Inflammation, the Immune System and Cancer

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We present an idiosyncratic analysis of inflammation and its interaction with the immune system and resulting cancerous changes. We focus on two elements; pentraxin 3 and the bromodomain, BET. The analysis attempts to summarize the impact of human environmentally generated chronic inflammation, such as that which results from obesity in Type 2 Diabetes, and the increased incidence of cancers. We do not attempt to present anything new but do try to connects a set of diverse dots in this field.

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1 INTRODUCTION

It is well know that inflammation is related to a multiplicity of cancers. Further many cancers are now treated by various immunotherapeutic means. In this note we examine inflammation in more detail and then focus on the actions of the innate immune system as both a factor in certain inflammatory cancers as well as a means to combat these cancers. The focus on the immune system is on the innate side, since this generally is the side where the fastest response is. This is in contrast to the significant progress on the use of the adaptive elements and the addition of T cell elements such as CAR-T cells or the use of Mabs for blocking such as PD-1 and T cell attacks.

We will focus this paper around a paper by Stallone et al. The authors note:

Pentraxin-3 (PTX3) is a member of the pentraxin family of innate immune regulators which includes C-reactive protein (CRP). PTX3 has been implicated in angiogenesis, proliferation and immune escape in cancer. In the present study, we evaluated PTX3 tissue expression and serum concentration as a biomarker to discriminate prostatic inflammation and benign prostatic hyperplasia (BPH) from prostate cancer (PCa), and to determine whether PTX3 status may predict progression from BPH to PCa. …We found reduced PTX3 tissue expression in patients with prostatic inflammation/BPH compared to patients who developed PCa.

In the latter group, there was an increase in PTX3 tissue expression between the first and second prostate biopsy. PTX3 serum levels were also higher in patients with PCa than in patients with inflammation/BPH. In contrast, there was no difference in serum PSA or CRP levels in these two groups. ROC curve analysis confirmed the reliability of PTX3 serum levels in predicting PCa development, identifying a cut-off value of 3.25 ng/ml with a sensitivity and a specificity of 89.3 and 88.5%, respectively. In summary, our results encourage further evaluation of PTX3 as a tissue biopsy and blood-borne biomarker to discriminate BPH from PCa.

We chose this as a reference point because it starts the process of examining several key factors:

1. PSA has taken a continual beating for its lack of specificity and sensitivity. Although it has managed to survive the endless assault by Government entities, it does have weaknesses.

2. BPH is generally accepted to be an inflammatory response and there is also the understanding that PCa is likewise. Where one sees progression from inflammation to malignancy, this is not always inevitable or even justified. For example, High Grade PIN has been assumed to be a progenitor of PCa. Yet we have shown that it can easily disappear and not turn into PCa in a reasonable time period, say ten years. Thus inflammation is suspect but not dispositive to cancers.

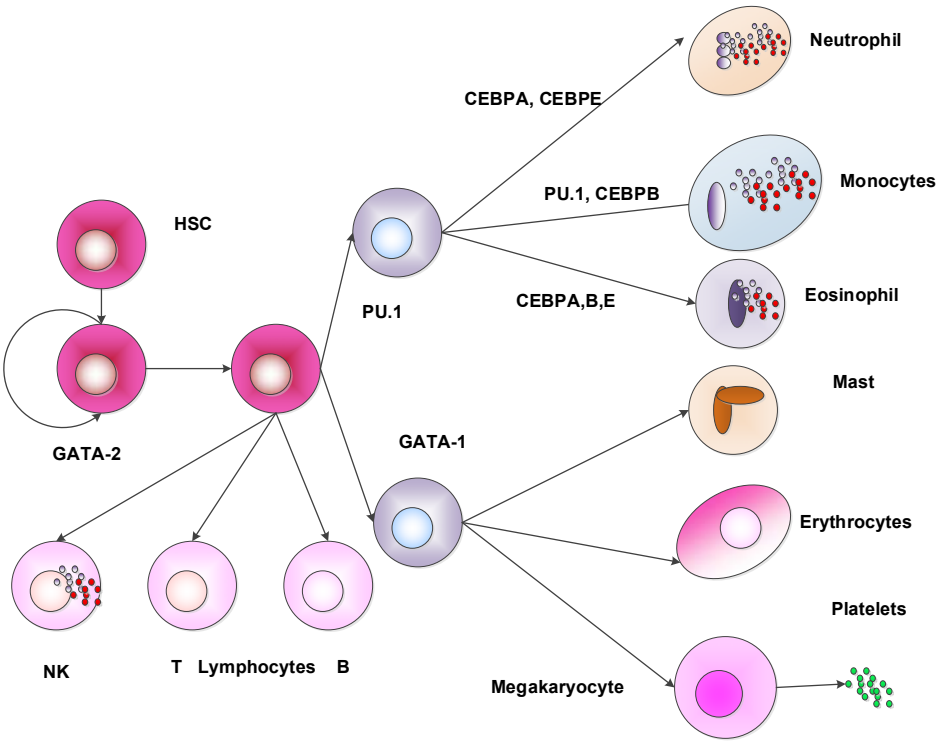
3. This referred to work focuses on the innate immune system, especially the humoral leg, and specifically pentraxins. Thus the work opens the windows on elements for which we have seen relatively little work.

4. Finally, in examining inflammation and the innate system as a cause, we also examine it as a tool to address a multiplicity of cancers.

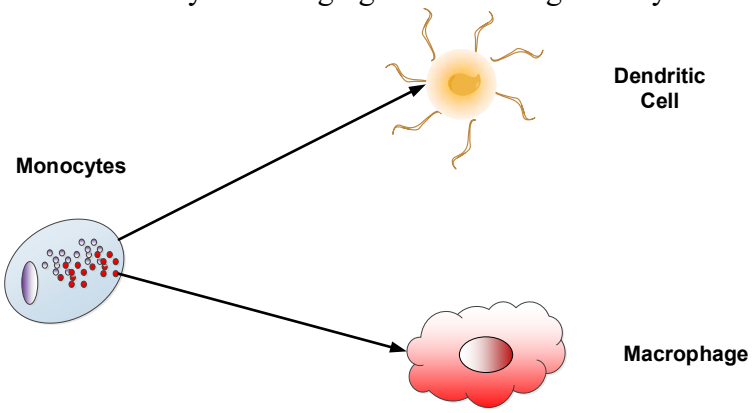
Yet we have also included a brief discussion on the bromo domain epigenetic factors which demonstrate another inflammatory element in malignant initiation.

1.1 Blood Cells and the Immune Elements

We start with a simple review of the primary blood cells and do this to set up a basis for the cell based elements in the innate system. In the adaptive system we have principally the B and T cells. They utilize the antigen presenting capabilities of the other cells and then via the B cells ability to deliver antibodies can then go on the attack using a variety of chemical drivers. In contrast the innate cells, those in the cellular part of the innate immune system go on the hunt alone. Such cells as NK cells, neutrophils and dendritic cells. We have seen the dendritic cells used in certain cancers such as prostate and the NK cells used in what is called the cytokine induced killer cells, CIK, in MDS, a hematological malignancy. Thus there is a developing cellular set of tools available. In contrast we also have the humoral part of the innate system, with such elements as the complement system, pattern recognition receptors and pentraxins.



Monocytes themselves subdivide into dendritic cells and macrophages, which are like "hunter-gatherers" working the human body and bringing back the antigens they find.



1.2 INFLAMMATION

Inflammation is the response of various cells to the presence of some factor which is deemed foreign and putatively a threat. As we will note, the human immune system responds in both the innate and adaptive manner to any perceived threat by a plethora of means. It sends out chemicals to attack the perceived invader and ultimately tries to identify the invader, kill it off, while remembering what it looked like so that the next time it can respond more quickly. Sometimes it works and other times it does not. But simply stated inflammation is the response of the immune system in some manner to something which activates it. This may sound a bit like circular reasoning by defining the process by its very existence, but that is simply what it is.

The problem with inflammation is that it can also cause more problems than what it solves. For example, H pylori can cause an inflammatory response in the stomach and the consequence is a MALT neoplasia, a cancer, resulting from this prolonged inflammatory response. Inflammatory responses release a variety of molecules whose goal is ultimately to rid the body of the invader. However, if a chronic situation is created where the invader is subdued but persists, the immune system can be keep in an "on" state resulting in the lasting presence of the secretions meant to kill off the invader. These powerful substances can then result in the activation or repression of homeostatic pathways resulting in the development and spread of neoplasia.[1]

[1] From Doan, Immunology 2nd Ed, Lippincott (New York), 2013

A. Cytokines: Low-molecular-weight soluble protein messengers that are involved in all aspects of the innate and adaptive immune response, including cellular growth and differentiation, inflammation, and repair. Originally called lymphokines and monokines to reject lymphocytic or monocytic origin, we now recognize that these substances are produced by a wide variety of leukocytes and non-leukocytes. A large number of cytokines have been identified, although the roles of many of them are not yet fully understood. Many cytokines are crucial in regulating lymphocyte development and in determining the types of immune responses evoked by specific responses.

1.3 INFLAMMATION AND CANCER

Inflammation may result in the formation of additional cells as well as the release of a variety of signalling elements. This release and activation can often set the path for malignant growth as well. Thus understanding inflammation is essential to understanding the development of many cancers.

As noted by Aggarwal et al:

Cancer is now generally believed to be a preventable disease. Only 5% to 10% of all cancers are caused by inheritance of mutated genes and somatic mutations, whereas the remaining 90% to 95% has been linked to lifestyle factors and environment (1). Almost 30% of all cancers have been attributed to tobacco smoke, 35% to diet, 14% to 20% to obesity, 18% to infections, and 7% to radiation and environmental pollutants. The underlying mechanisms by which these risk factors induce cancer are becoming increasingly evident. One process that seems to be common to all these risk factors is inflammation.

Inflammation is also common whenever cancer is seen or first discovered,

From Kundu et al:

Infection	**Cancer**
Kaposi's sarcoma herpes virus (KSHV)/Human herpes virus-8 (HHV8)	Kaposi's sarcoma
Endometriosis	Endometrial adenocarcinoma
Pelvic inflammatory disease	Ovarian cancer
Barrett's esophagitis	Esophageal cancer
Inflammatory bowel disease	Colorectal cancer
Chronic gastritis (usually with *H. pylori* infection)	Gastric cancer
Infection with Hepatitis virus B and C, hepatic fibrosis	Hepatocellular carcinoma
Telangiectatic features with inflammatory syndrome	Telangiectatic adenoma and hepatic malignancy
Thyroidtis	Papillary thyroid carcinoma
Asbestos	Malignant mesothelioma

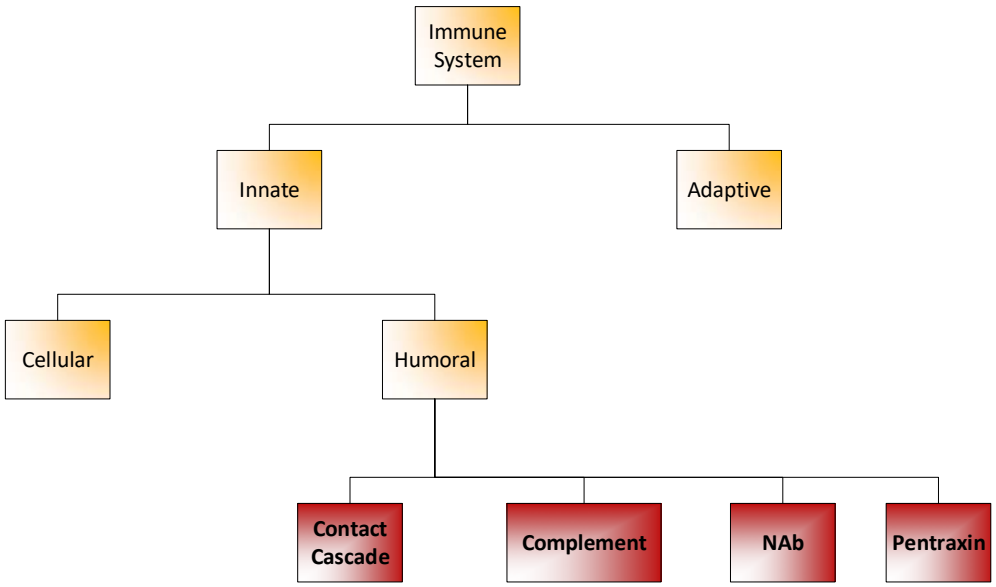
B. Chemokines: Low-molecular-weight cytokines known as chemokines (chemoattractant cytokines) stimulate leukocyte movement. Leukocytes are guided by chemokine concentration gradients to the site of an infection or inflammation (a process called homing). They are divided into four types based on the presence of certain structural motifs involving the numbers and intervals between cystine residues: C, CC, CXC and CX3C.

C. Adhesion molecules Often, leukocytes must interact directly to contact other cells under somewhat adverse conditions such as during rapid flow within the circulatory system or under weak ligand-receptor binding. Adhesion molecules provide stable cell-to-cell contact necessary for both innate and adaptive immune responses as well as for many other intercellular activities. Although a seemingly simple activity, the ability of cells to examine the surface of other cells and to establish stable contact with them is vital. For cells to communicate and for cell-surface receptors and ligands to interact, the cells must be able to establish and maintain relatively prolonged surface-to-surface contact. Types of adhesion molecules include integrins, selectins, and addressing.

Hemophagocytic lymphohistiocytosis (Epstein-Barr virus infection)	T cell lymphoma
Schistosomiasis	Bladder cancer
Primary sclerosing cholangitis	Cholangiocarcinoma
Chronic cholecystitis	Gall bladder carcinoma

1.4 IMMUNE SYSTEM

We layout the immune system as shown below. The first partition is on innate and adaptive. The former is fast and non-selective, to a degree, and the second is slower but generally highly selective and with memory. The innate can then be further divided into cellular and then humoral. Cellular innate consists of such cells as macrophages, neutrophils, dendritic cells, natural killer cells, and the like. The humoral system consists of the complement system (classic, alternative, mannose), the contact, the natural anti-body and the pentraxin.



1.5 THE CYCLE

What we will examine is the cycle between:

1. Irritant: Everything starts with something. Philosophically and physically this has a basis in observation. Thus before any inflammation we need a start, usually caused by some irritant. We know the immune system responds to a variety of antigens, whether they be polysaccharides, viral RNA, reactive oxygen species (ROS) or whatever else may induce the action of an immune system element. Thus it may be as complex as a viral infection or as mundane as a dietary complexity resulting in a proliferation of ROS.

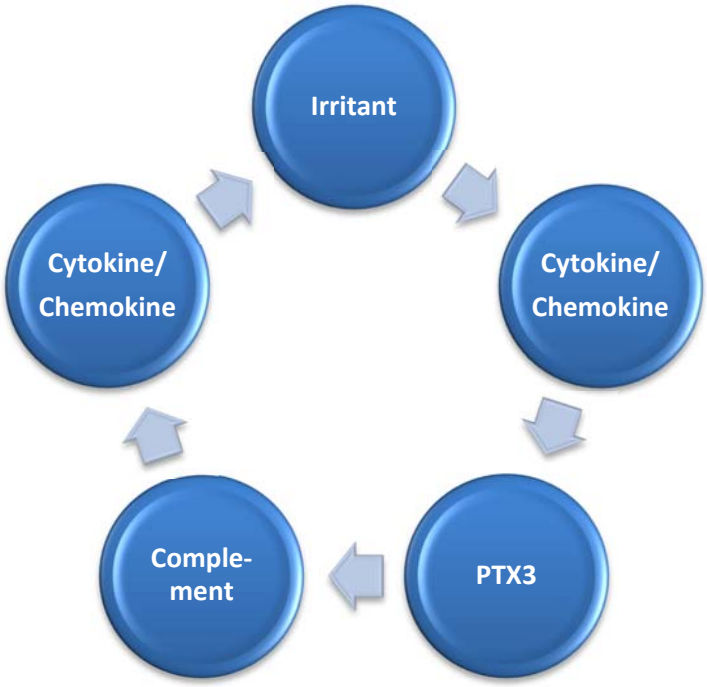
2. Innate System Response: Irritants may cause an immediate type of reaction most likely from the innate system.

3. Inflammation: Inflammation is the interaction of the cell, the irritant, and the elements of the immune system. Cells start responding as well as the immune elements: cells and pathway. There is an inflammation cascade which occurs. Generally the inflammation can result in an elimination of the irritant.

4. Chronic Inflammation: If the irritant is not eliminated or if it is ongoing such as ROS production due to dietary imbalances, then the result in a chronic inflammation. This is often the hallmark step leading to a cancer.

5. Dysplasia: The continual assault on the cells may result in blockage of genes or excess activation of growth factors. Also there may be significant methylation and resulting changes in promoters and expression.

6. Cancer: The final step may very well be the uncontrollable change to a malignancy.



2 IRRITANTS: REACTIVE OXYGEN SPECIES, AND EXAMPLE

There are a multiplicity of irritants that initiate an immune system response. We use here a simple one that may be a likely potential chronic source, namely the reactive oxygen species or oxidized radicals. There are many others such as tobacco smoke, alcohol, drugs, and various types of foods. The example of the reactive oxygen species, ROS, is but one but it exemplary of the type seen. Specifically, ROS are prevalent in those with Type 2 Diabetes resulting from obesity. As such, and in view of the growing number of people subject to this self-inflicted disease, this specific example is worthy of some detailed attention as a prime example.

Let us begin with a discussion of ROS. As Cleveland and Kasten note:

Reactive oxygen species are potentially dangerous by-products of cellular metabolism that have direct effects on cell development, growth and survival, on ageing, and on the development of cancer. They are generated by all aerobic organisms, but their production is a double-edged sword. On the one hand, they seem to be needed for signal-transduction pathways that regulate cell growth and reduction– oxidation (redox) status. But on the other, excessive amounts of these metabolites can start lethal chain reactions, which oxidize and disable structures that are required for cellular integrity and survival1.

Many tumour cells seem to have increased rates of metabolism compared with normal cells, which would typically lead to increased numbers of reactive oxygen species. So one way of treating cancer might be to design drugs to target the enzymes that regulate the levels of reactive oxygen species….

Reactive oxygen species are generated during the production of ATP by aerobic metabolism in mitochondria. The leakage of electrons from mitochondria during the electron-transport steps of ATP production generates the reactive oxygen species superoxide (O2-) and hydroxyl (OH -) radicals. These species can lead to the production of hydrogen peroxide (H2O2), from which further hydroxyl radicals are generated in a reaction that either depends on, or is catalysed by, Fe 2+ ions.

Cells have evolved a series of antioxidant systems to handle these dangerous natural by-products. These defence systems include intracellular superoxide dismutases (SODs), which convert O2- into H2O 2; enzymes that inactivate H2O2 or hydroxyl radicals; and enzymes that trap free radicals or transition metals (such as Fe 2+) that are a reservoir for electrons.

It is well known that these radicals can have drastic inflammatory effects on cells. As Rubin and Strayer note:

Hydroxyl radicals (OH•) are formed by

(1) the radiolysis of water,

(2) the reaction of H_2O_2 with ferrous iron (Fe_2) (the Fenton reaction) and

(3) the reaction of O_2 with H_2O_2 (the Haber-Weiss reaction).

The hydroxyl radical is the most reactive molecule of ROS and there are several mechanisms by which it can damage macromolecules. Iron is often an active participant in oxidative damage to cells by virtue of the Fenton reaction. Many lines of experimental evidence now suggest that in a number of different cell types H_2O_2 stimulates iron uptake and so increases production of hydroxyl radicals.

Lipid peroxidation: The hydroxyl radical removes a hydrogen atom from the unsaturated fatty acids of membrane phospholipids, a process that forms a free lipid radical. The lipid radical, in turn, reacts with molecular oxygen and forms a lipid peroxide radical. This peroxide radical can, in turn, function as an initiator, removing another hydrogen atom from a second unsaturated fatty acid. A lipid peroxide and a new lipid radical result and a chain reaction is initiated. Lipid peroxides are unstable and break down into smaller molecules. The destruction of the unsaturated fatty acids of phospholipids results in a loss of membrane integrity.

Protein interactions: Hydroxyl radicals may also attack proteins. The sulfur-containing amino acids cysteine and methionine, as well as arginine, histidine and proline, are especially vulnerable to attack by OH•. As a result of oxidative damage, proteins undergo fragmentation, cross-linking, aggregation and eventually degradation.

DNA damage: DNA is an important target of the hydroxyl radical. A variety of structural alterations include strand breaks, modified bases and cross-links between strands. In most cases, the integrity of the genome can be reconstituted by the various DNA repair pathways. However, if oxidative damage to DNA is sufficiently extensive, the cell dies.

The DNA damage is a key factor. ROS when present especially in cells which undergo more rapid duplication where the DNA is exposed to the ROS are thus subject to assault and change. The self-protective repair mechanisms of DNA may not be adequate to make all corrections by eliminating defective DNA or properly repairing single or double stranded DNA breaks[2]. Again Grivennikov et al note:

It has been suggested that an inflammatory microenvironment can increase mutation rates, in addition to enhancing the proliferation of mutated cells. Activated inflammatory cells serve as sources of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) that are capable of inducing DNA damage and genomic instability. However, it is not clear whether ROS and RNI produced and released by neutrophils or macrophages (mainly during acute inflammation) are sufficiently long lived to diffuse through the extracellular matrix, enter epithelial cells, cross their cytoplasm, enter the nucleus, and react with DNA packaged into chromatin.

[2] We have discussed the SS DNA and DS DNA repairs elsewhere. BRCA is the DSB repair protein and it is well known that in breast cancers and many others this lack of BRCA can lead to high malignancy. PARP is a SSB repair and it too, especially in prostate cancer, is a source of malignant transformation.

Alternatively, inflammatory cells may use cytokines such as TNF-a to stimulate ROS accumulation in neighboring epithelial cells. It has therefore been debated whether immune-mediated mechanisms as opposed to dietary and environmental mutagens are the critical driving forces behind tumor initiation

The last sentence is a compelling statement. It simply states that the body's own immune system, driven by ROS, may be a source of many of the damages imparted upon DNA. Further research as reported by Gorlach et al notes:

Within the last twenty years the view on reactive oxygen species (ROS) has changed; they are no longer only considered to be harmful but also necessary for cellular communication and homeostasis in different organisms ranging from bacteria to mammals. In the latter, ROS were shown to modulate diverse physiological processes including the regulation of growth factor signaling, the hypoxic response, inflammation and the immune response.

During the last 60–100 years the life style, at least in the Western world, has changed enormously. This became obvious with an increase in caloric intake, decreased energy expenditure as well as the appearance of alcoholism and smoking; These changes were shown to contribute to generation of ROS which are, at least in part, associated with the occurrence of several chronic diseases like adiposity, atherosclerosis, type II diabetes, and cancer.…

The research within the last twenty years on chemically reactive molecules containing oxygen, commonly called reactive oxygen species (ROS), has shown that these molecules are important for cellular communication and homeostasis in different organisms ranging from bacteria to mammals. Thereby, ROS were shown to modulate diverse physiological processes including the regulation of growth factor signaling, the hypoxic response, inflammation and the immune response in mammalian cells.

ROS are often simply called "free radicals" because their majority is characterized by at least one unpaired electron in their outer orbitals; however, peroxides like hydrogen peroxide may also give rise to the formation of oxygen radicals and are therefore also considered as ROS. Frequently the incomplete reduction of oxygen by one electron producing super oxide anion ($O2^{\cdot-}$) is the first step for the formation of most other ROS.

On the one hand we have managed to extend life span. On the other hand we have seen a dramatic increase in cancers due to longer life and more DNA repairs, but also as alleged the exposure to ROS in both environmental and dietary factors. As Alfadda et al note in their discussion of ROS in the context of the immune system:

Essentially, ROS are deeply involved in both arms of the immunological defense system, the innate and the acquired responses. Upon exposure to environmental pathogens, exaggerated ROS production as a part of the oxidative burst in activated phagocytes present in the local inflammatory milieu represents one of the first lines of defense mounted against the invading pathogens. Although rapid, this innate immunity is usually only partially effective, since certain fraction of pathogens might escape and proliferate, thereby producing a larger number of pathogens.

Acquired immunity will be initiated when pathogen-derived antigenic peptides that are the result of phagocytosis and digestion by activated phagocytes are presented to the T lymphocytes. As a result, the latter will proliferate and differentiate producing a large progeny of immunological effector cells that are capable of mounting an efficient and antigen-specific immune response. ROS are involved in the acquired immune response because excess ROS continue to be locally produced by the activated phagocytes and consequently enhance the intracellular signal transduction cascades within the T lymphocytes and thereby decrease their activation threshold

There is also the issue of which organs and/or cells are most susceptible to ROS compromise. Studies of the epithelial cells in the colon have been done extensively and putatively assigned high exposure. Yet the cells can easily defend themselves. Other cells may not be as well attuned to such defense.

3 INFLAMMATION AND CANCER

Inflammation has long been assumed to be a precursor and causative factor for cancers of all types. We first re-examine the nature of inflammation and then proceed to examine the relationship of inflammation to cancers.

We first address the issue of inflammation per se. As noted by Rubin and Styer:

Inflammation is a reaction, both systemic and local, of tissues and microcirculation to a pathogenic insult. It is characterized by elaboration of inflammatory mediators and movement of fluid and leukocytes from the blood into extravascular tissues. This response localizes and eliminates altered cells, foreign particles, microorganisms and antigens and paves the way for the return to normal structure and function. The clinical signs of inflammation, termed phlogosis by the Greek physician Galen, and inflammation in Latin, were described in classical times. In the first century AD , the Roman encyclopedist Aulus Celsus described the four cardinal signs of inflammation, namely, rubor (redness), calor (heat), tumor (swelling) and dolor (pain).

These features correspond to inflammatory events of vasodilation, edema and tissue damage. According to medieval concepts, inflammation represented an imbalance of various "humors," including blood, mucus and bile. Modern appreciation of the vascular basis of inflammation began in the 18th century with John Hunter, who noted dilation of blood vessels and appreciated that pus was accumulated material derived from the blood.

Rudolf Virchow first described inflammation as a reaction to prior tissue injury. To the four cardinal signs he added a fifth: functiolaesa (loss of function). Virchow's pupil Julius Cohnheim was the first to associate inflammation with emigration of leukocytes through the walls of the microvasculature. At the end of the19th century, the role of phagocytosis in inflammation was emphasized by the eminent Russian zoologist Eli Metchnikoff. Finally, the importance of chemical mediators was described in 1927 by Thomas Lewis, who showed that histamine and other substances increased vascular permeability and caused migration of leukocytes into extravascular spaces. More recent studies have elucidated the molecular and genetic bases of acute and chronic inflammation. ...

They continue with the description of Chronic Inflammation:

When acute inflammation does not resolve or becomes disordered, chronic inflammation occurs. Inflammatory cells persist, stroma responds by becoming hyperplastic and tissue destruction and scarring lead to organ dysfunction. This process maybe localized, but more commonly it progresses to disabling dis-eases such as chronic lung disease, rheumatoid arthritis, asthma, ulcerative colitis, granulomatous diseases, autoimmune diseases and chronic dermatitis. Acute and chronic inflammation are ends of a dynamic continuum with overlap-ping morphologic features: (1) inflammation with continued recruitment of chronic inflammatory cells is followed by (2) tis-sue injury due to prolongation of the inflammatory response and (3) an often disordered attempt to restore tissue integrity. The events leading to amplified inflammatory responses resemble those of acute inflammation in a number of aspects:

1. *Specific triggers, microbial products or injury, initiate the response.*
2. *Chemical mediators: direct recruitment, activation and interaction of inflammatory cells. Activation of coagulation and complement cascades generates small peptides that function to prolong the inflammatory response.*
3. *Cytokines, specifically IL-6 and RANTES, regulate a switch in chemokines, such that mononuclear cells are directed to the site. Other cytokines (e.g., IFN-) then promote macrophage proliferation and activation.*
4. *Inflammatory cells: are recruited from the blood. Interac-tons between lymphocytes, macrophages, dendritic cells and fibroblasts generate antigen-specific responses.*
5. *Stromal cell activation and extracellular matrix remodel-in occur, both of which affect the cellular immune response.*

As Grivennikov et al in a recent review of Inflammation and Cancers note:

The presence of leukocytes within tumors, observed in the 19th century by Rudolf Virchow, provided the first indication of a possible link between inflammation and cancer. Yet, it is only during the last decade that clear evidence has been obtained that inflammation plays a critical role in tumorigenesis, and some of the underlying molecular mechanisms have been elucidated. A role for inflammation in tumorigenesis is now generally accepted, and it has become evident that an inflammatory microenvironment is an essential component of all tumors, including some in which a direct causal relationship with inflammation is not yet proven.

Only a minority of all cancers are caused by germline mutations, whereas the vast majority (90%) are linked to somatic mutations and environmental factors. Many environmental causes of cancer and risk factors are associated with some form of chronic inflammation. Up to 20% of cancers are linked to chronic infections, 30% can be attributed to tobacco smoking and inhaled pollutants (such as silica and asbestos), and 35% can be attributed to dietary factors (20% of cancer burden is linked to obesity).

It appears that the human cells can withstand a certain amount of antigenic assault but that a chronic assault is oftentimes the driver for carcinogenic change. They continue with their list of basic facts:

1. *Chronic inflammation increases cancer risk.*
2. *Subclinical, often undetectable inflammation may be as important in increasing cancer risk (for instance, obesity-induced inflammation).*
3. *Various types of immune and inflammatory cells are frequently present within tumors.*
4. *Immune cells affect malignant cells through production of cytokines, chemokines, growth factors, prostaglandins, and reactive oxygen and nitrogen species.*
5. *Inflammation impacts every single step of tumorigenesis, from initiation through tumor promotion, all the way to metastatic progression.*
6. *In developing tumors antitumorigenic and protumorigenic immune and inflammatory mechanisms coexist, but if the tumor is not rejected, the protumorigenic effect dominates.*
7. *Signaling pathways that mediate the protumorigenic effects of inflammation are often subject to a feed-forward loop (for example, activation of NF-kB in immune cells induces*

production of cytokines that activate NF-kB in cancer cells to induce chemokines that attract more inflammatory cells into the tumor).

8. *Certain immune and inflammatory components may be dispensable during one stage of tumorigenesis but absolutely critical in another stag*e.

Inflammation can be causative to cancers. The above authors (Grivennikov et al) note:

It has been suggested that an inflammatory microenvironment can increase mutation rates, in addition to enhancing the proliferation of mutated cells. Activated inflammatory cells serve as sources of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) that are capable of inducing DNA damage and genomic instability. However, it is not clear whether ROS and RNI produced and released by neutrophils or macrophages (mainly during acute inflammation) are sufficiently long lived to diffuse through the extracellular matrix, enter epithelial cells, cross their cytoplasm, enter the nucleus, and react with DNA packaged into chromatin.

Alternatively, inflammatory cells may use cytokines such as TNF-a to stimulate ROS accumulation in neighboring epithelial cells. It has therefore been debated whether immune-mediated mechanisms as opposed to dietary and environmental mutagens are the critical driving forces behind tumor initiation. Nonetheless, p53 mutations, presumably caused by oxidative damage, were found in both cancer cells and in inflamed, but nondysplastic, epithelium in CAC, suggesting that chronic inflammation causes genomic changes. Chronic inflammation triggered by the colonic irritant dextran sodium sulfate (DSS) may induce DNA damage that gives rise to colonic adenomas. However, on its own DSS is a poor carcinogen.

However we also have many epigenetic factors as well. For example, methylation and acetylation play significant roles in activating and/or suppressing gene expressions. As to that the authors note:

Other findings implicate epigenetic mechanisms, including microRNA-based silencing and DNA methylation, in inactivation of tumor suppressors, such as INK4a and APC, and other changes that accompany tumor initiation. Recently, inflammation has been connected to epigenetic reprogramming by the JmjC-domain protein Jmjd3, which is encoded by an NF-kB target gene. In inflammation-associated intestinal cancer in Gpx1/2 knockout mice, inflammation induces DNA methyltransferase (DNMT)-dependent DNA methylation and silencing of a large cohort of Polycomb group target genes, some of which are also silenced by methylation in human colon cancer. However, it remains to be shown that any of these inflammation- induced epigenetic mechanisms actually make a critical contribution to tumor initiation, either in a suitable mouse model or through prospective analysis of human specimens.

We shall address this later as well as in an upcoming analysis of epigenetic factors.

4 INFLAMMATION AND THE IMMUNE SYSTEM

Inflammation has become well known as a source of a multiplicity of pathological states. Hansson has described how it is a major source of coronary debilitation:

Recent research has shown that inflammation plays a key role in coronary artery disease (CAD) and other manifestations of atherosclerosis. Immune cells dominate early atherosclerotic lesions, their effector molecules accelerate progression of the lesions, and activation of inflammation can elicit acute coronary syndromes. This review highlights the role of inflammation in the pathogenesis of atherosclerotic CAD. It will recount the evidence that atherosclerosis, the main cause of CAD, is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate, propagate, and activate lesions in the arterial tree.

In addition the following is a list of the immune system cells and their positive and negative effects.

Cell	*Anti-Tumor*	*Tumor Promoting*
Macrophages, dendritic cells, myeloid-derived suppressor cells	Antigen presentation; production of cytokines (IL-12 and type I IFN)	Immunosuppression; production of cytokines, chemokines, proteases, growth factors, and angiogenic factors
Mast cells		Production of cytokines
B cells	Production of tumor-specific antibodies?	Production of cytokines and antibodies; activation of mast cells; immunosuppression
CD8+ T cells	Direct lysis of cancer cells; production of cytotoxic cytokines	Production of cytokines?
CD4+ Th2 cells		Education of macrophages; production of cytokines; B cell activation
CD4+ Th1 cells	Help to cytotoxic T lymphocytes (CTLs) in tumor rejection; production of cytokines (IFNg)	Production of cytokines
CD4+ Th17 cells	Activation of CTLs	Production of cytokines
CD4+ Treg cells	Suppression of inflammation (cytokines and other suppressive mechanisms)	Immunosuppression; production of cytokines
Natural killer cells	Direct cytotoxicity toward cancer cells; production of cytotoxic cytokines	
Natural killer T cells	Direct cytotoxicity toward cancer cells; production of cytotoxic cytokines	
Neutrophils	Direct cytotoxicity; regulation of CTL responses	Production of cytokines, proteases, and ROS

4.1 ADAPTIVE SYSTEM

The adaptive immune system is now well studied and includes the complex interactions between B and T cells. We shall not provide any details herein and refer the reader elsewhere. (See Abbas et al).

4.2 INNATE SYSTEM

The innate immune system and an old system which predates the more complex adaptive system. It is that system which attempts to promptly mitigate any attack on the organism.

Innate immunity, the first line of defense against infections, is phylogenetically the oldest part of the immune system. It coevolved with microbes to protect all multicellular organisms from infections. Some components of the mammalian innate immune system are remarkably similar to components in plants and insects, suggesting that these appeared in common ancestors long ago in evolution.

For example, peptides that are toxic to bacteria and fungi, called defensins, are found in plants and mammals and have essentially the same tertiary structure in both life forms. A family of receptors that we will discuss in detail later in this chapter, called Toll-like receptors, recognize pathogenic microbes and activate antimicrobial defense mechanisms. Toll-like receptors are found in every life form in the evolutionary tree from insects up to mammals.

The major signal transduction pathway that Toll-like receptors engage to activate cells, called the NF-κB pathway in mammals, also shows remarkable evolutionary conservation. In fact, most of the mechanisms of innate immune defense that we will discuss in this chapter appeared very early in evolution, when the first multicellular organisms evolved, about 750 million years ago. An adaptive immune system, in contrast, is clearly recognizable only in vertebrates that appeared about 350 to 500 million years.

The following is a partial list of the Pattern Recognition Molecules, of the Innate Immune System[3]

PRR	*Location*	*Specific Examples*	*Ligands (PAMPs or DAMPs)*
TLRs	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS and peptidoglycans; viral nucleic acids
NLRs	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2	Bacterial cell wall peptidoglycans
		NLRP family (inflammasomes)	Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs	Cytosol of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
CDSs	Cytosol of many cell types	AIM2; STING-associated CDSs	Bacterial and viral DNA

[3] AIM2, Absent in melanoma; CDSs, cytosolic DNA sensors; CLRs, C-type lectin–like receptors; DAMP, damage-associated molecular pattern; DC, dendritic cells; MDA, melanoma differentiation-associated gene; NLRs, NOD-like receptors; NOD, nucleotide oligomerization domain; PAMP, pathogen-associated molecular pattern; RLRs, RIG-like receptors; SP-D, surfactant protein D; STING, stimulator of IFN genes; TLRs, toll-like receptors.

PRR	*Location*	*Specific Examples*	*Ligands (PAMPs or DAMPs)*
CLRs	Plasma membranes of phagocytes	Mannose receptor	Microbial surface carbohydrates with terminal mannose and fructose
		DC-sign	
		Dectin-1, Dectin-2	Glucans present in fungal and bacterial cell walls
Scavenger receptors	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
***N*-Formyl met-leu-phe receptors**	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Pentraxins	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins	Plasma	Mannose-binding lectin	Carbohydrates with terminal mannose and fructose
	Alveoli	Surfactant proteins SP-A and SP-D	Various microbial structures
Ficolins	Plasma	Ficolin	*N*-acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement	Plasma	Various complement proteins	Microbial surfaces

The innate system is composed of two general categories; cellular and humoral. Specifically:

Cellular: This is the use of the NK, macrophage, mast, and dendritic cells. Namely, the Cellular Innate system is composed of those cells which can be activated by the presence of some readily recognized antigen.

Humoral: The humoral arm of the innate system is that portion where various molecules, proteins, flowing in the blood stream and outside the blood stream can seek out and respond by themselves to an antigen. A classic example is the three complement cascades; classic,

alternative and lectin. These molecules by themselves can identify, attack, and destroy invaders. This part of the immune system is often overlooked as a tool in fighting malignancies. However, it is a tool used by the body to respond to inflammatory events, and in turn its over expression can often initiate cancers.

4.3 HUMORAL

The humoral system is composed of four general elements. From Abbas et al:

Several different kinds of molecules that recognize microbes and promote innate responses exist in soluble form in the blood and extracellular fluids. These molecules provide early defense against pathogens that enter the circulation or are present outside host cells at some stage of their life cycle.

The soluble effector molecules function in two major ways:

1. By binding to microbes, they act as opsonins and enhance the ability of macrophages and neutrophils to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins, and these receptors can efficiently mediate the internalization of the complex of opsonin and bound microbe and subsequent destruction of the ingested microbe.

2. After binding to microbes, soluble mediators of innate immunity promote inflammatory responses that bring more phagocytes to sites of infections, and they may also directly kill microbes.

3. The soluble effector molecules are sometimes called the humoral branch of innate immunity, analogous to the humoral branch of adaptive immunity mediated by antibodies. The major components of the humoral innate immune system are the complement system, collectins, pentraxins, and ficolins, …

They are:

1. Complement: This is the complex cascade of molecules which when activated attack and drill holes in the presenting pathogen.

2. Contact Cascade:

3. Naturally Occurring Antibodies

4. Pentraxins: These are proteins which can identify and initiate an attack on an invader. They may precede a Complement attack.

4.3.1 Complement

The complement element is a chain or reactions resulting in the MAC, a set of proteins which drill a hole in a cell and result in its demise.

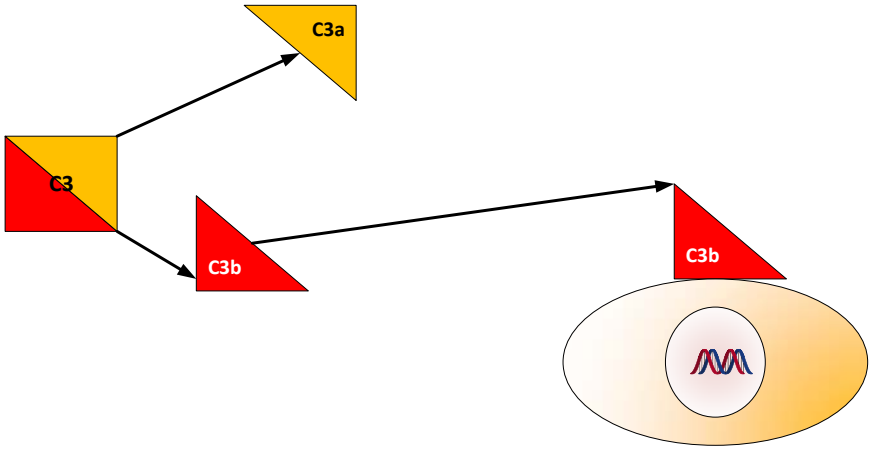
Now from Shishido et al:

Complement activation occurs by one of three initiation pathways, the classical, alternative, or mannose binding lectin (MBL) pathway. Each pathway contains a C3 convertase that cleaves C3 producing C3b and subsequently a C5 convertase. Cleavage of C5 by the C5 convertase results in C5b deposition and initiates the common terminal pathway. The terminal pathway forms the membrane attack complex (MAC), a pore in the cellular membrane, and lysis of the host or pathogenic cell. The action of the C3 and C5 convertases also produces potent anaphylatoxins, C3a and C5. Although not specifically part of the humoral immune response, complement receptor 3 (CR3) found on neutrophils and macrophages enhances the innate immune response by recognizing C3b opsonized pathogens.

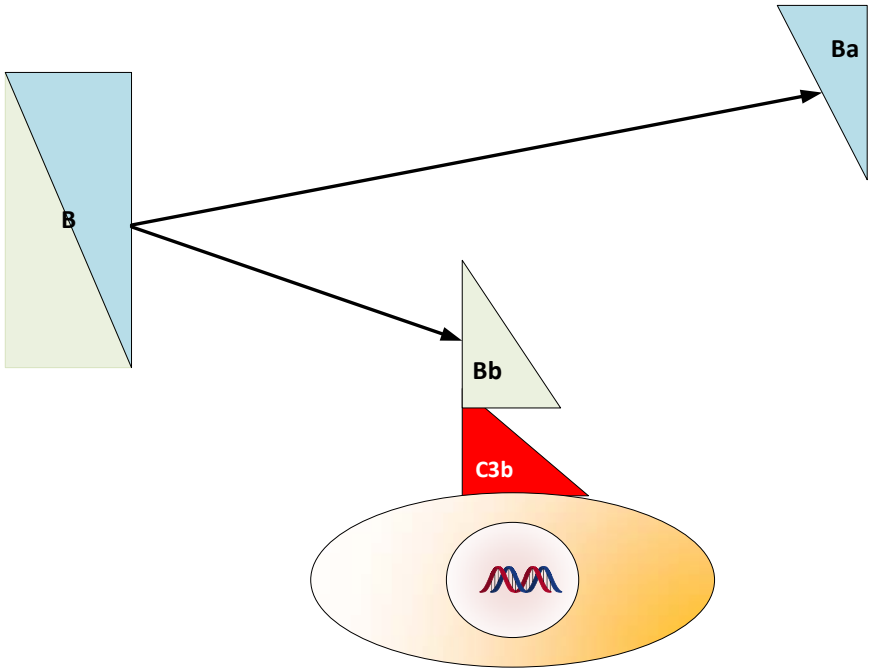
Recent evidence indicates that complement plays a significant role in directing the adaptive immune response as well as in tissue regeneration. Specifically, as part of the B cell receptor complex, CR2 recognition of cleavage products iC3b, C3dg, and C3d significantly increases Ab production. Thus, maintaining homeostasis requires tight regulation of the cascade. Regulation of this potentially damaging cascade occurs at multiple levels with soluble and membrane bound inhibitors including C1 inhibitor (C1INH), CD55, CD59, CD46, Factor H and related proteins.

TLR	Immune Cell Expression	PAMPs	DAMPs
TLR1+ TLR2	Cell surface	Triacylated lipoproteins (Pam3CSK4)	(TLR2 DAMPs listed below)
	Mo, MΦ, DC, B	Peptidoglycans, Lipopolysaccharides	
TLR2+ TLR6	Cell surface	Diacylated lipoproteins	Heat Shock Proteins
	Mo, MΦ, MC, B	(FSL-1)	(HSP 60, 70, Gp96)
			High mobility group proteins (HMGB1)
			Proteoglycans
			(Versican, Hyaluronic Acid fragments)
TLR3	Endosomes	dsRNA (poly (I:C))	mRNA
	B, T, NK, DC	tRNA, siRNA	tRNA
TLR4	Cell surface/	Lipopolysaccharides (LPS)	Heat Shock Proteins
	endosomes	Paclitaxel	(HSP22, 60, 70,72, Gp96)
	Mo, MΦ, DC, MC,		High mobility group proteins (HMGB1)
	IE		Proteoglycans
			(Versican, Heparin sulfate,
			Hyaluronic Acid fragments)
			Fibronectin, Tenascin-C
TLR5	Cell surface	Flagellin	
	Mo, MΦ, DC, IE		
TLR7	Endosomes	ssRNA	ssRNA
	Mo, MΦ, DC. B	Imidazoquinolines (R848)	
		Guanosine analogs (Loxoribine)	
TLR8	Endosomes	ssRNA,	ssRNA
	Mo, MΦ, DC, MC	Imidazoquinolines (R848)	
TLR9	Endosomes	CpG DNA	Chromatin IgG complex
	Mo, MΦ, DC, B,T	CpG ODNs	
TLR10	Endosomes	profilin-like proteins	
	Mo, MΦ, DC		

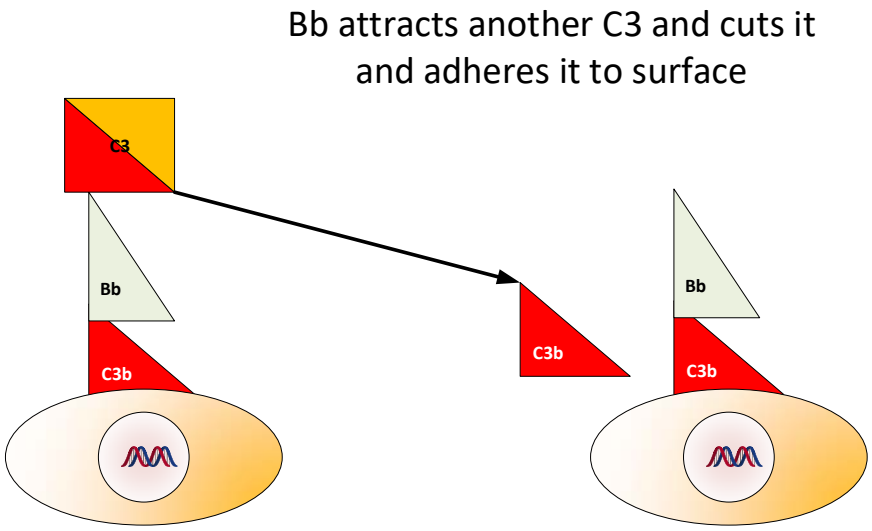
The following graphics depict the activation and actions of the complement system. First we have circulating C3 which can be broken into two active parts.C3b can attach to invading cells and this attachment starts the overall complement cascade. The alternative pathway is exemplary of this. One may ask how this could apply to invading cancer cells and we shall discuss this later.



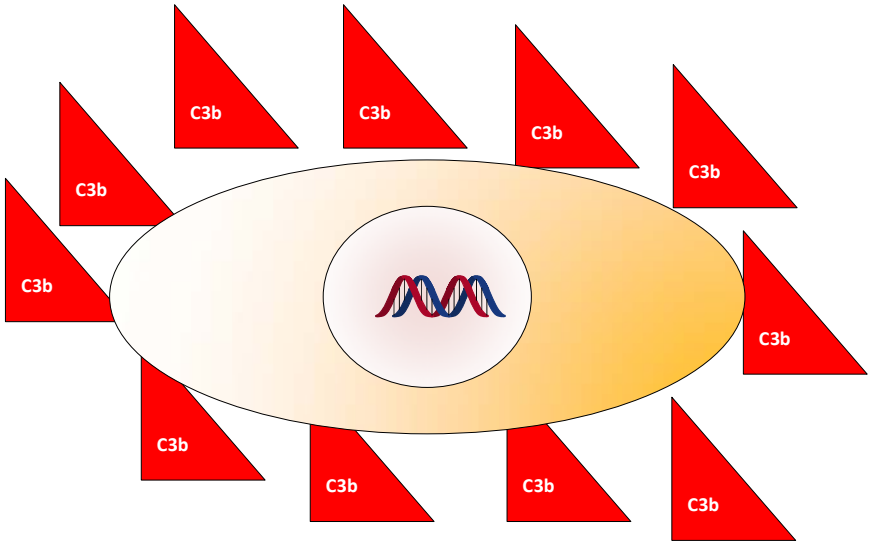
After the C3b is attached a B molecule binds to the c3B which itself is attached to the invader.



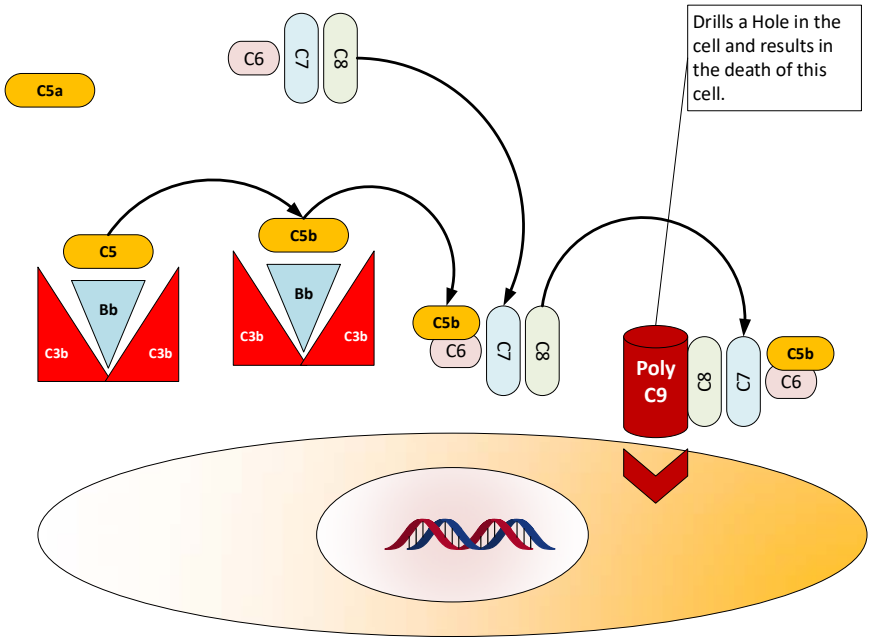
This initiates the C3b cascade wherein the C3 breaks apart and more and more C3b attach and cover the invader.



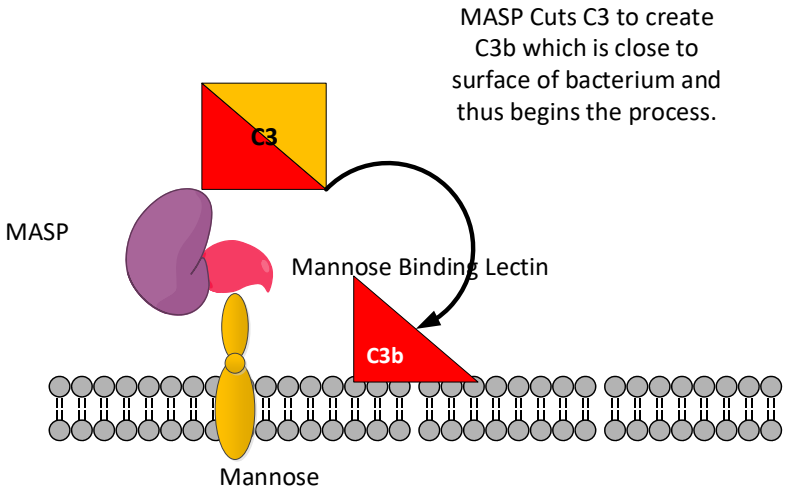
Finally the invader is totally covered with C3b.



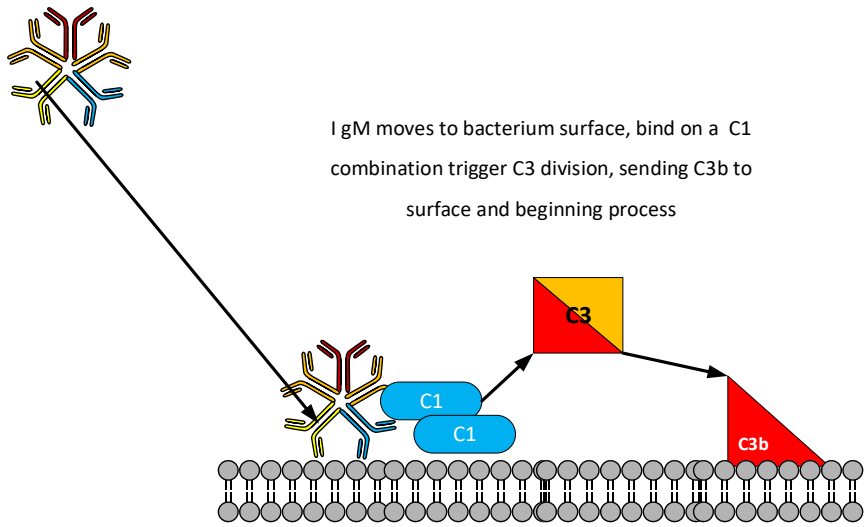
Finally the MAC or membrane attack complex is formed. This is an amalgam of a set of complement proteins which manages to attach to the invaders surface and drill holes through it that result in the death of the invader. It is a very powerful tool that can be used to seek out and destroy certain cells which have been targeted via the complement pathway.



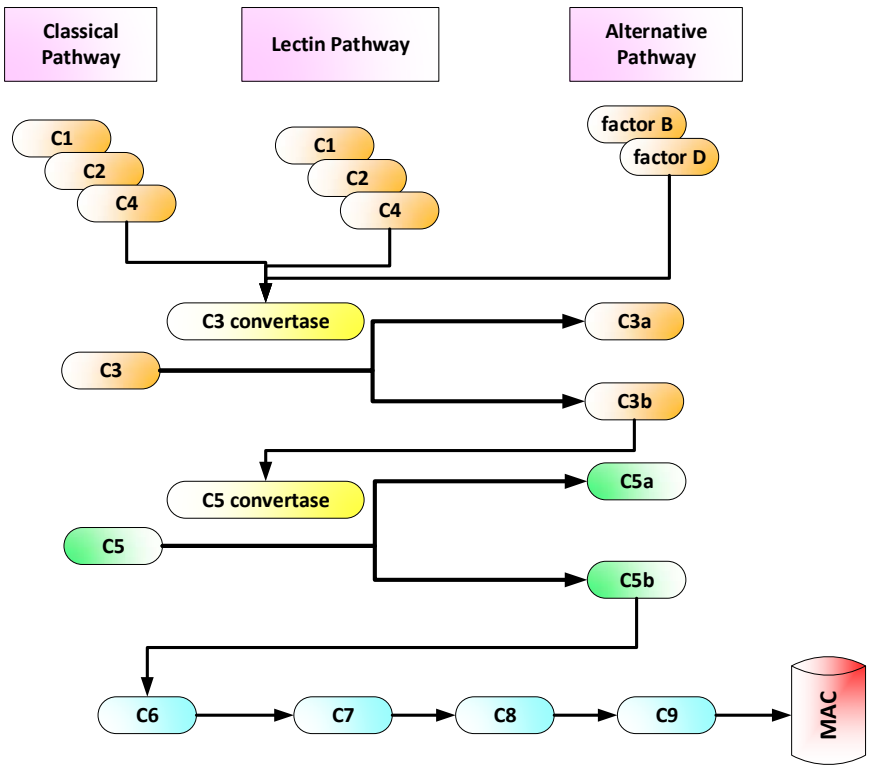
There are other ways in which complement works. The mannose pathway is shown below.



The third way, the classical, is shown below activated by an antibody.



We summarize these pathways below. Our main focus will be on the alternative. Mannose and classic require other surface molecules.



4.3.2 Contact cascade

Now from Shishido et al we have what the authors call the Contact Cascade:

The plasma also contains components of a second proteolytic cascade, the contact system. Factor XII (FXII; Hageman factor) of the contact system is proteolytically cleaved to FXIIa by negatively charged surfaces of damaged cells. FXIIa initiates the coagulation cascade leading to clot formation and cleaves prekallikrein to kallikrein for subsequent release of bradykinin.

Through an endothelial G-coupled receptor (bradykinin receptor 1; BKR1), bradykinin induces vasodilation, neutrophil chemotaxis and vascular permeability. Furthermore, the bradykinin degradation product, des-arg9-bradykinin regulates the adaptive response and alters the blood-brain barrier through a second receptor, bradykinin receptor 2 (BKR2).

Importantly, both FXIIa and kallikrein activate the complement cascade independent of known complement initiators. Several components of the activated contact system including, FXa, FXIa and plasmin, cleave C5 and C3 producing C5a and C3a. The complement inhibitor, C1INH, also inhibits FXIIa indicating multiple interactions between the two pathways. These data suggest crosstalk between two cascades of humoral innate immune response.

4.3.3 Naturally occurring antibodies

Now from Shishido et al:

Produced primarily by B1 B lymphocytes, NAb are germline encoded Ab with restricted epitope specificities and are produced in the absence of external antigen stimulations. NAb are usually of the IgM isotype but may include IgG and IgA isotypes as well.

Natural IgM Abs mediate clearance of cellular debris, aging or apoptotic cells by oponization and recruitment of complement components. As part of the innate immune response, NAb recognize a wide range of pathogens, albeit with low affinity and modulate the adaptive immune response by interacting with B, T and dendritic cells [10]. Finally, NAb are potent initiators of the complement cascade suggesting additional interactions between components of the innate humoral response.

4.3.4 Pentraxins

The fourth and a significant part of the humoral innate system is the pentraxin. We shall focus on this element and discuss its usefulness as both a prognostic element and target for therapy. Now again from Shishido et al:

As a family of evolutionarily conserved multimeric pattern recognition proteins, pentraxins are acute phase proteins which are rapidly synthesized and serve as markers of infection, inflammation, and tissue damage. Each pentraxin contains a common domain in the C terminus.

The presence or absence of additional domains divides the family into long or short pentraxins, respectively. The short pentraxins include C-reactive protein (CRP) and serum amyloid P protein (SAP), both of which are produced by the liver.

Produced by a multitude of cell types, pentraxin 3 (PTX3) is the primary long pentraxin active in humoral innate immunity. Similar to NAb, pentraxins recognize and bind multiple pathogens as well as intrinsic ligands, including apoptotic cells and extracellular matrix components. Macrophages and other innate immune cells recognize pentraxins, CRP, SAP and PTX3, via Fcy receptors. Binding of pentraxins to a target facilitates clearance of pathogens and cell debris by complement activation indicating additional interactions between components of the innate immune response. Overall, pentraxins are multifunctional and nonredundant components of the humoral innate immune response.

Therefore, pentraxins play a critical role in human disease by interacting with multiple components of the humoral response.

We shall detail the pentraxins later in this report.

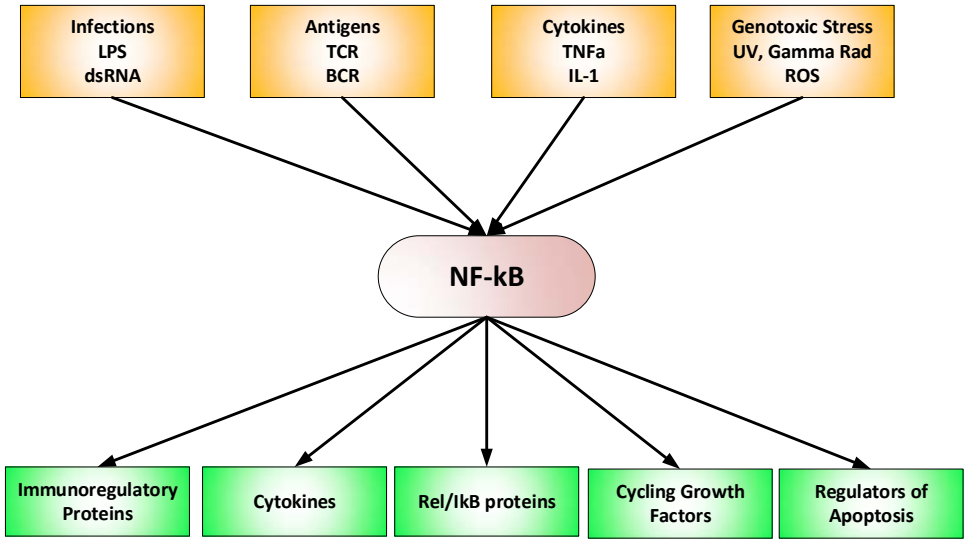
These four humoral innate elements have received some attention but they may be worth a considerable amount more as regards to the link between inflammation and cancer.

5 NF-KB AND ITS IMPLICATIONS

The NF-kB dimer is a powerful transcription factor that plays a role in the function of the immune system and in dealing with inflammation. It also has a significant role in cancer development. We briefly summarize this significant factor and highlight the key elements that relate to the conjunction between inflammation and cancer.

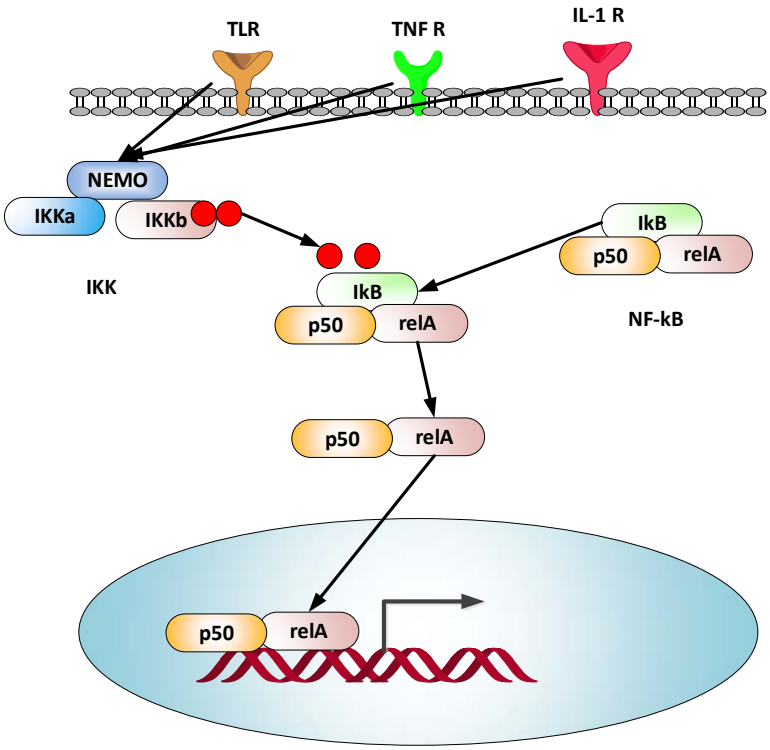
From Gorlach et al we have:

The activation of NF-κB is closely linked with ROS generation during inflammation and obesity. ROS were found to mediate inhibitor of NF-κBα (IκBα) kinase (IKKα and IKKβ) phosphorylation and release of free NF-κB dimers. Tumor necrosis factor α (TNFα), a bona fide NF-κB activator, was shown to mediate a redox- dependent activation of protein kinase A which subsequently phosphorylated Ser276 on RelA (v-rel avian reticuloendotheliosis viral oncogene homolog A). By contrast, the NF-κB member p50 was found to have reduced DNA binding activity when oxidized at Cys62.

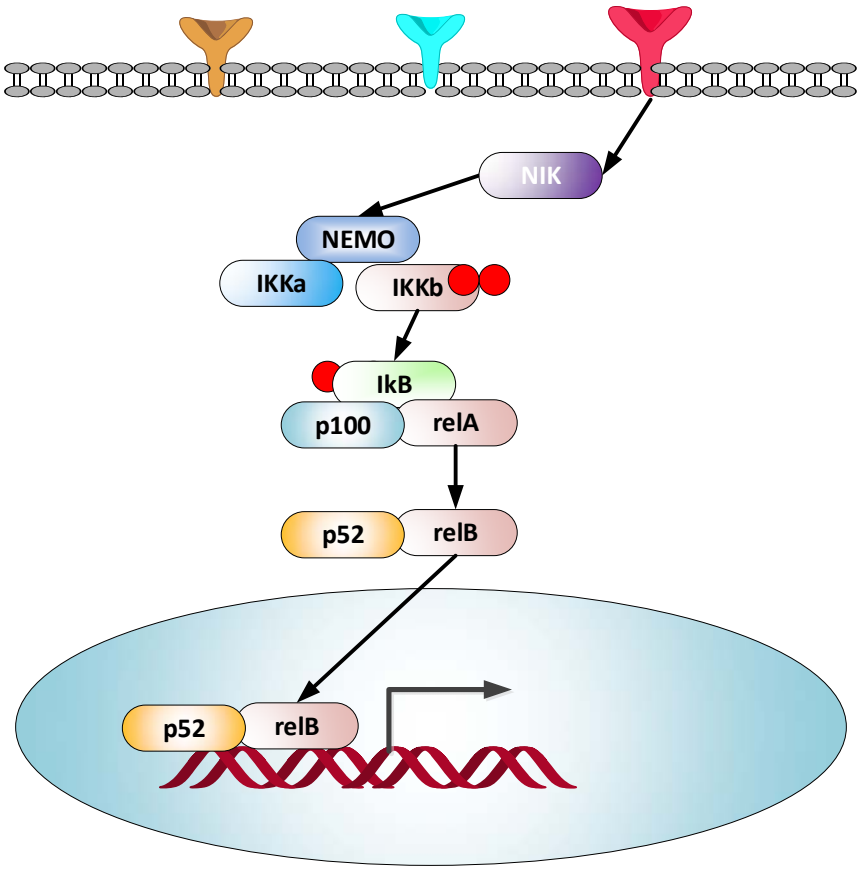


Note that NF-kB can be activated by the very things that are part of the inflammatory response. In turn, NF-kB as a promoter can then release more of this drivers, increase growth factor expression, and stop normal apoptosis. NF-kB is one of the most significant intracellular drivers that connects inflammation, the immune system, and unregulated growth.

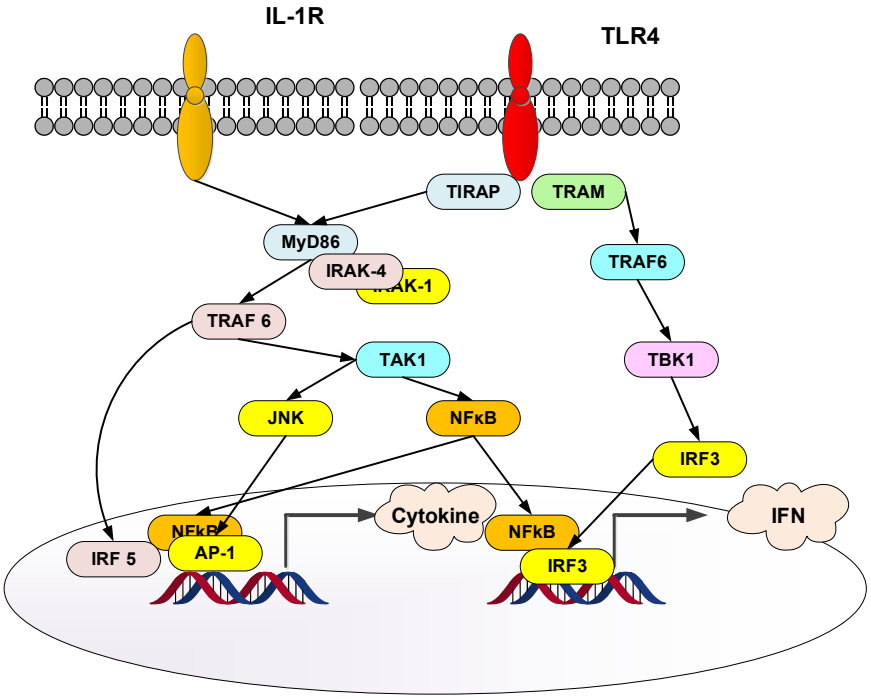
Now as we noted NF-kB is a dimer, namely a combination of two proteins which in turn, when activated, result in a molecule which in a very effective promoter. We show this below with a set of examples:



Here in the above is one of the dimer expressed, namely a relA along with a p50. Below we show the relB and the p52 expression.



The integrated combination is shown below.



To understand NF-kB we need to see how it functions as a powerful promoter. From NCBI we have the following description[4]:

NF-kappa-B is a ubiquitous transcription factor involved in several biological processes. It is held in the cytoplasm in an inactive state by specific inhibitors. Upon degradation of the inhibitor, NF-kappa-B moves to the nucleus and activates transcription of specific genes. NF-kappa-B is composed of NFKB1 or NFKB2 bound to either REL, RELA, or RELB. The most abundant form of NF-kappa-B is NFKB1 complexed with the product of this gene, RELA. Four transcript variants encoding different isoforms have been found for this gene.

From Nature we have the following description[5]:

The canonical pathway is induced by tumour necrosis factor-alpha (TNFalpha), interleukin-1 (IL-1) and many other stimuli, and is dependent on activation of IKKbeta. This activation results in the phosphorylation (P) of IkappaBalpha at Ser32 and Ser36, leading to its ubiquitylation (Ub) and subsequent degradation by the 26S proteasome. Release of the NF-kappaB complex allows it to relocate to the nucleus. Under some circumstances, the NF-kappaB–IkappaBalpha complex shuttles between the cytoplasm and the nucleus (not shown).

IKK-dependent activation of NF-kappaB can occur following genotoxic stress. Here, NF-kappaB essential modifier (NEMO) localizes to the nucleus, where it is sumoylated and then ubiquitylated, in a process that is dependent on the ataxia telangiectasia mutated (ATM) checkpoint kinase. NEMO relocates back to the cytoplasm together with ATM, where activation of IKK-beta occurs. IKK-independent atypical pathways of NF-kappaB activation have also been described, which include casein kinase-II (CK2) and tyrosine-kinase-dependent pathways.

The non-canonical pathway results in the activation of IKK alpha by the NF-kappa B-inducing kinase (NIK), followed by phosphorylation of the p100 NF-kappa B subunit by IKK alpha. This results in proteasome-dependent processing of p100 to p52, which can lead to the activation of p52–Rel B heterodimers that target distinct kappa B elements. Phosphorylation of NF-kappa B subunits by nuclear kinases, and modification of these subunits by acetylases and phosphatases, can result in transcriptional activation and repression as well as promoter-specific effects.

Moreover, cooperative interactions with heterologous transcription factors can target NF-kappa B complexes to specific promoters, resulting in the selective activation of gene expression following cellular exposure to distinct stimuli.

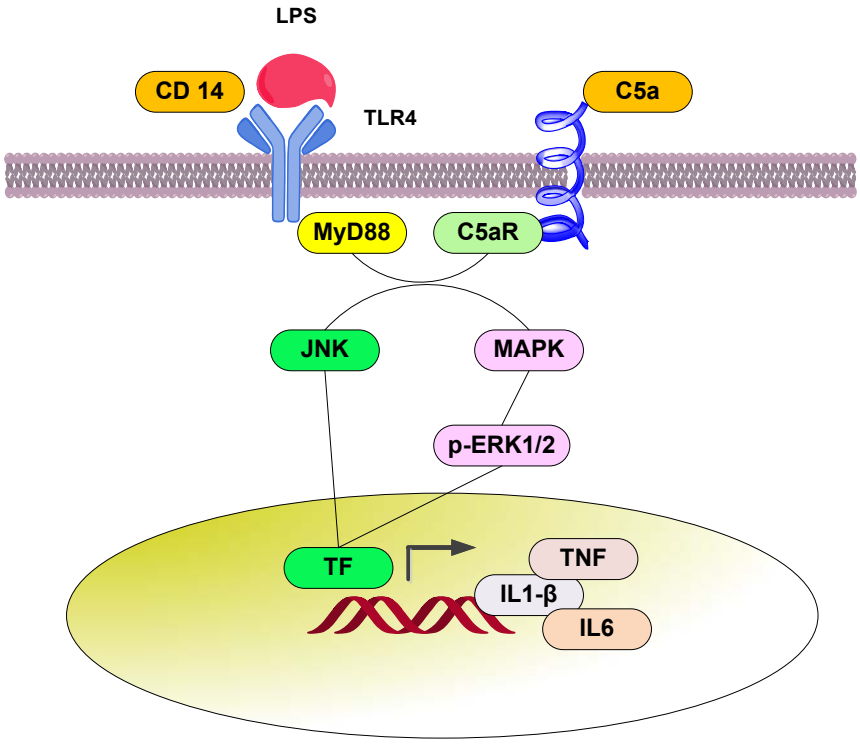
As Merle et al discuss when examine the Complement system they state:

[4] https://www.ncbi.nlm.nih.gov/gene/5970

[5] https://www.nature.com/nrm/journal/v8/n1/box/nrm2083_BX1.html

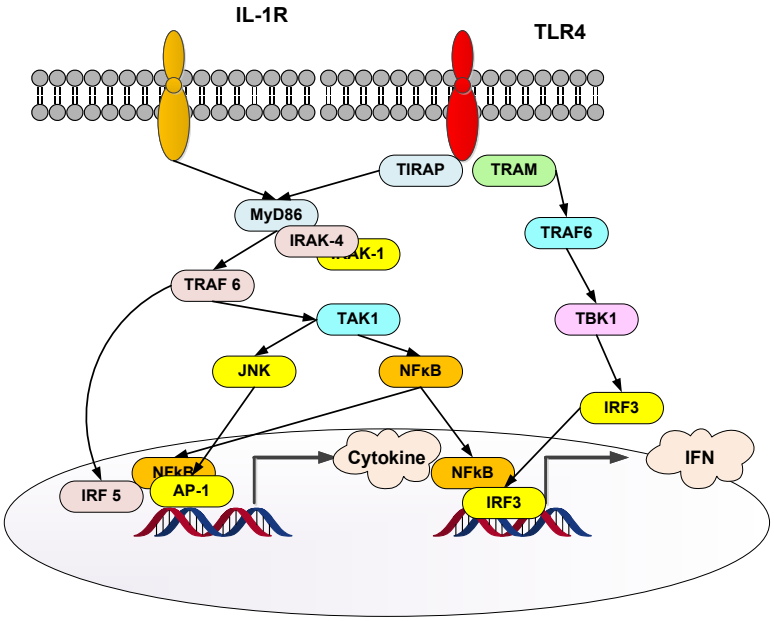
C3a and C5a are able to induce potent inflammatory pathways via their receptors C3aR and C5aR. The implication of intermediates such as NF-kB, MAPK, and c-Jun N-terminal kinase (JNK) in their transduction pathways suggests a potential crosstalk with other pathways, such as those of TLRs. Indeed, complement is involved in TLR-induced inflammation.

They show in the following Figure how this does function:



C5a/C5aR signalingpathwaycancooperatewithTLR-4activationby LPS on macrophages. Intermediate signaling pathways JNK and MAPK are activated and thus lead to proinflammatory effect by TNF-α, IL6, and IL1-β synthesis. On dendritic cells (DCs),TLR-4 and C5aR cooperate in different manner between mice and human. In vivo experiments have demonstrated an implication in Th1cells expansion, whereas in human, an anti-inflammatory role of TLR-4/C5aR collaboration has been described by an antagonized effect on IL-12 and IL-23 synthesis by DC.

Thus, when examining the effects of the complement proteins one must also examine the interactions with other receptors. Further details on this interaction are shown below. Here we show the Toll like receptors, TLR as initiations. These are powerful initiators in the innate response.



As Amiri and Richmond state:

Nuclear Factor-kappa B (NF-κB) is an inducible transcription factor that regulates the expression of many genes involved in the immune response. Recently, NF-κB activity has been shown to be upregulated in many cancers, including melanoma. Data indicate that the enhanced activation of NF- κB may be due to deregulations in upstream signaling pathways such as Ras/Raf, PI3K/Akt, and NIK. Multiple studies have shown that NF-κB is involved in the regulation of apoptosis, angiogenesis, and tumor cell invasion, all of which indicate the important role of NF-κB in tumorigenesis. Thus, understanding the molecular mechanism of melanoma progression will aid in designing new therapeutic approaches for melanoma.

They continue:

Constitutive activation of NF-κB is an emerging hallmark of various types of tumors including breast, colon, pancreatic, ovarian, and melanoma. In the healthy human, NF-κB regulates the expression of genes involved in normal immunologic reactions (e.g. generation of immunoregulatory molecules such as antibody light chains) in response to proinflammatory cytokines and by-products of microbial and viral infections. NF-κB also modulates the expression of factors responsible for growth as well as apoptosis. However, increased activation of NF-κB results in enhanced expression of proinflammatory mediators, leading to acute inflammatory injury to lungs and other organs, and development of multiple organ dysfunctions as well as cancer.

They then summarize NF-kB's role as:

***3.1. Apoptosis resistance and cell proliferation**: In processes such as tumor initiation and promotion where prolonged survival of cells is a crucial event, NF-κB plays an important role as*

a mediator of inhibition of apoptosis. In melanoma, NF-κB has been shown to activate expression of anti-apoptotic proteins such as tumor necrosis factor receptor-associated factor 1 (TRAF1), TRAF2, and the inhibitor-of apoptosis (IAP) proteins c-IAP1, c-IAP2, and melanoma inhibitor of apoptosis (ML-IAP), survivin as well as Bcl-2 like proteins…

***3.2. Invasion and metastasis**: In invasion and metastasis of melanoma, NF-κB may regulate the production of prostaglandins via cyclooxygenase-2 (COX-2), which has been shown to be overexpressed in melanoma [44,45]. It was shown that COX-2 is expressed in the majority of primary malignant melanoma, as well as in five human malignant melanoma cell lines….*

However as Liu et al (2006) state:

Malignant melanoma is the most lethal skin cancer, whose ability to rapidly metastasize often prevents surgical cure.

Furthermore, the systemic treatment of melanoma is largely ineffective due to the intrinsic resistance of melanoma cells to numerous anticancer agents. Increased survival of melanoma cells is primarily attributed to the constitutive activation of the transcription factor nuclear factor kB (NF-kB), which regulates the expression of many anti-apoptotic, pro-proliferative and pro-metastatic genes.

Canonical activation of the NF-kB pathway occurs when NF-kB switches its localization from the cytoplasm, where it is maintained inactive by assembly with the inhibitor IkB protein, to the nucleus, where NF-kB regulates gene expression. NF-kB activation relies upon the phosphorylation dependent ubiquitination and degradation of IkB mediated by the IkB kinase (IKK) complex and b-Trcp E3 ubiquitin ligases.

Consequently, both IKK activity and the levels of b-Trcp regulate the extent of IkB degradation and hence NF-kB activation. The genetic basis that underlies the elevated NF-kB activity in malignant melanoma largely remains elusive.

Constitutively active IKK has been demonstrated to sustain NF-kB activation in human melanoma cells, resulting in induction of the chemokine CXCL1. CXCL1, in turn, is capable of activating IKK and NF-kB and promoting cell survival and tumorigenesis …. However, the original genetic alterations that initiate this feed-forward mechanism in melanoma remain unclear.

One of the major oncogenic events described in the genesis of malignant melanoma is constitutive activation of the Ras-regulated RAF-MEK-ERK mitogen-activated protein kinase (MAPK) pathway. This is achieved most frequently by activating mutations in either BRAF (e.g. V600E substitution) or, less frequently, in N-RAS … Recent evidence indicates that oncogenic BRAF activity is essential for human melanoma cell growth and survival …

However, despite prior reports that RAF can activate NF-kB …, the mechanism(s) by which $BRAF_{V600E}$ ($BRAF_{VE}$) may elicit NF-kB signaling in melanoma cells have not yet been elucidated.

Activation of the canonical NF-kB pathway depends on both IKK activity, which has been shown to be elevated in human melanomas….

Liu et al conclusion is speculative but telling:

Taken together, these data support a model in which mutational activation of BRAF in human melanomas contributes to constitutive induction of NF-κB activity and to increased survival of melanoma cells.

Again we have the issue of speculation as to where and why the mutations occur. Here they speculate about the BRAF mutation resulting in the antiapoptotic control with NF-κB.

6 PENTRAXIN 3

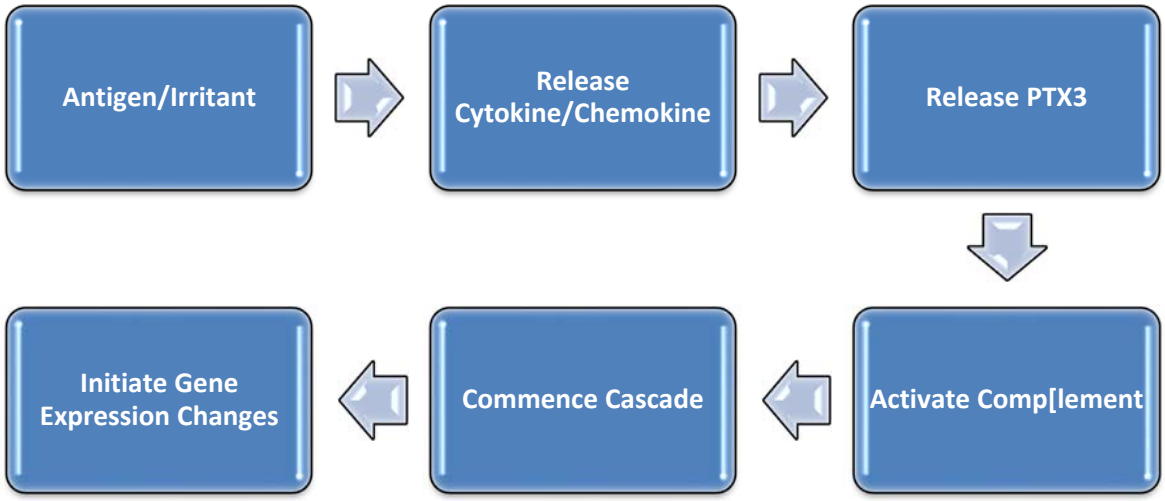
Pentraxin is one of the four generic elements of the humoral innate system. Generally the emphasis is on complement but Pentraxin presents a powerful element not only to monitor progress of inflammation but also to assess the impact and finally as a putative therapeutic target for cancer management. We examine PTX3 as an example of the immune system itself playing a part in the context of inflammation response and the resulting changes that impact malignant behavior.

6.1 THE PTX3 GENE AND ITS EXPRESSION

As NCBI notes[6]:

This gene encodes a member of the pentraxin protein family. The expression of this protein is induced by inflammatory cytokines in response to inflammatory stimuli in several mesenchymal and epithelial cell types, particularly endothelial cells and mononuclear phagocytes. The protein promotes fibrocyte differentiation and is involved in regulating inflammation and complement activation. It also plays a role in angiogenesis and tissue remodeling. The protein serves as a biomarker for several inflammatory conditions.

As noted above the PTX3 produced can be a powerful marker. This is especially the case with a multiplicity of inflammatory processes.



[6] https://www.ncbi.nlm.nih.gov/gene/5806

6.2 IMPACT

We now examine the impact that PTX has. From Abbas et al we have:

PTX3 is

(i) produced by several cell types, including DCs, macrophages, and endothelial cells,

(ii) in response to TLR ligands and inflammatory cytokines, such as TNF, and may be considered an acute-phase reactant.

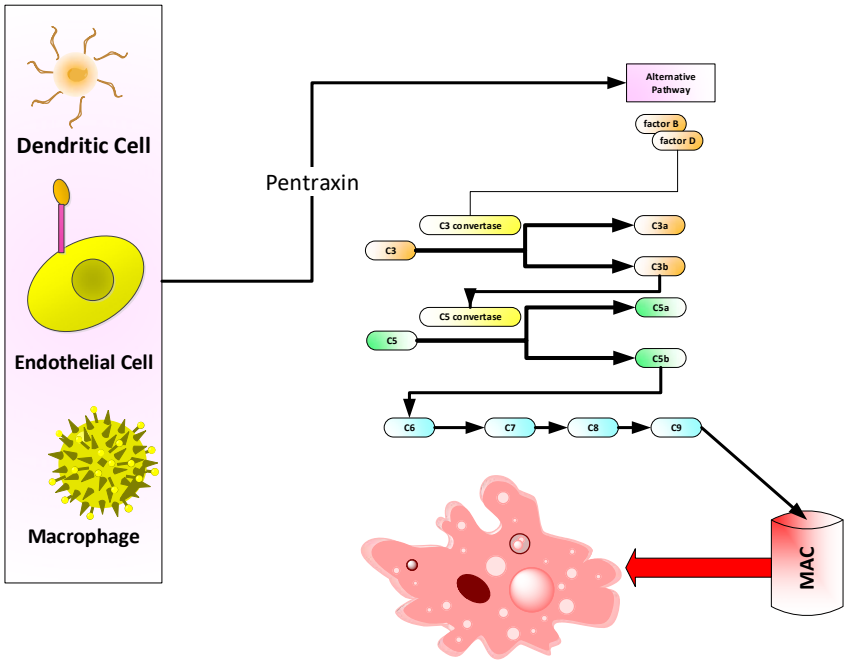
(iii) PTX3 is also stored in neutrophil granules and released as neutrophils die.

(iv) PTX3 recognizes various molecules on fungi, certain bacteria, and viruses, as well as apoptotic cells, and

(v) activates the classical complement pathway.

Studies with knockout mice reveal that PTX3 provides protection against these microbes, including the fungus….

We demonstrate this process below:



The use of PTX3 is the activation of the classical complement pathway. It should be recalled, however, that complement does require an Ab presence of some sort.

Now let us consider PTX3 from the perspective of Bonavita et al:

Inflammation is an essential component of the tumor microenvironment that sustains tumor development and growth. The role in cancer-related inflammation of innate immunity cells recruited in the tumor has been clarified in preclinical models. In contrast, the role of the humoral arm of the innate immune system, which includes biochemically heterogeneous molecules such as Complement components, collectins, ficolins and pentraxins, is still under investigated.

The long pentraxin PTX3 represents a functional paradigm of humoral innate immunity. By interacting with selected microbial moieties and playing opsonic activity via Fcγ receptors, and activating and regulating the Complement cascade, PTX3 acts as a functional ancestor of antibodies. PTX3 plays non-redundant roles in resistance against selected microbial pathogens and in regulating inflammatory and tissue repair responses. PTX3 is highly conserved in evolution and genetic evidence is consistent with a role of PTX3 in antimicrobial resistance in humans. ....

We also showed that PTX3-deficiency was associated to increased DNA damage, as demonstrated by increased Trp53 mutations, oxidative DNA damage and expression of DNA damage (DDR) markers, in line with the hypothesis that cancer-associate inflammation contributes to genetic events that cause cancer and to the genetic instability of tumors.

We finally showed that PTX3 promoter and regulatory regions were highly methylated in selected human mesenchymal and epithelial tumors, in contrast to the normal counterpart. In particular, in colorectal cancer, PTX3 epigenetic modifications occurred early in progression already at the level of adenomas. PTX3 methylation was responsible of silencing of PTX3 protein expression. Indeed, treatment of colorectal cancer cells with a methylation inhibitor (5-Aza-2'- deoxycytidine) was sufficient to restore the histone modifications associated to transcriptional activation and the interaction of transcription factors responsible of PTX3 expression (e.g. NF-kB, c-Jun, c-Fos) with their binding sites in the PTX3 promoter region, and rescued PTX3 protein expression in response to an inflammatory stimulus.

The following relates to the production of PTX3[7]:

PTX3 is rapidly induced by several stimuli in different cell types. Peripheral blood leukocytes and myeloid dendritic cells (DCs) release PTX3 in response to proinflammatory cytokines (IL-1 and TNF-α) and agonists of TLR or following stimulation with microbial components, including LPS, lipoarabinomannan, and Outer membrane proteins (Omp).

PTX3 production is also stimulated by the anti-inflammatory cytokine IL-10 and by high-density lipoproteins (HDLs). Polymorphonuclear cells (PMNs) store PTX3 in lactoferrin-positive

[7] Bottazzi, Barbara . An Integrated View of Humoral Innate Immunity: Pentraxins as a Paradigm (Annual Review of Immunology Book 28) (Kindle Locations 179-193). Annual Reviews. Kindle Edition.

granules. Following microbial recognition by cellular pattern-recognition receptors, PTX3 is promptly released from PMN granules and localizes in neutrophil extracellular traps, where it likely contributes to the generation of an antimicrobial microenvironment essential to trap and kill microorganisms. Other cell types can produce PTX3 in response to appropriate proinflammatory stimulation, such as vascular ECs; smooth muscle cells (SMCs); fibroblasts; adipocytes; chondrocytes; stromal, mesangial, and epithelial cells; and cells of the granulosa.

Different signaling pathways can affect PTX3 production, depending on the cell type and/or the stimuli:

The NF-κB pathway is involved in regulation of PTX3 production in a model of acute myocardial ischemia and reperfusion in mice (41), whereas induction of PTX3 by HDL requires activation of the PI3K/Akt pathway through a G-coupled lysosphingolipid receptor.

Induction of PTX3 by TNF-α in alveolar epithelial cells is mediated by the JNK pathway.

PTX3 expression can be regulated by the chimeric transcription factor obtained by the fusion of the gene encoding the N terminus of the FUS (fused in liposarcoma) gene in frame to the coding region of the CHOP gene.

The involvement of NF-kB is significant. The dimers in this promoter pathway can be readily activated and thus significant cellular proliferation can occur.

From Stallone et al:

Pentraxins, a superfamily of evolutionary conserved proteins, are essential components of the humoral arm of the innate immune system and play a pivotal role in vascular biology. Pentraxin-3 (PTX3), the prototype of long pentraxins, differs from short pentraxins for gene organization, cellular source and ligand-binding capacities.

Like short pentraxins, PTX3 facilitates dysregulation of mitogenic signalling pathways, sustains cellular proliferation, angiogenesis, insensitivity to apoptosis, cancer cell invasion and migration, and tumour escape from immunosurveillance.

Unlike short pentraxins, such as C reactive protein (CRP), PTX3 is not produced by hepatocytes but synthesized by a variety of cell types at the site of inflammation, whereby it seems to regulate complement activation. Recent findings suggest an insidious relationship between complement and cancer in terms of cellular proliferation and regeneration as well as angiogenesis. Considering that chronic inflammation is found in as much as 80% of PBxs and the potential role of PTX3 in inflammatory-related carcinogenesis, the aim of present study was to determine whether PTX3 prostate tissue expression and serum levels could predict progression from chronic prostate inflammation to PCa.

From Shishido et al:

Complement products such as C1q, C3, C3a, C4, C5 and the MAC are detectable in the tumor microenvironment . These activated complement proteins have three mechanisms for complement-mediated destruction of tumor cells:

a) complement-dependent cytotoxicity (CDC) ,

b) indirect Ab-dependent, cell-mediated cytotoxicity , which can be complement receptor-dependent and

c) CR3-dependent cellular cytotoxicity (CR3- DCC) , which is relatively rare with tumors.

Complement components are deposited in various tumor types, indicating that activation of complement may contribute to immunosurveillance of malignant cells. Complement proteins C5b-9 are deposited on the cellular surface of breast cancer cells and papillary thyroid carcinoma cells…

Tumor cells have natural mechanisms for self-protection against the complement system, specifically MAC and the cytotoxic activation of CR3. Extracellular protectors such as membrane and soluble complement inhibitors are released by tumor cells into the microenvironment and interfere with complement cascade activation and limit the quantity of complement deposition.

Membrane complement inhibitors, including CD35 (CR1), CD46 (MCP) and CD55 (DAF) control the activation of complement at the level of C3. These serve as an important mechanism of self-protection, making the cells insensitive to the action of complement. Although the complement system regulates inflammation and the innate immune response, complement proteins also aid in tumor growth and immunosuppression. …

Complement anaphylatoxins may alter cellular differentiation resulting in immune suppression. In healthy individuals, myeloid-derived suppressor (MDS) cells differentiate to macrophages, dendritic cells and neutrophils. However, when trapped in the intermediate stage of differentiation, MDS cells may mediate tumor-induced immune suppression. …

These complement stimulated MDS cells also prevent the activation of CD4+ and CD8+ T cells, inhibit Natural Killer cell cytotoxicity, stimulate cytokine production for tumorigenesis and increase angiogenesis. The complement system proteins such as C5a provide inflammatory protection in the tumor microenvironment. Tumor-associated antigens are known to modulate trans-membrane signaling that is required for proliferation, invasion and metastasis of tumor cells.

The presence of NAb against tumor-associated antigens, such as gangliosides of melanoma cells, correlate with increased patient survival . Tumor-reactive Ab exist in healthy wildtype animal blood samples (IgM) and peripheral blood concentrations of NAb increase shortly after initial tumor development and prior to detection of circulating antigens. NAb may have a direct cytotoxic effect on tumor cells, while also inducing a bystander effect during a humoral anti-tumor response.

Thus, NAb recognize tumor-modified cell surfaces that develop during tumorigenesis and activate complement to destroy nascently transformed cells. Tumor cells are able to utilize NAb to escape immunosurveillance.…

Tumor cells use the innate immune system and NAb to avoid immunosurveillance and elimination. NAb are important for the recognition and elimination of precancerous and cancerous cells. Such tumor-reactive NAb are expressed in multiple tumor types, including melanoma , lung , breast , head and neck and ovarian cancers. …

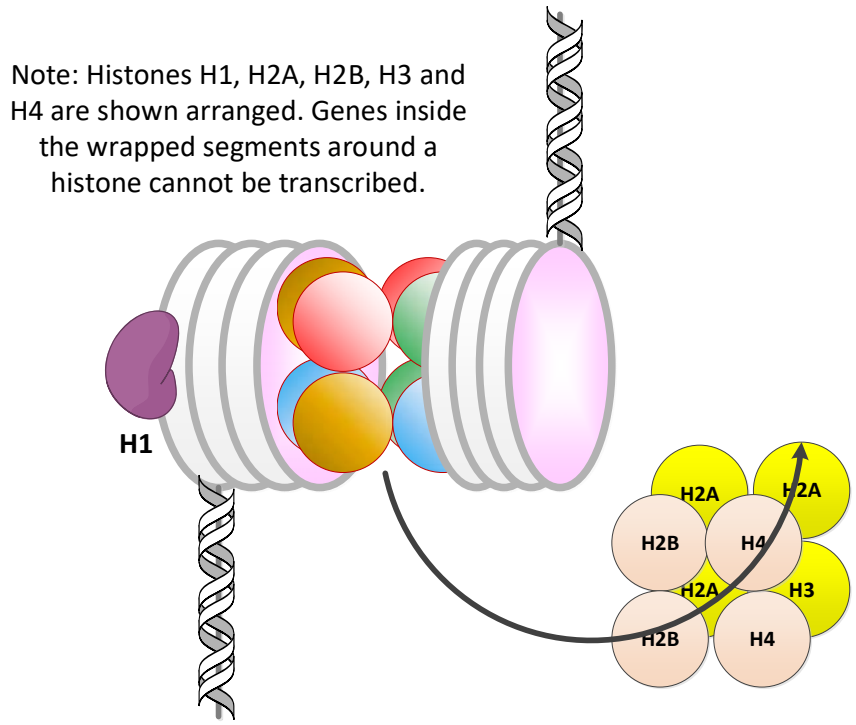
The humoral innate immune response and NAb specifically have an important role in the recognition and elimination of neoplastic cells.

7 EPIGENETICS AND ITS IMPACT

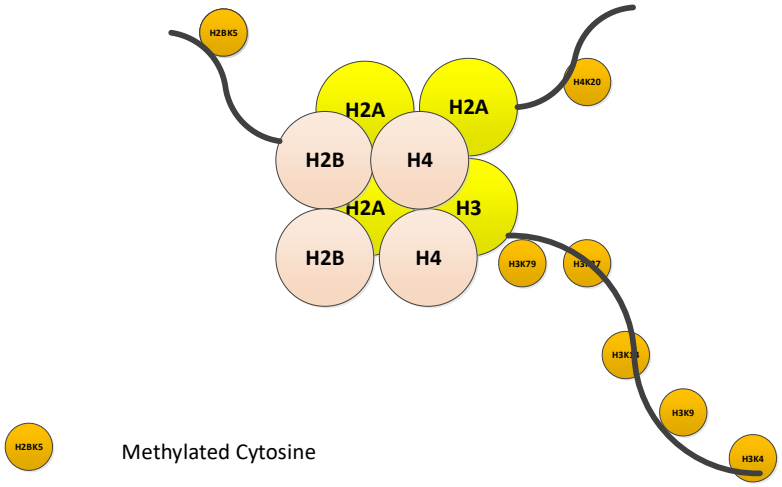
There is an increasing interest in epigenetic influences and cancer. Such effects as methylation result in gene promoter interference and gene expressions. It is not only the methylation of CG islands and the histone methylation and acetylation, but miRNAs and lncRNAs which add up to a significant and complex changing of what the DNA can and should accomplish. There are a plethora of definitions for epigenetics and Dawson et al use one, we shall look at an expanded view where epigenetics represents any effect resulting from the interference of gene expression. Thus the miRNA and methylation and acetylation effects are merely some of the many epigenetic effects. Epigenetic effects are also an important result of inflammation and as a result we will consider some examples to drive the point.

7.1 HISTONES

Let us begin with a brief discussion of histones. We demonstrate this below. The histones are a complex of eight proteins around which is wrapped the double stranded DNA. The DNA to be effective must be unwrapped.



Now the histones unwrap the DNA for expression. The histones have tails and these tails are protein segments that can be methylated or acetylated. The methylated tails would appear as below:



These tails when so methyl/acetyl ated can perform differently than before. Or equally of the methyl or acetyl element is blocked the histone and subsequently the DNA functions differently.

7.2 BROMODOMAINS

One area of epigenetic effects is the impact of and on bromodomains, specifically BET[8]. BETs can block the acetyl element on a histone tail thus blocking expansion and expression of DNA. The BET can be activated via an inflammatory response. Thus the BET represents an alternative to the previous DNA expression modifications. Namely this is an example of a DNA expression modification via inflammation and in turn via an epigenetic control[9].

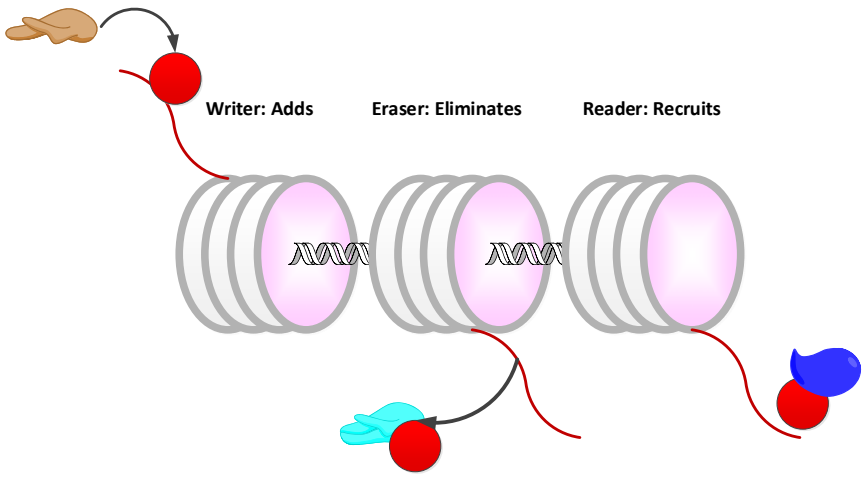
From Dawson et al there are proteins which can modify the elements on the histone tails. This can be accomplished in three ways as we graphically depict below:

[8] Bromodomain and extraterminal (BET) proteins: A family of proteins (BRD2, BRD3, BRD4, and BRDT) characterized by tandem bromodomains that interact with acetylated histones and influence gene expression, cell-cycle regulation, and development.

Bromodomains: Regions within proteins capable of recognizing acetylated histones. Proteins containing bromodomains are involved in transcription, DNA repair, replication, and chromosome condensation.

Acetylation: A reaction that results in the addition of a functional acetyl group to an organic compound. Deacetylation is the removal of the acetyl group. Acetylation is a post-translational chemical modification of both histones and nonhistone proteins.

[9] See Gray, Epigenetic Cancer Theory for a discussion. Also see Armstrong, Epigenetics, pp 62-63.



As Belkina and Denis note:

The bromodomain is a highly conserved motif of 110 amino acids that is bundled into four anti-parallel α-helices and found in proteins that interact with chromatin, such as transcription factors, histone acetylases and nucleosome re-modelling complexes. Bromodomain proteins are chromatin 'readers'; they recruit chromatin-regulating enzymes, including 'writers' and 'erasers' of histone modification, to target promoters and to regulate gene expression.

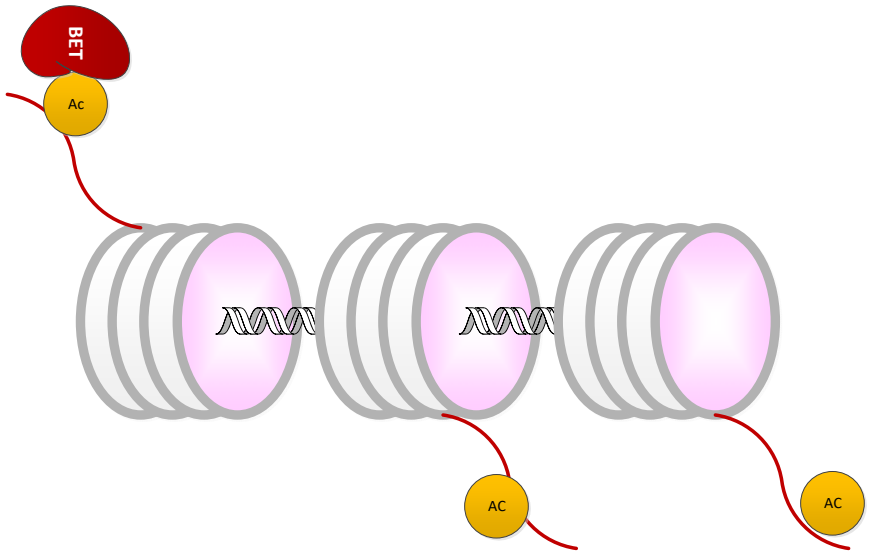
Conventional wisdom held that complexes involved in chromatin dynamics are not 'druggable' targets. However, small molecules that inhibit bromodomain and extra-terminal (BET) proteins have been described. We examine these developments and discuss the implications for small molecule epigenetic targeting of chromatin networks in cancer.

There is recent evidence that, in addition to BRD2, BRD4 can also co-activate pro-inflammatory genes that depend on NF-κB transcription, through interaction with acetylated RELA. A full account of the interplay of BRD2, BRD3 and BRD4, and how they co-activate NF-κB and cooperate with SWI/SNF complexes to regulate the transcription responses of genes that encode important pro-inflammatory cytokines, such as TNF and IL-6, awaits exposition. These data are potentially relevant to the links between unresolved chronic inflammation or irritation and increased cancer risk, a long-established association. For example, the bowel inflammation that is characteristic of Crohn's disease and related conditions is strongly linked to colorectal cancer.

It is possible that inflammation promotes certain obesity-associated cancers that are resident in or near to inflamed white adipose tissue in insulin-resistant obese subjects. The role of unresolved, chronic inflammation and metabolic dysfunction in obesity-associated cancers is a considerable public health problem, and new epigenetically acting drugs such as the BET protein inhibitors might provide a novel pathway for treating or preventing obesity-associated cancer. Additional preclinical studies are required to more firmly establish the mechanisms

underlying hypotheses that the anticancer and anti-inflammatory properties of BET protein inhibitors usefully combine in a chemo-preventive strategy for the obesity-associated cancers.

The bromo domain is that set of 4 protein sequences which is common across the class. This common domain has the ability to attach itself and act as a histone editor as we depicted above. Thus as shown below the BET using the bromo domain can attach to an acetyl element on the histone tail and dramatically change the way genes are then expressed.



The above authors continue:

The mechanistic links between inflammation and cancer, and between inflammation and insulin-resistant obesity, ground an overarching hypothesis that, at the transcriptional level, chronic inflammation in obesity exacerbates risk for both metabolic complications and cancer. The ongoing and anticipated dire consequences of the world wide epidemic of obesity highlight the translational importance of this discovery, because about 90% of type 2 diabetes is attributable to obesity.

Chemoprevention of obesity-associated cancers by uncoupling NF-κB driven cytokine gene expression with small-molecule BET protein inhibitors would represent an innovative, epigenetically based approach to protect obese subjects who are at risk of both diabetes and cancer. Targeting one set of processes with a BET protein inhibitor might confer benefit by targeting other, apparently orthogonal transcriptional networks that are actually fundamentally related. An extraordinary interconnectedness of chromatin-dependent transcription programmes is thus revealed.

One could argue that the insulin resistant obesity, of which we are seeing epidemic proportions occurring, especially in youth, could be a set up for multiple malignancies. The BET elements may very well be key initiators of the process.

From Sahai et al,

There is increasing interest in inhibitors targeting BET (bromodomain and extraterminal) proteins because of the association between this family of proteins and cancer progression. BET inhibitors were initially shown to have efficacy in hematologic malignancies; however, a number of studies have now shown that BET inhibitors can also block progression of non-hematologic malignancies.

…we summarize the efficacy of BET inhibitors in select solid tumors; evaluate the role of BET proteins in mediating resistance to current targeted therapies; and consider potential toxicities of BET inhibitors. We also evaluate recently characterized mechanisms of resistance to BET inhibitors; summarize ongoing clinical trials with these inhibitors; and discuss potential future roles of BET inhibitors in patients with solid tumors. …

Epigenetic changes that occur during cancer progression are increasingly recognized as a potential target for therapeutic intervention. Bromodomains (BRDs) are evolutionarily conserved protein interaction modules that bind to acetylation motifs present in histones and enable recruitment of transcription factors and other chromatin regulators during the precise sequence of events involved with RNA transcription.

*The BET (**BRD and extra-terminal**) family of proteins regulates the transcription of genes involved in several human diseases and includes family members BRD2, BRD3, BRD4, and the testis-specific BRDT. Significantly, BRD4 has been established as a key regulator of transcriptional elongation by recruiting the positive transcription elongation factor b (P-TEFb) complex to chromatin.*

BRD4 also mediates the formation of the active form of P-TEFb, which in turn phosphorylates and activates RNA polymerase II. BRD4 is enriched in large numbers of enhancer regions, and also in some large super-enhancer regions, and mediates expression of key transcription factors important for cancer development and progression. BET inhibition displaces BRD4 from these super-enhancers and blocks expression of certain key oncogenes, such as MYC. Besides binding histones, BRD proteins can also regulate cellular function by binding to a number of other proteins.

The following is a list of some of the bromodomain impacts and related malignancies.

Gene in Bromodomain	Cancer
KAT3A (CBP) H2AK5 H2BK12–K15 H3K14–K18 H4K5–K8	Acute myeloid leukemia, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, non-Hodgkin's lymphoma, and transitional-cell bladder cancer
KAT3B (p300) H2AK5 H2BK12–K15 H3K14–K18 H4K5–K8	Colorectal, breast, pancreatic, acute myeloid leukemia, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, transitional-cell bladder cancer
SMARCA4 (BRG1)	Lung, rhabdoid, medulloblastoma, breast, prostate, pancreas
SMARCA2 (BRM)	Squamous-cell carcinomas of the head and neck
BRD1 PHD finger	Acute lymphoblastic leukemia
BRD3	NUT midline carcinoma
BRD4 NUT midline carcinoma	NUT midline carcinoma
TRIM33 PHD finger	Papillary thyroid
PBRM1 Renal, breast	Papillary thyroid

For example in Shtivelman et al:

The recent demonstration of preclinical efficacy of inhibiting bromodomain and extraterminal (BET) proteins in different malignancies may be applicable in CRPC. BET domain protein BRD4 was shown to interact with the N-terminal domain of AR, and the BET domain inhibitor JQ1 disrupts AR transcription program in vitro and inhibited growth of CRPC in mouse models in vivo, presenting a new epigenetic approach

Thus the bromo domain is one of many examples where epigenetic effects can give rise to gene expression deviations. These in turn result in malignant expression.

8 EXAMPLES

PTX3 has a significant use as a marker in many cancers.

8.1 PROSTATE

We have already demonstrated the usefulness in PCa. From Tony et al we have:

The term proteomics was introduced as an analogy to that of 'genomics' [108]. While genomics involves the study of the genes that code for a protein, proteomics is focused on studying the proteins themselves—thus providing a clearer reflection on cellular activity [109]. Proteomic-based experiments can be used to characterize any alterations in protein expression during disease progression.

The emerging field of proteomics has had a tangible impact on biomarker discovery in PCa. A useful cancer protein biomarker would be a protein measurable in body fluids or tissues that could reflect the presence of cancer and provide information on the cancer's stage, aggressiveness and how well the patient is responding to therapy.

For such a biomarker to be clinically applicable, however, it must also meet the following criteria: (i) the protein must be easy to measure at a reasonable cost; (ii) elevated levels of the protein must provide information that would not be available without that protein and (iii) the information obtained from measurement of the protein can be used to guide clinical decision making. Due to the complex nature of cancer, uniformity is non-existent among each histologic cancer type and within each individual tumor. As such, examination of combinations of potential protein biomarkers as panels is believed to provide greater promise for improved PCa diagnosis and monitoring. This trend is reflected in the most recent publications related to PCa associated biomarker discovery

They then include the following Table for a broad summary wherein PTX3 is but one:

Title	Marker(s)
Prostate stromal cell proteomics analysis discriminates normal from tumour reactive stromal phenotypes	Proteins including TAGLN, VDAC1, VDAC2, ALDH1A1
Novel potential serological prostate cancer biomarkers using CT100+ cancer antigen microarray platform in a multi-cultural South African cohort.	41 antigen biomarkers including GAGEI, ROPN1, SPANXA1, PRKCZ, MAGEB1, p53, S15A, S46A, FGFR2, COL6A1, CALM1
Quantitative proteomic study of human prostate cancer cells with different metastatic potentials	SETDB1
Phosphoproteome analysis demonstrates the potential role of THRAP3 phosphorylation in androgen-independent prostate cancer cell growth.	THRAP3
Glycosylation status of serum immunoglobulin G in patients with prostate diseases	Glycosylation changes in IgG
Interlaboratory study on differential analysis of protein glycosylation by mass spectrometry: the ABRF glycoprotein research multi-institutional study 2012	Glycoforms of PSA
An integrative proteomics and interaction network-based classifier for prostate cancer diagnosis	3 proteins (PTEN, SFPQ, HDAC1)
Identification of novel serological tumor markers for human prostate cancer using integrative transcrip tome and proteome analysis	IMPDH2
Identification of phosphorylated proteins involved in the oncogenesis of prostate cancer via Pinl-proteomic analysis	TFC
Urinary CD14 as a potential biomarker for benign prostatic hyperplasia—discovery by combining MALDI-TOF-based biostatistics and ESI-MS/MS-based stable-isotope labeling	CD14
Proteomics-based signature for human benign prostate hyperplasia and prostate adenocarcinoma	15 proteins (TPM1, PHB, KRT8, TUBB2, DES, Glycerol 3 phosphate, P4HB, EHHADH, HSPA5, KRT18, SERPINA1, CKB, HSPA8, ATP5B, ANXA4
CD90/THY1 is overexpressed in prostate cancer-associated fibroblasts and could serve as a cancer biomarker	CD90/THY1
Proteomic analysis of pancreatic secretory trypsin inhibitor/tumor-associated trypsin inhibitor from urine of patients with pancreatitis or prostate cancer	PSTI
In vivo chemoresistance of prostate cancer in metronomic cyclophosphamide therapy	3 proteins (TXN, CTSB, ANXA3)
The cancer-related Runx2 protein enhances cell growth and responses to androgen and TGF-beta in prostate cancer cells	Runx2
Proteomic analysis of conditioned media from the PC3, LNCaP, and 22Rvl prostate cancer cell lines: discovery and validation of candidate prostate	4 proteins (FST, CXCL16, PTX3, SPON2)

8.2 LUNG

In the work by Diamandis et al:

PTX3, but not KLK11 or progranulin, is a new serum biomarker for lung carcinoma. Its diagnostic sensitivity and specificity is similar to other clinically used lung cancer biomarkers. More studies are needed to establish if PTX3 has clinical utility for lung cancer diagnosis and management….We conclude that PTX3 is a novel biomarker for lung carcinoma, which displays comparable sensitivity and specificity to other currently used lung cancer biomarkers. It appears that the observed serum elevations of PTX3 are associated with inflammation and cancer cell apoptosis around the tumor microenvironment.

More studies will be necessary to establish if PTX3 has clinical utility in lung cancer, either alone or as a member of a biomarker panel. In such studies, inclusion of patients with benign lung diseases, in order to further assess the specificity of these biomarkers, would be important. As mentioned earlier, PTX3 may also be elevated in other malignancies such as prostate cancer and liposarcomas.

8.3 HEAD AND NECK

From Chang et al:

Overexpression of the epidermal growth factor (EGF) receptor (EGFR) is associated with enhanced invasion and metastasis in head and neck squamous cell carcinoma (HNSCC). Long Pentraxin PTX3 is involved in immune escape in cancer cells. Here, we identified PTX3 as a promoting factor that mediates EGF-induced HNSCC metastasis.

EGF-induced PTX3 transcriptional activation is via the binding of c-Jun to the activator protein (AP)-1 binding site of the PTX3 promoter. PI3K/Akt and NF-kB were essential for the PTX3 activation. EGF-induced PTX3 expression was blocked in c-Jun- and NF-KB-knockdown cells. EGF-mediated PTX3 secretion resulted in the enhancement of cell migration and invasion, and interactions between cancer and endothelial cells.

The tail-vein injection animal model revealed that depletion of PTX3 decreased EGF-primed tumor cell metastatic seeding of the lungs. In addition, fibronectin, matrix metalloproteinase-9 (MMP9) and E-cadherin were essential components in EGFR/PTX3-mediated cancer metastasis. In conclusion, PI3K/Akt and NF-KB-dependent regulation of AP-1 mediates PTX3 transcriptional responses to EGF. Autocrine production of EGF-induced PTX3 in turn induces metastatic molecules, activating inflammatory cascades and metastasis.

8.4 PANCREAS

From Kondo et al:

Pentraxin family members, especially PTX3, may be used as promising biomarkers in the prognosis of pancreatic carcinoma patients….Inflammatory responses have decisive roles at different stages of tumour development, including initiation, promotion, malignant conversion,

invasion, and metastasis, and affect immune surveillance and response to therapy. The invasive capacity of malignant cells has been observed to increase in the presence of inflammatory cytokines, including TNF-alpha, interleukin (IL)-1beta, and IL-6, as well as transcription factors, including AP-1, NF-kB, and STAT3.

In a previous study, we identified C-reactive protein (CRP), which is produced via IL-6 and TNF-alpha stimulation in the liver, as an important factor in the prognosis of pancreatic carcinoma. In other studies, long pentraxin (PTX3), a member of the pentraxin family, which includes CRP and whose members may have a significant role in tumour inflammatory and malignant behaviours, was reported to be overexpressed in several malignancies, including liposarcomas and lung cancer.

These findings indicate that reduction of the key inflammatory mediators may be an important means of promoting antitumour activity. In a previous study, we had observed direct secretion of PTX3 from pancreatic carcinoma cell lines in vitro. Building on this finding, we aimed to determine the biological significance of PTX3 in pancreatic cancer via further in vitro study of several pancreatic carcinoma cells lines, as well as prospective clinical investigation of the clinical significance of plasma PTX3 expression in chemotherapy naive pancreatic carcinoma patients. We found that PTX3 expression might be a promising biomarker for pancreatic carcinoma prognosis

9 OBSERVATIONS

We have examined the issue of inflammation and the impact on the immune system and cancer. These have been issue of concern for at least a century. We know how powerful the immune system can be, both for good and for adverse events. Inflammation also has been a major concern and we are just beginning, in my opinion, to understand its causes and effects. Obesity is a major problem, and within the context of obesity is the issue of inflammation, especially for those who have Type 2 Diabetes. Thus there is a clear need to understand inflammation, especially as we are able to deal with many cancers, and most likely cancer may then become a co-morbidity of a high costs for those who are obese.

In this paper we have used a rather idiosyncratic approach to the subject. On the one hand we focus on the innate immune system, and specifically pentraxins. Secondly we look at pentraxin and in contrast BET, bromodomain, effects and cancers. The reason why is simply that both relate directly to inflammation and specifically obesity. Thirdly we examine these across a broad spectrum of organs and finally we have included some discussions on the free radical problem. They all fit nicely since free radicals are a result of obesity and Type 2 diabetes, the resulting inflammation is chronic, the chronicity drives the innate system via pentraxins and the epigenetic elements via bromodomain proteins. The next result is a dual assault on genes, DNA expression and major epigenetic defects via histone acetyl defects.

9.1 USE OF THE COMPLEMENT SYSTEM

The innate system, complement and pentraxins, has been proposed as a means to attack cancer especially in an inflammatory environment. As Macor and Tedesco note:

Direct killing of tumor cells by the membrane attack complex (MAC) represents one of mechanisms used by the C(omplement) system to control tumor growth. However, C may also exert its antitumor activity through additional non-cytotoxic effects. Thus C3b deposited on tumor cells and subsequently converted into iC3b promotes binding of these cells to the C receptors CR1 and CR3 expressed on human leukocytes. Although CR1 and CR3 fail to trigger the killing of tumor cells following their interaction with their respective ligands, C3b and iC3b, evidence collected both in vitro and in vivo indicate that the adhesion of iC3b-coated tumor cells to phagocytes and natural killer (NK) cells expressing CR3 (CD11b–CD18) results in C-dependent cell cytotoxicity (CDCC) provided that a second signal is delivered to tumor cells by anti-tumor Abs (Fc-FcR) that mediate Ab-dependent cellular cytotoxicity (ADCC).

These data suggest that the C system plays an important role in immunotherapy of cancer and acts as an additional weapon in support of the standard therapy provided by surgery, chemotherapy and radiation against tumor cells particularly in the control of the minimal residual disease. Optimal conditions are required for C to be effective, which include the level of expression of tumor antigens present on the surface of tumor cells, the class of Abs and the reduced expression of C inhibitors.

These data suggest that the C(omplement) system plays an important role in immunotherapy of cancer and acts as an additional weapon in support of the standard therapy provided by surgery, chemotherapy and radiation against tumor cells particularly in the control of the minimal residual disease. Optimal conditions are required for C to be effective, which include the level of expression of tumor antigens present on the surface of tumor cells, the class of Abs and the reduced expression of C inhibitors.

9.2 MARKER AND TARGET?

We can thus ask if we have a cause and effect and if so do we have a control mechanism. As we have seen great strides in immunotherapy via PD-1 and like Mabs and controls we could anticipate a similar result with the targeting of the innate and epigenetic drivers.

10 REFERENCES

1. Abbas, et al, Immunology, 9th Edition, Elsevier (Philadelphia) 2018.
2. Aggarwal et al, Targeting Inflammatory Pathways for Prevention and Therapy of Cancer: Short-Term Friend, Long-Term Foe, Clin Cancer Res 2009;425 15(2) January 15, 2009.
3. Akira, S., et al, Pathogen Recognition and Innate Immunity, Cell 124 2006.
4. Alfadda, et al, Reactive Oxygen Species in Health and Disease, Hindawi Publishing Corporation Journal of Biomedicine and Biotechnology Volume 2012
5. and localizes in extracellular traps
6. Armstrong, L., Epigenetics, Garland (New York) 2014.
7. Asangani, et al, BET Bromodomain Inhibitors Enhance Efficacy and Disrupt Resistance to AR Antagonists in the Treatment of Prostate Cancer, Mol Cancer Res; 14(4) April 2016
8. Belkina, A., G. Denis, BET domain co-regulators in obesity, inflammation and cancer, Nature Reviews, Cancer, Volume 12, July 2012, 465
9. Bonavita, et al, PTX3 acts as an extrinsic onco-suppressor, Oncotarget, Vol. 6, No. 32, 2015
10. Bottazzi, Barbara . An Integrated View of Humoral Innate Immunity: Pentraxins as a Paradigm (Annual Review of Immunology Book 28) (Kindle Locations 19-20). Annual Reviews. Kindle Edition.
11. Chang et al, PTX3 gene activation in EGF-induced head and neck cancer cell metastasis, Oncotarget, Vol. 6, No. 10 2015
12. Cleveland and Kasten, Cancer: A radical approach to treatment, Nature, Sept 2000.
13. Clevers, At the Crossroads of Inflammation and Cancer, Cell, Vol. 118, 671–674, September 17, 2004
14. Dawson et al, Targeting Epigenetic Readers in Cancer, NEJM, august 16, 2012
15. Diamandis, E., et al, Pentraxin-3 Is a Novel Biomarker of Lung Carcinoma,
16. Fakona, S., et al, Cancer Immunotherapy, the beginning of the end of cancer, BMC, 2016.
17. Garraway, L., E. Lander, Lessons from the Cancer Genome, Cell Mar 2013.
18. Gorlach et al, Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? RedoxBiology6(2015)372–385
19. Gray, S., Epigenetic Cancer Therapy, Academic (New York) 2015.
20. Grivennikov, S. et al, Immunity, Inflammation, and Cancer, Cell 140, 883–899, March 19, 2010.
21. Halliwell, B., J. Gutteridge, Free Radicals in Biology and Medicine, Oxford (New York) 2015.
22. Hansson, G., Inflammation, Atherosclerosis, and Coronary Artery Disease, NEJM, 2005.

23. Harijith et al, Reactive oxygen species at the crossroads of inflammasome and inflammation, Frontiers in Physiology, Sept 2014

24. Hayden, M., S. Ghosh, NF-kB in Immunology, Cell Research, 2011.

25. Hoesel, B., J. Schmid, The Complexity of NF-kB Signalling in Inflammation and Cancer, Mol Can, 2013

26. Hussain, P., C. Harris, Inflammation and cancer: An ancient link with novel potentials, Int. J. Cancer: 121, 2373–2380 (2007)

27. Inforzato, A., et al, The "sweet" side of a long pentraxin: how glycosylation affects PTX3 functions in innate immunity and inflammation, Front in Immun, Jan 2013.

28. Jaillon, S., et al, The humoral pattern recognition receptor PTX3 is stored in neutrophil granules, JEM The Rockefeller University Press, Vol. 204, No. 4, April 16, 2007 793–804

29. Karin, M., L. Staudt, NF-kB, CSHL Press (New York) 2010.

30. Kondo et al, Clinical impact of pentraxin family expression on prognosis of pancreatic carcinoma, British Journal of Cancer (2013) 109, 739–746

31. Kundu et al, Inflammation: Gearing the journey to cancer, Mutation Research 659 (2008) 15–30

32. Li, K., Promising Targets for Cancer Immunotherapy, Int Jrl Mol Sci, 2017.

33. Macor, P., F. Tedesco, Complement as Effector System in Cancer Immunotherapy, Immun Letters 2007.

34. Marcelino, E., et al, Mast Cell Functions, JHC 2014.

35. McGarty, T., Cancer Immunotherapy: A Systems Approach, DRAFT 2017, www.telmarc.com

36. Merle, N., yet al, Comnplement System, Pts I and II, Front Immunology June and May 2015.

37. Ozcan, et al, Biochemistry of Reactive Oxygen and Nitrogen Species, in Sivakumar Joghi Thatha Gowder, ISBN 978-953-51-2200-5, Published: November 11, 2015 under CC BY 3.0 license.in Basic Principles and Clinical Significance of Oxidative Stress

38. Rajalingam, R., Human Diversity of Killer Cell Immunoglobulin like Receptors and Disease, Korean Jrl Hematology, Dec 2011.

39. Rubin, R., D. Strayer, Pathology, Lippincott (New York) 2012.

40. Sahai et al, Targeting bet bromodomain proteins in solid tumors, Oncotarget, Vol. 7, No. 33, 2016.

41. Shishido, S., et al, Humoral innate immune response and disease, Clin Immunol. 2012 August ; 144(2): 142-158

42. Shtivelman et al, Molecular pathways and targets in prostate cancer, Oncotarget, Vol. 5, No. 17, 2014.

43. Stallone, et al, Pentraxin 3: A novel biomarker for predicting progression from prostatic inflammation to prostate cancer, AACR Journal, Published OnlineFirst on June 20, 2014; DOI: 10.1158/0008-5472.CAN-14-0369.

44. Steiner, M., et al, The Evolution of the Human Basophil Biology from Neglect towards Understanding their Immune Functions, BioMed Res Inter, 2016.

45. Tonry et al, The Role of Proteomics in Biomarker Development for Improved Patient Diagnosis and Clinical Decision Making in Prostate Cancer, Diagnostics 2016, 6, 27;

46. Turvey, S., et al, Innate immunity, Journal of Allergy Clin Immunol, Volume 125, Number 2

47. Ueno, H., et al, Dendritic Cell subsets in Health and Disease, Immuno Rev 2007.