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РЕГУЛЯЦИЯ ИММУННОЙ СИСТЕМЫ ПРИ СТАРЕНИИ: В ФОКУСЕ — ЭПИГЕНЕТИЧЕСКИЕ МЕХАНИЗМЫ

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Regulation of the Immune System in Aging: Focus on Epigenetic Mechanisms

Резюме

Эпигенетика изучает процессы, приводящие к изменению активности генов без изменения последовательности ДНК. Эпигенетические механизмы, такие как метилирование ДНК и модификации гистонов, формируются в период эмбрионального развития, а эпигенетические профили стабильно наследуются при митозе, обеспечивая дифференцировку клеток и их дальнейшую судьбу в процессе развития. Под действием внутренних и внешних факторов, таких как метаболический профиль, гормоны, питание, наркотики, курение и стресс, эпигенетические механизмы активно модулируются и, в этом смысле, образ жизни может существенно влиять на эпигеном, а следовательно, и на профиль экспрессии генов и функцию клетки. Показано, что развитие и функции клеток как врожденной, так и адаптивной иммунной системы, также регулируются эпигенетическими механизмами, а негативные эпигенетические изменения являются отличительной чертой старения и онкологических заболеваний. Учитывая эти данные, можно полагать, что возрастные изменения профиля эпигенетических меток могут привести к снижению иммунной функции и способствовать увеличению заболеваемости у пожилых людей. Поэтому, чтобы обеспечить здоровую старость, необходимо лучше понять, как избежать эпигенетических изменений, которые связаны со старением иммунной системы. В данном обзоре мы попытались обобщить последние достижения в этой области исследований и рассмотреть возможность их использования в качестве средств диагностики, профилактики и лечения заболеваний.

Ключевые слова: *иммунное старение, эпигенетика, метилирование ДНК, модификации гистонов, окружающая среда, возрастные заболевания*

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Abstract

Epigenetics studies processes leading to changes in the activity of genes without changing the DNA sequence. Epigenetic mechanisms, such as DNA methylation and histone modifications, are formed during embryonic development, and epigenetic profiles are stably inherited in mitosis, providing cell differentiation and their further fate in the development process. Under the influence of internal and external factors such as metabolic profile, hormones, nutrition, drugs, smoking and stress, epigenetic mechanisms are actively modulated and, in this sense, a lifestyle can significantly affect the epigenome, and consequently, the gene expression profile and function of cells. It is shown that the development and function of cells of both congenital and adaptive immune systems are also regulated by epigenetic mechanisms, and negative epigenetic changes are a distinctive feature of aging and cancer. Given these data, it can be assumed that age-related changes in the profile of epigenetic labels can lead to a decrease in immune function and contribute to an increase in morbidity in the elderly. Therefore, to ensure healthy aging, better understanding of how to avoid epigenetic changes that are associated with aging of the immune system is needed. In this review, we tried to generalize the latest achievements in this field of research and consider the possibility of using them for diagnosis, prevention and treatment of diseases.

Key words: *immune aging, epigenetics, DNA methylation, histone modifications, environment, age-related diseases*

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Introduction

Aging is a process of gradual failure of all normal physiological functions of the body [1] that eventually ends in death. This process is understudied, although many different theories exist, including the following: accumulation of genetic damage [2]; free radicals [3]; cell apoptosis [4]; immunological theory [5], and others that are aimed at shedding light upon its origin. Unfortunately, none of them can fully explain all aspects of this complex biological process. In humans, aging is characterized by decreased immune function, chronic inflammation, sarcopenia, and, most importantly, increased susceptibility to several diseases such as cancer, cardiovascular, metabolic, and neurodegenerative diseases. Despite their systemic nature, these phenotypes are the result of changes in different cellular processes, such as the response to DNA damage, mitochondrial and proteasome function, and regulation of cell death. Dysregulated transcription that develops in aging leads to significant changes in gene expression. Studies have shown that these changes in transcriptome, known as "epigenetic drift," are significantly affected by modifications of the epigenetic mechanisms of regulation of gene activity.

Epigenetic Mechanisms of Regulation of Gene Activity

As is known, all cells of an organism have an identical genome. However, they are very different in cytomorphological traits and function. These differences are a consequence of "epigenetic processes" in cells that can modulate the pattern of gene expression. Epigenetic processes are direct hereditary changes in gene expression with no alterations in the DNA sequence when active or silent states of genes are controlled by adding or removing chemical modifications in chromatin [6]. These modifications include the following: 1) DNA methylation; 2) various post-translational modifications of histone (acetylation, methylation, phosphorylation, etc.); and 3) binding of messenger RNAs to non-coding RNAs (ncRNAs). In this review, we will discuss the latest advances in investigating the role of epigenetic mechanisms in the regulation of gene activity in cell aging, including immune cells, focusing on the significance of two types of epigenetic modification—DNA methylation and histone modifications.

DNA Methylation: In mammals, DNA methylation is mainly associated with the attachment of methyl groups (-CH3) to cytosine residues in CpG sites. This reaction is catalyzed by DNA methyltransferases (DNMT)—DNMT1, 3A and 3B—that transfer a methyl group from S-adenosyl methionine (SAM) on the 5th carbon of cytosine forming 5-methylcytosine (5mC). In mammalian cells, methylation usually develops in CpG islands, and such methylated sites amount to 70–80% [7]. Hypermethylation of gene promoter regions usually induces transcriptional silencing, while hypomethylation stimulates gene expression.

Histone Modifications: In nucleosomes, which are the basic structural units of chromatin, not only a DNA

molecule but also histone proteins can carry chemical modifications that are fundamental for chromatin-dependent gene regulation [8]. Post-translational modifications (PTMs) of histones regulate chromatin structure, thereby affecting internucleosomal interactions, and recruit proteins and complexes that not only affect gene transcription but also mediate processes such as DNA replication, DNA repair, alternative splicing, and recombination [6]. PTMs of histones such as acetylation, methylation, ubiquitination, phosphorylation, sumoylation, and poly-ADP-ribosylation develop mainly in the N-terminal tails of histones that are readily available for covalent post-translational modifications and constitute a potential "histone code"—a hypothesis which argues that histone modifications can result in activation or repression of gene transcription [9]. For example, acetylation and phosphorylation reduce the positive charge of histones and thus weaken the interaction between histones and DNA, thereby facilitating the access of transcription factors to DNA transcription mechanisms. As a result of these and other PTMs of histones, the chromatin structure changes, leading to the activation or suppression of target genes due to modulation of the binding of transcriptional agents to their respective promoter elements of the nucleus [10].

Changes in Epigenetic Marks with Aging

DNA Methylation: It has been demonstrated that global DNA hypomethylation develops in advanced years. Repetitive DNA sequences that are usually "silenced" by epigenetic marks become expressed with age and may be partially responsible for the loss of heterochromatin during aging [11]. Besides, aging is accompanied by hypomethylation of the promoters of certain genes, for example, IL17RC, which induces its transcription and leads to the expression of inflammatory chemokines and cytokines [12]. At the same time, the promoters of some other genes become hypermethylated and abnormally "silent." Regarding 5-hydroxymethylcytosine (5hmC) generated during active demethylation of 5-methylcytosine, it was demonstrated that although the global level of 5hmC in the brain increases with aging in mice and humans, its level decreases in other tissues, such as blood. Despite that age-related changes in DNA methylation develop more frequently in CpG islands, tissue-specific changes were also found in other genomic regions. In their comprehensive study of DNA methylation, Yuan et al. [13] showed that, in addition to hypermethylated CpG islands, a large number of differentially methylated sites were found in regions with low CpG content. These authors also identified large aging-related hypomethylated blocks similar to those described in cancer cases. Based on the study of the genomic profile of whole blood methylation in 656 individuals across a wide age range (from 19 to 101 years), a quantitative model was constructed not only for more accurate determination of the biological age of a person but also for predicting his/her risk of death from senile diseases and, in particular, the probability of cancer development [14].

Histone Modifications: Global DNA hypomethylation seen in advanced years has been closely associated with changes in histone modification patterns. Moreover, with aging, changes were observed in the activity, functions, and number of enzymes of the epigenetic apparatus. For example, the genes identified as hypermethylated were associated with bivalent chromatin domains (genes carrying two types of histone H3 marks—active and inactive—are located on these domains) in embryonic stem cells, and those with repressive histone marks H3K27me3 and H3K9me3—in differentiated cells [15]. With age, there was a global loss of histones, as well as an imbalance between activating and repressive histone marks. There was a decrease in acetylated H3K9 and trimethylated H3K27 marks in old cells. Histone lysine methyltransferase expression also decreased with age, contributing to a decrease in the level of H3K9me3 marks and loss of heterochromatin. Age-related decrease in HP1 and DNMT expression may contribute to DNA demethylation in heterochromatin. Another change that may contribute to a more open chromatin state is increased H4K16Ac level with replicative age, as described in human fibroblast culture. H4K16 is a target of histone deacetylase SIRT1, which is associated with the rate of aging and genome maintenance in different organisms.

Changes with aging develop not only in canonical histones but also in the attachment of histone variants that do not depend on replication. The replication-independent histone variant H3.3 becomes more common with age not only in non-replicating cells (such as neurons) but also in other cells; this fact also contributes to the greater availability of chromatin for transcription apparatus. Another replication-independent histone variant associated with aging is H2A.Z; its knockout leads to premature aging in fibroblasts [16]. Finally, the macroH2A variant of histone H2A is associated with aging. An age-related increase in macroH2A level has been described both during replicative aging of human fibroblast culture and in many other tissues of aged animals [17].

Effect of Environment and Lifestyle: The classic study conducted by Fraga et al. [18] demonstrated, on the one hand, significant differences in the level of epigenetic marks in elderly monozygotic twins and, on the other hand, their general indistinguishability in very young twins. An even more interesting thing in this study was that the greatest differences in epigenetic marks

were found in elderly twins who lived together for a little time. The data obtained on the dominant effect of the environment on the variability of phenotypic traits are confirmed by studies in the human population, which showed that genetic factors are responsible for only 20–30% of the variability observed in identical twins, and most of the variability is due to the epigenetic drift that develops throughout the life of twins [19]. These studies also demonstrate how age and various environmental factors impact on changes in epigenetic marks. Generally speaking, these modifications of epigenetic marks alter the state of chromatin, making it more open and accessible to transcriptional regulators and leading to abnormal gene transcription and genomic instability. Therefore, they were proposed as key regulators of the aging process that contribute to the development of age-related diseases and even as predictors of chronological age.

It should be emphasized that the epigenome acts as a molecular interface between the genome and the environment. This means that lifestyles, including eating habits, exercise, stressors, smoking, alcohol or drug abuse, and exposure to chemicals, can alter the epigenetic landscape, affecting the structure and function of chromatin, thereby contributing to the development of phenotypes of age-associated diseases. It was found that physical exercise results in the reconstruction of epigenetic marks in human skeletal muscles and adipose tissue. Increased cardiorespiratory performance and endurance and decreased low-density lipoprotein levels that are observed during physical exercise were accompanied by demethylation of CpG islands, in contrast to methylation changes observed in aging. At the same time, such bad habits as smoking [20] and alcohol abuse [21] had negative effects on the processes associated with changes in epigenetic marks. For example, prenatal smoking affected blood cell DNA methylation in children of smoking mothers. Epigenetic changes caused by chronic exposure to cigarette smoke contributed to the sensitization of bronchial epithelial cells to malignant transformation. Tobacco smoking caused changes in DNA methylation in the cells of both innate and adaptive immune systems. Alterations in fetal DNA methylation were also associated with alcohol abuse of the mother.

The way how many of these environmental factors obtained in both human and animal models have an impact on aging is associated with oxidative stress. Although severe acute or chronic stress effects accelerate aging, contributing to the accumulation of cellular damage due to the depletion of defense mechanisms, moderate stress, on the contrary, slows down this process, activating defense mechanisms and preventing and/or eliminating such cellular damage [22]. Recent research has demonstrated the relationship between cellular stress and epigenetic changes. As is known, reactive oxygen species (ROS) lead to oxidized DNA damage, which can contribute to changes in DNA methylation. In this regard, the results of studies on 8-hydroxy-2'-deoxyguanosine (8-oxo-dG), one of the main oxidative products of DNA damage, and its level in tissues are especially noteworthy. It was demonstrated that this modified nucleotide is accumulated in the DNA of various organs and tissues of mammals with age [23]. Also, reducing caloric intake, which is known to slow aging and increase longevity, caused a significant decrease in the level of 8-oxo-dG in the DNA of all tissues in mice [24]. It was also established that ROS can interfere with TET (ten-eleven translocation)-mediated DNA demethylation [25].

Sirtuins, i.e., histone deacetylases that catalyze the removal of the acetyl group of histones, play a key role in responses to various stresses, such as oxidative or genotoxic stress. Sirtuins modify histones and alter chromatin conformation, making DNA packaging denser and less accessible for transcription factors, leading to transcriptional repression. However, this is not the only function of sirtuins. In the event of DNA damage caused by ROS, sirtuins move to these damage sites and participate in its restoration. O'Hagan et al. [26] demonstrated that this process can result in stable aberrant epigenetic and gene transcriptional changes similar to those observed in cancer diseases. In mouse embryonic mesenchymal fibroblasts, elevated levels of hydrogen peroxide induced SIRT1 to move from repressed DNA sequences to DNA breaks in order to facilitate repair; this resulted in transcriptional changes similar to those seen in the brains of aged rats. However, in response to environmental stress, sirtuins appear to promote cell survival, thereby extending the replicative and chronological lifespan. This assumption is based on the following: 1) calorie reduction, which induces SIRT1 deacetylase, increased the viability of mammalian cells; 2) sirtuin activity was a prerequisite for increasing physical activity and increasing life expectancy with calorie reduction [27]; 3) the health and survival of mice on a high-calorie diet improved after treatment with resveratrol, which activates SIRT1 [28]. This evidence of the important role of sirtuins in extending the lifespan of various model organisms under caloric restriction apparently shows that epigenetic mechanisms play an essential role in this process. In this regard, new and known compounds were tested as "calorie restriction mimetics," including sirtuin-activating compounds such as resveratrol. Compounds that inhibit histone acetylation, such as spermidine, also helped to increase lifespan.

As mentioned earlier, ROS can modify TET-mediated DNA demethylation [25]. Increasing endogenous antioxidants and reducing calorie intake reduces high 5hmC levels in the brains of aged mice. Demethylase activity

of TET enzymes can be stimulated with nutrients such as ascorbic acid. Since the activity of many epigenetic enzymes depends on the intracellular levels of metabolites (methionine, iron, ketoglutarate, NAD^+, acetyl coenzyme A, SAM), cell metabolism controls epigenetic modifications and can regulate longevity [29].

Other studies in human cohorts have demonstrated that life-threatening stressors, especially during early development, can cause long-term changes in the epigenome. Human and animal studies also demonstrated that stress and glucocorticoids can induce long-term changes in DNA methylation both at the genomic level and at the level of individual gene loci.

Epigenetic Regulation of Immune System

The most important feature of the immune system is its ability to distinguish what is "friendly" from what is "hostile" and then attack and neutralize the "hostile" (potentially pathogenic agents or substances) in order to protect the body from harmful effects. Protection from a potentially dangerous environment is provided by several populations of immune cells using both innate and adaptive mechanisms. However, these immune cells can exercise their protective functions to the full extent only under strictly controlled regulation of the differentiation of hematopoietic cells from which they originate. A growing number of studies show the critical role of epigenetic mechanisms in the development and differentiation of cells of the immune system and the pathologies associated with them. It is widely known that immunocompetence, i.e., the functional state of the immune system that provides effective protection of the body against infectious agents, tumor cells and chemicals with antigenic properties, becomes defective with age. It turns out that one of the leading causes for this is the repression of immune cell differentiation genes, along with the activation of autoimmunity genes due to changes in DNA methylation.

Cells of the Innate Immune System: The innate immune system, which includes macrophages, neutrophils, dendritic cells (DCs) and natural killer cells (NKs), is the first line of defense against pathogenic agents. Macrophages and dendritic cells are professional antigen-presenting cells (APCs) that can capture antigens for processing and presentation to lymphocytes. When activated, resident macrophages can act either directly, thereby destroying their targets, or indirectly by initiating an acute inflammatory response by producing cytokines, chemoattractants, and inflammatory mediators, as well as recruiting neutrophils, monocytes, and DCs. Activated macrophages produce different factors in response to the extracellular environment and can acquire functionally different phenotypes: classically activated M1 and alternatively activated M2. Activated M1 macrophages are induced by cytokine interferon-gamma (IFN-γ) as well as bacterial products and have a pro-inflammatory profile, playing an important role in host defense. Unlike M1 macrophages, M2 macrophages are induced by interleukin-4 and -10 (IL-4 and IL-10) as well as helminth products and have an anti-inflammatory profile that promotes tissue repair. Since mature cells of the immune system should quickly respond to pathogens, the contribution of epigenetic mechanisms to the regulation of genes involved in such response was described to a large extent. In this context, it was established that epigenetic mechanisms were involved in modulating the polarization of macrophages mainly through the presentation of histone marks in the enhancers of specific genes.

The fact that inflammation is regulated by epigenetic mechanisms was first demonstrated in the study conducted by Sakkani and Natoli [30]. They found that the loss of H3K9 methylation in the promoter regions of cultured human monocytes after exposure to bacterial lipopolysaccharide (LPS) endotoxin induced inflammatory cytokines such as IL-8 and macrophage inflammatory protein 1-alpha (MIP-1α). Innate immune cells have a certain degree of specificity through the presentation on their surfaces of pattern recognition receptors (PRRs) targeted at the recognition of molecular structures associated with pathogens. Recent studies demonstrate that, in contrast with previous ideas, cells of the innate immune system can form the memory of past stimuli. This phenomenon, called "trained immunity," allows the cells of the innate immune system to change their response to repeated stimuli, reacting more strongly or reacting to a larger number of microbes compared to the baseline level [31]. This immunological "memory" includes changes in transcriptional programs made by reprogramming epigenetic marks. For example, metabolic changes in monocytes triggered by β-glucan from *Candida* are associated with increased levels of active histone marks, trimethylation of H3K4, and acetylation of H3K27, which leads to increased production of cytokines IL-6 and TNF, inflammation, and the development of "trained immunity" [32]. Macrophages re-stimulated by LPS induce a more attenuated inflammatory response while maintaining an intact antimicrobial response. Foster et al. [33] demonstrated that genes involved in LPS tolerance lost active histone marks H3K4me3 and H4Ac in their promoters during re-stimulation with LPS, while intolerant genes, on the contrary, retained these active marks. Under certain stimuli, epigenetic mechanisms also regulate the differentiation of human monocytes into DC. For example, the increase in CD209 expression observed during differentiation was shown to be a result of the acquisition

of H3K9Ac and the loss of H3K9me3, H4K20me3, and DNA methylation in its promoter [34].

T Lymphocytes: Age-related decline in the function of the immune system, called "immune aging," is accompanied by changes in epigenetic marks. Kuwahara et al. [35] demonstrated that CD4 T-cell senescence and homeostasis of cytokines were controlled by maintaining histone acetylation at the Bach2 gene locus (encodes a protein of the same name, i.e., a transcription factor), which was stimulated by binding to *menin*, a nuclear protein. Also, genomic instability in the thymus, which increases with age, was associated with the loss of heterochromatin markers, including H3K9me3, with a corresponding reduction in the expression of the SUV39H1 gene. This suggests that aging is stimulated by DNA hypomethylation, which is observed particularly in aging but not in immortal cells, and inhibition of DNA methylation leads immortal cells to stop the cell cycle.

Cells of the innate immune system present antigens to both B lymphocytes and T lymphocytes, activating them for proliferation and differentiation into effector cells. APCs activate T-cell receptor and costimulatory molecules of naive T cells, initiating T-cell differentiation by activating the nuclear factor of activated T cells (NFAT) and the production of interleukin-2 (IL-2). Activation of naive T cells triggers the synthesis and secretion of IL-2 and the simultaneous expression of its receptor on the cell surface. By interacting with its own receptor, IL-2 provides rapid multiplication and subsequent differentiation naive T cells into mature effector cells. Naive and resting CD4 + T cells do not express IL-2. However, this cytokine is expressed in T cells upon antigenic stimulation. Murayama et al. [36] demonstrated that demethylation of a single specific CpG site in the enhancer region of the human IL-2 gene was sufficient for IL-2 transcription and, more interestingly, this single epigenetic change was the memory that CD4 + T cells had encountered an antigen.

Peptide antigens are presented to T cells by APCs in combination with the major histocompatibility complex (MHC). Cytotoxic T cells that express CD8 recognize antigens presented by normal cells in regard to MHC class I molecules, and can directly kill infected cells. Activated CD8 + T cells have increased levels of H3Ac in the IFN-γ promoter and enhancer. This epigenetic modification is maintained through the memory of CD8 + T cells and provides a faster and stronger cytotoxic response to additional antigen stimulation. MHC class II molecules are MHC molecules involved in the presentation of CD4 + antigen to T-helper cells. A class II transactivator (CIITA) is a key factor that controls MHC-II expression; both CIITA expression and CIITA-dependent MHC-II expression are epigenetically regulated. An analysis of chromatin availability in peripheral blood mononuclear cells defined the memory of CD8 + T cells as a subpopulation with the deepest chromatin remodeling with aging.

After antigen recognition, naive T lymphocytes differentiate into effector T-helper cells (Th1, Th2, and Th17) or regulatory (Treg) CD4 + T cells depending on the cytokine environment, and coordinate specific immune responses by generating different sets of cytokines. Differentiation towards the Th1 profile is induced by IFN-γ, IL-12, or IL-15, while differentiation towards the Th2 profile is induced by IL-4, IL-10, or IL-13; both pathways include the regulated expression of several effector genes. Transforming growth factor beta and IL-6 are responsible for the induced differentiation of naive T cells into Th17 cells. CD4 + T cell differentiation into these different profiles is tightly regulated in order to provide specific cytokine profiles; changes in epigenetic marks are essential for completing this process. The IFNG gene promoter, which is hypermethylated in naive human T cells, is demethylated during differentiation into the Th1 profile. Specific histone marks were identified throughout the entire IFNG locus: H4Ac and H3K4me3 in Th1 cells, and H3K27me2 and H3K27me3 in Th2 cells. Naive and Th1 cells had a highly methylated IL-4-gene promoter, while Th2 cells had a partially demethylated IL-4-intron 2. Th17 cells were characterized by the expression of IL-17 cytokine and RAR-related orphan receptor C (RORC). Demethylation of both IL-17A and RORC locus correlates with gene expression in human Th17 cells; active histone marks H3Ac and H3K4me3 were found in the IL-17 locus. Demethylation of the Foxp3 locus, as well as histone hyperacetylation, was shown to be important for maintaining stable expression of the transcription factor FOXP3 (has an impact on the development and functioning of regulatory T lymphocytes) and stabilization of the regulatory phenotype in Treg cells.

B Lymphocytes: After binding to an antigen and induction by T-helper cells, B cells differentiate into antibody-secreting plasma cells. Antibodies bind to a specific antigen, leading to better recognition and destruction of pathogens (e.g., bacteria, viruses, and tumor cells) by activating complement and/or interacting with lytic cells. During B-cell differentiation, lineage-specific genes are expressed, while genes associated with multipotent progenitors and alternative lines are suppressed. Complex epigenetic regulatory mechanisms coordinate the differentiation and function of B cells, including monoallelic V(D)J rearrangement and determination of antibody diversity. The key transcription factor involved in B-cell commitment is the PAX-5 (paired box 5) transcription factor, which, in addition to the expression of this cell being regulated by epigenetic mechanisms, recruits chromatin-modifying proteins to regulate the

expression of its targets. For example, the CD79a-gene promoter, which is hypermethylated at the precursor stage, is demethylated at the early stages of B-cell differentiation, followed by the action of histone acetyltransferase recruited by Pax5, which allows gene expression [37]. Pax5 can also interact with chromatin-modifying enzymes to repress genes specific to other lines [38]. V(D)J rearrangement and determination of antibody diversity are essential for the production of effective antibodies and require activation-induced cytidine deaminase (AID) expressed by B cells at certain stages of differentiation. In naive B cells, the AID-gene promoter is hypermethylated and the gene is not expressed. When B cells are activated, the AID gene becomes demethylated and acquires higher levels of the active histone H3Ac mark. The uptake of this histone mark by active promoters and distal enhancers is also critical for changes in gene expression during B-cell differentiation into plasma cells. Blimp-1, a transcriptional repressor that maintains plasma cell identity, has its epigenetically induced expression and epigenetically downregulates mature B-cell gene expression by recruiting histone modifiers. After V(D)J rearrangement processes and determination of antibody diversity, B cells can differentiate into memory B cells that acquire additional epigenetic marks in addition to those obtained upon activation of B cells. Various epigenetic modifications, as well as epigenetic enzymes, such as the enhancer of zeste homolog 2, monocytic leukemia zinc finger protein histone acetyltransferase, and DNMT3a, are observed in resting and activated B cells that demonstrate that the epigenome of B cell memory may contribute to faster and more efficient activation than the epigenome of naive cells.

Involvement of Epigenetic Modifications in the Aging of Cells of the Immune System

Age-related defects are observed in all cells of the immune system and affect their activation and cytokine production.

Innate Immune Cells: It was shown that many immune responses are inhibited with aging. However, several responses are hyperreactive. Age-related epigenetic changes seem to affect monocyte differentiation since the hypomethylation of genes associated with differentiation was observed in older hematopoietic progenitor cells (HPCs) compared to progenitor cells from umbilical cord blood. This is possibly due to a decrease in pluripotency and decreased HPC differentiation potential in elderly donors. At the same time, methylation *de novo* in a subgroup of genes associated with the repressive Polycomb complex was observed in older HPCs, which could contribute to a decrease in the phenotypic plasticity of old stem cells. Moreover, according to Kramer a. Challen [39], epigenetic dysfunction may be a precursor of hematological disease in elderly individuals. In the elderly, epigenetic mechanisms also contribute to decreased expression of MHC-II in macrophages. Although the number of NK cells in the elderly increases, their cytotoxic activity decreases. The regulation of DNA methylation by IFN-γ and IL-2 appears to contribute to this defective NK-cell function. With aging, there is an imbalance between inflammatory and anti-inflammatory responses, which can be described by high levels of inflammatory mediators such as IL-6 and tumor necrosis factor alpha (TNF-α), even with no acute infection or another physiological stress (a process known as "subclinical systemic inflammation") [40]. Expression of TNF-α increases with aging; this is associated with the demethylation of its promoter. This epigenetic modification contributes to increased levels of TNF-α and IL-1α, thereby initiating subclinical systemic inflammation associated with resting neutrophils in elderly donors.

The primary cause of morbidity of elderly individuals worldwide is diseases of the circulatory system and inflammatory lung diseases. In this regard, hypomethylation of the promoter of inflammatory genes such as toll-like receptor 2, carnitine O-acetyltransferase, and coagulation factor III were associated with decreased lung function [41]. Zinc is an essential trace mineral for the development and functioning of the immune system. Its deficiency, which often comes with aging, contributes to a wide range of immune defects, including increased inflammatory response by inducing demethylation of the IL-6 promoter. Using C-reactive protein (CRP) as an inflammatory biomarker, Ligthart et al. [42] conducted a meta-analysis of large-scale associative studies of DNA methylation in chronic subclinical inflammation. In that study, the authors demonstrated that several inflammation-associated CpG sites were associated with the expression of adjacent genes, and that many of these CpGs showed associations with cardiometabolic phenotypes and cases of coronary heart disease. These genes also include the AIM2 gene that plays a critical part in innate immune responses (it is involved in the host's defense mechanisms against bacterial and viral pathogens); it was found to be hypermethylated and expressed at low levels in samples with low CRP levels.

T Lymphocytes: Involution of the structure and function of the thymus, which is characterized by the decreased number and functional defects of naive thymic T cells, is another process that contributes to immune aging. The analysis of CD4 + T-cell methylome in neonates and centenarians performed by Heyn et al. [43] revealed that these immune cells had the same changes in DNA methylation observed in other tissues during

aging—global DNA hypomethylation and higher variability of DNA methylation.

More recently, a comprehensive analysis of transcriptome, methylome, and the totality of all miRNAs in the same CD4 + T cells conducted by Zhao et al. [44] revealed a potential link between gene transcription and DNA methylation for age-related or immune genes, indicating the involvement of DNA methylation in the regulation of transcription associated with the development and functions of T cells during aging. Mice with heterozygous *Dnmt1* null mutation had hypomethylated DNA and were phenotypically normal. However, they showed signs of immune aging and early development of autoimmunity compared with normal mice of the same age. In the analysis of naive CD4 + T cells in 74 healthy (19 to 66 years) individuals [45], Dozmorov et al. identified sites that were hypomethylated with age and demonstrated a specific enrichment of active T-cell enhancers H3K27Ac and H3K4me1 with marks. This indicates a progressive age-related shift in T-cell epigenomes towards a pro-inflammatory and T-cell activated phenotype, which may contribute to increased autoimmunity with age. It was also demonstrated that the elderly with higher levels of autoantibodies had T cells with demethylation and overexpression of the same genes as in patients with lupus [46]. Progressive loss of the co-stimulatory molecule CD28 in CD4 + T-lymphocytes during aging is associated with impaired immune response. A unique DNA methylation landscape in CD28 null T cells that leads to the expression of genes associated with inflammation was recently described. Another recent study revealed two CpG sites in the promoter region of the KLF14 gene (transcription factor), which is involved in CD4 + T-cell differentiation through suppression of FOXP3 (transcription factor that functions as a regulator of the development and functioning of regulatory T cells); they demonstrated stable methylation in early age and a sharp increase in methylation at the end of life in peripheral whole blood, monocytes, and isolated CD4 + T cells [47]. Dysfunctional Treg cells are thought to be involved in immune aging and increased susceptibility to age-related diseases by suppressing T-cell responses. Garg et al. [48] demonstrated that a large number of Treg cells observed in aged mice was associated with the hypomethylation of the FoxP3 enhancer, which caused its increased expression. They also demonstrated that Treg cells from aged mice produced more IL-10 and were more effective in reducing the co-stimulatory molecule CD86 on Dcs; they also modulated the extracellular redox environment by suppressing T-cell proliferation.

Immune aging is also described by the loss of naive T cells and central memory cells, as well as the proliferation of effector memory cells in the CD8 + T-cell compartment. The transition to a more differentiated state of chromatin openness that provides DNA accessibility for transcriptional regulators was observed in naive T cells and central memory cells in the elderly; there was also loss of chromatin availability on gene promoters in old naive cells, which was partly mediated by the loss of nuclear respiratory factor 1 (NRF1). An analysis of methylation data of peripheral blood mononuclear cells (PBMCs) among the Italian population performed by Horvath et al. [49] revealed that the biological age of centenarians was less than their chronological age. While studying the profile of immune cells among "the Nikoyans" (a population from Costa Rica characterized by longevity), McEwen et al. [50] found that this population had a significantly higher number of predicted CD8 + T naive cells and a lower content of CD8 + T memory cells compared to "non-Nikoyans," which indicates a younger profile of immune cells. Also, they demonstrated that the epigenetic characteristic of longevity in Nikoyans is a lower variability in their DNA methylation compared to non-Nikoyans.

B Lymphocytes: Considering the role of epigenetic mechanisms in the differentiation and functioning of B cells, age-related epigenetic changes may be responsible for decreased humoral immunity in the elderly. Loss of function of B cells and their precursors, reduction of immunoglobulin diversity and affinity, and shifts in the ratio of naive and committed subpopulations of peripheral B cells are typical for the aging of the immune system. Hematopoietic stem cells (HSCs) lose their ability to differentiate with age, and epigenetic modifications are critically involved in these changes. Aged mice had HSCs with aberrant gene expression profiles due to epigenetic deregulation. With aging, defects were observed both in the early and late stages of the maturation and differentiation of B cells.

Conclusion and Perspectives

According to the review data, a lot of new information was obtained in recent years regarding the understanding of the role of epigenetic mechanisms in the regulation of genome activity, including genes associated with the immune system. After analyzing and summarizing this information, we can draw the following basic conclusions: 1) epigenetic mechanisms modulate chromatin states and determine gene expression profiles; 2) epigenetic mechanisms play a critical role in the development and functioning of immune system; 3) strictly regulated functioning of the immune system is essential to maintain a healthy state of the body; 4) the environment modifies epigenetic marks throughout the entire life cycle; and 5) epigenetic marks are potentially reversible.

As for the role of epigenetic modifications in the aging of body cells and, particularly, immune cells, despite the

abundance of data on their involvement in aging processes, there are still gaps and questions in this area that require thorough investigation. In particular, the epigenetic changes causally related to the aging process, and how and via what mechanisms they cause immune aging should be determined. Solving these problems undoubtedly requires great efforts. However, these efforts are justified. Given that epigenetic marks are potentially reversible, knowledge about how the environment contributing to the development of age-related pathology modulates the immune system via epigenetic mechanisms can lead to the development of innovative strategies for the prevention and management of human diseases.

Finally, since several age-related epigenetic changes are similar in different tissues, they can already potentially be used as biomarkers of phenotypes of age-related diseases in biological samples such as blood or saliva. However, most importantly, considering that both internal and external factors change epigenetic marks throughout life, we should clearly realize that a healthy lifestyle that positively modulates our epigenome could be the most effective method of preventing age-related diseases and ensuring healthy aging today.

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