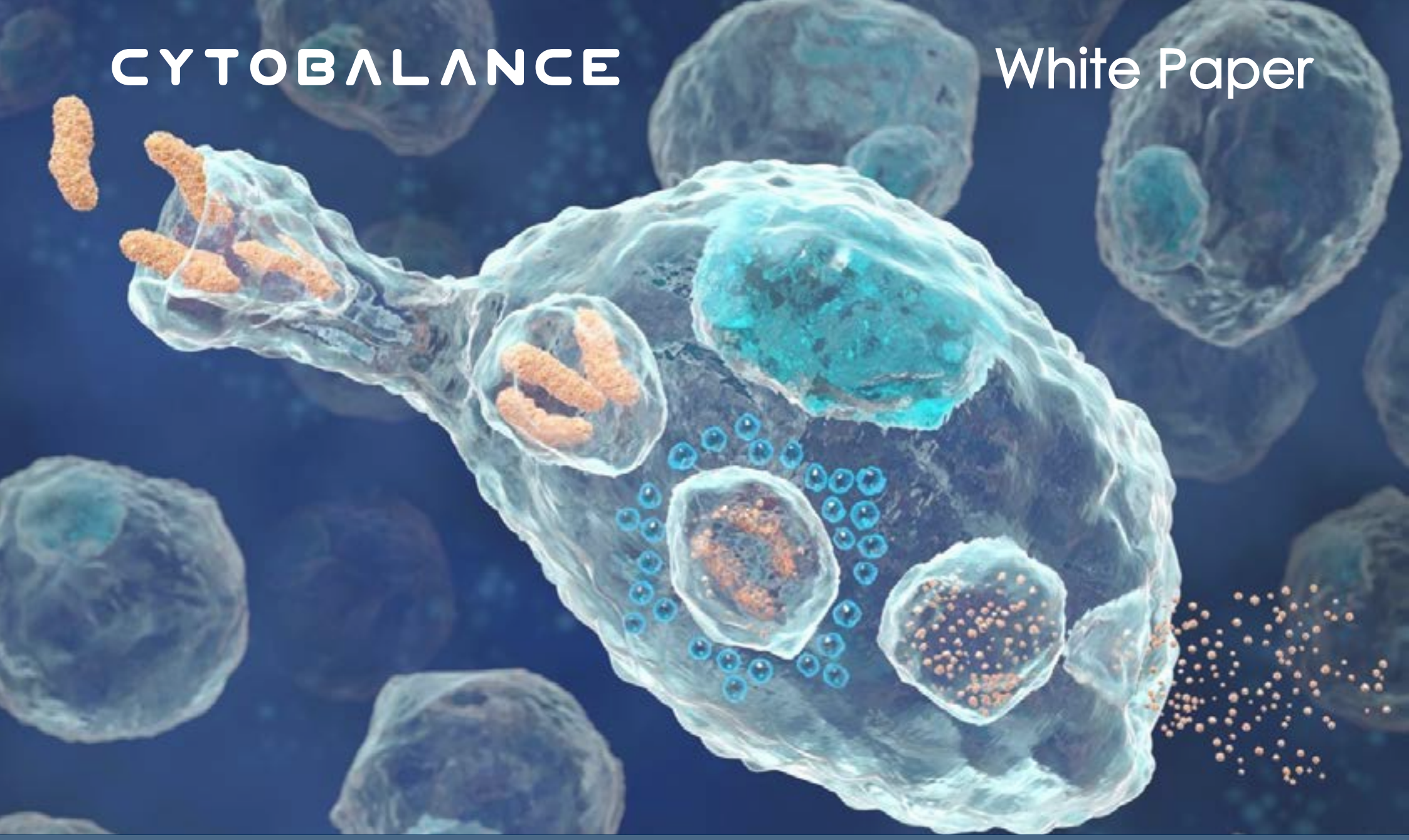
CYTOBALANCE

White Paper



PROINFLAMMATORY CYTOKINES FOR EVALUATION OF CHRONIC INFLAMMATION

INTRODUCTION

For many years, inflammation has been referred to as the response of the organism to tissue injury and infection. However, lots of things have changed since Aulus Cornelius Celsus (ca 25 BC – ca 50 AD) first definition of acute inflammation as a state characterized by the presence of swelling, pain and – hence the name, derived from "flame" – redness and warmth.

In XIX century the father of modern pathology, Rudolph Virchow, concluded that there were various inflammatory processes. Nowadays it is known that inflammation can be induced by tissue stress and malfunction too, yet in the absence of infections or overt tissue damage. In particular, low-grade, chronic, inflammation is triggered by sentinel immune cells that monitor for tissue stress and malfunction; it has been associated with a number of lifestyle factors (such as smoke, but also unhealthy diets, sleep deprivation and low levels of physical activity) and conditions (such as prehypertension and cancer), and new associations are constantly emerging. It is currently recognized that inflammation is present when there is an increase of the concentration or the activity of elements involved in innate responses, such as inflammatory cytokines or the transcription factor NF-kB – a master regulator of inflammation.

HOW INFLAMMATION IS REGULATED

Low-grade inflammation is not accompanied by the classic signs of the acute process (swelling, pain, redness and warmth). However, the ultimate goal of every type of inflammation is return tissues to their normal state, and acute and chronic inflammation share many of effector molecules and cells involved in this task. Among other factors, proinflammatory cytokines such as such as TNF-□, IL-1, 6, and 12, and interferon (IFN) play essential roles. Produced by activated inflammatory cells, their signaling is regulated by different mechanisms, among which suppressor of cytokine signaling (SOCS) activity is particularly important. It act on NF-kB, signal transducers and activators of transcription (STAT) 1 and 3, and other transcription factors, and its presence further activates inflammatory cells.

NF-kB activation by inflammatory signals results in the coordinate expression of multiple proinflammatory and innate immune genes (including IL-1, 4 and 6, and TNF- α) by translocation of NF-kB to the nucleus, where it binds to DNA. However, NF-kB activation is not solely proinflammatory: depending on the context, it can also participate in anti-inflammatory responses. It acts as a proinflammatory transcription factor when activated during the onset of inflammation, but it is crucial for the activation of anti-inflammatory genes during inflammation resolution. Most of NF-kB activated genes overlap with target genes activated by inflammatory stimuli through STAT1 and STAT3. The former is essential for IFN-γ signaling and promotes NF-kB-induced programmed cell death (apoptosis); conversely, STAT3 is activated by IL-10 and promotes cell proliferation and survival, suppressing NF-kB pathway.

Also, the increase of the concentration of the acute phase protein CRP (C-reactive protein), produced by liver cells in response to circulating proinflammatory cytokines such as IL-6, is regarded as a synonym for inflammation, even when only modest. In particular, low-grade inflammation is associated with CRP levels minimally elevated (3-10 mg/l) compared with those associated with tissue injury or infection-arising; such modest CRP increases are found in a consistent proportion of western population (e.g., in 30% of Americans) and are stable markers of inflammation usually unaffected by physiological or pathological processes other than underlying inflammation.

Whilst limited and short-lived expression of cytokines is beneficial, the longtime course of cytokines expression during chronic inflammation is detrimental and leads to chronic diseases, including cancer.

INFLAMMATION AND AGING

According to the research field that studies the molecular link between aging and chronic diseases related to age (the so-called "geroscience") inflammation is one of the mechanisms shared by age-related diseases. In particular, chronic, low-grade inflammation occurring in the absence of infection that takes place during aging is called "inflammaging".

Inflammaging is primarily driven by endogenous signals, including the presence of cell debris, misplaced self molecules and misfolded or oxidized proteins. Macromolecular damage, metabolism, epigenetics, stress, proteostasis, and stem cell regeneration are all interconnected factors influencing this low-grade inflammation state, which is characterized by a chronic activation of the innate immune system which can become damaging.

There are several cellular and molecular mechanisms involved: cellular senescence; the dysfunction of mitochondria (the power stations of the cell);

defective mechanisms of autophagy and mitochondria degradation; the activation of the intracellular multiprotein complex that detects biological and non-biological stressor (the inflammasome); the dysregulation of the system controlling protein degradation; the activation of the response to DNA damage; changes in the composition of the microbiota (dysbiosis); and nutrient excess and overnutrition, which can generate a specific type of chronic inflammation called "metaflammation" associated with metabolic diseases such as obesity and type 2 diabetes. All this stimuli converge on a small number of sensors which trigger the innate immune response causing inflammation and an adaptive metabolic response. This process is critical for survival until middle age, but with aging the inflammatory response usually increases, becoming detrimental – and eventually leading to inflammation – in post-reproductive age. The increase of senescent cells and their accumulation and the hyperactivation of immune response play an important role in this phenomenon; moreover, according to the garbaging theory, the age-related progressive impairment of cell debris elimination systems largely sustains inflammaging.

The inflammatory tone can increase progressively during several years or decades, depending on genetics, anatomical features, immunological history, and lifelong lifestyle habits. Nutrient excess and overnutrition fuel inflammaging; metaflammation contributes to the onset of insulin resistance, activating inflammatory responses that affect organs such as brain, muscle, pancreas and liver, and adipose tissue.

Lipids play a central role in metaflammation, increasing oxidative stress and cytokines such as IL-6. After a high-fat meal blood levels of lipopolysaccharide (LPS, the endotoxin associated with sepsis) increase too; this generates a state called metabolic endotoxaemia associated with low-grade inflammation and with the development and the progression of cardiometabolic diseases. Finally, high-fat diets can alter gut microbiota, further increasing LPS production; dysregulation in meal timing contributes to this metabolic and inflammatory dysregulation, whereas nutrient-dense diets induce the increase of adipocytes size, until they reach a structurally critical condition that contributes to metaflammation.

Representing the boundary between diet, metabolism and the innate immune response, gut microbiota has a crucial role in inflammaging. Moreover, it undergoes profound remodeling with age, losing diversity and relative abundance.

This characteristic decrease in gut microbiota is associated with almost all age-related diseases. Even if in the case of extreme longevity (> 105 years) longevity-adapted and possibly beneficial subdominant species (such as *Akkermansia, Bifidobacterium* and Christensenellaceae) can increase, centenarians' gut microbiota changes typically consist in an increase in Proteobacteria (minor components of the gut microbiota that in some circumstances can induce pathology) and a decrease in bacteria producing butyrate (a short-chain fatty acid that protects against inflammatory bowel diseases), and correlate with the increase in pro-inflammatory cytokines IL-6 and IL-8 levels.

The aging process and inflammaging are different in different individuals, depending both on early critical immunological experiences such as intrauterine stimuli, type of birth, neonatal feeding and the early use of antibiotics and on events in adulthood (such as infections and diet). Clinically manifest diseases accelerate the rate of aging; metabolic age-related diseases could then be considered manifestations of aging acceleration, and metabolic history of an individual influences the risk of developing age-related chronic metabolic diseases. Aging progresses also during the so-called prodromic phase preceding the overt manifestation of age-related pathologies. Specific biomarkers, such as cell-free mitochondrial DNA (cf-mtDNA) and cytokines can help the analysis of metabolic aging, distinguishing healthy and unhealthy aging.

In the presence of a variety of pathological conditions, cell damage and death increase the amount of cf-mtDNA that can be found in the blood. This cf-mtDNA – which seems to be a familial or genetic trait – is an example of inflammatory garbage and is present in large amounts in the blood of elderly individuals and nonagenarians.

THE ROLE OF MITOCHONDRIAL DNA

Mitochondrial DNA (mtDNA) is among inflammation-causing agents. Primary factors in the liberation of mtDNA from mitochondria are cellular stress and necrosis; moreover, several studies suggested other mechanisms, such as programmed necrosis (necroptosis) and liberation of free extracellular mitochondria from platelets.

Plasma mtDNA levels are strongly variable between individuals. Almost 20% of this variability is explained by a familiar component; other factor potentially influencing it are mutation or drugs affecting intracellular mtDNA copy number, immune mechanisms controlling the response to inflammatory triggers, and the propensity to cell death, that can be enhanced in response to chronic viral infections that

are common in old people, such as cytomegalovirus infection in southern Europe populations.

Mitochondria evolutionarily derive from energy-producing bacteria which have been engulfed by ancestral cells approximately 2 billion years ago. That is why mitochondrial constituents (including freely circulating mtDNA) are immunogenic in a way similar to bacterial molecules – and that is why when released into the cytosol and extracellular environment they trigger innate immune responses, promoting inflammation.

Elevated circulating mtDNA is associated with chronic organ-specific and systemic illnesses. For example, in diabetes mtDNA levels are higher in patients with coronary artery disease than in patients without coronary artery disease, and in obese people with steatohepatitis mitochondria enclosed in microparticles can be detected in plasma. mtDNA levels correlate with autoimmune diseases too, for example with rheumatoid arthritis, granulomatosis with polyangiitis, and systemic lupus erythematosus (SLE). In SLE a defect in mitochondrial clearance leads to abnormal extrusion of oxidised mtDNA, and anti-mtDNA antibodies are found; mtDNA released from inflammatory cells is higher and forms entities proinflammatory in nature.

In general, the proinflammatory action of mtDNA depends on its oxidization; the highly oxidative extracellular environment typical of chronic inflammatory diseases may overwhelm antioxidant systems, further enhancing its inflammatory potential. Deregulation of local mechanisms for mitochondrial homeostasis contributes to organ specific pathologies, whereas mtDNA release into the circulation generates a more systemic effect. However, failure of local mechanisms may give rise to more wide-ranging consequences too, such as SLE or other autoimmune diseases.

Moreover, mtDNA plasma levels gradually increases after the fifth decade of life. This association is not a mere epiphenomenon: the increase in plasma mtDNA can contribute to the onset and maintenance of the proinflammatory status typical of inflammaging.

Highest mtDNA plasma levels correlate with highest amount of proinflammatory cytokines, in particular TNF-α and interleukin (IL) 6, and experiments conduced in vitro demonstrated that after 16 hours of treatment at the highest concentration measured in oldest people mtDNA is able to increase peripheral blood monocytes response to LPS. It has also been suggested that the transfer of altered mtDNA between cells may play a role in Alzheimer's disease, and mtDNA is significantly higher in a number of cancers, including ovarian, testicular, prostate, bladder, renal cell and lung cancer.

mtDNA can bind a receptor able to sense DNA of bacterial and viral origin, Toll-like receptor 9 (TLR9). Activation of TLR9 induces cytokine production and immune responses. This receptor is expressed both by immune cells and by other type of cells, namely intestinal epithelial cells.

Moreover, mtDNA-mediated inflammation can be driven by inflammasome and stimulator of interferon genes (STING) pathways too. Inflammasome activation by mtDNA requires a priming step of TLR activation and leads to the maturation of IL-1β and IL-18. Finally, mtDNA can activate a STING-mediated interferon (IFN) response; aberrant mtDNA-STING signaling has been involved in inflammatory diseases such as SLE.

Given the relevance of mtDNA-mediated inflammation to many inflammatory diseases and the possibility of measuring mtDNA and use it as a biomarker, testing freely circulating mtDNA levels offers an opportunity to stratify individuals based on their inflammatory potential. Moreover, interfering with mtDNA increase represents a possible strategy to reduce a harmful immune activation. Among plausible approaches are augmenting mtDNA clearance, targeting cytosolic mtDNA release, diverting the cellular response following mitochondrial damage and reducing mtDNA inflammatory potential (for example by reducing its oxidation).

THE ROLE OF INFLAMMATION IN CANCER

The role of immune system in cancer development is being extensively studied. Years of research led to the conclusion that immunity serves both as a tumor suppressor and as an initiator or promoter of tumors. Among factors involved is chronic inflammation; in fact, more than 20% of all cancers are initiated or exacerbated by inflammation. For example, hepatocellular carcinoma, gastric cancer and colon cancer have all been associated with chronic inflammation. Gastritis, the inflammation of the lining of the stomach, correlates with gastric cancer, whereas colitis, the inflammation of the colon, associates with colon cancer. And in a mouse model, K-Ras oncogene mutation leads to pancreatic ductal adenocarcinoma development only if chronic pancreatitis is present.

Also, non-infectious causes of chronic inflammation, such as hormonal changes, cigarette smoke and other toxicants (for example adhesives, air fresheners, cleaning products and glue) increase cancer risk; it is estimated that almost 20% of smokers with inflammation of bronchial tubes mucous membrane (a bronchitis)

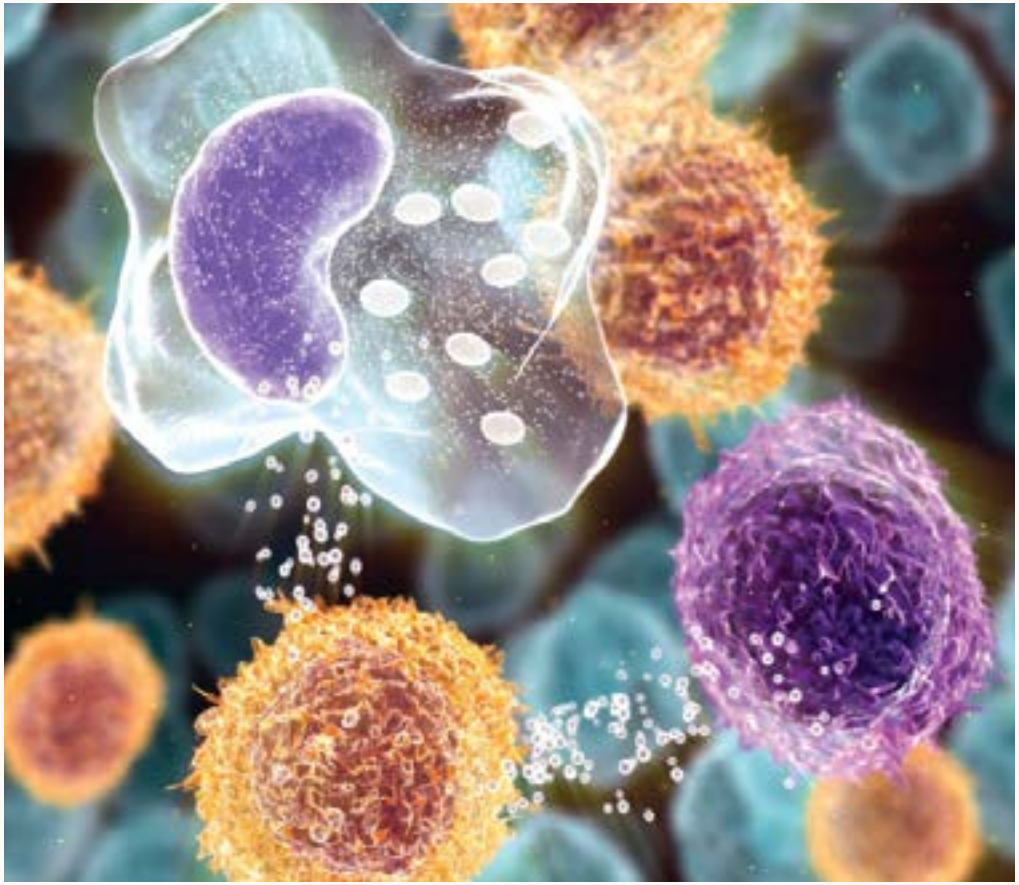
can develop cancer. Moreover, specific variants of inflammatory genes such as tumor necrosis factor (TNF) and interleukin (IL)-1 and mutations in genes playing important roles in innate immunity have been associated with cancer.
On the other hand, non-steroidal anti-inflammatory drugs (NSAIDs) use has been associated with a 30-60% reduction of colon cancer incidence.
In general, proinflammatory cytokines have been implicated in inflammation-associated tumors. This association is supported by epidemiological, genetic and pharmacological studies. Inflammation could contribute to cancer initiation through mutations in genes involved in cell division, survival or senescence, and to cancer development promotion through cellular and extracellular signals making cells resistant to growth-inhibitory signals, apoptosis or antitumor immunity. It acts both via an extrinsic pathway (that is, via chronic conditions associated with smoldering, non-resolving inflammation) and an intrinsic pathway (that is, genetic events that orchestrate the generation of cancer-related inflammation), playing a crucial role in several aspects of tumor development, including cellular transformation, cell survival, cell proliferation, invasion, metastasis formation and angiogenesis.
In fact, chronic inflammation is associated with the production of reactive oxygen and nitrogen species (ROS and RNS) from both inflammatory cells – recruited by inflammatory factors such as infectious organisms and physicochemical or endogenous elements – and epithelial cells. These highly reactive molecules damage DNA, proteins and lipids, promoting cancer development. Among mutagenic DNA lesions are 8-oxodG (8-oxo-7,8-dihydro-2'-deoxyguanosine) and 8-nitroguanine; they are generated by the presence of highly reactive peroxynitrite deriving from nitric oxide reaction with superoxide, and their presence was associated, for example, to cholangiocarcinoma related to *Opisthorchis viverrini* (liver fluke) infection. Moreover, hydrogen peroxide generated from superoxide reacts with iron to produce hydroxyl radical, a highly reactive species that can attack not only DNA but also proteins, causing carbonylation, an irreversible and irreparable modification. Inflammation contributes to lipid peroxidation too, and reactive lipids can further interact with and damage DNA and proteins. All these lesions result in molecules disfunction, damage tissues and activate stem cells for tissue regeneration. Moreover, ROS and RNS damage stem cells too, resulting in mutations accumulation and, eventually, cancer stem cells generation. Cancer stem-like cells DNA damage associated with inflammation leads to the development of cancers with aggressive clinical features. Finally, inflammation-related oxidative stress can form a vicious cycle, playing a critical role in cancer, and chronic inflammation is also associated with immune-suppression, a cancer risk factor.
Another key event of inflammation-related carcinogenesis is DNA methylation – the addition of a chemical group (methyl) to specific DNA sequence. ROS and RNS induce global DNA hypomethylation, resulting in genomic instability – a factor associated with cancer. But beside oxidative stress, also inflammatory cytokines such as IL-6 contribute to carcinogenesis affecting DNA methylation. In fact, under an inflammatory microenvironment proinflammatory cytokines affect the transcription of the gene encoding for DNA methyltransferase 1 (DNMT1), a protein involved DNA methylation. Among methylation sites are cytosine-phosphate-guanine (CpG) sites in tumor suppressor genes promoters and tumor suppressor microRNA-targeting oncogenes promoters. Normally, these sites are hypomethylated and tumor suppressor genes and microRNA are expressed; inflammation-associated alteration of DNMT1 expression enhances their methylation and downregulates their expression, an event that can represent the first step of carcinogenesis.
Inflammation causes genome instability too. It is associated with mutations in mismatch repair family members (proteins involved in error correction after DNA replication) or with their silencing, promoting a form of genetic instability called microsatellite instability. Moreover, it promotes chromosome instability and DNA double strand breaks.
Finally, tumors arise in the stroma, which includes innate immune cells producing a number of factors that can influence tumorigenesis by promoting growth and survival of cancer cells, angiogenesis, tissue invasion and metastases. Moreover, tumor-associated macrophages can produce immunosuppressive cytokines (such as IL-10 and transforming growth factor (TGF)-β) and other molecules that negatively influence T cells. That means that ongoing inflammatory responses can also suppress antitumor immune responses.
Aberrant activation of NF-kB and/or STAT3 is found in over 50% of all cancer. This activation renders both premalignant and fully transformed cells resistant to apoptosis, and accelerates their rate of proliferation, increasing tumor growth.
The role of NF-kB in cancer initiation, development and progression is dual; its activation has been observed in several types of cancer, and inflammation-inducing carcinogens (such as cigarette smoke) have been shown to activate it, but there is evidence for a role in tumor suppression too. Besides activating proinflammatory genes expression, NF-kB promotes tumor cells proliferation by

inducing proto-oncogenes expression and is fundamental in allowing cancer cells to avoid detection by immune cells. However, it is capable of switching from tumor suppressor to tumor promoter depending on the progression of tumorigenesis.
Also, STAT3 plays a dual role. It is constitutively activated in many primary tumor and human cancer cell lines, and accumulating evidence suggests that it is an oncogenic transcription factor. Its activation involves IL-6 production from inflammatory cells, and blocking its function in cancer cells leads to tumor cells apoptosis. However, STAT3 mediates the anti-inflammatory effect of IL-10 too, repressing NF-kB and STAT1 inflammatory activity. Moreover, it upregulates IL-10 and another anti-inflammatory mediator, TGF-β; therefore, blocking STAT3 in tumor cells promotes tumor-specific immune responses. Thus, suppression of STAT3 could be an effective way to prevent tumor development.

INFLAMMATION BIOMARKERS UPREGULATED IN CANCER

Several biomarkers of inflammation have been associated with cancer. Among them is CRP, a commonly used inflammatory marker whose production is one of the processes regulated by proinflammatory cytokines. Normally, CRP is present at very low levels difficult to detect in healthy individuals; however, it rapidly increases with inflammation.
Serum CRP levels positively associate with cancer too. In particular, CRP correlates with progressive disease and decreased survival in esophageal, gastric, colorectal, liver, pancreatic, ovarian and other cancers. Moreover, elevated CRP is associated with a higher risk of developing any cancer. For example, prediagnostic levels of CRP have been associated with increased colorectal cancer incidence and mortality, and with breast cancer risk.
In breast carcinoma, in which cytokines may be secreted both by inflammatory cells and tumor and stroma cells, a significantly cancer-affecting interplay exists between tumor cells and inflammatory molecules. The production of inflammatory cytokines such as TNF-α by tumor-associated macrophages (TAMs) may contribute to their tumor-promoting activities. TNF-α serum concentration has been associated with more advanced tumor phenotype. Moreover, elevated levels of IL-6 may contribute to disease progression, and elevated levels of IL-1β directly correlate with a more advanced disease.
In general, TNF is considered an important tumor-promoting cytokine in several cancers. It is produced during the initiation of inflammatory responses, plays a critical role in chronic inflammation maintenance, and is involved in tumor growth, angiogenesis, tissue remodeling, and metastasis.
Elevated expression of IL-17 (a proinflammatory cytokines whose levels increase in the presence of metastases) and of another cytokine playing an important role in its expression, IL-23, has been detected in colon, ovaries, lung, breast, stomach, skin, liver, and head and neck cancer. Moreover, in small cells lung cancer IL-17 expression has been associated with tumor stage and metastases number, and it could be a new prognostic biomarker.
Another potent angiogenic factor in non-small cell lung carcinoma is IL-10. In gastric cancer it seems to contribute to tumor progression through the suppression of antitumor immunity. A similar effect may be attributed to IL-12 and IL-18, whereas IL-1α correlates with metastases, IL-1β and IL-18 stimulate the proliferation of gastric cancer cell, and IL-6 correlates with tumor stage, depth of tumor, lymphatic and venous invasion, metastasis and unfavorable outcome. Finally, transforming growth factor β (TGFβ) seems to play a complex role in direct tumor suppression and progression that may depend on cancer stage and cell type and on its systemic immunosuppressive and anti-inflammatory properties. In general, several polymorphisms in genes encoding for inflammatory cytokines have been implicated as risk factors for gastric cancer.
Cytokines like TNF-α, IL-1β, IL-6, and IL-8 are upregulated in epithelial ovarian cancer; TNF-α, IL-6 and IL-8 have been involved in angiogenesis and metastases, and TGF-β can promote the transcription of genes involved in invasion and migration. IL-6 and 8 may contribute to chemoresistance too, and increased levels of cytokines correlate with decreased survival.
IL-1β, 6, 8, 10, TGF-β, and TNF-α are involved in pancreatic cancer too. Inflammatory cytokines can be upregulated and lead to exacerbation of tumor progression. They are increased in chronic pancreatitis (a condition associated with pancreatic cancer) and in cancer early stages. IL-8 levels in chronic pancreatitis are 9.3 times more elevated than in normal pancreas tissue, and in pancreatic cancer some polymorphisms in IL-6 gene make its levels increase. IL-1β is involved in tumor growth and metastasis. And TNF-α can be used as a target for pancreatic cancer treatment. Instead, IL-10 plays a dual role: it prevents growth of the tumor, but it is upregulated in patient with pancreatic cancer and correlates with tumor stage and prognosis.
Elevated expression of IL-6, 17 and 23 has been linked to adverse colorectal

cancer prognosis and to a more aggressive disease, and IL-6 participates in the growth of multiple myeloma cells too. Its elevated expression is also linked to increased risk of colorectal cancer, and in general its expression in cancer correlates with poor prognosis. Together with TNF, IL-6 promote tumor development through direct effects on premalignant cells and by creating a tumor-promoting environment.



EXPLOITING LIFESTYLE AGAINST INFLAMMATION: THE ROLE OF NUTRACEUTICALS

Lifestyle factors can modulate the production of inflammatory molecules, but not only, they can also induce the production of ROS, which in turn can induce inflammation by regulating molecules such as proinflammatory transcription factors.

In particular, nutraceuticals plays an interesting role in suppressing inflammatory pathways. They are food constituents with potential health benefits others than their nutritional value; in fact, their name is derived from "nutrition" and "pharmaceuticals". Usually they are products isolated from foods and sold in form of dietary supplements. However, there is no agreement on a unique definition, and sometimes the term "nutraceutical" refers to both dietary supplements and functional foods.

Nutraceuticals potentially useful against inflammation include caffeic acid phenethyl ester (CAPE), capsaicin, emodin, epigallocatechin gallate (EGCG), gugglesterone, sanguinarine, deguelin, quercetin, ginseng, ginger, vitamins C and D, gamma linoleic acid (GLA), gentianine and bromelain. Amomg others, curcumin and resveratol seem particularly interesting.

Curcumin belongs to the class of polyphenols, secondary plant metabolites with both anti-inflammatory and anticarcinogenic, antimutagenic and antioxidant properties. It is derived from the turmeric spice (*Curcuma longa*), and it is the yellow component of curry. It can hinder cancer cells production and promote apoptosis by decreasing the production of p53, a protein mutated in over 50% of cancers, and of NF-kB. In a murine model of ovarian cancer it was find to suppress STAT3 pathway, which is recognized as an important link between inflammation and cancer.

Curcumin exerts its anti-inflammatory action through multiple mechanisms that modulate the production and the activity of several molecules playing a role in inflammation. Curcumin downregulates inflammatory transcription factors, cytokines and protein kinases. It hinders NF-kB translocation into the nucleus, and in human myeloid leukemia cells it was found to inhibit the DNA binding of p65, the NF-kB subunit responsible for its strong activating potential resulting in the transcription of several proinflammatory genes; it is also known to prevent phosphorylation and degradation of IkBα, a central point in NF-kB activation, and exhibits anti-inflammatory properties by suppressing proinflammatory cytokines, including interleukins such as IL-6 and IL-12 and TNF-α; in the case of IL-6, the effect is more evident with higher systemic inflammation degrees.

Curcumin can also directly bind and inhibit cyclooxygenase COX-1 and COX-2 – the pharmacological targets of nonsteroidal anti-inflammatory drugs (NSAIDs) – and matrix metalloproteinases (MMPs) – a family of enzymes expressed in pathological conditions involving inflammation. Its supplementation leads to a significant decrease in CRP levels.

Finally, curcumin acts as an anti-inflammatory through its antioxidant action too. It suppresses ROS production, counteracts free oxygen radicals and inhibits lipid peroxidation.

Curcumin ingestion and pharmacologic use are labeled safe by United States FDA (Food and Drug Administration). Both curcumin and turmeric extracts are non-mutagenic and non-genotoxic; standardized powder and extract are safe for human use even at high doses of 1.5 grams a day of curcumin and for periods up to 6 months. Adverse effects, including abdominal pain, nausea and dyspepsia, are mild and similar to placebo ones.

Also, resveratrol is a polyphenolic compound (specifically, a flavonoid). It is found in the fruits of different blueberry species (*Vaccinium myrtillus, V. angustifolium, V. ashei* and *V. corymbosum*), in other berries, in grapes, in peanuts and in other plant sources; plants produce it in response to environmental stress, to which it promotes resistance.

Resveratrol has been associated with reduced risks of many lifestyle-related diseases. Nowadays, it offers a preventive option against different conditions, including inflammation and cancer.

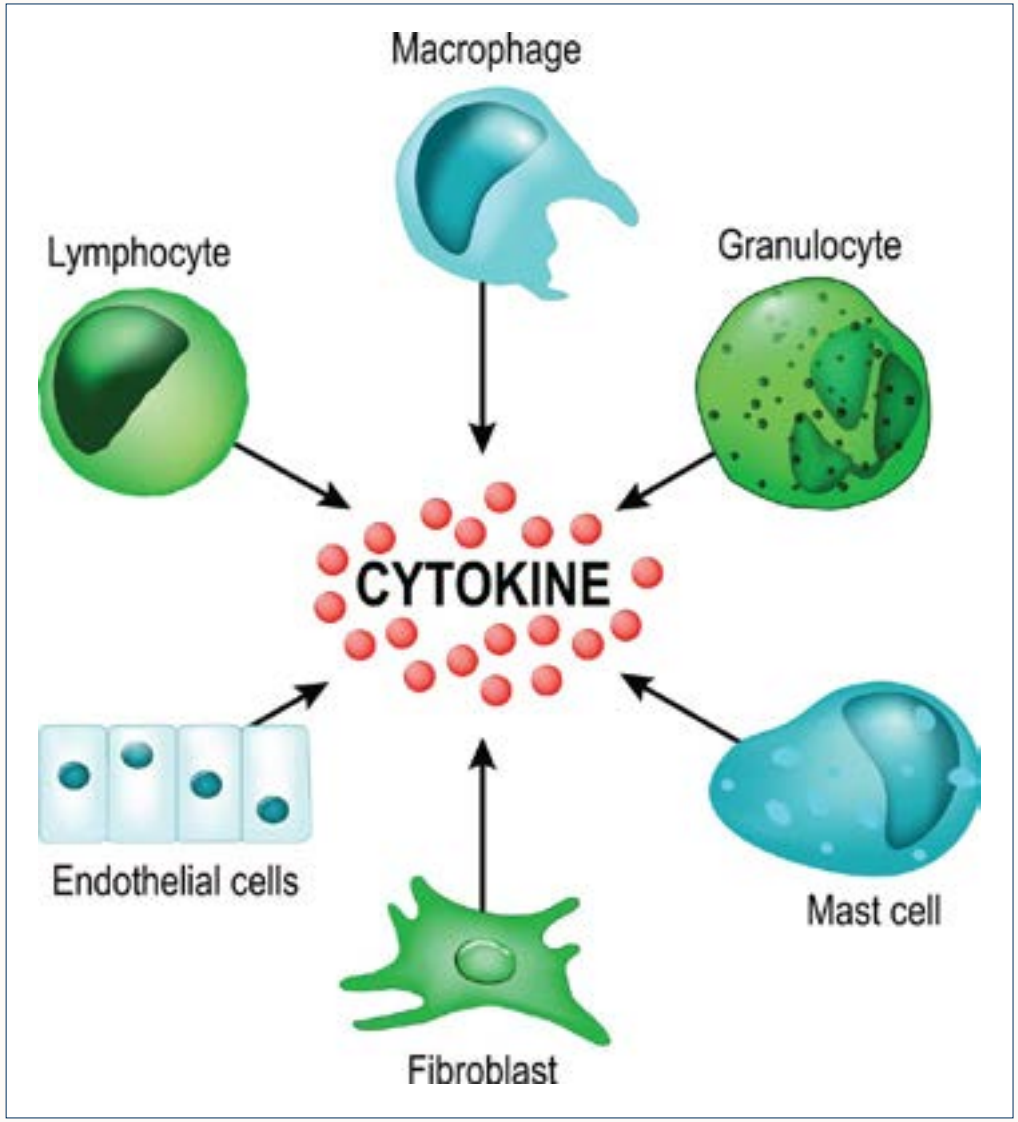
Resveratrol downregulates the inflammatory response by inhibiting proinflammatory mediators and transcription factors. It is the phytochemical characterized by the strongest activity similar to sirtuins, chemical exerting an inhibitory action against COX-1. Moreover, it exerts interesting effects potentially useful against cancer; it acts on oxidative stress and apoptosis, and can influence angiogenesis too.

As curcumin, resveratrol inhibits Nf-kB. Moreover, it lowers the expression levels of key inflammatory factors both transcriptionally and at the protein level; among others, it downregulates IL-6, IL-12 and TNF-α, and suppresses STAT3 and MMPs.

FRONTIERS IN ANTIINFLAMMATORY TREATMENT: REMOVING CYTOKINES FROM BLOOD

The demand for scientific tools to eliminate or at least significantly reduce residual cancer risk factors such as inflammation has been increasing day by day by healthy people (even if not included in classic risk groups). Among newest tools for solid cancer prevention is CYTOBALANCE, Bioscience Institute instrument to find out and correct an increase of pro-inflammatory cytokines in the blood.

The procedure is aimed at controlling disease development predisposing or



concurring conditions in healthy individuals, that is without diagnosed pathologies nor symptoms or genetic alterations making possible to hypothesize a pathway of genetic instability prodromic of cancer development, but in which it could be possible to identify or prevent physiologic or pre-pathologic conditions predisposing to the development of such alterations. Through a periodic monitoring of cytokines blood levels, CYTOBALANCE allows the detection of the increase of one or more inflammation mediators.

Monitoring is based on a fluorescence immunoassay allowing significant benefits compared to traditional technologies. It is completely automatized and allows the testing of multiple samples for multiple cytokines simultaneously. Sample size is strongly reduced (25 µl), and there is no need of manual steps increasing the risk of reduced reproducibility. Automatic control systems and a triple measurement-based quantification further increase analysis reproducibility. Cross-reactivity is eliminated by a complex microfluidic system. Finally, fluorescence-based detection enhances test sensitivity.

Among cytokines tested are IFN-α, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-17A, TNF-α and GM-CSF (granulocyte-macrophage colony-stimulating factor, a cytokine that regulates immune cells numbers and function). Moreover, CRP levels are tested too; testing of CRP is recognized as an easy and inexpensive tool to identify high cancer risk and people that may benefit from interventions like prophylactic therapy with anti-inflammatory drugs.

If at least one of tested levels exceeds attention threshold, blood can be drown from the subject to proceed to cytokine removal through Direct Absorption of Lipoproteins (DALI). Filtered blood is then reinfused to the subject.

Blood drawing and cytokine removal can be part of a continuous process, with removal cycle duration varying depending on starting cytokine levels. Alternatively, they can be separated processes; cytokine levels are tested after blood reinfusion, and the operative cycle is repeated until proinflammatory cytokine levels drop below attention thresholds.

After removal cycles completion, a new cytokine testing is programmed on the basis of starting detected cytokine levels, subject history, and other factors determined by cytokine removal experts. Lifestyle-based strategies (including taking dietary supplements) are suggested to reduce inflammation mediators levels.

Maintaining inflammation mediator levels below inflammation-associated thresholds is expected to increase genetic stability, reducing disease risk factors.

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