enter host cells by molecules that dock onto the cell surface; one form of defence against viruses is to interfere with this docking mechanism by antibodies. Once inside a host cell the virus is hidden from most components of body defence and we rely on natural killer cells and cytotoxic T cells (described below) to combat the infection. Antibiotics have no effect on growth of viruses but for some types of virus there are now useful anti-viral agents eg acyclovir for the cold sore virus.

Bacteria and fungi are about one thousandth the size of a human cell and grow independently of host cells usually in the spaces and fluids between our cells. Some bacteria can divide into two bacteria every 20 minutes and so a single bacterium can become more than 15 million in 8 hours. This rapid division is why bacterial infections require prompt treatment (see summary). Bacteria and fungi damage us in many ways. One way is the secretion of toxins such as diphtheria toxin which was the commonest cause of childhood death before the advent of immunisation and antibiotics. Most bacteria can be fairly easily grown in the laboratory allowing identification of the cause
of infection and testing for sensitivity of the bacteria to antibiotics – an essential component to management of bacterial infections. There is a growing number of very effective anti-fungal agents.

**Mechanical barriers to infection** The skin provides a barrier; when this is intact it provides a relatively effective protection – but it is easily breached by cuts, burns, insect bites, skin diseases and doctors (eg. lines for venous access). The far larger absorptive (mucosal) surfaces of the intestine and lungs are inevitably more fragile because they have to allow nutrients and gases to pass through them. Moist mucus is effective at trapping small airborne dust particles and droplets containing bacteria or the spores of fungi. Cilia waft mucus and contained micro-organisms towards the main bronchi and trachea where it is removed by coughing. Previous lung infections can leave scarring that impairs this mechanism. Similarly in the gut, a single cell layer thick, mucus protects the intestinal wall and injury is rapidly repaired.

**Drainage and irrigation** Irrigation (tears, urine, saliva, bile, mucus and sebaceous secretions) are an important means by which infection is inhibited. These secretions contain substances that inhibit the growth of micro-organisms; lysozyme in tears is bactericidal and acid in the stomach helps sterilise food. Drainage of these secretions is important - for example blockage to the outflow of urine impairs renal function and there is a considerable danger that static urine will become infected.

**Normal bacterial flora** There are large numbers of bacteria and fungi (flora) on the skin, in the mouth and in the large intestine. Normally these are non-invasive micro-organisms that do not cause disease. The presence of this normal flora helps to inhibit invasion by harmful bacteria. Loss of the normal flora as a result of antibiotics or excessive use of antiseptic solutions can provide an opportunity for harmful bacteria to colonise the vacated surfaces. Overgrowth by fungi (oral or vaginal thrush) is commonly associated with the use of antibiotics which selectively kill the competing bacterial flora.

**Good public health and hygiene** Clean water supply, good nutrition and reasonable standards of hygiene in preparing food have made an immense contribution to the reduced incidence of infection and increased good health and longevity enjoyed by developed countries.

**Factors associated with increased susceptibility to infection in myeloma** Impairment of any of the protective systems listed above increases susceptibility to infection. This is particularly important for individuals with myeloma in whom the internal defences described below are often not working properly. A particular problem with myeloma is skeletal disease that can cause thoracic pain from ribs and vertebrae, and immobility. This impairs breathing, movement of mucous and coughing and thus increases risk of lung infection. Effective pain relief is very important but should not be such that it makes you sleepy and impairs the drive to deep breathing. Chemotherapy damages the repair processes at the mucosal surfaces of lungs and gut.

**Internal defences (innate and adaptive immunity)**
The surfaces of the body provide a barrier against infection. If these barriers are penetrated a wide variety of immune mechanisms utilising proteins and cells act to control and eradicate infection. These mechanisms are either innate (present from birth and unchanging) or adaptive (derived from selection and expansion of lymphocytes).
White blood cells are the cells of innate and adaptive immunity. All blood cells have a common origin from blood-forming stem cells. These stem cells give rise to red cells (prevent anaemia), platelets (prevent bleeding) and all the white cells that make up the different cell types of immunity. In contrast to red cells and platelets white blood cells are in transit to tissues and spend only a matter of hours or less in the blood. The main types of white blood cells are

Granulocytes; (also called polymorphs) these cells are part of innate immunity. There are three main types of granulocyte – basophils, neutrophils and eosinophils. Basophils give rise to mast cells which promote immune and inflammatory responses. Excessive activation of mast cells leads to symptoms that characterise allergic diseases such as hay fever and asthma. Eosinophils bind to and can kill the larval forms of certain parasitic worms and are also involved in allergic processes. Neutrophils seek out, phagocytose (engulf and eat) and kill (by digestion) rapidly dividing bacteria. Only a small proportion of people with myeloma have reduced numbers of neutrophils in their blood at diagnosis. However immediately following high dose chemotherapy production of blood cells is halted. While red cells and platelets can be replaced effectively by blood transfusion, the very short life-span of neutrophils makes replacement by transfusion generally impractical. In this period of few neutrophils in the blood (neutropenia) the individual is at high risk of developing septicaemia (rapidly dividing bacteria in the blood) and would require intravenous antibiotics until neutrophils numbers recover. Along with red cells and platelets, neutrophil numbers are measured in the Full Blood Count test – hence our desire to carry out FBCs so often in people receiving chemotherapy. Other treatments like steroids can impair neutrophil function.

Monocytes / macrophages and dendritic cells; these cells are found in all tissues and like neutrophils phagocytose micro-organisms and are part of the innate immune system but they also present material to lymphocytes providing a crucial link between innate and adaptive immunity. An important role of macrophages is to remove dying cells; if this capacity is exceeded when large numbers of neutrophils are combating bacteria at a site of infection then the dead neutrophils build up to form pus. The presence of pus indicates a bacterial infection. Neutrophils play little part in fighting viruses and there is thus little pus formation at the site of virus infection. Some bacteria, despite being phagocytosed by neutrophils and macrophages can evade the internal killing mechanisms of these phagocytes and actually divide inside them. These bacteria include salmonella and tuberculosis; they can only be eradicated with the help of T lymphocytes.

Natural Killer cells – NK cells are part of innate immunity, important to combating viral infections and cancer

Lymphocytes are the components of adaptive immunity and comprise T and B lymphocytes. The purpose of B lymphocytes is to evolve into plasma cells that secrete antibody

Innate versus adaptive immunity

The main components of innate immunity are summarised in the table below and together interact to prevent or eradicate infection by most infectious agents that might be encountered but not all. Different components of innate immunity interact with each other; for example the ability of neutrophils to recognise bacteria is enhanced if the bacteria are made more easily recognised by the presence of complement proteins.
<table>
<thead>
<tr>
<th>Component of non-adaptive immunity</th>
<th>Main mechanisms of action</th>
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<tbody>
<tr>
<td>Mast cells (concentrated under the body surfaces)</td>
<td>Release factors which increase blood flow and blood vessel permeability bringing components of immunity to the site of infection</td>
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<tr>
<td>Neutrophils and macrophages (phagocytes)</td>
<td>Engulf (phagocytose) and destroy micro-organisms</td>
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<tr>
<td>Eosinophils</td>
<td>Secrete factors which kill protozoa and worms</td>
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<tr>
<td>Natural killer cells</td>
<td>Destroy virus infected cells</td>
</tr>
<tr>
<td>Complement (a system of enzymes and control proteins)</td>
<td>Activate mast cells, attract phagocytes, make micro-organisms more easily recognised by phagocytes, directly kill micro-organisms</td>
</tr>
<tr>
<td>Acute phase proteins (C-reactive protein, Mannan binding protein)</td>
<td>Activate complement and attract phagocytes</td>
</tr>
<tr>
<td>Cytokines - interferon</td>
<td>Toxic to viruses</td>
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The other type of immunity is **adaptive immunity**; it is not effective when a new micro-organism is first encountered but over a period of a week or two adapts to provide new defence systems against that particular micro-organism. This newly learnt defence system is remembered providing defence against re-infection by that micro-organism. Adaptive immunity is totally derived from T and B lymphocytes and once induced can eliminate almost any infection.

**How innate and adaptive immune mechanisms recognise micro-organisms.**

The mechanisms by which the innate immune system recognises micro-organisms are based on less than a hundred receptors in the cellular and protein components of the innate immune system interacting with molecules on micro-organisms surfaces. The ability of any individual neutrophil to recognise a wide range of bacteria is identical to that of any other neutrophil and relies on a small range of receptors that can interact with a wide range of different micro-organisms.

- The efficiencies of these interactions are essential to ensure that micro-organisms are recognised and destroyed.
- But it is essential that these interactions are entirely confined to micro-organisms otherwise the innate immune system may make a mistake and attack ones own body.

The compromise between these two problems is that the innate immune system only recognises what is obviously different from self with the danger that micro-organisms may hide those differences thus requiring the more sophisticated recognition mechanisms of adaptive immunity. These are provided by T and B lymphocytes in the form of T and B cell receptors. The part of the micro-organism that these receptors bind to is called antigen and so they are sometimes called antigen specific receptors.

During the process of making lymphocytes from stem cells, lymphocytes rearrange genes for receptors that recognize antigen. This is a random process that generates an enormous diversity (10,000,000,000) of receptors from a few genes. Each lymphocyte bares...
receptors for only one target and can thus only recognize a few of the millions of different types of micro-organisms. However as there are more than $10^{11}$ lymphocytes in the human body the total lymphocyte pool contains cells with receptors for almost any micro-organism that could occur. Presented with a new micro-organism the frequency of lymphocytes that can recognize it is as low as one in a million but over a period of days each of these few lymphocytes proliferate to produce many thousands of progeny all bearing the same antigen receptor as the parent cell (a clone). These expanded clones of T and B lymphocytes baring receptors highly specific for a given micro-organism evoke efficient resolution of infection and prevent re-infection by the same micro-organism for many years (memory).

Because the antigen receptor gene rearrangement process is random, many of the lymphocytes produced in the body bare receptors that react with self; there are strict selection mechanisms to eliminate these self-reactive cells before they mature to effector cells (cells that can kill). **Lymphocytes undergo selection** as they develop and when they first express their receptors for antigen; some are positively selected for their potential to recognize non-self or altered self (tumour cells) – others that recognise normal self are eliminated. **If these self reactive cells are not eliminated autoimmune disease occurs.**

Selected lymphocytes do not distribute throughout the body, instead they use the blood and lymphatic vasculature as highways to recirculate between the different secondary lymphoid organs (lymph nodes, spleen, tonsils and mucosa associated lymphoid tissue). There are processes by which antigen is brought from the tissues to secondary lymphoid organs – for instance lymphatics carry antigen to lymph nodes. The few lymphocytes that recognize an arriving antigen, are activated to proliferate (clonal expansion) and differentiate to become effector cells of the immune system.

**Myeloma and normal plasma cells**

The part of the **B cell receptor that binds antigen is surface immunoglobulin (antibody).** Subsequent B cell proliferation and maturation generates plasma cells that secrete this antibody – this is the only effector function of B cells. Antibody neutralizes toxins (eg. from diphtheria and tetanus bacteria) and prevents viruses from docking onto our cells. Antibody also makes micro-organisms more easily recognized by neutrophils and other phagocytes and activates complement proteins that can be directly toxic to the micro-organism. There are different classes of antibody; immunoglobulin M (IgM) is made by plasma cells in the lymph nodes and spleen and is the first antibody class to be secreted in an antibody response. IgA is made by plasma cells in the mucosal surfaces and by plasma cells in the bone marrow. IgG is predominantly made by plasma cells in the bone marrow. All three classes of antibody are found in the blood and throughout the tissues. IgA and IgM are also secreted into the mucus at the mucosal surfaces, reinforcing that barrier to infection.

Bone marrow plasma cells are derived from B cell proliferation in the lymphoid organs, particularly in areas called germinal centers. It is perhaps the enormous extent to which B cells proliferate and clonally expand in germinal centers that predisposes to mistakes in replicating their DNA as the cells double in size and divide. It is these mistakes that give rise to malignant cells – **myeloma cells.** The changes in their DNA are retained by them
and there progeny and cause the clone to expand uncontrollably. The antibody that they secrete is unique to the clone and thus called **monoclonal antibody (also M-protein or paraprotein)**. The monoclonal antibody can only bind one very specific antigen and is useless in combating most micro-organisms.

**Myeloma and antibody deficiency**
Most people diagnosed with myeloma have a high blood level of monoclonal antibody from the myeloma clone and reduced levels of normal polyclonal antibodies that protect us from infection. Although that makes one susceptible to a wide range of bacteria and viruses, in practice it mainly increases risk of infection by particular types of bacteria. These are bacteria that encapsulate themselves in polysaccharide (sugar coat) and thereby are hidden from the innate immune system. That makes the production of antibody against the polysaccharide capsule essential to flag up the presence of the bacteria to the innate immune system that can only recognize the bacteria once antibody is bound to their surface. The commonest of these bacterial infections are pneumococcal and haemophilus influenzae bacteria along with E. coli. Lung and other respiratory tract infections are the main sites of these bacterial infections.

Total antibody levels and functional antibody levels (levels against particular micro-organisms) are usually measured at diagnosis, giving an assessment of the severity of the antibody deficiency. Some babies have an inherited total absence of antibody production with consequent recurrent, severe lung infections. This can be reversed by antibody replacement therapy. Consequently antibody replacement therapy has been tested in patients with myeloma. Unfortunately randomized placebo controlled trials have shown little benefit to giving antibody replacement therapy. This lack of benefit in myeloma may partly reflect the amounts and time to achieving therapeutic levels of antibody but mainly illustrates that many components of immunity are compromised in myeloma and addressing just one is not sufficient.

The range of types of bacteria that cause infection in myeloma are mostly susceptible to **prophylactic antibiotics (taken continuously to prevent bacterial infection)**. A small trial of prophylactic antibiotics early after diagnosis of myeloma has shown promise but requires confirmation in larger studies – one of which is on-going. **Vaccination** is unlikely to improve and may actually reduce immunity if given during chemotherapy. Once chemotherapy has finished vaccination against the flu virus is recommended; for other protein vaccines there is some evidence of their effectiveness whilst less so for pure polysaccharide vaccines – this requires further investigation.

**Myeloma and T cells- infection, transplantation and immunotherapy**
T cell receptors do not bind free antigen. The antigen must be first processed into small peptides by antigen presenting cells and then these peptides are displayed on the antigen presenting cell surface in the clefts of major histocompatibility complex (MHC) molecules. The T cell receptor recognizes this peptide/MHC complex which activates T cell effector function. The three types of T cell effector function are:-

- **Cytotoxic T cells** (CD8 positive) that recognise and destroy virus infected cells
- **T inflammatory cells** (CD4 positive T\(_H^1\)) that help macrophages in the eradication of intracellular infection like tuberculosis
- **T helper cells** (CD4 positive T\(_H^2\)) that help B cell responses to antigen

MHC molecules are classified as MHC Class I and MHC Class II molecules.
- CD8 T cells interact with MHC Class I to fight viral infection and cancer cells
- CD4 T cells interact with MHC Class II to help macrophages and B cells

MHC Class I molecules are found on the surface of most cells in the body and constantly present peptides derived from digestion of their own internal proteins; in this way cells that are infected by virus immediately advertise that infection by presenting viral peptides in MHC class I on their surface. This MHC Class I / viral peptide complex is recognised by CD8 positive cytotoxic T cells specific for that viral peptide. The cytotoxic T cell then induces the virus containing cell to commit suicide – a process called apoptosis. Through apoptosis the contained virus is eliminated, albeit at the expense of the host cell.

MHC class II molecules are restricted to a few cell types in the body (mainly macrophages and B cells) and present peptides derived from proteins internalised from outside of the cells. This MHC Class II peptide complex is recognised by CD4 positive T helper cells that are thus triggered to effector function
- CD4 T helper type 1 cells help macrophages eliminate bacteria that they have phagocytosed but cannot kill eg. TB
- CD4 T helper type 2 cells help B cells that have internalized antigen through their surface antibody proceed to proliferation and maturation to plasma cells

There is enormous variation (polymorphism) in the form of different individuals MHC molecules and thus their ability to bind antigens from particular micro-organisms. This largely accounts for the great variation in severity of infection between individuals infected by the same micro-organism. It is that variation that ensures that whatever new micro-organism appears or evolves some individuals will be able to resist it relatively easily and thus ensure survival of the species. It is also this variation in MHC molecules (also called human leukocyte antigens -HLA) and their key role in antigen presentation that renders them the main antigenic target for immune responses in organ and stem cell transplantation.

**General immunosuppression in myeloma** Reduced T cell immunity increases risk of infection by viruses and bacteria like TB that grow inside macrophages; it also reduces antibody responses. Myeloma itself reduces antibody responses much more than T cell function. However chemotherapy, high dose steroids and some of the newer biological agents do affect T cell function and can render individuals susceptible to viruses some bacteria and a variety of micro-organisms that can only cause infection when T cell immunity is suppressed (opportunistic infections). This susceptibility to infection is compounded and widened by the effects of these agents on the innate immune system as well. For people in whom myeloma has damaged kidney function there are two further problems. Firstly impaired kidney function in turn leads to a reduction in immune function. Secondly all patients with myeloma are at increased risk of dehydration which
is often triggered by infection. This can lead into a vicious circle because dehydration itself further damages kidney function.

**Vaccination against myeloma** Myeloma cells are different to normal plasma cells because of defects in their DNA that enable them to proliferate uncontrollably. These differences produce abnormal proteins mostly proteins inside the cell that normally control cell proliferation. In the same way that virus infected cells can advertise (in MHC class I molecules) to cytotoxic T cells that they contain viral proteins so myeloma cells can advertise to cytotoxic T cells that they contain ‘malignant proteins’ and should be destroyed. An alternative protein target is the antibody binding site for antigen (the idiotype) that is unique to the myeloma clone. An alternative strategy is to destroy all plasma cells – malignant myeloma cells and normal plasma cells alike. This is not as drastic an approach as it might seem because the normal polyclonal antibody lasts for several weeks and can be replaced with therapeutic antibody. Furthermore new polyclonal plasma cells will be generated from the B cell pool.

**Summary**

1. Rapidly improved understanding of how normal bone marrow plasma cells are made will provide new treatment strategies for myeloma
2. Myeloma and its treatment impair immunity which whilst in itself does not cause obvious symptoms it significantly increases the risk of infection
3. In myeloma our physical barriers against infection may be damaged both by the disease and the treatment for it. Deep breathing and ability to cough without pain are important in reducing the risk of lung infection
4. Viruses are the commonest cause of coughs and colds in people around us. White mucoid sputum indicates a viral infection whilst coloured, purulent sputum indicates a bacterial infection. In myeloma the greatest increase in risk of serious infection is for bacterial infection and when sputum is purulent antibiotics should be started immediately
5. It is important to drink plenty of fluid to prevent dehydration
6. Development of a fever requires an immediate visit to the hospital irrespective of time of day or night