



Clinical Diagnosis of Pathological Conditions using Cytokines



SureScreen Diagnostics Ltd
Cutting Edge Biotechnology

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In this bulletin we explore a relatively recent branch of medical diagnosis which monitors the chemical messengers in your body to determine what is going wrong with your health. These messengers, called **cytokines**, synergise the daily running of your body. An understanding of cytokine activity **helps us to identify a potential health problem, monitor the development of a disease and target it with the appropriate treatment.**

It is perhaps surprising that the human body is able to function at all. Far beyond the complexities of the most advanced technologies known to man, our body consists of around **50 trillion cells**, our gut is host to a **quadrillion bacteria** (that's a thousand billion, with typically 500 species present), and our brains contain more neural connections than **the number of all the atoms in the universe.**

Controlling this massive collection of cells is the most complicated biochemical factory imaginable, where raw materials (metals, non-metals, carbohydrates, proteins, vitamins and oxygen) are converted into complex chemicals and energy, at the same time generating waste as by-products (uric acid, solid waste and carbon dioxide). This process is aided by friendly bacteria in the gut, as well as outside stimuli such as sunshine.

Your body has the ability to manufacture many of the missing ingredients, but those key nutrients that cannot be made are classed as **'essential nutrients'** and they must be obtained from a dietary source.

CYTOKINES - OUR CHEMICAL MESSENGERS

This chemical factory is kept working efficiently by a series of chemical messengers that maintain the flow of information with each of the 50 trillion cells that make up a human being. These messengers are mostly **proteins, peptides or glycoproteins**, but because they pervade the whole body they are known collectively as **Cytokines** (from the Greek cyto = cell and kinos = moving).

ESSENTIAL AMINO ACIDS
Soleucine
Lysine
Leucine
Methionine
Phenylalanine
Threonine
Tryptophan
Valine

Hormones do a similar job as well, but as we learn more about the intricate detail of cell chemistry messengers, we have come to realise there is some overlap between what we know as cytokines and what we have traditionally considered as hormones.

The main difference is that hormones exist at the nano-molar level (that is, less than one hormone in a thousand million parts) that vary very little, while cytokine messengers are usually found at the picomolar level (that is, one cytokine in a million million parts) but can rapidly increase a thousand fold or more during disease or trauma. This can happen because hormones are made by specific organs, whereas virtually every nucleated cell in the body has the capacity to manufacture several cytokines itself.

This capacity to make cytokines can produce a fast response in the body. The sudden expansion of cytokines that occurs in trauma cases has a drastic and sometimes life-threatening effect, particularly where the resulting inflammation triggers even more cytokine activity in a **cytokine cascade**, which can be fatal.



Causes of cytokine cascades include infections, allergic reactions, cancer, and HIV complications, but can also include anaphylaxis from bee stings, peanut allergies and the like. The photograph to the left shows the dramatic effects of an allergic reaction to a peanut.

USING CYTOKINES FOR DIAGNOSIS

Your complex system of chemical messengers is finely tuned to ensure each of those 50 trillion cells receives optimum conditions to thrive and be replaced, but sometimes the system fails due principally to disease, stress, inadequate nutrition, cell damage or an error in converting a message into a particular cell action, known as faulty signal transduction. Such events can result in inflammation, cell damage or cancer. Sometimes these conditions can also trigger abnormal levels of other cytokines.

The study of cytokines is a recent development in disease diagnosis, and SureScreen's Jim Campbell attended the First Symposium on Cytokine Medicine, held in Manchester, in February 2003. Cytokine analysis shows considerable promise in **detecting a disease state or tracking its progress**, and some of the latest treatments aim to reduce or modify the levels of inflammatory cytokines in the body; especially those responsible for **rheumatoid arthritis, Crohn's disease** and so on.

WHICH CYTOKINES ARE INVOLVED IN DISEASE?

Leading clinical chemists from neuropharmacology are now dissecting this complex chemical factory to find trends that identify the cause of disease, its progression, and the monitoring of a cure. There are no bad cytokines, but there is unbalanced cytokine production which leads to disease development. For example, low production of Interferon gamma could be associated with depression and the accumulation of cancerous or infected cells. High production of Interleukin 17 is linked to the development of auto-immune disorders such as diabetes. High production of Interleukin 12 is the core of a number of inflammatory diseases such as rheumatoid arthritis and irritable bowel syndrome. Cytokines such as Interleukin 1b and Tumour Necrosis Factor alpha are involved in the development of sickness syndrome. Of the many chemical messengers in the body, three cytokines are proving to be very helpful in understanding life-threatening disease. These are **Bcl-2 gene expression, P53 gene expression and P53 protein level.**

'Three cytokines hold the key to understanding life threatening diseases'

THE CHEMICAL FACTORY

Before we can understand the importance of cytokines to our daily health, it is helpful to learn a new vocabulary. Understanding just a few of the terms used by a clinical biochemist will enlighten the uninitiated to take a peek into this fascinating subject, as well as providing

some key words for further study.

The term **Gene expression** often appears in cytokine analysis, but it simply means a product that is produced under the instruction of one of your genes. For example, proteins are produced in the cells according to the instructions of the related genes, from chemicals called amino acids. The human genome project has identified around 25,000 genes which we believe produce some one million proteins in your body. The study of proteins that a cell manufactures is called **proteomics**, and information about what genes do under various conditions is studied by a system of **expression profiling.**

The genetic code that your cells contain is known as **the genotype** of the cell. The genetic constitution of an organism is all that is required for it to replicate. On the other hand, traits that develop once they have been modified by your surroundings, diet, and lifestyle are referred to as the **phenotype.** These environmental pressures can result in diversity between organisms that are not genetic, for example the variations in bird nest building techniques, or the variety of blood groups found in human populations. It is not often appreciated that these external factors significantly affect your health through the manipulation of the genes and cytokines that control your cells.

ESSENTIAL ELEMENTS
Calcium (Ca)
Chloride (Cl)
Chromium (Cr)
Cobalt (Co)
Copper (Cu)
Iodine (I)
Iron (Fe)
Magnesium (Mg)
Manganese (Mn)
Molybdenum (Mo)
Nickel (Ni)
Phosphorus (P)
Potassium (K)
Selenium (Se)
Sodium (Na)
Sulphur (S)
Zinc (Zn)

These variations can develop as a result of **wild gene isoforms** which are very minor mutations of your normal genes. Gardeners refer to these minor mutations as 'a sport' or deviation, and they might for example affect flower shape or colour. Mutations occur all the time, in fact viruses play an important part in maintaining genetic diversity by implanting pieces of DNA into their host cells.

As your 50 trillion cells age, they are programmed to self destruct and initiate a repair procedure which ensures they are regenerated with cells having the same genetic code. This programmed cell death is called **apoptosis.** Between 50 and 60 billion cells in your body die each day and are usually replaced by new cells that are copies of their originals. But trivial deviations occur and form part of the ageing proc-

ess. **Defective apoptotic processes** have been implicated in an extensive variety of diseases. Excessive apoptosis causes atrophy or wasting, whereas an insufficient amount results in uncontrolled cell proliferation, causing growth defects **such as cancer**.

Carcinogenesis or **oncogenesis** literally mean the development of cancer cells. Mutation of the genetic material in any of the 50 billion cells that are replaced in your body daily might result in uncontrolled cell division and the rapid proliferation of cells resulting in **benign tumours**. More than one mutation is necessary for carcinogenesis. In fact, a series of several mutations to certain classes of genes is usually required before a normal cell will transform into a cancer cell. Only mutations in those certain types of genes which play vital roles in cell division, apoptosis, and DNA repair will cause a cell to lose control of its cell proliferation. Cytokines control the whole process and trigger a mop-up action as defective cells are identified and are absorbed by white blood cells, called **phagocytes**.

Benign tumours do not spread to other parts of the body or invade other tissues, and they are rarely a threat to life unless they compress vital structures or are physiologically active, for instance, those parts of the body producing a hormone. When the proliferation continues out of control **malignant tumours** form which can invade other organs, spread to distant locations in a process known as **metastasis** and become life-threatening. This is the process generally referred to by the public as **cancer** and by doctors as a **malignant neoplasm**.

CELL DEATH PROGRAMMING

Apoptosis is controlled by regulators which have either an inhibitory effect on programmed cell death (anti-apoptotic) or block the protective effect of inhibitors (pro-apoptotic). Of these, **the Bcl-2 cytokines** are some of the most important. **There are pro-apoptotic Bcl-2 family proteins such as Bax, Bik, Bad, Bid and Bim. There are also anti-apoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-X_y**

ESSENTIAL VITAMINS

- Vitamin A (retinol)
- Vitamin Bp (choline)
- Vitamin B₁ (thiamin)
- Vitamin B₂ (riboflavin)
- Vitamin B₃ (niacin)
- Vitamin B₄ (adenine)
- Vitamin B₅ (pantothenic acid)
- Vitamin B₆ (pyridoxine)
- Vitamin B₇ (biotin)
- Vitamin B₉ (folic acid)
- Vitamin B₁₂ (cobalamin)
- Vitamin C (ascorbic acid)
- Vitamin D (calciferol)
- Vitamin E (tocopherol)
- Vitamin K (naphthaquinoids)

Tumour protein 53, or p53, is a tumour suppressor protein controlled by the human *TP53* gene located on chromosome 17. *p53* regulates the cell cycle and it has been described as "The Guardian of the Genome", the "Guardian Angel Gene", and the "Master Watchman", referring to its role in conserving stability by preventing genome mutation.

If the *p53* gene becomes non-active, tumour suppression is severely reduced. People who inherit only one functional copy of the *p53* gene may develop tumours in early adulthood, a disease known as **Li-Fraumeni syndrome**. The *p53* gene can also be damaged in cells by mutagens (chemicals, radiation, or viruses), increasing the likelihood that cell division get out of control. **More than 50 percent of human tumours** contain a mutation or deletion of the *p53* gene. Analysing blood to determine its status is therefore very useful.

Increasing the amount of *p53* might seem a good way to treat cancer or prevent it from spreading, but it can cause premature aging. Loss of *p53* creates genomic instability that most often results in aneuploidy (chromosome abnormality). Certain pathogens can also affect the *p53* protein that the *TP53* gene expresses. One such example is the **human papilloma virus (HPV)**. There are almost 200 types of HPV and some cause warts while others are implicated in cancers of the cervix and penis.

So, armed with this information, we can start to explore some of these markers and how they might be used to detect disease, record its progress, and monitor the success of treatment. The following is just a selection of what is currently available to monitor disease in this way by our laboratory, and each patient might be offered a selection of analytical techniques to monitor their own individual case.

Three assays have shown to be very valuable in this process and will usually be included in any testing regime.

Anti - apoptotic Bcl - 2/ Pro - apoptotic Bax Genes Expression

An indicator of the ability of cancer and other abnormal cells to survive.

This blood test shows the likelihood of tumour growth without destruction by measuring the ratio of the anti-apoptotic Bcl - 2 against pro-apoptotic Bax genes expression. Protein Bcl - 2 inhibits apoptosis, (programmed cell death), by preventing the release of apoptosis inducing factor (AIF), and cytochrome C from mitochondria. Protein Bax promotes apoptosis by triggering the release of AIF and cytochrome C from mitochondria. If the Bcl - 2 level is higher than the Bax level, then apoptosis should be prevented. The ratio of Bcl - 2 to Bax in the cell can determine whether or not the cell initiates apoptosis or survives. Many cancer cells over express Bcl - 2, thus preventing apoptosis, allowing malignant growth to continue.

Anti p53 Antibodies

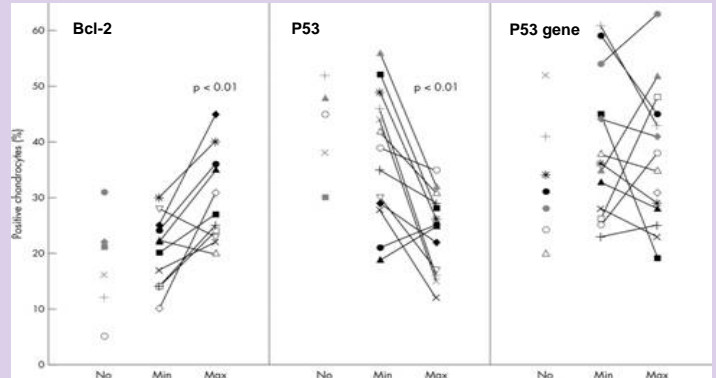
An indicator of the ability of cancer cells to survive.

This blood assay is able to provide information relating to the presence of a wild, mutated or deleted *p53* gene, which is associated with the suppression of tumour development.

p53 is a tumour suppressor gene that functions as an inducer of apoptosis and is a regulator of the Bcl-2 gene, which expresses protein inhibiting programmed cancer cell death. In cancerous cells, the *p53* gene is one of the most mutated genes that express mutant forms of the *p53* protein in the nucleus. This mutant *p53* protein is not able to induce apoptosis of cancerous cells and they will continue to divide, thus increasing cancer growth. Also, cancerous cells with a deleted (absent, for unknown reasons) *p53* gene will continue to proliferate, thus increasing cancer growth.

TYPICAL RESULTS AND INTERPRETATIONS

The higher the level of Bcl-2, the greater the risk of cancerous cell development, requiring a corrective programme to decrease Bcl-2 levels. Increased



levels of *p53* good protein (wild) shows that appropriate treatment will be effective or may be unnecessary, while higher levels of mutated *p53* protein means that treatment is necessary. A *p53* gene expression will show if a correction programme is necessary to activate the *p53* gene by suitable medication.

These analyses provide a valuable insight into the chemical imbalances that might be driving a tumour and reducing the ability for cells to undergo apoptosis, since cancer must start by the formation of a precancerous lesion. There are a number of other cytokines that may prove beneficial in monitoring disease. Some of these are described below.

C - Reactive Protein (CRP) Level

A marker of the response to infected or impaired cells and tissue.

This assay is able to provide information related to the presence of active inflammation or tissue destruction.

CRP is a protein which is not normally present in blood to any great extent. However, elevated levels of CRP have been found in the blood in virtually all diseases associated with active inflammation or tissue destruction. Rheumatoid diseases, acute infectious processes, advanced and wide spread malignancy, post myocardial infarction or surgery are particularly characterised by high levels of CRP.

Since CRP is at a maximum in the active stage of inflammation, and rapidly decreases during recovery, the estimation of CRP is of considerable prognostic value. **CRP is considered to be one of the best markers for heart disease and monitoring during and after a heart attack.**

Fas Ligand (Fas L) Gene Expression

An indicator of the ability of cancer cells to destroy cells of the immune system.

This assay is able to obtain information related to the cancer cell defence mechanism protecting against its own death. Fas Ligand is the special protein molecule, which is able to trigger apoptosis in the Fas sensitive cells. The immune system T cells and B cells, as well as Natural Killer (NK) cells are Fas sensitive cells.

On their surface there is a special receptor molecule called Fas or CD95. When Fas Ligand interacts with the Fas receptor, the destructive mechanism of the immune system cells is triggered. Activated T cells, B cells and NK cells have an increased number of Fas receptors, which make them more Fas sensitive. There is evidence that some cancer cells produce significant amounts of Fas Ligand. This is able to trigger destruction of the cells of the immune system, which would normally attack cancer cells. This means that cancer cells avoid destruction whilst some of the cells of the immune system could be destroyed. This phenomenon is known as the 'immune escape' of cancer cells.

Interferon Gamma (IFN - gamma) Gene Expression

A marker for the presence of Natural Killer (NK) and Th1 cells.

This assay is able to assist in the evaluation of the processes relating to the destruction of cancer and other abnormal cells. It has been designed to estimate the Interferon Gamma gene expression as part of the characterisation of the state of the defence system.

Production of IFN - gamma is a function of NK cells and T cells (both cytotoxic and the Th1 type). Interferon gamma is a lymphokine which has a number of properties such as anti-viral and anti-proliferative. It is able to activate the macrophages in order to destroy tumour cells by releasing reactive oxygen intermediates and Tumour Necrosis Factor-alpha. In addition, IFN-gamma is capable of down regulating (inhibiting) anti-apoptotic Bcl-2 protein followed by the induction of cell destruction.

'50 – 60 billion cells die every day and are replaced by new copies'

Interleukin 1 Beta (IL-1beta) Gene Expression
A marker of the presence of macrophages, Th1 cells, inflammation and oxidative stress.

The interleukins are a special group of cytokines that are produced by leukocytes, the white cells in your body that are your primary defence system. This assay is able to provide information related to the activation of cellular immunity. It is also designed to estimate the risk of inflammation and oxidative stress. IL-1beta, originally known as Lymphocyte Activating Factor (LAF), activates T lymphocytes, which then proliferate and secrete

Interleukin 2. IL-1beta is also known as endogenous pyrogen is able to mediate oxidative stress and is produced mainly by macro phages and monocytes.

Interleukin 2 (IL-2) Gene Expression
A marker of the presence of naive T and Th1 cells.

This assay is able to assist in the estimation of specific immune response to cancer (anti-tumour immunity) and also various types of pathological conditions. It is designed to estimate the Interleukin – 2 gene expression as part of the characterisation of the Th1/Th2 immunological balance.

IL-2 is produced by the embryo CD4+ T cells and Th1 cells and is a cytokine, which plays a central role in immune responsiveness by promoting the activation and proliferation of lymphocytes, that have been primed by antigens (antigen specific T – lymphocyte proliferation). IL-2 also stimulates the proliferation of B cells, augments NK cell activity and inhibits granulocyte macrophage colony

IL – 5 is a cytokine, that promotes the development and the activation of eosinophils. It is produced mainly by Th2 cells, in response to the stimulation from parasite derived antigens and allergens. In addition, various transformed B cells, Reed – Sternberg cells in Hodgkin's disease and activated eosinophils can express IL – 5. Some inflammatory processes (e.g. asthma or various allergic responses) are associated with Th2 cell activation followed by an elevated production of Interleukin 5, together with Interleukin – 6.

Interleukin 6 (IL – 6) Gene Expression
A marker of infection or trauma.

This assay is able to provide information relating to both viral and bacterial infections, trauma and cancer development.

IL – 6 is a cytokine, which is produced by a variety of cells, including macrophages and Th2 cells. Normal cells do not produce this cytokine constitutively, but its expression is induced by a variety of cytokines, bacterial or viral infections. IL – 6 has numerous properties, it acts on a wide range of tissues, exerting growth-induction, growth-inhibition or differentiation, depending on the nature of the target cells. IL – 6 is involved in other important processes, such as the proliferation of T cells, the induction of IL – 2 expression and B cell differentiation. The changed expression of IL – 6 is associated with infections, autoimmune, proliferative and neoplastic diseases and inflammatory responses to infections or trauma.

Interleukin 10 (IL – 10) Gene Expression
A marker of cellular immunosuppression and infection.

This assay is able to assist in the estimation of the level of Interleukin – 10 gene expression as part of the characterisation of the Th1/Th2 immunological balance.

IL – 10 is a cytokine, that is produced by the regulatory CD4T cells and has the ability to suppress the functions of macrophages, Th1 cells and NK cells. It is associated with the promotion of Th2 cell development and consequently with the regulation, proliferation and differentiation of B cells.

IL – 10 has immunosuppressive properties by inhibiting the production of Th1 specific cytokines and the maturation of dendritic cells and is able to exert strong anti – inflammatory activities. The expression has been shown to be elevated in parasitic infections (such as schistosoma mansoni, leishmania, toxoplasma gondii and trypanosoma), mycobacterial infections (such as mycobacterium leprae, mycobacterium tuberculosis and mycobacterium avium) and retroviral infections.

'Supplementation with minerals is cost effective but often Ignored'

Matrix Metalloproteinase 2 (MMP – 2) Gene Expression

An indicator of the ability of cancer to be invasive and form metastasis. This assay is able to assist in the prognosis of the progression of cancer.

Matrix metalloproteinases (MMP's) are a family of zinc dependent endopeptidases, which can degrade the major components of the extra-cellular matrix. Cancer cells subvert MMP's activity to promote invasion of the surrounding tissues, as well as metastasis to distant tissues. MMP's by releasing growth factors sequestered in the extra-cellular matrix are also thought to promote the growth of these tumour cells once they are changed. The inhibition of one of the MMP enzymes can lead to the dissemination of cancer, in other words the suppression of its progression and metastasis.

Interleukin – 12 (IL – 12) Gene Expression.

A marker of the presence of B cells, macrophages and possible stimulation of Th1 cells.

This assay is able to assist in the estimation of the formation of the anti-tumour immunity and also to provide information related to the presence of bacterial and viral infections. It is designed to estimate the Interleukin – 12 gene expression as part of the characterisation of the Th1/Th2 immunological balance.

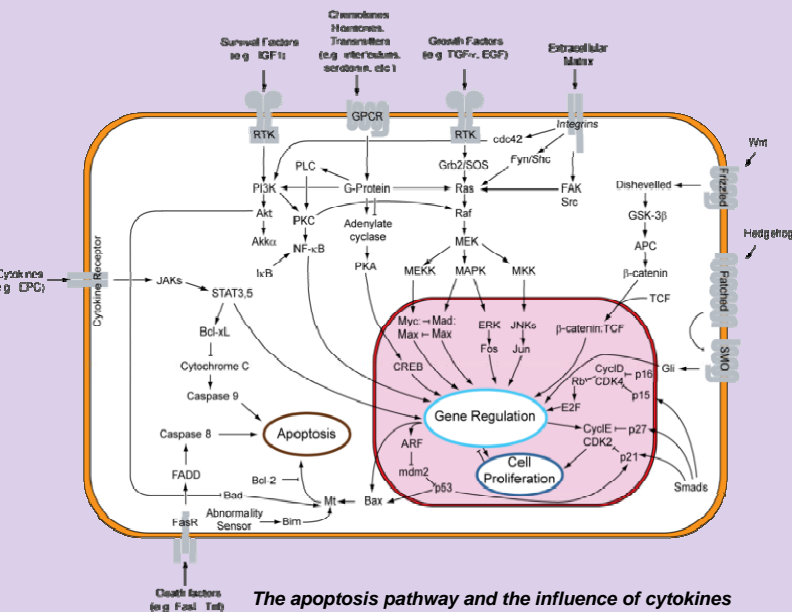
IL –12, which is also known as the natural killer cell stimulatory factor or the cytotoxic lymphocyte maturation factor, is a cytokine which can have multiple effects upon T cells and NK cells. IL –12, produced by macrophages, is associated with the promotion of Th1 cell development, followed by cell – mediated immune response. In addition, IL –12 has been shown to be a pro – inflammatory cytokine produced by phagocytic cells, B cells and some anti-gen – presenting cells that modulate adaptive immune responses.

The critical role of Interleukin 12 in several pathologies has been demonstrated. Significant elevations were measured in autistic patients and sufferers of multiple sclerosis. Auto-immune diseases, cancer and chronic inflammatory reactions can all be accompanied by an increase in the level of IL – 12. Additionally, a large number of bacterial & viral infections are capable of changing the expressed levels of Interleukin 12.

Soluble p185 Her – 2 Protein Level

An indicator of the ability of cancer cells to metastasize.

This assay is able to provide information related to very early oncogenic changes and is able to assist in the monitoring of tumour spread, post-operative relapse and metastatic risk.



formation. IL-2 displays anti-tumoural effects. Researchers in Russia have findings showing that after 3 months of injecting IL-2 into lung tumours, the cells were eradicated naturally by the body.

Interleukin 4 (IL-4) Gene Expression
A marker of the presence of naive T and Th2 cells

This assay is able to assist in the estimation of factors preventing the formation of the anti-tumour immunity and also to provide information related to the presence of some bacterial infections. It is designed to estimate the Interleukin 4 gene expression as part of the characterisation of the Th1/Th2 immunological balance.

IL-4 is produced mainly by the Th2 cells, as well as Mast cells. This cytokine exerts numerous effects on various haematopoietic cell types. It can promote survival, growth and differentiation of both T and B lymphocytes, mast cells and endothelial cells. In addition, IL-4 can inhibit the production of Tumour necrosis factor – alpha and Interleukins 1 and 6, by macrophages.

Interleukin – 5 (IL – 5) Gene Expression
A marker of the presence Th2 cells.

This assay is able to assist in the estimation of the factors preventing formation of the anti-tumour immunity and also to provide information related to the presence of parasite infections or allergens. It is designed to estimate the Interleukin – 5 gene expression as part of the characterisation of the Th1/Th2 immunological balance.

p185 protein is a transmembrane growth factor receptor, which belongs to a class of oncogenes related to tyrosine protein kinase, which possess tumourigenic or transformation activity. It is involved in all cell growth and cell transformation. It is possible to detect increased levels of p185 in the blood of individuals who will subsequently develop cancer up to 60 months before clinical diagnosis.

Survivin Level

A marker of the ability of cancer cells to inhibit their own death.

This assay is able to assist in the estimation of cancer cells to survive the normal destructive process and to continue the growth process.

Survivin is a protein, which is produced in many fetal tissues, such as the kidneys, liver, lungs and brain in addition to transformed cell lines and most cancers. Survivin is not found in terminally differentiated adult tissues. Whilst a critical role for Survivin in tumour growth has been established, overproduction of Survivin in rapidly growing cells may explain its abundance in tumours.

Transforming Growth Factor – Beta (TGF - beta) Gene Expression

A marker of the presence of cells with immunosuppressive properties.

This assay is able to assist in the estimation of the level of TGF - b gene expression as part of the characterization of the state of the defence system.

TGF - beta is a multi-functional protein, which is produced mainly by mammalian cells. TGF - beta is capable of influencing differentiation, proliferation and a variety of cellular functions. This protein generally slows immunosuppressive and anti-inflammatory activities but is able to stimulate the macrophages and monocytes. TGF - beta overproduction is linked to tumour growth, perhaps as an indirect result of its other actions.

Tumour M2 – Pyruvate Kinase (TM2 – PK)

A marker of active tumour cells.

Pyruvate Kinase (PK) or pyruvic acid kinase is an enzyme which catalyses the transfer of a phosphate group from phosphoenolpyruvic acid to adenosine diphosphate (ADP) with the formation of an 'energy' molecule – adenosine triphosphate (ATP). This reaction is very important in the glycolytic pathway. This enzyme has an absolute requirement for magnesium and is inhibited by calcium. Whilst the conventional form of pyruvate kinase is a tetrameric molecule, a dimeric isoform of PK is over produced by a wide range of different tumours. This appears to be linked to the different metabolic requirements shown by tumour cells, which leads to these cells using a metabolic shortcut to 'save energy' for cell multiplication. In this process the proportion of pyruvate kinase present as the dimer form is increased. Therefore the level of the dimer, namely Tumour M2 – PK, is significantly increased in tumour cells.

In rare cases however, a somewhat increased TM2 – PK level could be associated with the presence of impaired glucose utilization which may not be cancer cells, eg. chronic inflammatory processes such as diabetic nephritis.

Tumour Necrosis Factor – Alpha (TNF - alpha) Gene Expression

A marker of the presence of macrophages, Th1 cells and neurotoxicity.

This assay is able to assist in the estimation of the formation of anti-tumour immunity and also to provide information related to the presence of pathogenic micro-organisms and inflammatory processes. It is designed to measure the tumour necrosis factor – alpha gene expression as part of the characterisation of the state of the defence system.

TNF - alpha, also known as cachetin, is a cytokine which is produced mainly by monocytes and macrophages. Endotoxins and Interferon – gamma promote the production of TNF - alpha and it functions as a modulator of the immune response by activating dendritic and Th1 cells. It may play a role in the pathogenesis of many disease states and, in particular, inflammatory diseases. In high quantities, this cytokine acts as a pyrogen and neurotoxin by increasing the permeability of the blood/brain barrier and destroying the serotonin containing cells of the nervous system.

TNF-alpha is responsible for the inflammatory response in rheumatoid arthritis and Crohn's disease.

Telomerase - Number of Cells with Active Telomerase

An indicator of the presence of cancer cells or their precursors

This assay is able to provide information related to the very early oncogenic changes prior to the definitive formation of any tumour and clinical diagnosis. When the tumour is formed, this assay is able to assist in the prognosis of cancer regression or progression.

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Specific structures found at the ends of chromosomes in eukaryotes are known as telomeres. These telomeres protect chromosome ends from undergoing fusion, rearrangement and translocation. In somatic cells the telomere length is progressively shortened with each division due to the inability of the DNA polymerase complex to replicate the 5' end of the lagging strand.

Telomerase is a unique enzyme, which is a ribonucleoprotein that synthesises and directs the telomeric repeats on to the 3' end of existing telomeres using its RNA component. Telomerase activity becomes suppressed through the ageing process but activation of telomerase is regarded as essential to most cancer.

Telomerase activity has been shown to be specifically expressed in immortal cells, cancer and germ cells where it compensates for telomere shortening during its DNA replication and this stabilises telomere length. The expression of telomerase activity in cancer cells is a necessary step for tumour development and growth. This means that there is a specific association of human telomerase activity with cancer and it is usually high in cancer patients. It is considered that the regression of telomerase activity could be one of the mechanisms for cancer regression.

Low telomerase activity in the blood of cancer patients can be associated with the tumour being in an encapsulated form, remission of cancer and the inability to create tumour immunogenicity, eg. recognition of cancer cells by the immune cells. High telomerase activity in the blood of cancer patients can be associated with a risk of further tumour development, the destruction of the tumour during effective treatment and two to four weeks after such a treatment.

To correlate the level of telomerase activity with one of these indicators, additional information is necessary. The special packages for cancer (p 4) are able to provide this information.

- The concentration of total high molecular protein.
- The concentration of 'bonded' protein which could be part of the antibody – antigen complexes, which result from immunostimulation or could be associated with adhesion molecules involved in the cells aggregation.
- The concentration of total small peptides in blood.

The latter is of particular importance as some of these peptides can have a negative affect upon the nervous and immune systems. Total peptides can serve as an indicator of gut problems.

Tumour Necrosis Factor – Beta (TNF - beta) Gene Expression

A marker of the presence of Th1 cells.

This assay is able to assist in the estimation of the formation of cell-mediated immunity. The tumour necrosis factor - beta (TNF - beta), also known as lymphotoxin alpha, and the tumour necrosis factor - alpha are two closely related proteins that bind to the same cell surface receptor and show many common biological functions.

TNF - beta plays a central role in lymphoid development and in normal host resistance to infection and to the growth of malignant tumours, serving as an immunostimulant and as a mediator of the inflammatory response. It is produced by activated T, B and NK cells, astrocytes and human myeloma cells. Excessive production has been found to play a very significant role in a number of autoimmune disorders including multiple sclerosis, insulin dependent diabetes and rheumatoid arthritis.

CONCLUSIONS

Our knowledge of cytokines and their role in the metabolic pathway of cancer and other diseases is still relatively recent, but already their **analysis provides a valuable insight into disease and its progression.** These analyses can be provided by our laboratory, accompanied by their interpretation, meaning cell activity and associated illnesses can be detected and monitored in far greater depth compared to traditional methods. We see Cytokines as the next break through in monitoring health, whether you are a clinician establishing the condition of a patient or a private individual monitoring their condition.

Next issue – Urinalysis

Peanut allergy photo courtesy of Dr. H Morrow-Brown from his website: www.allergiesexplained.com maintained by SureScreen.

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