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The Role of Epigenetics in Aging and Autoimmunity

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Abstract

The decline in immunocompetence with age is accompanied by the increase in the incidence of autoimmune diseases. Aging of the immune system, or immunosenescence, is characterized by a decline of both T and B cell function, and paradoxically the presence of low grade chronic inflammation. There is growing evidence that epigenetics, the study of inherited changes in gene expression that are not encoded by the DNA sequence itself, changes with aging. Interestingly, emerging evidence suggests a key role for epigenetics in human pathologies, including inflammatory and neoplastic disorders. Here we will review the potential mechanisms that contribute to the increase in autoimmune responses in aging. In particular, we will discuss how epigenetic alterations, especially DNA methylation and histone acetylation, are accumulated during aging and how these events contribute to autoimmunity risk.

Keywords

aging; immunity and epigenetics

Introduction

The incidence of autoimmune disorders appears to be higher in the elderly, even though immunological response to antigenic stimulus declines with age. Interestingly, some inflammatory diseases, such as polymyalgia rheumatica and temporal arteritis, are almost nonexistent in younger persons. Epidemiologic data show that the overall incidence and prevalence of autoimmunity rise with increasing age, with the notable exception of an earlier peak in women of childbearing age (1). Additionally, an age-associated increase in both organ specific and non-organ specific autoantibodies in humans and mice has been consistently reported.

Decreased T cell memory, exhaustion of the naïve T cell population with involution of the thymus, defective humoral immunity, and a chronic inflammatory state referred to as “inflamm-aging” (2) are also commonly observed in the elderly. Inflamm-aging results in both decreased immunity to exogenous antigens and increased autoreactivity, whereby the beneficial effects of inflammation devoted to the neutralization of harmful agents early in life become detrimental late in life. Although aging is inexorable, it is worth noting that immunosenescence does not represent an unavoidable and progressive decline of all immune functions. Indeed, the immune system in the elderly is the result of a continuous remodeling process where although some functions are reduced, others remain unchanged or even increased (3). This raises the fundamental question of whether one or more unifying

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mechanisms can explain the essential changes in the immune and inflammatory responses in aging.

Cumulative new experimental evidence suggests that epigenetic changes may be critical determinants of cellular senescence and organismal aging (4). This review will focus on the features of immunosenescence in autoimmune conditions and examine the role of epigenetic changes as an attractive mechanistic link between aging and autoimmunity.

Immunosenescence and Autoimmunity

It is well documented that a significant fraction of elderly people have low-affinity autoantibodies in their serum, and the prevalence of autoantibodies associated with systemic autoimmune diseases increases with age. For example, rheumatoid factors (RFs) are present in up to 5% of young, healthy individuals, and increases up to 5 times in elderly persons (1). In most instances, the RF titer is low and is not associated with any inflammatory rheumatic disease. Similarly, the prevalence of antinuclear antibodies (ANAs) is higher in healthy individuals over 70 years of age compared to healthy younger adults (1). A number of hypotheses have been proposed to explain the relationship between aging and the development of autoimmunity. For example, a striking feature of the aging process is the involution of the thymus. Reduced thymic output has been postulated to induce compensatory autoprolieration of T cells, which can then lead to premature T cell senescence and contribute to immune system abnormalities associated with autoimmunity and aging. Additionally, alterations in apoptosis in T cells may be an important mechanism of autoimmune disease and immunosenescence. Expansion of CD4⁺ and CD8⁻ senescent T cells that have lost the expression of CD28 emerge in normal aging and in several autoimmune diseases including diabetes mellitus, rheumatoid arthritis (RA), and multiple sclerosis (5). These cells are resistant to apoptosis, are potent producers of proinflammatory cytokines, acquire cytolytic capability, have shorter telomeres than their CD28⁺ counterparts, and often expand to large clonal populations including autoreactive T cells. This is particularly striking in RA, where the increased levels of CD4⁺CD28⁻ T cells correlate with more severe disease (5). Highly elevated levels of CD4⁺ CD28⁻ T cells have also been found in patients with acute coronary syndromes (6). This is of particular interest, as the increased mortality of RA patients can be attributed to coronary atherosclerosis and its complications (7). Thus, changes in the immune system in aging may contribute to both the occurrence of autoimmune diseases and their associated vascular complications.

Although aged animals have normal numbers of B cells in the periphery and are capable of mounting robust humoral responses, the antibodies produced are generally of lower affinity and are less protective than those produced by young animals (8). Careful dissection of splenic B cell subsets also revealed significant alterations in sub-population distribution as mice age (9). Specifically, the percentage of naïve follicular B cells declines dramatically, whereas subsets of antigen-experienced cells increase, including poly/self-reactive subtypes such as marginal zone (MZ) and CD5⁺ B1-like cells, and memory B cells. Additionally, an increase in lifespan of mature B cells in the periphery of aged mice has been reported (10). It is therefore possible that autoimmunity may increase with aging by accumulation of long-lived B cells with specificity against “neoantigens” that form during one's life. Others have also postulated that self-reactive memory B cells may become reactivated later in life (recall memory) due to age-associated reduction in immune tolerance, loss of tissue integrity leading to the exposure of neo-self antigens, or re-exposure to similar environmental agent(s) that result in aberrant autoimmune response through molecular mimicry (8).

Furthermore, aging has been associated with a shift from Th1 to Th2 cytokine profile in response to stimulation, with concomitant greater IL-4 production. However, it should be

noted that the skewing from a naïve to memory T cell phenotype could at least in part account for the observed age-related changes in T cell cytokine profile. It has been postulated that the age-associated shift to Th2 cytokines could augment B cell-mediated autoimmune disorders by enhancing the production of autoreactive antibodies (8). Moreover, this remodeling of the cytokine network seems to be responsible for the inflamm-aging phenotype observed in aging.

Repeated observations have shown that elevated levels of circulating inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α) and C-reactive protein (CRP) exist in healthy adults of advancing age (11), and correlate with increasing overall mortality, and/or frailty (12, 13). Maggio et al. reported that IL-6 levels are associated with disability in community-dwelling elderly persons and are predictive of future disability in nondisabled older persons (14). Of interest, it has been shown that activity of nuclear factor kappa B (NF- κ B), a transcription factor that controls many proinflammatory genes, increases with aging (15, 16). Interestingly, participation in regular physical activity lowers CRP, and IL-6 levels (17, 18). Importantly, chronic systemic inflammation is related to several aging-related diseases, including coronary heart disease and stroke, diabetes mellitus, Alzheimer's disease, and several autoimmune disorders, such as lupus, RA, and Sjogren's syndrome. In RA, excess levels of pro-inflammatory cytokines, such as TNF- α , IL-6, IL-1b, and/or leukotriene B4 (LTB4), are known to cause or contribute to the inflammatory syndrome that ultimately damages the joints (19). Therefore, elevated levels of pro-inflammatory cytokines in the elderly could not only contribute to the overall pro-inflammatory state in many of those individuals, but also provide the susceptibility background for the development of autoimmune diseases in aging.

Evolution of Epigenetic Control during Life

Epigenetic effects occur over the full course of a human life span. They are reproduced during DNA replication and are stably transmitted to the daughter cells. On the whole, epigenetic modifications act as control gates, open during differentiation and growth, and are susceptible to changes in environmental exposure during adult life. Somatic epigenetic inheritance is critical in the development of multicellular eukaryotic organisms. Differentiation of mature cells is characterized by a selective repression of gene transcription governed by epigenetic mechanisms. Epigenetic mechanisms have many layers of complexity, including DNA methylation by DNA methyltransferases (Dnmts), histone modifications such as methylation, acetylation and phosphorylation, structural modifications of chromatin, and microRNAs as well as other noncoding regulatory RNA (20). DNA hypomethylation in CpG-rich, promoter-associated regions termed CpG islands, and acetylated histones allow active transcription, while DNA hypermethylation and histone hypoacetylation promote gene silencing.

Genomic Imprinting and X-Chromosome Inactivation

Apart from development and differentiation, epigenetic modifications play a critical role in genomic imprinting and X-chromosome inactivation (21). In the normal cell imprinted genes (expression restricted to male or female germ line and tissue-specific genes) are silenced by methylation mechanisms. Imprinted genes are differentially methylated during gametogenesis depending on their parental origin. For example, the fetal growth factor insulin-like growth factor 2 (Igf2) is maternally imprinted, whereas the H19 gene, which is involved in silencing Igf2 expression, is paternally imprinted (22). Although relatively few in number, imprinted genes are thought to be vital to human embryonic and post-natal development. Abnormal demethylation of these important genes leads to biallelic expression and contributes to the development of multiple diseases (23). For example, loss of imprinting at the Igf2 locus is implicated in higher incidence of cancer and in Beckwith-

Wiedemann syndrome, an overgrowth condition associated with an increased risk of childhood cancer (22).

X chromosome inactivation refers to the transcriptional silencing of one of the two X chromosomes in mammalian female cells, resulting in equal male and female gene dosing for X-linked genes. X inactivation occurs early in embryogenesis in female somatic cells and is stably maintained in the silenced state for the lifespan of the organism. Compared to its active counterpart, the inactive chromosome X (Xi) expresses the *Xist* gene, which encodes a non-translated RNA that is expressed only from the Xi, and is characterized by a series of epigenetic chromatin modifications including histone H3 methylation, histone H4 hypoacetylation, enrichment of variant histone macroH2A, and DNA methylation, all of which are associated with gene silencing and synergize to maintain Xi (24, 25). Maintenance of imprinting and X inactivation has been shown to depend on the continuous activity of Dnmt1 (26).

Epigenetic Changes in Aging

Although DNA methylation patterns are largely established *in utero*, it has been estimated that the frequency of epigenetic changes over a lifetime may be one to two orders of magnitude greater than the rate of somatic mutations. In an interesting international study, Fraga et al. reported that histone H3 and H4 acetylation profiles and DNA methylation patterns diverged in the peripheral blood lymphocytes of older monozygotic twins compared with younger ones, affecting their gene-expression portraits and providing evidence that epigenetic variants accumulate during aging independently (27). Part of the explanation for the aging-associated epigenetic drift observed may be related to differences in environmental exposure (26), as twin pairs who have spent less of their lifetime together also have greater epigenetic differences. Total genomic 5-methyl-cytosine has been found to generally decrease during aging in various organisms (28-30), and appears to be inversely proportional to the maximum life span potential (30). A recently published longitudinal study of 718 elderly individuals between 55 and 92 years of age demonstrated that repetitive element methylation, particularly in Alu sequences, decreases throughout aging (31). It has been postulated that the reduction of Dnmt1 activity, the predominant maintenance DNA methyltransferase, with age contributes to the decrease in global DNA methylation (32, 33). However, although genome-wide levels of methylation decrease with age in mammals, methylation levels can be either increased, decreased or unchanged depending on the specific strain of mice, tissue or gene examined (recently reviewed in (34)). While age-associated change in DNA methylation is well documented, little information is available on site specific histone modifications in aging. Yoon et al. reported that mouse RhoB is epigenetically regulated in a tissue-specific manner by histone H3 modification, but not by CpG methylation (35). The total abundance of the trimethylated form of histone H4 at lysine 20 (H4K20Me) has also been reported to increase with age in rat liver and kidney, possibly resulting in transcriptional repression (36). In a recent study, Kawakami et al. examined changes in histone H3 modifications with age in rat liver. They found that histone H3 Lys9 acetylation (H3K9ac) was decreased and H3 Ser10 phosphorylation (H3S10ph) was increased with age significantly, supporting the notion that heterochromatin accumulates with tissue aging, at least at some sites (37). Epigenetic control for both X-inactivation and genomic imprinting as a function of age was examined in young (2 months), middle-age (13 months) and old (24 months) mice held in a controlled environment (38). The authors reported that normal aging is associated with a tissue-dependent loss of allelic silencing of the X-linked ATPase copper-transporting type7a (*Atp7a*) gene and at the imprinted locus *Igf2*, confirming the loss of silencing of selected X-linked genes. Loss of imprinting of *Igf2* with aging was further confirmed in normal human prostate tissues and found more extensive in men with associated cancer (39).

Environmental Factors and Epigenetic Inheritance

In addition to age, epigenetic changes have also been observed to occur in response to environmental exposure (40). Accumulating literature show that diet in particular can influence the biochemical pathways of methylation processes by modulating the availability of methyl donors, including folate, choline, and methionine, as well as through their effects on methyltransferase activity (reviewed in (26, 41, 42)). Miller et al. found that mice fed a long-life diet containing very low levels of methionine early in life live longer, have lower risk of developing cataracts, and have relative preservation of their immune function, supporting an important role of methyl diet and possible mechanism for the life-extending effect of caloric restriction (43). Interestingly, several groups have shown that maternal dietary manipulation of methyl donors (either deficiency or supplementation) can have a profound impact on the phenotype of the offspring through epigenetic mechanisms. This was first demonstrated in the agouti mouse model. The *agouti* imprinted gene encodes a paracrine signaling molecule that stimulates the production of yellow pigment. Methyl donor supplementation of agouti dams during pregnancy causes the agouti gene to be hypermethylated, altering the coat color of the offspring and leading to a healthier and longer life span (44, 45). The prenatal diet-modifying effect on the germ-like allele is permanent and continues into multiple generations (46). In a similar study, Waterland et al. show that methyl donor supplementation of female mice before and during pregnancy increases methylation of the gene *Axin Fused* which determined whether offspring had permanently kinked tails (47). Additionally, Lylicrop et al. reported that feeding pregnant rats a protein restricted diet induces hypomethylation at specific CpG dinucleotides of the peroxisomalproliferator-activated receptor alpha (PPAR α) promoter in livers of the offspring (48). A recent study examining the effect of pre-natal maternal methyl donor supplementation showed that this approach can increase the methylation of runt-related transcription factor 3 (Runx3), a gene that plays an important role in reactive airway disease, and can modify the heritable risk of asthma through at least two generations (49). Interestingly, Heijmans et al. recently reported the first evidence that early-life environmental exposure can cause epigenetic changes in humans that persist throughout life (50). Comparing individuals exposed to famine prenatally during the Dutch Hunger Winter in 1944-45 to same-sex siblings and unrelated individuals born before or conceived after the famine period, they showed that periconceptional exposure to famine is associated with lower methylation of the IGF2 gene six decades later.

It is worth noting that many companies are marketing over-the-counter nutritional supplements that purport to slow down age-associated DNA hypomethylation. Because of the close link between the DNA methylation, methionine and folic acid cycles, these nutritional supplements typically include folic acid, vitamin B6, Zinc, choline, and S-adenosylmethionine (SAM-e), a coenzyme involved in methyl group transfers. However, there is very little human data to support the contention that such approach has any beneficial effect on the immune system.

Cross-talk between Epigenetics, Aging and Late-life Diseases

Growing evidence supports epigenetic dysregulation as a potential explanation for the higher incidence of autoimmune and neoplastic diseases associated with increasing age. Epigenetic mechanisms linking aging to cancer include hypermethylation of the promoter of tumor suppressor genes such as RB1, p16, and Wnt associated factors, aberrant Dnmt activity, loss of genomic imprinting, and chromosomal translocations in hypomethylated DNA sequences (41, 51, 52). Hypomethylation of proto-oncogenes has also been reported in leukemia and colorectal cancer as well as in liver tumors (53). The fact that these epigenetic changes often occur early during tumor formation suggest that they have a pathologic role and are not simply a consequence of tumor development. Additionally, several groups have reported

that microRNAs (miRNAs), a new class of small non-protein-coding RNAs that negatively affect gene expression at the post-transcriptional level, are regulated by epigenetic mechanisms, exhibit changes in expression during aging, and have an important role in cancer and metastasis (54, 55). New evidence indicates a role of miRNA in aging and growth control. For example, reducing the activity of *C. elegans* lineage 4 (*lin-4*) miRNA shortens lifespan, whereas overexpression of *lin-4* extends lifespan (55, 56). Interestingly, two groups reported that the expression pattern of miR-155 and miR-146 miRNAs is altered in synovial tissue in RA, suggesting a potential role of miRNA in the pathogenesis of other disorders acquired with age (57, 58).

The role of epigenetics in the pathogenesis of individual autoimmune diseases is discussed in separate articles and will not be reviewed here. However, it should be noted that epigenetic modifications may also provide a mechanistic link between immunosenescence and autoimmunity. In this context, we and others have investigated the role of lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) in the development of autoimmunity and aging. LFA-1 is an integrin adhesion molecule involved in T cell activation, whose expression increases progressively throughout life (59). Interestingly, LFA-1 is overexpressed in T cells from patients with systemic lupus erythematosus (SLE), with the extent of the overexpression directly relating to disease activity and responsible for T cell autoreactivity in vitro, and a lupus-like disease in vivo (60-62). Additionally, inhibiting T cell DNA methylation with the DNA methyltransferase inhibitor 5-azacytidine increases LFA-1 overexpression. Furthermore, Kevil et al. reported that in MRL/MpJ-*Fas*^{Lpr} mice which develop a systemic autoimmune disease with similarities to SLE, loss of LFA-1 significantly protected mice from the development of murine lupus, as measured by attenuated autoantibody formation, and inhibited development of glomerulonephritis, and increased survival compared to control MRL/MpJ-*Fas*^{Lpr} mice (63). Dr Richardson's group demonstrated that regions flanking the promoter of the ITGAL gene, which encodes for the CD11a chain of LFA-1, demethylate during aging, providing evidence that age-dependent decreases in T cell DNA methylation may contribute to the changes in T cell function and gene expression that occur in aging (64). Since anti-DNA antibodies are induced by LFA-1 overexpression, these changes may contribute to the development of anti-nuclear antibodies with aging.

A myriad of evidence has emerged for a prominent role of epigenetic regulation of genes in immune responses and age-related differential expression, including cytokines such as interleukin-2 (IL-2) (65) and interferon- γ (IFN- γ) (66). Sacca et al. have shown that both the acetylation of H4 and the phosphoacetylation of H3 that take place following a proinflammatory signal result in the increased recruitment of NF- κ B, a key player in the induction of inflammatory responses, to the promoter of several cytokines and chemokines. Constitutive activation of NF- κ B has been observed in various tissues during aging and seems to be the culprit of inflamm-aging (67). One of the major NF- κ B sensitive genes is IL-6, whose level increases with age as previously mentioned. Interestingly, IL-6 has been shown to regulate the promoter activity of DNMT-1 (68), as well as the enhancer of zeste homolog 2 (ezh2), a histone methyl transferase (69). Indeed, the number of genes that have been shown to be regulated by epigenetic mechanisms has expanded exponentially with the availability of microarray genome scanning technology (70).

We recently investigated the effect of DNA methylation in the development of autoimmunity in aging, by examining the effect of Dnmt1 deficiency on the development of autoimmunity in aging mice (71, 72). While homozygous Dnmt1 knockout is embryonically lethal, young heterozygous animals have globally hypomethylated DNA and are phenotypically normal (73). When allowed to age, the heterozygous animals have lower titer of autoantibodies and less age-dependent lymphocytic infiltration in the liver and salivary

glands than their genetically intact siblings. Interestingly, 18 months old Dnmt1 deficient mice showed less signs of T cell immune senescence, and maintained their T cell immune function better than the control animals. T cells from the old Dnmt deficient mice showed slower development of memory T cells, higher IL-2 and better T cell proliferation response. However, despite these differences, the longevity between the two groups was the same. Surprisingly, the Dnmt1 deficient mice also developed jejunal apolipoprotein AII amyloidosis in aging, reminiscent of the spontaneous development of age-associated amyloidosis that has been described in a mouse model of accelerated senescence (74). While both the Dnmt1 heterozygous knockout and the control mice have decreased Dnmt1 expression with aging, the expression of the *de novo* methyltransferases Dnmt3a and 3b increased with aging in T cells from control animals. Furthermore, the aged Dnmt1 deficient mice have increased expression of the methylcytosine binding protein MeCP2, thus providing a potential mechanism explaining the sustained DNA methylation level in these mice.

Epigenetics: a novel therapeutic intervention?

One of the fundamental and clinically relevant aspects of epigenetic alterations in diseases is the possibility of reversion by using various enzymatic inhibitors. Experimental evidence unequivocally shows that treatment with Class I and Class II histone deacetylase (HDAC) inhibitors extends lifespan of several organisms ((75, 76)). However, scant data is currently available on the mechanism involved in this process. Kypreou et al. (77) reported that treatment of peripheral blood phytohemagglutinin-stimulated lymphocytes from donors of different age groups (young, mid-aged, senior and elderly) with trichostatin A (TSA), an HDAC inhibitor, resulted in hyperacetylation of histone H4 with increasing donor age and subsequent induction of H1 linker histone variant H1^o protein synthesis. Interestingly, upregulation of H1^o expression has been observed in aged post-mitotic senescent fibroblast cultures (78). Additionally, Happel et al. recently published that dephosphorylation of two H1 somatic subtypes and an increase in heterochromatin formation occurred as a function of in vivo aging of human peripheral blood lymphocytes (79). A recent study indicated that in TSA-treated mice, the in vivo production and suppressive function of Foxp3⁺ CD4⁺ CD25⁺ regulatory T cells (Tregs) were increased (80). The mechanism of TSA action involves increased acetylation of both histones and Foxp3 protein. However, the contribution of Tregs to immunosenescence remain unclear as controversial reports are available on the correlation between circulating CD4⁺ CD25⁺ Treg prevalence and age, as well as the association of aging with loss of Treg suppressive function ((81, 82), and reviewed in (83)).

In recent years, mounting interest has focused on the NAD⁺ dependent unique Class III HDACs, the sirtuin family. In yeast, Sir2 has been shown to mediate the effects of caloric restriction on the extension of life span and high levels of Sir2 activity promote longevity (84). An aging-like phenotype and shortened lifespan have been observed in SIRT6-deficient mice (85). Michishita et al. reported that knockdown of SIRT6, a histone H3 lysine 9 deacetylase, leads to telomere dysfunction and premature senescence of human fibroblasts (86). Interestingly, Kawahara et al. recently showed that SIRT6 repressed NF- κ B transcriptional activity and that the target genes involved overlap with those associated with human aging (87). Given the fact that blockade of NF- κ B in the skin of aged mice can induce the reversal of many features of aging (88), the key role of NF- κ B in inflammation as a chemical modulator of sirtuin activity could emerge in novel drugs of therapeutic relevance to age-related inflammatory disorders. Of interest, young sirt1-null animals develop an autoimmune-like condition that resembles SLE and those that survive up to 2 years of age have a disease resembling diabetes insipidus (89). More studies will definitely be needed to understand the functional significance of all these observations in the general

immune dysfunction of the elderly and their higher predisposition to immune-mediated diseases.

Conclusions

In recent years, epigenetic changes in multiple organisms have been recognized as an important part of normal and pathological aging. Epigenetics, particularly DNA methylation and histone modifications, has emerged as a key biological process linking genetic and environmental factors in human diseases. An important characteristic of epigenetic traits is that while they are both stable and heritable, they are at the same time alterable by the environment. Drugs targeting epigenetic pathways are being introduced in cancer therapy. Inhibitors for Class I and Class II HDACs are under intensive study as transcriptionally based anticancer therapies, and they have just begun to be evaluated experimentally for inflammatory diseases. Many genes, diseases, and environmental substances are part of the epigenetics picture. Determining the contribution of the diverse elements that define and affect the aging phenotype to increase incidence of autoimmune disease in the elderly will help elucidate potential strategies for treating age-associated pathology.

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