

Review Article

Persistent Circulating Immune Complexes: Potential Source of Epimutation and Cancer Poor Prognosis

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Abstract: Estimation of serum level of circulating immune complexes and its use for monitoring treatments have been carried out extensively in various disease conditions including autoimmune diseases and cancer, but little or no work has considered persistent circulation of immune complexes consequent to physiological anomalies that could mediate epimutation and subsequent epigenetic cell alteration and tumourigenesis. This review looked into the immuno-physiological activities of circulating immune complexes to expose its possible epigenomic consequences and potential role in epimutation. The environmental link between epigenetic cell alteration and formation of circulating immune complexes makes this review a unique one but on the other hand, gives room for concern. Immune complexes have strong capacity to stimulate various immune responses, yet the immunological activities of these circulating immune complexes are over looked or under estimated. Immune complexes is a normal immunological phenomenon but its persistence and subsequent deposition could induce endogenous assaults that would continuously and adversely perturb the epigenomic activities by fuelling chronic inflammation, activating transcription factors (NFkB), generation of reactive oxygen species (ROS) and frequent release of cytokines leading to epigenetic cell alteration especially in developing countries where environmental pollution is a serious factor.

Keywords: Circulating Immune Complexes, Epimutation, Epigenome, Persistence, Poor Prognosis, Tumourigenesis, Cancer

1. Introduction

Circulating Immune complex (CIC) is the binding of the antigen with its corresponding antibody by electrostatic van der Waals force, to form an interlock [1]. The formation of immune complexes (IC), due to the interaction of foreign substances (endogenous and exogenous antigens) with specific antibodies, is a physiological process which constitutes an essential part of man's normal immune defence mechanisms against environmental factors and are usually eliminated by the mononuclear phagocytes system (MPS) without development of pathological changes [1]. In any normal immune response, the half-life of CIC is transitory in nature. Continued presence of CIC over extended periods,

however, is a cause of consequence of some pathological condition or infection [2, 3]. Elevated levels of CIC have been found in a variety of diseases including neoplasia [4]. Serum CIC levels in cancer patients have been used for early diagnosis, metastatic spread, tumour burden, degree of aggressiveness, therapeutic response as well as prognosis [5, 6]. In most cancers, the antigenic part of the circulating immune complexes have been shown to originate from the disease affected tissues. As the disease progresses the shredding of the antigen by these tissues also increases and it is thought to increase the level of CICs [6, 7]. Circulating immune complexes may persist due to chronic infections, persistent low grade infections, continuous exposure to environmental pollutions and stress, personal attitude to

hygiene leading to continuous ingestion of contaminated food and drinks. No work has been done, implicating persistence of immune complexes in epimutational processes. Research has shown that epimutations are primarily genomic abnormality with 90–95% of cases attributed to environmental factors [8]. Epimutation is a gene mutation due to obstruction in normal epigenomic DNA marking which obstructs the process of turning genes on and off. This would hinder gene expression and cause phenotypic cell alteration [8]. Pursuing the cause of this review therefore is a pursuit against environmental challenges inducing regular immunological responses to generate immune complexes which are referred to in this study as immunological risk factors suspected to induce epimutation and subsequent tumourigenesis. This could be a peculiar problem for some developing countries where environmental pollutions is eminent.

Deposition of circulating ICs in tissues, mainly in capillary beds, promoting inflammation and tissue damage, is the most relevant pathological mechanism underlying IC-mediated diseases [9]. Continuous exposure to infection as may be experienced in some developing countries [10], would enable persistent circulation of immune complexes and subsequent continual activation of immune cell and release of pro-inflammatory cytokine leading to chronic inflammation. Prolonged subclinical inflammation and associated necrotic cell death also cause release of intracellular molecules that alert the immune system to danger, evoking responses leading to epithelial regeneration, angiogenesis, proliferation, and ultimately tumorigenesis [11]. Immune complex formation then could be a very serious challenge amongst different groups of people in various parts of the world, considering different levels of environmental conditions and pollutions. Environmental, as used by cancer researchers, means any cause that is not inherited genetically, not merely pollution [8]. The term environment refers not only to air, water, and soil but also to substances and conditions at home and at the workplace, including diet, smoking, alcohol, drugs, exposure to chemicals, sunlight, ionizing radiation, electromagnetic fields, infectious agents, etc. Lifestyle, economic and behavioural factors are all aspects of our environment [8]. This review considers that highly polluted environment would continue to mount serious challenge to immune responses and create disparity in immune cell and molecular activations and subsequent cytokine secretion in individuals at a particular point in time.

Thus, this review, considers Immune Complex (IC) formation as an environmentally (exogenous) and systemically (endogenous) induced immunological risk factor that would continually: fuel chronic inflammation; generate reactive oxygen species thus inducing persistent oxidative stress; induce persistent molecular interaction such cytokines pleiotropic activities, perturbation of epigenomic process and subsequent epimutational phenotypic changes.

2. Sources of Immune Complexes

Two major sources of antigens fuelling circulating immune complex (CICs) formation include: (1) Endogenous antigens-

Individuals can produce antibody against self-components (auto/endogenous antigens); antigen compounds of one's own cells/tissues. (2) Exogenous antigens- Mediated injury antigen may be exogenous such as microbial antigens, chemical toxin, radiation.

2.1. Endogenous (Assaults) Sources

The sources of endogenous CICs formation involve physiological processes such as inadequate removal of apoptosed cells [12]. Apoptosis produces cell fragments called apoptotic bodies that under go efferocytosis (phagocytic cells are able to engulf and quickly remove apoptotic bodies before the contents of the cell can spill out onto surrounding cells and cause damage) [12]. Another possible means of endogenous antigens that form immune complexes include the continual response of the body's immune system, which overloads the ability of the body to remove the immune complexes that formed, expression of mutated gene products [13], cell senescence and normal cell metabolic processes, proliferation of cancer cells and breakdown of tissue structure due to injuries, resulting from persistent inflammatory response. These have lead to release into circulation sequestered antigens (endogenous and/or auto-antigen) [13]. It is important to note that continual progression of cancer cells and persistence of tissue injury, would continually induce the activation of the apoptotic pathway and activation of cytotoxic T cell activities, thereby generating more cell debris (apoptotic bodies and/or cell fragments). The increase in these cell fragments may overwhelm the phagocytic cells thereby frustrating efferocytosis [14]. Antibodies are formed against this debris leading to increase in immune complex formation, as well as fuelling the persistent circulation and possible deposition of immune complexes. Tissue damage caused by trauma, infection or inflammation is associated with the release of endogenous proteins that signal impending danger to the host. The terms “damage-associated molecular patterns (DAMPs)” or “alarmins” have been used to collectively describe endogenous proteins that signal tissue and cell damage which may be present in the absence of microbial pathogens [15]. Any influence that can persistently generate damage associated molecular pattern (DAMP) or alarmin, such as stress (situations enabling physical or psychological trauma that could obstruct normal body homeostasis), could as well sustain immune complexes in the system [15]. Inflammatory mediators play important roles in the development and progression of cancer. Cellular stress damage, inflammation, and necrotic cell death cause release of endogenous damage-associated molecular pattern (DAMP) molecules or alarmins, which alert the host of danger by triggering immune responses and activating repair mechanisms through their interaction with pattern recognition receptors [15]. Recent studies show that abnormal persistence of these molecules in chronic inflammation and in tumour microenvironments underlies carcinogenesis and tumour progression, indicating that DAMP molecules and their receptors could provide novel targets for therapy [15].

2.2. Exogenous (Assaults) Sources

The combined effects of a low-grade persistent infection (such as occur with a parasite such as *Plasmodium species*, or in viral hepatitis), together with a weak antibody response forms chronic immune complexes (ICs) with the eventual deposition of the complexes in body tissues [16]. Continuous exposure to these exogenous substances would be a source to retain pathological level of immune complexes in circulation. Due to constant exposure to many infectious agents or foreign pathogens, with continuous infection and re-infection as may occur in some developing countries [10], IC accumulation may reach a plateau, deposit on organs and constitute a great immunological risk factor to epimutagenesis.

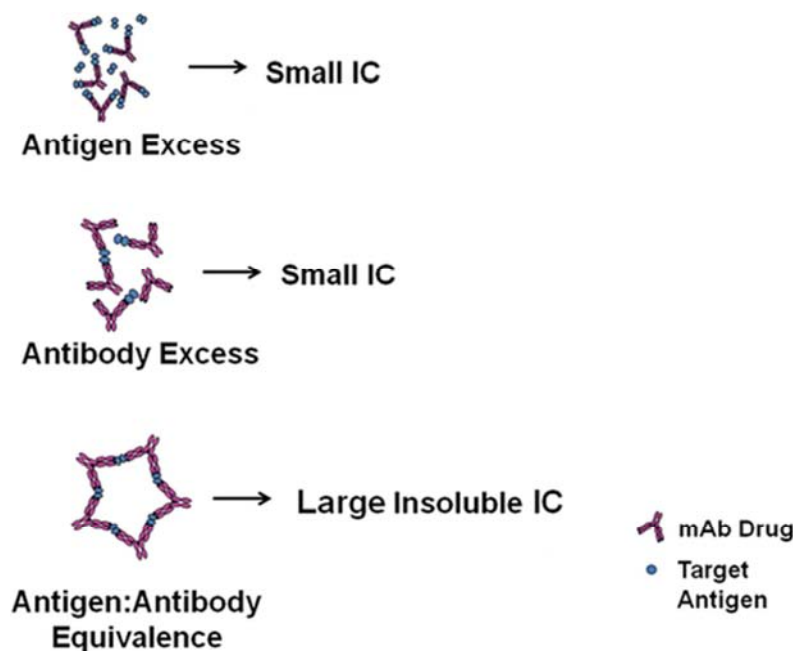


Figure 1. Immune Complex lattice formation at different molar ratios of antigen and antibody. When antigen or antibody is in great excess, small soluble complexes form. When antigen and antibody are in molar equivalence, large, insoluble complexes form. As antigen/antibody ratios approach molar equivalence, ICs are larger but remain soluble. IC = immune complex [19].

3. Physiology of Immune Complexes Deposition

Mart, (1982) reported that concentration of immune complex at any giving time in circulation depends on the rate of immune complex formation and rate of removal [21]. The rate of immune complex formation in turn depends on the rate of antibody synthesis and rate of availability of specific antigen. The rate of immune complex removal in turn depends on the rate of removal by mononuclear phagocyte system (MPS), and on the deposition of immune complex on tissues [21]. In cases with inefficient clearance by the mononuclear phagocytes system (MPS) only, pathological consequences will be expected, in particular by immune complexes formed with moderate excess of antigen (soluble immune complexes [19]. Additionally, the fate of circulating immune complexes (CIC) has been examined by injection of preformed IC prepared with IgG class of antibodies [19]. These

Epidemiological studies suggest that chronic infections can pre-dispose patients to secondary infections [17]. Since many pathogens causing chronic pathology are co-endemic, one could argue that the high rate of co-infection is simply due to enhanced co-exposure [18]. Although the geographical overlap of pathogen spread cannot be excluded as a potentially contributing factor. Mathematical models suggest that chronic infections, such as malaria and human immunodeficiency virus (HIV), actively contribute to the increased rate of infection with unrelated pathogens [18]. Continuous exposure to these exogenous substances would be a source to retain pathological level of immune complexes in circulation. Hence it becomes imperative to measure the rate of formation.

investigations have shown that removal of CIC by MPS depends on the lattice of the immune complexes (IC), the status of the MPS, the nature of the antigen in IC and characteristic of the antibody in IC [19, 21]. The lattice of IC is defined as the number of antigen and number of antibody molecules in a given complex. When mixture of large latticed antibody defined as containing more than two antibody molecule and small lattice, defined as containing one or two ab molecules were injected into the mice, rabbit or monkeys, the large latticed complexes (above ag2-ab2) were removed relatively quickly while the small latticed complexes (either ag2-ab2, ag2-ab1, ag1-ab1) persisted longer in circulation [19]. The disappearance of large latticed complexes after the initial extravasations was best described by single exponential components; the disappearance of the small latticed complexes was best described by two exponential components, reflecting equilibrium between intravascular and extravascular spaces and catabolism. As increasing doses of IC were given, the clearance velocity of large latticed

complexes and specific hepatic uptake of the materials reached a plateau, suggesting saturation of MPS with large latticed complexes [19, 21]. This phenomenon could be the

same when infection and reinfection continuously occur, or where environmental stress challenges are overwhelming.

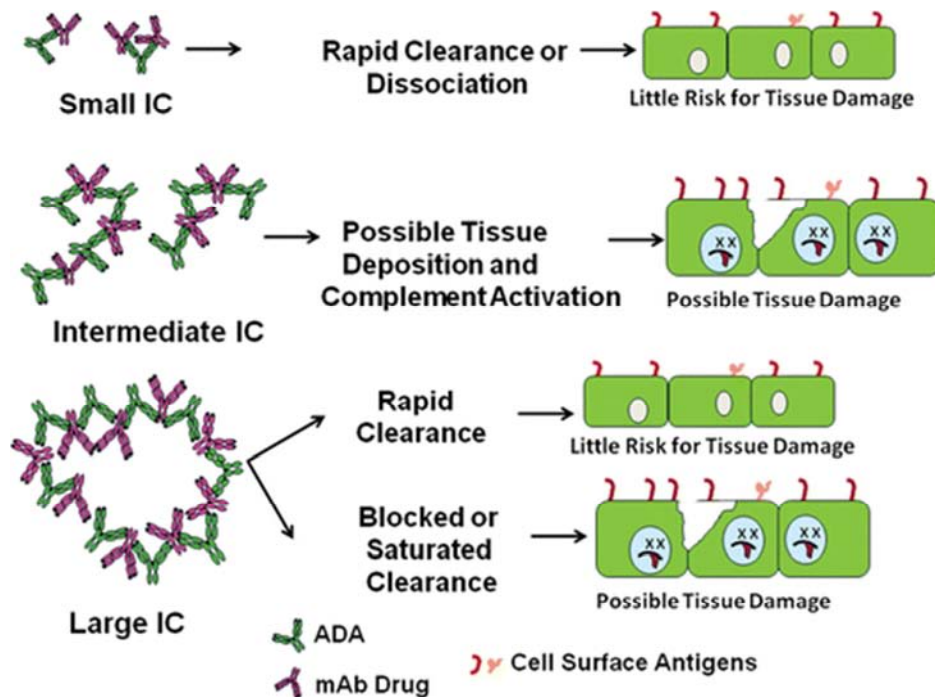


Figure 2. Effect of IC lattice formation on IC tissue deposition, tissue deposition, and pathogenicity. Small ICs, which may form under great antigen or antibody excess, generally remain in circulation or are rapidly cleared or dissociated and pose little risk for tissue damage. Intermediate-sized IC can deposit in tissue and activate complement. Large ICs are generally rapidly cleared through the phagocytic system, but when clearance mechanisms are blocked or saturated, large ICs can deposit in tissue, activate complement, and cause tissue damage. IC = immune complex [19].

4. Immune Complexes Mediated Immunological Activities

During the primary response, naive B cell differentiation and antibody (Ab) production occur several days after antigen encounter. In contrast, following secondary antigenic exposure, B cells expand with a shortened lag phase and produce larger quantities of Abs. The difference between the primary and secondary exposures is the presence of memory B cells and pre-existing Ag-specific Abs. These antibodies (Abs) can form immune complexes (ICs) with the incoming antigen (Ag), and it is known that ICs can induce the production of higher antibody titres than antigen alone [22].

One possible mechanism of IC-mediated enhancement is the activation of complement cascade [22]. Immune complexes, particularly those containing the Antibody isotypes IgG2a and IgG3, are able to activate the classical complement pathway [22]. Because the complement receptor CD21 is part of the B cell co-receptor complex, this could lead to enhanced B cell activation. In addition, ICs are able to bind to a variety of cell types, particularly dendritic cells through FcγRs [22]. For dendritic cells, engagement of FcγRs leads to cell activation, which results in enhanced Antigen presentation and increased expression of co-stimulatory molecules [22]. Because ICs are able to activate naive cells with enhanced kinetics, and because ICs are present after

secondary Antigen encounter, it seems likely that ICs activate naive cells to participate in secondary responses. The formation of circulating immune complexes is the physiological consequence of antibody responses to different antigens, including microorganisms and intricate mechanisms for immune complex clearance have developed in mammals. Thus, immune complex formation is a physiological event, but their accumulation in the tissue or circulation, as seen in such disorders as rheumatoid arthritis or systemic lupus erythematosus, can be considered pathological. Immune complex accumulation leads to a broad spectrum of pro-inflammatory effects, including complement activation with release of phlogistic C3a and C5a peptides and cytokine secretion from FcγR-expressing cells [23]. Immune Complex formation is generally followed by one or more secondary reactions, all of which enable the body to neutralize and clear microorganisms and non-self molecules (in the form of IC after antibody binding) that have penetrated the various body barriers. Inactivation and elimination of these "invaders" prevents their deposition (localization) where they might multiply (in the case of microorganisms) or induce specific damage (toxins or enzymes). IC formation followed by these secondary reactions (such as complement fixation) enhances Mononuclear Phagocytic System (MPS) clearance mechanisms and prevents interaction with specific sites in the body that could be damaged by deposition. This entire dynamic process must be very efficient under normal

circumstances, because although we are constantly exposed to and challenged by foreign pathogens, IC do not normally accumulate in blood or organs [24]. However, Immune complexes have been found to be immunosuppressive in a variety of experimental systems and have been demonstrated in other parasitic diseases, such as malaria, trypanosomiasis, schistosomiasis, and onchocerciasis [25].

Immune Complexes interact with receptors for the Fc portion of Immunoglobulins the Fc Receptors (FcRs) which are expressed by many cells of the immune system. Ligation of FcRs, specific for IgG, termed Fc γ Rs, on myeloid cells induces cell activation which include phagocytosis of opsonized pathogens, Ab-dependent cell-mediated cytotoxicity (ADCC), release of proinflammatory mediators and reactive oxygen intermediates, and production of several cytokines and chemokines [26]. Circulating Immune Complexes (CICs) first localize within the vasculature and then translocate into extravascular tissue, attracting immune cells [27, 28]. Immune Complexes deposited intravascularly can directly engage circulating leukocytes. Both soluble and insoluble immune complexes can activate infiltrating immune cells such as T cells, neutrophils, mast cells and macrophages that then release inflammatory mediators (cytokines and prostanoids) capable of activating the endothelium and their ability to recruit more cells [29]. Based on these, this review revealed that persistent circulation of immune complexes is a potent source of acute and chronic inflammation which continues to fuel the inflammatory pathways that leads to persistent perturbation of cell epigenesis.

The ability of CICs to classically and continuously activate immune responses is considered in this review as neglected immunological response that can induce epimutation and as such deserves serious attention. Circulating immune complexes (CICs) are now viewed as regulators of both cellular and humoral immune responses by virtue of their capacities to interact with antigen receptor bearing lymphocytes and sub-population of T and B cells as well as with macrophages and neutrophils having FC receptors [7].

Persistent circulation of immune complexes could continue to exacerbate immune cell activities and subsequent production of cytokine such as Interleukin-1, Tumor necrosis factor- α (TNF- α), that has the potentials to induce translocation of Nuclear factor kappa-B (NF- κ B), leading to chronic inflammation and subsequent epigenetic cell alteration and DNA damage. Immune complexes (IC) induce a number of cellular functions, including the enhancement of cytokine production from monocytes, macrophages and plasmacytoid dendritic cells [30].

5. Immune Complex Mediates Chronic Inflammation

Inflammation is a pattern of response to injury, in which cells and exudates accumulate in irritated tissues and tend to protect from further damage. It may be classified as acute or chronic. Chronic inflammation may result from failure of the

recovery phase of acute inflammation, or may occur as a distinct process from the outset, because of the nature of the irritant, from an autoimmune response to a self - antigen, or may be caused by an innately chronic irritant of low intensity that persists. Examples in the first category include persisting infections by pyogenic bacteria, e.g. in anatomical locations such as bone (osteomyelitis) where elimination of the organism may be inefficient [31]. Chronic inflammation is most appropriately defined in terms of the process, in which continuing inflammation and attempted tissue healing by repair occur simultaneously. Although it is often defined simply in terms of time course, with lesions of over 6weeks' duration traditionally being regarded as chronic, any such definition is entirely arbitrary. Essentially there are two forms of chronic inflammation: either the chronic reaction supervenes on the acute reaction or may in fact develop slowly with no initial acute phase (ab initio) [32]. Chronic inflammation ab initio can have many causes including local irritants, poor circulation, some micro-organisms or immune disturbances. At a microscopic level, chronic inflammation is sometimes defined in terms of the pattern of cellular response, although this is variable and not altogether reliable. Recruitment and activation of macrophages is typical of chronic inflammation and is often accompanied by recruitment of cell types participating in an immunological response, notably T lymphocytes. Significant destruction of tissue may occur. Repair, which involves induction of granulation tissue, may lead to subsequent scarring [31]. The chronic inflammatory response is regulated by the coordinated action of various cytokines and growth factors. Many common and clinically important diseases result from chronic inflammation. Although it shares many characteristics of the acute inflammatory response, chronic inflammation is a biologically distinct pattern of response to an irritant. It may be divided into non-granulomatous and granulomatous chronic inflammation; the term granuloma refers to a localized collection of activated macrophages and their derivatives [31].

We have earlier emphasized on infection and reinfection as enabling exogenous source of persistent circulation of immune complexes. Immune complex is an irritant that can persist and cause chronic inflammation and can evade elimination and continue to activate immune responses. Inflammation is an immune response to infection and tissue injury, characterized by the release of a complex regulatory network of mediators all aimed at combating the infectious or noxious agent, repairing damaged tissue, and restoring homeostasis [32]. In chronic inflammation, this response is exaggerated or sustained. Long-term inflammation is thought to lead to cancer because of the dysplastic degeneration of repaired epithelium by the continuous release of reactive oxygen and nitrogen species, which cause DNA damage resulting in genomic instability and providing a proliferative advantage for cells carrying mutations [33]. Recent evidence also indicate these chemokines and proinflammatory cytokines such as tumor necrosis factor α (TNF α), interleukin 6 (IL-6), and IL-23 play pleiotropic roles in tumor progression [34]. The Cytokines and growth factors are secreted by

inflammatory cells and stromal cells in the tumor microenvironment during chronic inflammation; they engage their cognate receptors to activate downstream kinases and transcription factors such as NF- κ B, STAT, and HIFs. These TFs can up-regulate the expression, and enhance the activity of Noxs. They can also regulate target genes involved in cell cycle control, apoptosis, metabolism, angiogenesis, and metastasis. On the other hand, Nox-derived ROS can oxidize redox-sensitive cysteine residues in the catalytic domain of PTPs and inactivate their ability to limit the propagation of cytokine-derived signals. ROS can also directly oxidize the cysteine residues in some TFs and regulate their transcriptional activity [35].

Transcription factors such as nuclear factor- κ B (NF- κ B) and signal transducer and activator of transcription 3 (STAT3) are activated in many tumors and serve as important molecular links between inflammation and cancer. Nuclear factor- κ B activation seems to play a complex role because NF- κ B inhibition also induces certain tumors, and these contradictions have been incompletely understood [32]. Immune complexes could also result from binding of auto antigens to its corresponding auto antibody. Additionally, we hypothesize that microbial antigen that finds its way into the system, induce immune complex formation and may remain in circulation as a complex antigen even when the microorganism itself has been attenuated either by use of incomplete dosage of antibiotic or by self medication or use of resistible antimicrobial drugs. Therefore immune complex can be a vehicle for chronicity of some microbial infection, thereby inducing chronic inflammation. The distinctive features of this process are best appreciated by comparing it with the acute inflammatory response. In contrast to acute inflammation, where the host response leads to elimination of the irritant, followed by recovery involving tissue regeneration or repair, chronic inflammation is characterized by inflammation and repair occurring concurrently, rather than consecutively. Note that repair is always a feature of chronic inflammation because it is associated with irritants that cause destruction of tissue architecture. Because the irritant fails to be eliminated in chronic inflammation (either because of its innate characteristics or because of an ineffective host response) it may cause continuing tissue damage in its own right. In addition, most persistent irritants (including immune complexes) are recognized as foreign antigens by the host immune response, which contributes to the chronic inflammatory process and may add to the tissue destruction. This is well illustrated in diseases such as tuberculosis and hepatitis B, where the inciting agents persist in the host and continue to evoke a chronic inflammatory response [31]. Depending on the nature of the irritant, deferent profiles of inflammatory mediators and growth factors (collectively referred to as cytokines) are generated locally, giving rise to deferent morphological patterns of chronic inflammation. The systemic effects of inflammation are more pronounced in chronic inflammatory diseases and may contribute significantly to the clinical consequences [31].

We suggest that persistence of circulating immune

complexes is a potent source of acute and chronic inflammations which continues to fuel the inflammatory pathways that could result to perturbation of cell epigenomic engagements. Persistence of these immunological processes especially in individuals in high environmentally polluted area may occur, leading to chronic inflammatory response. In the 2-stage carcinogenesis model, cancer development begins when somatic cells incur irreversible DNA sequence alterations (initiation stage). Subsequently, continuous or repeated stimuli lead to a sustained induction of cellular proliferation, bringing about changes in the cellular microenvironment that favour tumour formation (promotion stage). While the cancer initiation stage is better defined (e.g., mutation of oncogenes, tumor suppressor genes, and other key regulators of cell proliferation), the promotion stage, in which many cell types interact via secreted factors, is more complex. Chronic inflammation is an important tumor promoter [36], and can possibly be induced by persistent circulating immune complexes.

An acute inflammatory response begins when neutrophils infiltrate sites of injury by responding to chemical cues elicited by pro-inflammatory cytokines and chemokines. Subsequent resolution or healing is associated with release of anti-inflammatory cytokines. In contrast, chronic inflammation is a response to persistent injury and/or infection and involves lymphocytes, plasma cells, macrophages, and neutrophils. Neutrophils are critical immune effector cells that cause tissue damage in immune complex (IC)-mediated diseases, such as immune-mediated glomerulonephritis, arthritis, and other autoimmune disorders. ICs form repeatedly in the blood in response to foreign or self-antigens, tissue injury, or infection. However, excessive accumulation of ICs within the vasculature and surrounding tissue underlies the pathogenesis of a variety of human diseases [37]. IC deposition in tissue is associated with neutrophil accumulation, and neutrophil adhesion to ICs triggers robust reactive oxygen species generation, primary granule release, and generation of cytokine and lipid mediators, which can contribute to tissue inflammation [37]. Thus, a tight regulation of neutrophil recruitment at sites of IC deposition is required to limit IgG-mediated tissue injury. Leukocyte adhesion receptors on neutrophils and the endothelium, such as the selectins, integrins, and members of the IgG superfamily intracellular adhesion molecules (ICAM family members), have been implicated in neutrophil recruitment during an inflammatory response. However, an understanding of the molecular requirements for neutrophil recruitment in the context of deposition is still in its infancy. FcRs, receptors for IgG-containing ICs, contribute to the pathogenesis of several immune-mediated diseases in mice, including nephrotoxic nephritis, lupus nephritis, autoimmune skin diseases, and arthritis [38]. In all of these models, Fc deficiency is associated with a reduction in neutrophil accumulation. Neutrophil recruitment in these models may result from the engagement of ICs by FcR on mast cells and macrophages, which leads to the release of endothelial-activating agonists chemokines and cytokines. Subsequent

endothelial cell activation, IC-mediated activation of complement, and subsequent generation of anaphylatoxins C3a and C5a may mobilize neutrophils, and/or FcRs on neutrophils may directly facilitate recruitment to deposited ICs [27, 37].

Cytokines such as Tumor necrosis factor- α , elevated in autoimmune diseases, not only activate the endothelium but also have the potential to “prime” neutrophil responses [38]. In the past several years, studies have suggested that Fc gamma receptors (Fc γ Rs) play primary roles in diseases initiated by antibodies [39]. Fc γ Rs are members of the immunoglobulin gene superfamily that binds the Fc binding domain of IgG and are widely expressed in the hematopoietic system. Currently, 2 groups of Fc γ Rs are recognized on cells of the human immune system: the high-affinity Fc γ RI, which binds monomeric IgG, and the low-affinity receptors Fc γ RII and Fc γ RIII, which preferentially bind complex IgG. The low-affinity Fc γ Rs are present in multiple isoforms, Fc γ RII (CD32) A and B and Fc γ RIII (CD16) A and B. The Fc γ Rs are further classified as activating or inhibitory. Signals from these receptors are transmitted via immune-receptor tyrosine-based activation (ITAM) or inhibitory (ITIM) motifs, respectively. On crosslinking of the receptors by Immune Complexes (ICs), the tyrosine residues in the ITAM motifs are phosphorylated by src family tyrosine kinases, which initiate a cascade of signaling events that trigger neutrophil effector responses. The exception is the uniquely human Fc γ RIIB, which anchors to the outer leaflet of the neutrophil plasma membrane through a glycosylphosphatidylinositol linkage and does not contain or interact with ITAMs containing adaptors. It may signal by associating with Fc γ RIIA and the integrin complement receptor 3 (Mac-1), which serve as signaling partners, and/or localizing to membrane rafts enriched in signaling molecules like Src protein kinases. The ITIM motif is present in the cytoplasmic domain of the single-chain, inhibitory Fc γ RIIB. Colligation of activating and inhibitory receptors on the same cell by ICs inhibits ITAM-triggered activation, thus providing a higher threshold for activation of cells [36]. Human neutrophils express Fc γ RI, which is upregulated on the surface by cytokines such as interferon- γ , and contain low levels of Fc γ RIIB, but this is not consistently reported [40]. Fc γ RIIB is present only in granulocytes and its surface expression is 4- to 5-fold higher than Fc γ RIIA. Neutrophil activation by inflammatory mediators leads to rapid Fc γ RIIB shedding and modulates Fc γ RIIA expression and function [41]. Thus, the repertoire or activity of human Fc γ Rs is regulated at an inflammatory site, which in turn may determine the magnitude of neutrophil effector responses. Neutrophil accumulation is an early and consistent feature of IC-mediated diseases. The importance of Fc γ Rs in this process is shown by the reliable reduction in neutrophil recruitment observed in Fc γ chain-deficient mice in various models of inflammation [42]. Similarly, complement deficiency or blockade of the complement components C5a, C5aR, or C3 leads to a reduction in neutrophil accumulation in select models of IC-mediated disease [43]. The prevailing paradigm is that tissue-resident

cells (mast cells and macrophages) sense ICs through Fc γ Rs and complement receptors. This results in the elaboration of secondary mediators such as tumor necrosis factor- α and chemokines [42], which activate endothelial cells. Tumor necrosis factor- α priming of neutrophils enhanced IC-induced leukocyte recruitment [43], which may aid in localizing neutrophil influx to sites of IC inflammation. Once soluble ICs lodged in the capillaries reach the underlying tissue, activation of resident cells and subsequently the endothelium leads to the expression of leukocyte adhesion molecules and chemokines which enhance neutrophil recruitment. Once neutrophils are recruited, they can signal cytotoxic functions through Fc γ R and/or complement receptors that promote tissue damage. In vitro, engagement of Fc γ RIIA promotes phagocytosis, degranulation, and reactive oxygen species generation. Fc γ RIIIB crosslinking induces calcium mobilization and triggers degranulation and leukotriene release, but it has not been consistently shown to induce other neutrophil cytotoxic responses [43]. With pro-inflammatory activities of Immune Complexes, their persistence in circulation is considered inimical to good health and a pathway to development of chronic inflammation, DNA Strand Breaks and epigenetic cell alteration as discussed below.

6. Immune Complex Mediates Molecular Interactions

TNF- α has been reported to cause excessive free radical generation within cultured myocytes, endothelial cells, hepatocytes and cholangio-carcinoma cells. Proposed mechanisms involve upregulation or direct activation of several RONS producing enzymes including NADPH oxidase and inducible nitric oxide synthase (iNOS), altering levels of intracellular glutathione and damaging components of oxidative metabolism in the mitochondria resulting in excessive reactive oxygen species (ROS) production [44, 45]. Elevation of intracellular RONS and redox imbalance may therefore be responsible for TNF- α /TNFR signalling-induced DNA strand breaks, and as proposed in endothelial dysfunction [43]. The ability of immune complexes to activate immune cells and generate cytokines such IL-1 and TNF- α , is a potent pathway to DNA damage and continuous generation of these cytokines have been shown to cause DNA damage. Damage was observed as DNA strand breaks in the alkaline comet assay, detecting both single- and double-stranded breaks and apurinic sites, and by formation of γ H2AX foci, which specifically detects double-stranded breaks. Micronuclei formation in erythroblasts, indicative of clastogenicity measured in circulating NCEs, was significantly elevated 48-h post-TNF- α injection. Within the peripheral blood, DNA single- and double-stranded breaks accompanied by oxidative base damage were most evident in CD4⁺ and CD8⁺ T cells after 1-h TNF- α treatment and to a lesser extent CD19⁺ B cells versus CD11b⁺ cells or the eluate, indicating differing susceptibility to DNA damage. DNA

single- and double-stranded breaks measured by the alkaline comet assay and immunostaining for γ H2AX foci were significantly higher in T cells; however, only DNA double strand breaks were significantly higher in CD19⁺ B cells T-cell lymphomas, due to persistent systemic genotoxicity to T cells and peripheral lymphoid organs, which manifested significant amounts of DNA damage [46]. Research has shown that elevated levels of circulating cytokines characteristic to intestinal inflammation such as TNF- α and their downstream mediators are partially responsible for inducing DNA damage [47]. In this review, we maintain that the persistent circulation of immune complexes is encompassing. This is because many exogenous and endogenous proteins or molecules including microbial agents would end up in fuelling immune complex formation, the tissue injury that resulted due to presence of these exogenous and endogenous insults, may equally end up in immune complex formation if the immunological mechanism for the removal of the cell debris are incompetent or rather overwhelmed by the frequency of the tissue damage and continuous exposure of individual to infectious agents. All these factors are potent reasons for immune complex persistence. Therefore elevated levels of cytokines and persistence of cytokines in circulation, may be continually fuelled by presence of immune complexes.

Presence of circulating immune complexes is a potent mediator of TNF- α secretion from innate immune cells. TNF- α acts by binding to the TNFRs, which results in recruitment of various signal transducers activating caspases, AP-1 and NF- κ B. TNFR signalling induces NADPH oxidase and iNOS, alters glutathione levels and causes mitochondrial disruption, all of which have the capacity to produce RONS and induce DNA damage. Earlier research demonstrated that NF- κ B signalling is involved in induction of DNA damage [47]. We hypothesized that persistence Immune complexes can mediate the translocation of NF- κ B into the nucleus by inducing the generation of cytokines (IL-1, TNF- α) that can cause phosphorylation of I κ Bs kinase and subsequent release of NF κ B from the cytosol into the nucleus. Extracellular RONS can also induce TNFR signalling in the absence of TNF- α . One of the key molecules that link chronic inflammation and

cancer is represented by the NF- κ B family of transcription factors [48, 49]. In particular, different mouse studies provide strong and direct genetic evidence that the classical, IKK- β dependent NF- κ B activation pathway is indeed a crucial mediator of tumor promotion [50, 51]. This pathway is triggered by bacterial and viral infections, as well as by pro-inflammatory cytokines, such as TNF- α and IL-1, all of which activate the IKK complex [52]. This complex, phosphorylates the NF- κ B inhibitors I κ Bs, thereby targeting them for proteosomal degradation and freeing NF- κ B to enter the nucleus and mediate transcription of target genes. It is worth noting that many of the genes able to mediate alterations characterizing a tumour cell are under the transcriptional control of NF- κ B (For example, the activity and expression of cyclin D1, CDK2 kinase, c-myc, p21, p53 and pRb), which are involved in the control of cell cycle and are altered in several types of cancer, are NF- κ B-dependent [53, 54]. The expression of numerous cytokines, that are growth factors for tumour cells (IL-1 β , TNF, IL-6, EGF) are also regulated by NF- κ B. Tissue invasion and metastasis, two crucial events of tumor progression, are regulated by NF- κ B-dependent genes, including metalloproteases (MMPs), urokinase type of plasminogen activator (uPA), IL-8, the adhesion molecules VCAM-1, ICAM-1 and ELAM-1. NF- κ B is also involved in the regulation of angiogenesis, the process by which tumor cells promote neo-vascularization. Finally, altered expression of genes involved in suppression of apoptosis (i.e. Bcl-2 family members and IAP proteins), a key feature of cancer cells, is often due to deregulated NF- κ B activity. Concerning this last point, several pathogenic bacteria, particularly those that can establish a persistent intracellular infection, activate NF- κ B in the host cell and suppress cell death, thus creating a niche in which the bacterium can survive, in spite of the attempts of the host immune system to destroy the infected cell [55]. As a consequence, the suppression of apoptosis by a pathogen might also allow a partially transformed cell to evade the self-destructive process and to progress to a higher level of transformation.

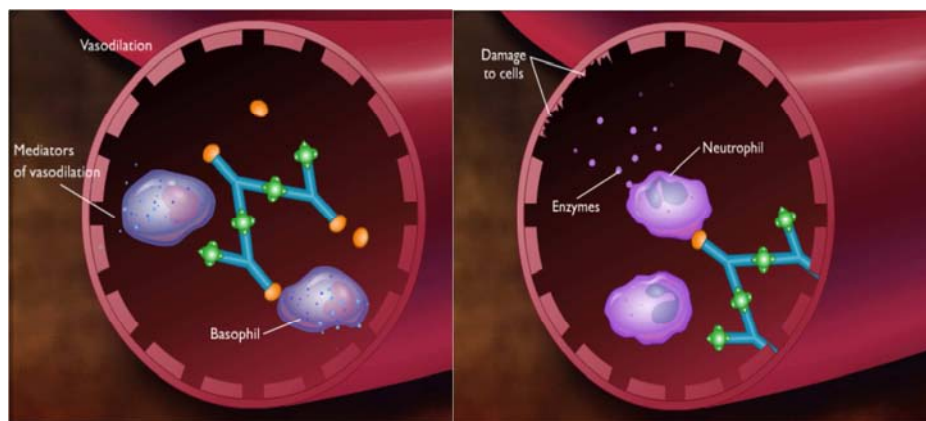


Figure 3. [20] Immune complexes in blood vessels bound by complement at the Fc portion inducing chemotaxis, thus mediating vasodilation and subsequent cell damage. Persistence of such development can increase the level of immune complexes and induce persistence chronic inflammation, formation and perturbation of the normal epigenetic flow in the cells.

7. Immune Complex Mediates Oxidative Stress

Profound changes in neutrophil responsiveness to these complexes occur after cytokine priming. It has been established that under appropriate conditions these neutrophils can actively release large quantities of reactive oxidants and discharge the contents of their granules extracellularly. If such large scale release of these toxic molecules occurred in vivo, then it is likely that local antioxidants and antiproteases would become saturated and tissue damage would ensue [57].

It is increasingly proposed that reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a key role in human cancer development [57], especially as evidence is growing that antioxidants may prevent or delay the onset of some types of cancer. ROS is a collective term often used by biologists to include oxygen radicals [superoxide (O_2^-), hydroxyl (OH), peroxy (RO) and alkoxy (RO) and certain non-radicals that are either oxidizing agents and/or are easily converted into radicals, such as HOCl, ozone (OS), peroxynitrite (ONOO $^-$), singlet oxygen (1O) and HO. RNS is a similar collective term that includes nitric oxide radical (NO), ONOO $^-$, nitrogen dioxide radical (NO), other oxides of nitrogen and products arising when NO reacts with O_2^- , RO and RO. HO, NO and O_2^- react quickly with very few molecules, whereas OH reacts quickly with almost anything. RO, RO, HOCl, NO, ONOO $^-$ and OS have intermediate reactivity [58].

Circulating Immune Complex Liganding of Fc receptors (on neutrophils, monocytes or macrophages) and mannose receptors (on macrophages) increases their O_2 uptake, called the respiratory burst. These receptors activate a membrane-bound NADPH oxidase that reduces O_2 to O_2^- (superoxide). Superoxide can be reduced to OH $^\cdot$ (hydroxyl radical) or dismutated to H_2O_2 (hydrogen peroxide) by superoxide dismutase. O_2^- , OH $^\cdot$, and H_2O_2 are activated oxygen species that are potent oxidizing agents in biological systems which adversely affect a number of cellular structures including membranes and nucleic acids [56]. Furthermore, at least in the case of neutrophils, these reactive oxygen intermediates can act in concert with a lysosomal enzyme called myeloperoxidase to function as the myeloperoxidase system. During phagocytosis glucose is metabolized via the pentose monophosphate shunt and NADPH is formed. Cytochrome B which was part of the specific granule combines with the plasma membrane NADPH oxidase and activates it [58]. The activated NADPH oxidase uses oxygen to oxidize the NADPH. The result is the production of superoxide anion. Some of the superoxide anion is converted to H_2O_2 and singlet oxygen by superoxide dismutase. In addition, superoxide anion can react with H_2O_2 resulting in the formation of hydroxyl radical and more singlet oxygen [58]. The result of all of these reactions is the production of the toxic oxygen compounds superoxide anion (O_2^-), H_2O_2 , singlet oxygen (1O_2) and hydroxyl radical (OH $^\cdot$).

Mutagenesis by ROS/RNS could contribute to the initiation of cancer, in addition to being important in the promotion and progression phases. For example, ROS/RNS can have the following effects. (1) Cause structural alterations in DNA, e.g. base pair mutations, rearrangements, deletions, insertions and sequence amplification. OH is especially damaging, but 1O , RO, RO, HNO, O, ONOO $^-$ and the decomposition products of ONOO $^-$ are also effective. ROS can produce gross chromosomal alterations in addition to point mutations and thus could be involved in the inactivation or loss of the second wild-type allele of a mutated proto-oncogene or tumour-suppressor gene that can occur during tumour promotion and progression, allowing expression of the mutated phenotype [56]. (2) Affect cytoplasmic and nuclear signal transduction pathways [63]. For example, HO (which crosses cell and organelle membranes easily) can lead to displacement of the inhibitory subunit from the cytoplasmic transcription factor nuclear factor B, allowing the activated factor to migrate to the nucleus [59]. Nitration of tyrosine residues by ONOO $^-$ may block phosphorylation. (3) Modulate the activity of the proteins and genes that respond to stress and which act to regulate the genes that are related to cell proliferation, differentiation and apoptosis. For example, HO can stimulate transcription of c-jun [60].

8. Immune Complexes and Perturbation of Epigenome

Epigenome is a group of chemical compounds or proteins that can attach to the DNA (assembly of genome). When this attachment is done, the DNA is said to be marked. Marking of DNA does not affect the DNA protein sequence, instead, it changes the way the cell in question yields to DNA instructions. Marking of DNA influences such action as turning a gene on or off, in other words enabling gene expression especially during cell development or growth. Thus epigenomic activities (epigenesis) are normal phenomena required for growth and development of different parts of the body. Therefore, alteration of the marking of DNA is called epimutation, while influences that can induce epimutation are referred to as epimutagens. This influences include most environmental influences. This work considers persistence of immune complexes as epimutagen emanating as immunological risk factor and can induce epimutation (epigenetic cell alteration). Thus, this review reveals the possible mechanisms and various pathways through which circulating immune complexes can induce epigenetic cell alteration. This review infers that epigenetic cell alteration and formation of immune complexes are sequel to environmental stress.

Broadly speaking, epigenetics refers to stimuli-triggered changes in gene expression due to processes that arise independent of changes in the underlying DNA sequence. Some of these processes have been elucidated and include

DNA methylation, histone modifications and chromatin-remodeling proteins [61] and DNA silencing by noncoding RNAs (ncRNA). These changes may involve chemical modifications of the DNA itself, such as DNA methylation or modifications of proteins that are closely associated with DNA, such as the histones that bind and compact DNA into chromatin packages. Research has shown that all recognized epigenetic marks (including DNA methylation, histone modification, and microRNA (miRNA) expression) are influenced by environmental exposures, including Infectious agents, diet, tobacco, alcohol, stress, genetic factors, which play important roles in the etiology of cancer. Some of these epigenetic modifications change the

expression of tumor suppressor genes and oncogenes and, therefore, may be causal for tumorigenesis [61].

Epigenetic damage, such as aberrant DNA methylation, aberrant histone modification and miRNA expression is well recognized as a major driving force in cancer development and progression. The ability to activate immune cell, leading to secretion of cytokines, is hypothesized to be the major mechanism for regulation of DNMTs. We have earlier stated that persistence circulation of immune complexes is a strong factor for activation of immune responses. We have also earlier discussed the ability of immune complexes to continue to induce the persistent generation of cytokines and reactive oxygen species and reactive nitrogen species.

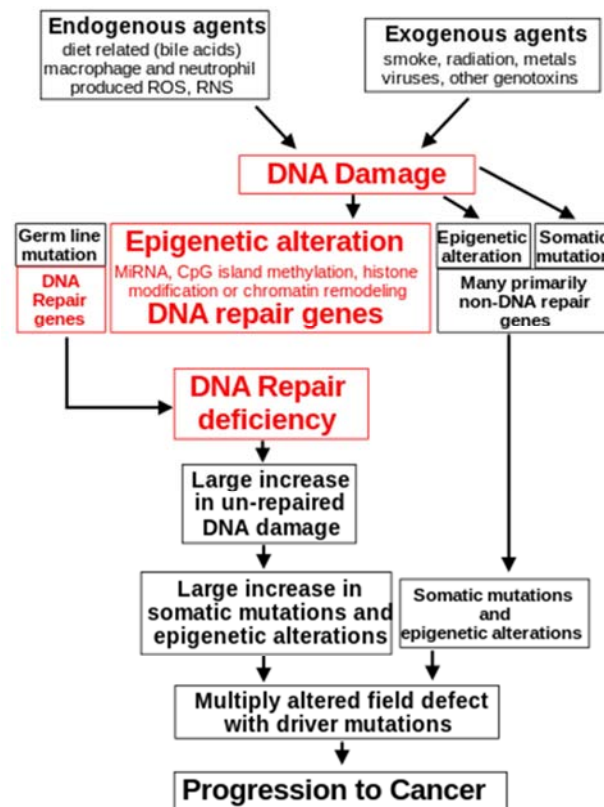


Figure 4. [56] Endogenous and exogenous sources of antigens can induce immune complex formation and thus retain such antigens, enabling complement activation, engagement of the Fc portion of the immune complexes by immune cells, induce generation of ROS and subsequent DNA damage or epimutation and activate molecular mechanisms enabling inflammation related cancer.

Immune Complexes are produced continuously in response to infection, tissue injury, foreign antigens environmental chemicals. The link between immune complexes to inflammation and oxidative stress is of great importance in this review. The association between inflammation and oxidative stress is well documented [62], with studies of inflammatory conditions or infections reporting elevated levels of 8-hydroxy-2-deoxy Guanosine (8-OH-dG). The endogenous reactions that are likely to contribute to ongoing DNA damage are oxidation, methylation, depurination and deamination [63]. Nitric oxide or, more likely, reactive products derived from it, such as NO, ONOO⁻, NO and HNO₂, are mutagenic agents, with the potential to produce nitration, nitrosation and deamination reactions on DNA bases [64].

Methylation of cytosines in DNA is important for the regulation of gene expression, and normal methylation patterns can be altered during carcinogenesis. Conversion of guanine to 8-hydroxyguanine, a frequent result of ROS attack [65], has been found to alter the enzyme-catalysed methylation of adjacent cytosines, thus providing a link between oxidative DNA damage and altered methylation patterns. The chemistry of DNA damage by several ROS has been well Characterized in vitro [70], although more information is needed about the changes produced by RO[•], RO₂[•], O[•], ONOO⁻ and several of the RNS. Different ROS affect DNA in different ways, e.g. O[•] and HO[•] do not react with DNA bases at all [61]. OH[•] generates a multiplicity of products from all four DNA bases and this pattern appears to be a diagnostic

of OH attack. By contrast" O selectively attacks guanine [59]. The most commonly produced base lesion, and the one most often measured as an index of oxidative DNA damage, is 8-hydroxyguanine (8-OHG). It is sometimes measured as the nucleoside, 8-hydroxydeoxyguanosine (8-OHdG) [59]

While the mutagenic effects of oxidative DNA damage are largely well recognized, emerging work is broadening the number of routes by which these lesions may affect the cell, being suggestive of epigenetic effects exclusive of mutation. When exposed to oxidants, mammalian cells express stress-induced genes or genes encoding antioxidant defences. Such adaptive responses to oxidative assaults are not surprising and are seen with other, nonoxidative assaults. DNA methylation is an epigenetic event that affects cell function by altering gene expression and refers to the covalent addition of a methyl group, catalyzed by DNA methyltransferase (DNMT), to the 5-carbon of cytosine in a CpG dinucleotide. DNA is wound around clusters of eight histone proteins (H1–H8), and together, histones and DNA make up chromatin. Histones not only keep DNA organized, but they are also known to help regulate expression of genes. Specifically, modifications to histone proteins, such as methylation and acetylation, are thought to help keep genes active or silent, thus comprising a kind of code to be read by transcriptional regulators. Epigenetic alterations of genomic DNA play a critical role in many important human diseases, especially in cancer. Core mechanism for epigenetic alterations of genomic DNA is hypermethylation of CpG islands in specific genes and global DNA hypomethylation. Methylation of CpG islands involves the course in which DNA methyltransferases (Dnmts) transfer a methyl group from S-adenosyl- L-methionine to the fifth carbon position of the cytosines. Region-specific DNA methylation is mainly found in 5'-CpG-3'dinucleotides within the promoters or in the first exon of genes, which is an important pathway for the repression of gene transcription in diseased cells [65]. Global DNA hypomethylation is likely caused by methyl-deficiency due to variety of environmental influences, and has been proposed as a molecular marker in multiple biological processes such as cancer. It is well demonstrated that the decrease in global DNA methylation is one of the most important characteristics of cancer. Thus, the quantification of global methylation in cancer cells could provide very useful information for detection and analysis of this disease.

Immune complex is an evolving immunological product that can induce ROS production which would in turn attack DNA and cause breakage. This immunological pathway through the persistence of circulating immune complexes remains an unattended pathway of DNA damage or epimutational changes. The immunological activities of circulating immune complexes may directly or indirectly induce DNA damage or epigenomic obstruction and impair its repairs in developing lymphocytes that have remained continuously challenged with reactive oxygen species emanating from its activities.

Persistent circulation of immune complexes is considered in this review as endogenous genotoxic substances that can

induce epigenetic cell alteration and thus obstruct gene expression. The rate of immune complex formation would likely increase as precursor B and T cells, assemble functional immunoglobulin (Ig) and T cell receptor (TCR) genes, an indication of effective adaptive immunological response via recombination of numerous variable (V), diversity (D), and joining (J) gene segments. Although this combinatorial process generates significant diversity, genetic reorganization is inherently dangerous. Thus, V (D)J recombination must be tightly regulated to ensure proper lymphocyte development and avoid chromosomal translocation that cause lymphoid tumors [66]. It is very pertinent to find out how Immune Complexes, their persistence in circulation may induce genotoxicity, affect regulation of antigen receptor gene assembly, their possible effect on RAG endonuclease activity and the expected repairs of the double strand breaks (DSBs). The perturbing effect of persistent CIC on promoters and enhancers that enhance accessibility of RAG endonuclease to antigen receptor genes for generation of DSBs and their subsequent repair in lymphocytes may hamper the process of DNA repairs. Thus we consider Immune Complexes as a endogenous pathological agent that can alter DSBs and DNA repairs inducing mutation. Thus the role of immune complexes due to its immune-provocative approach becomes very questionable. This review tends to project that the immunological activities of circulating immune complexes as they persist in the system, being a two edged sword, may hamper the DNA repair process and induce aberrant resolution. This may also occur in tissues of various organs of the body, leading to carcinoma [66].

Through alterations in the accessibility of antigen receptor gene segment by RAG endonuclease, a misnomer may occur in the process of arranging for RAG endonuclease to have access to antigen receptor gene segments. In this regard, we hypothesize that persistent circulation of immune complexes and persistent generation of cytokine activities and genotoxic effect of ROS due to presence of CIC, may induce the *cis*-acting transcriptional regulatory elements such as promoters and enhancers that promote or enhance this accessibility, to bring about alterations in DNA methylation, chromatin structure, and nuclear positioning that affect the ability of RAG to access the appropriate regions of antigen receptor loci.

9. Conclusion

Persistent circulation of immune complexes could be leading factor in so many insidious sicknesses with unknown origin, this includes cancers. High levels of immune complexes have been seen in individuals in many undeveloped countries where environmental pollution is high. This fact is attributed to persistence of immune complexes which under normal physiological and immunological functions should be eliminated. Couple with stress, low hygienic level and low standard of living, persistence of immune complexes may induce tumourigenesis and exacerbate cancer. Many poor cancer prognosis have been reported in many developing countries where circulating immune complexes are increased in

the human system, while cancer menace is being reduced in the developed countries where circulating immune complexes are reduced in the system.

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