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The role of immune system in pathogenesis of primary hypertension**

Udział mechanizmów odpornościowych w patogenezie pierwotnego nadciśnienia tętniczego

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S u m m a r y

Most of human studies on immunity in hypertension have been performed in adults so their pathogenic significance still remains obscure because of the numerous confounders such as smoking, drugs use, metabolic disorders as well as generally high inflammatory background created by overall excess of the effector/memory lymphocyte populations over the naïve ones. PH children are regarded as the best clinical model of development of cardiovascular disease not influenced by other factors. Both blood pressure elevation and vascular inflammation in the PH children were associated with a systemic low-grade inflammation that correlated with: a) the changes in the metabolism of adipose tissue: increase in leptin and decline in adiponectin serum levels, b) altered distribution of fat tissue with relative increase of visceral fat over subcutaneous fat, c) serum elevation of oxidative stress components and certain pro-inflammatory cytokines. The PH children were also characterized by: a) accelerated biological maturation expressed as the difference between bone age and chronological age, b) changes in expression profile of genes of renin-angiotensin system and metalloproteinases as well as leukocyte surface markers such as AdipoR1 receptors, c) elevation of T-reg cell numbers and increase in transition of T-reg into Th17 cells. Altogether, these results strongly suggest that PH in both children and adults is strongly associated with immune cells activation but the origin of the stimulating agents still remain unknown.

S t r e s z c z e n i e

Większość badań układu odpornościowego u ludzi z pierwotnym nadciśnieniem tętniczym wykonano u osób dorosłych, stąd ich znaczenie patogenetyczne jest wątpliwe z powodu licznych obciążeń, niezwiązanych bezpośrednio z chorobą pierwotną, takich jak stosowanie używek, leków, obecność zaburzeń metabolicznych i innych uwarunkowań, powodujących zwiększoną aktywację mechanizmów odpornościowych, manifestujących się przewagą limfocytów o fenotypie komórek efektorowych i pamięci immunologicznej (profil prozapalny), a mniejszą populacją limfocytów dziewiczych (profil niezapalny).

Dziecięca postać PN (nadciśnienie pierwotne) stanowi najbardziej przydatny model badawczy chorób sercowo-naczyniowych z powodu nieznacznego tylko obciążonia innymi czynnikami współistniejącymi. Zwiększenie ciśnienia tętniczego oraz zapalenie naczyń u dzieci z PN związane są z obecnością wykładników chronicznego, podostrego zapalenia systemowego, którego aktywność korelowała: a) ze zmianami aktywności metabolicznej tkanki tłuszczowej manifestującymi się wzrostem poziomu leptyny i spadkiem adiponektyny, b) ze zmianami w zakresie dystrybucji tkanki tłuszczowej, c) ze wzrostem stężenia surowiczych komponent stresu oksydacyjnego oraz niektórych cytokin prozapalnych.

Dzieci z PN charakteryzują się także: a) przyspieszeniem wieku biologicznego, wyrażonego jako różnica pomiędzy wiekiem kostnym a wiekiem chronologicznym, b) zaburzeniami w zakresie ekspresji genów systemu renina-angiotensyna oraz genów kontrolujących ekspresję receptorów dla adipokin i metaloproteinaz w leukocytach krwi obwodowej, c) zmianami w zakresie ekspresji szeregu receptorów komórkowych leukocytów takich jak AdipoR1, wzrostem populacji limfocytów o fenotypie komórek T-reg (regulatorowych) i ekspresją interleukiny 17.

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Wyniki te wskazują, że PN u dzieci i dorosłych jest silnie związane z wieloma parametrami aktywacji układu odpornościowego, lecz pochodzenie czynnikow stymulujących ciągle pozostaje niewyjaśnione.

INTRODUCTION

The role of immune mechanisms in pathogenesis of hypertension was suggested by several early findings including: a) a functional thymus requirement for hypertension development, b) a presence of markers of systemic inflammation as well as agonistic antibodies against angiotensin II receptors and adrenergic receptors in the serum of hypertensive patients, c) a correction, amelioration or prevention of experimental hypertension by suppression of T cell driven inflammation in the target organs.

At present, primary hypertension (PH) both in humans and animal models is considered as a condition of low-grade, chronic, systemic inflammation often associated with metabolic syndrome components (dyslipidemia, insulin resistance, obesity, etc.), and acute phase response manifested by elevation of cytokines (IL-6, TNF-alfa) and C-reactive protein serum levels (1-4). Blood pressure elevation in the course of hypertension depends on innate and adaptive immune immune responses that result in inflammation in the kidney, arteries and central nervous system.

INNATE IMMUNITY IN HYPERTENSION

The innate immune system is responsible for fast and non-specific immediate inflammatory response recruited to eliminate infection or in response to tissue injury. The system consists of cells (granulocytes, monocytes, macrophages, dendritic cells, mast cells, NK lymphocytes) and many soluble factors (interferons, acute phase proteins, cytokines, chemokines, defensins, and complement fragments).

The cells of innate immune system express pattern recognition receptors (PRRs) which recognize pathogen associated molecular patterns (PAMPs) that are expressed and shared by large groups of pathogens or damage associated molecular patterns (DAMPs) that represent structures of damaged cells.

Dendritic cells (DCs) infiltrate the kidney and arterial walls in many hypertension models. Their numbers increase in perivascular tissue and adventitia in large vessels (aorta) and in medium sized vessels (mesenteric arteries) together with other immune cells including CD4+ and CD8+ T cells and macrophages. In the kidney, immune cells are located preferentially around renal arteries. The reason why DCs and other immune cells accumulate in peri-vascular space are unknown but possibly sympathetic nerve endings present in these areas play some role in this phenomenon (5). DCs are the most efficient antigen-presenting cells and promote a differentiation of T cells towards many different functional phenotypes (6). The number of activated DCs in perivascular space increased in angiotensin II and DOCA-salt hypertension. They expressed high levels of several co-stimulatory molecules engaged in antigen presentation to T cells (7).

Co-stimulators are the molecules that are engaged in providing the second signals necessary for optimal T cells activation following their stimulation by the first signals (the ones generated by recognition of antigens presented to T cells by DCs, macrophages and B cells which act as antigen-presenting cells – APCs). One of the best characterized co-stimulatory pathway is the CD28/B7 in which CD28 represents a surface protein constitutively expressed by over 90% of mature CD4+ T cells and about 50-70% of CD8+ T cells. The CD28 molecule interacts with its B7 ligands (CD80 and CD86) expressed by activated and/or resting APCs, respectively). Pharmacological inhibition of CD28/CD80 pathway, lack of this pathway in CD80/CD86 knock-out mice or engrafting wild type bone marrow in CD80/CD86 deficient mice restored the hypertensive response to angiotensin II (7).

It has recently been found that DCs played a causal role in hypertension development by increased formation of ROS and oxidative modifications of proteins by highly reactive gamma-ketoaldehydes (isoketals). DCs from angiotensin II infused mice produced increased amounts of O_2^-, accumulated isoketal protein adducts, and released cytokines such as IL-6, IL-1 beta and IL-23 and had elevated expression of costimulators such as CD80, CD86. They promoted T cell proliferation, especially CD8+, and polarized T cells to an inflammatory phenotype involving production of IL-17, IFN-gamma and TNF-alfa. DCs from hypertensive mice primed a hypertensive response in recipient mice to low-dose of angiotensin II.

Scavenging isoketals prevented these parameters of DCs activation and ameliorated hypertension. Exposure of DCs to the prooxidant agent tert-butyl hydroperoxide (t-BHP) promoted their ability to suport CD8+ T cell proliferation and hypertension, thus mimicking the effect of angiotensin II *in vivo*. Likewise, DCs pulsed with isoketal-modified proteins from renal homogenates potently stimulated T cell proliferation, while DCs pulsed with other oxidized lipid products did not. The authors also noted that plasma F-2 izoprostanes which are formed in concert with isoketals are elevated in humans with treated hypertension and very markedly elevated in patients with resistant hypertension. Isoketal-modified proteins were also elevated in circulating monocytes and DCs from human with hypertension and that these increased with severity of hypertension. The authors conclude that hypertensive stimuli activates DCs by promoting the formation of isoketals and suggest that reducing isoketals has potential as a treatment strategy for this disease (8).

These observations directly suport the earlier assumption that vascular inflammation that results in hypertension is initiated by neo-antigens formation in the target tissues such as vasculature and kidney. These antigens are subsequently recognized by macrophages and dendritic cells via PRRs. Antigen presen-

tation to responder T cells (in draining lymph nodes) results in their further activation, proliferation and differentiation into numerous types of different effectors, responsible for vascular inflammation (9).

This scheme implies the role of both innate (macrophages, dendritic cells) and adaptive immunity (T cells) in hypertension development. These data also imply the development of memory adaptive immune responses to vascular neoantigens (autantigens) that develop in the onset of the disease that support the idea of autoimmune reactivities against certain vascular antigens, e.g. HSP-70 proteins (10, 11).

MACROPHAGES AND MONOCYTES

Macrophages like DCs infiltrate the kidney and periadventitial areas of the peripheral vessels (aorta and medium sized arteries). Hypertension induced infiltration of monocytes/macrophages into the vessel wall, brain, heart and kidney represents the main component of vascular inflammation and inflammation-induced organ damage in many experimental models including polygenic, monogenic, renovascular and mineralocorticoid hypertension. Macrophage infiltration in the kidney appears to be most prominent in forms of hypertension associated with an activated renin-angiotensin-aldosteron system.

On the contrary, a reduction in macrophage infiltration is associated with improvement of hypertension in several animal models including spontaneously hypertensive rats (SHRs) (12), Dahl salt-sensitive rats (13), hypertension induced by angiotensin II (14) and aldosteron (15), salt-dependent hypertension (16) and autoimmune hypertensive renal disease (17).

Elimination of circulating monocytes in mouse model by action of diphtheria toxin (18), deficiency in macrophage population in osteopetrotic mice with mutation in colony – stimulating factor gene (19) and treatment of mice with the CCR2 or MCP-1 antagonists that blocks chemokine receptors (CCR-2) or chemokine itself (MCP-1), protected mice from angiotensin II or DOCA induced hypertension (20, 21).

Macrophages are also main targets for adrenergic and cholinergic regulations of the immune reactions in course of hypertension. The spleen macrophages of spontaneously hypertensive rats (SHRs) primed with nicotine (cholinergic, anti-inflammatory signal) or angiotensin II (pro-inflammatory signal) remain highly reactive in response to pro-inflammatory activities induced by second stimulation via certain TLRs (TLR-7,8) as measured by IL-6, and TNF-alfa production. These effects are observed only in the hypertensive rats but not in the control, normotensive rats, and appeared before the onset of hypertension indicating on impaired response to cholinergic signals in the pre-hypertension stage (22).

There is still unclear if macrophage infiltration into vessel walls or target organs represents “primary” or “secondary” alterations in the immune system. So far unidentified “primary” changes of the immune system may result in subsequent inflammatory response in the vessel wall and/or the kidney vasculature leading then to blood pressure elevation. There are some data suggesting that certain immune defects may preceed hypertension development in SHRs (23) but this remains difficult to confirm in human.

“Secondary” leukocyte alterations induced either by hypertension per se (via mechanical stress) and/or by factors that cause hypertension (e.g. angiotensin II) may also lead to the infiltration and/or activation of leukocytes, particularly macrophages, in target tissues where these cells contribute to the development of organ injury by realeasing pro-inflammatory agents including soluble mediators (cytokines, chemokines, oxygen radicals, etc.) and acting as cytotoxic cells for many vascular targets or other toxic mediators (24, 25).

From the practical reasons, the pathological diagnosis of hypertensive kidney disease is difficult to study in patients with hypertension. Macrophage analysis in atherectomy speciments is often confounded by the presence of atherosclerosis. Kidney biopsies may not be representative if the infiltration is as focal as it is in experimental hypertension (26). Diagnosis is often doubted by pathologists and a marked macrophage infiltration may even suggest the presence of other primary immunological kidney disease. A true “control” tissue without hypertension or atherosclerosis is hard to obtain. Therefore studies in human patients have been performed with the use of peripheral blood monocytes. There is a lot of data concerning this issue. In general, they indicated that hypertension was associated with the presence of activated monocytes in periphery, which showed: a) decreased sensitivity to blocking action of glucorticosteroids *in vitro* (27), b) increased adherence to endothelium *in vitro* and elevated production of IL-1 and TNF-alfa (28), TGF-beta (29), MCP-1 as well as TF (30) upon stimulation, c) elevated expression of some surface receptors related to endothelial adhesion and transmigration, CD11b, CD11c, ICAM-1, CCR2 and CCR-5 (31), d) increased expression of TLR-4 but not TLR-2 (32), e) increase in ROS production (33), f) increased expression of transient receptor potential canonical type 3 channels (TRPC3) that was associated with increased monocyte migration (34), g) up-regulation of bradykinine receptors 1 and 2 (35).

In addition, monocytes have some phenotype and functional characteristics which are strictly related to their role in blood pressure regulation. They inlude: a) expression of AT1 receptor for angiotensin II (36); it enables monocyte activation by this peptide that results in high production of several pro-inflammatory cytokines and has an influence on several monocyte functions related to their endothelial transmigration and differentiation to tissue macrophages, b) expression of renin angiotensinogen, and aldosteron (37), c) expression of adrenergic and cholinergic receptors which enable monocyte interaction with neurotransmitters (acetylocholina, noradrenaline, dopamine) that are engaged in blood pressure regulation (38) as well as abilities for their production (39) d) expression of leptin, and adiponectin receptors that enable monocytes to interact with these adipokines (40, 41).

The latter point is especially interesting. Fat mass is linked mechanistically to the cardiovascular system through adipokines action, including leptin. Leptin increases blood pressure via hypothalmic-sympathetic pathways and also stimulates many pro-inflammatory activities such as monocyte migration through extracellular matrix, up-regulation of monocyte scavenger receptor expression, increased uptake of oxidized low-density lipoprotein (LDL), cytokines secretion, adhesion to endothelium and other immune functions that contribute to vascular inflammation.

Many of these functions depend on pro-inflammatory monocytes with high expression of CD16 receptor. It has been found that leptin promoted the development of these cells (40). Circulating numbers of $CD14^{++}/CD16^{++}$ monocytes which are primary producers of TNF-alfa were positively related to plasma leptin concentrations with a stronger correlation in man. *In vitro*, recombinat human leptin induced CD16 expression in a dose related manner, with a stronger influence on monocytes from man but without any sex related differences in total leptin receptor expression, relative expression of long vs short receptor's isoforms, or soluble leptin receptor concentration in plasma. The number of circulating $CD14^{++}/CD16^{++}$ monocytes was positively correlated with systolic blood pressure and intima-media thickness, and negatively related to carotid compliance. These observations suggest that leptin promotes the development of CD16 positive monocyte population in a sex-specific manner and that these subpopulations are associated with diminished vascular function (40). These data are in agrement with our earlier studies that indicated on positive correlations between circulating leptin concentrations, blood pressure and vascular inflammation parameters in children with PH (41).

It shoud be pointed out that all these data have been obtained in adults. Our studies on PH children showed increased levels of serum leptin concentrations that correlated with certain indices of vascular inflammation and blood pressure elevation (42, 43). However, the PH children did not show any significant changes in the peripheral distribution of monocyte subsets characterized according to their surface receptors expressing profiles, including CD14/CD16 or other monocyte markers such as leukocyte integrins, Toll receptors, complement receptors, HLA-DR and CD54 (ICAM-1) (data not shown). We only found slightly increased CD14 mRNA levels in leukocytes of PH children but without any correlations with vascular inflammation and organ damage indices (43). These observations indicate that changes in monocyte subsets distribution may appear relatively late in the course of hypertension and probably represent so called "secondary alterations" of immune system possibly related to action of some inflammatory factors produced in the course of the disease.

Another adipokine which play a role in hypertension is adiponectin. In contrast to leptin, its serum levels correlates negatively with fat mass. In general, adiponectin exerts several anti-inflammatory effects. We found that serum adiponectin levels in the PH children were inversly related to the levels of systemic inflammatory markers, oxidative stress components, target organ damage, and fat tissue distribution profile (visceral/vs peripheral fat) (41-43).

The adiponectin exerts its action via interaction with 2 cellular receptors (AdipoRs 1 and AdipoRs 2). With regard to the immune system, the highest AdipoRs expression have been observed in the cells of innate immune system (monocytes neutrophils, and dendritic cells), with a relatively low expression in lymphocytes (44). We found that PBLs of PH children showed either high (AdipoRs(+) patients) or low AdipoR expression (AdipoR(−) patients) profiles, which were strictly related to different disease severity and TOD. Only AdipoR(+) patients presented with increased cIMT and had severe ambulatory hypertension, alongside low serum adiponectin concentrations. Strikingly, neutrophil (but not monocyte) AdipoRs protein expression levels were negatively related to serum adiponectin concentrations but positively related to the severity of hypertension. These changes occurred independently of other studied systemic factors, including biochemical, anthropometric, and immune parameters (45).

In contrast to neutrophil, monocyte AdipoRs expression levels was independent of serum adiponectin concentrations but showed negative correlations with some serum immune mediators (sCD14, TNF-alpha), anthropometric parameters, and LMVI.

These data indicate that neutrophil AdipoRs up-regulation in the PH children was associated with early stages of vascular injury and possibly may represent leukocyte defect that appear in conditions of low adiponectin levels (45).

These findings extended previous reports suggesting the role of neutrophils in the pathogenesis of PH. Adult PH patients exhibited an increased neutrophil count (46) and activated neutrophil function, including up-regulation of several receptors expression and rapid release of neutrophil primary granules on short incubation with PBS or TPA (47). Neutrophils normally show AdipoR expression levels that are equal to those of monocytes,as found also in our present study, and both of them react with adiponectin. Adiponectin modulates both neutrophil phenotype and functions, but its significance in the PH development still remains unknown.

T CELLS ENGAGEMENT IN HYPERTENSION AND ROLE OF THE CENTRAL NERVOUS SYSTEM

Immune reactions which initiate hypertension represent T cell driven vascular inflammation: a) mice lacking recombinase-activating gene 1 (Rag1-/- mice) and therefore deprived of T and B cells did not exhibit elevated vascular superoxide production and did not develop hypertension in response to several stimuli including Ang II and DOCA; adoptive transfer of T cells but not B cells restored the ability for hypertensive responsiveness, b) wild type mice treated with angiotensin II had elevated levels of circulating memory/effector T cells expressing CCR5 and CD44 markers, c) severe combined immunodeficiency mice did not develop hypertension and had reduced albuminuria

and renal damage, d) Dahl salt-sensitive rat with Rag-1 gene deletion did not develop hypertension, albuminuria and renal damage (48).

T cells action is enhanced by sympathetic drive which originate from central nervous system that responds to a variety of signals from periphery. T cell response in this system proved to be independent of the experimental model of hypertension. T cell mediated responses occur in mice treated with angiotensin II, DOCA-salt, or nor-epinephrine and in the salt sensitive and genetic rats models (49).

Lack of ATII receptors on peripheral T cells did not protect mice from Ang II-induced hypertension and kidney damage. It suggests that T cells response in hypertension is not induced by direct angiotensin II action but strongly depends on the central signals which sensitize the T cells to the action of different stimuli.

Animal studies indicate that inflammatory responses in the course of hypertension are strictly controlled by central nervous system. Brain inducing signals involve: a) systemic action of circulating agents (angiotensin II, salt, cytokines, etc.) that activate circumventricular organs of the forebrain, hypothalamus, and brainstem centers, resulting in integration of baroreceptors input and sympathetic outflow to the kidney and splanchnic circulation, b) the action of so called "inflammatory reflex" in which local tissue stimulants so called "danger signals" (cytokines, prostaglandins, in vessels, kidney, etc.) activate vagus nerve to send afferent signals to the brain resulting in an increase in sympathetic outflow.

Resulting sympathetic outflow (efferent signals) increases norepinephrine (NE) release in the secondary lymphoid organs (spleen, lymph nodes) that in turn acts on a subset of CD4 memory T cells. These cells express beta adrenergic receptors (betaAR), and are located adjacent to adrenergic nerve endings in the spleen and lymph nodes. Interaction of NE with betaARs induces production of parasympathetic mediator, acetylocholine (Ach). Ach interacts with macrophages and dendritic cells via alfa-7 nicotinic receptors providing inhibitory signals that suppress inflammatory response. This scheme provides balance between anti- and pro-inflammatory immune responses and represents an important mechanism of cooperation between autonomic nervous system and immune system (49, 50).

Autonomic dysfunction with an increase in sympathetic and decrease in parasympathetic activities is often related to higher mortality in cardiovascular diseases and is associated with the development of hypertension. Clinical observations also indicate that hypertensive children present altered sympathetic activity what suggests that changes in the sympathetic signaling occurs early in the onset of human hypertension (51, 52).

T CELLS SUBSETS AND MEDIATORS ENGAGED IN HYPERTENSION

The most important subset of effector T cells engaged in inflammatory reaction and elevation of blood pressure is Th17 T cell subset. Th17 produce IL-17 and are engaged in many autoimmune diseases including rheumatoid arthritis, inflammatory bowel disease, psoriasis, sclerosis multiplex, SLE, etc. IL-17 exerts its effect by stimulation of production of chemokines and adhesion molecules in tissue. This leads to accumulation of other inflammatory cells in the vascular wall (kidney, peripheral vessel wall), promotes reactive oxygen species production in the vascular smooth muscle cells and decreases nitric oxide synthesis by endothelial cells. It causes vasoconstriction, sodium retention and hypertension (53). Hypertensive adult patients with acutely increased blood pressure had elevated numbers of IL-23 and IL-17 expressing CD4+ T cells and increased serum levels of these cytokines (54). These data seem to confirm the role of IL-23 – IL-17 axis in hypertension development what was previously observed in animals models. We also noted increased population of CD4 T cells bearing IL-17 in hypertensive children (42, 43, and unpublished data). The number of Th17 cells was greater in those patients whose leukocytes did not express adiponectin type 1 receptors (AdipoR1) (unpublished results). This indicates that decreased AdipoR1 expression may limit leukocyte responsiveness to adiponectin. This in turn may increase synthesis of pro-inflammatory IL-17 by T cells. Signals which lead to differentiation of naive CD4+ T cells into proinflammatory Th17 cells require IL-6 and TGF-beta.

Inflammation is controlled by T-regulatory cells (T-regs) which limit the extent of the response and prevents the tissue damage (in this case arteriolar wall causing hypertension). T-regs can stop inflammation by acting on T cells, B cells, macrophages, dendritic cells and NK lymphocytes. In the mouse model of angiotensin or aldosteron II-induced hypertension, adoptive transfer of T-regs limited hypertension, reduced inflammatory mediators and immune cells in kidney, decreased generation of superoxide and immune cell infiltration in vascular and perivascular tissue as well as reduced small artery stiffness (55). Furthermore, adoptive transfer of T-regs to angiotensin II infused or aortic constriction mice decreased cardiac damage, prevented cardiac fibrosis, and improved electric remodelling but with no blood pressure lowering (56, 57).

The mechanisms of T-regs action that result in blood pressure regulation include: a) suppression of innate and adaptive immune responses by a variety of mechanisms (cytotoxicity by cell to cell contacts, soluble mediators action, etc.), b) regulation of endothelium-dependent relaxation responses through the action of IL-10 and other cytokines (58).

Significance of T-regs in immune regulation in hypertension and other cardiovascular diseases is still obsure, especially in humans. They are generated during immune responses against mosty unknown antigens that result in low-grade inflammation in hypertension and other cardiovascular diseases (59). We found that CD4 T cells from children with PH had increased T-regs population in comparison with normotensive, age and BMI matched children (43, and unpublished).

There is strictly controlled relation between T-regs and Th17 cells. In the presence of TGF-beta but of low

IL-6 concentrations, naive T cells preferentially polarize into T-regs, and Th17 cells may transit into T-regs. However, when cytokines milieu contains mainly pro-inflammatory cytokines such as IL-6 and low TGF-beta concentrations, then T cells preferentially polarize into Th17, and T-regs may lose FOXP-3 expression and transit into Th17 cells. It suggests that type of inflammatory reactions generated during initial phases of responses to vascular antigens may decide about the immune response profile (60). Accordingly, IL-6 accumulates in the kidney, especially the glomeruli, of patients with chronic kidney disease and hypertension, to a greater extent than in normotensive patients with CKD (61).

IMMUNE SENESCENCE AND HYPERTENSION

Immunosenescence is a proces in which cells of immune system gradually loose their ability for replacement of dying cells (apoptosis) by new ones. This process is caused by decrease in telomer length in aged cells. There is an inverse relationship between telomer length and cellular aging; short telomeres force their cells to enter senescence. Human telomers contain guanine-reach repetitive sequences which are gradually lost in each mitotic division because the DNA polymerase is unable to replicate linear chromosomes in a process known as telomere erosion. This process functions as a mitotic clock for which the length of the telomers represents the number of cell divisions sustained by the cells (62).

In contrast to somatic cells, lymphocytes have a robust capability to proliferate that enables these cells to undergo clonal expansion following antigen recognition. Proliferating lymphocytes overexpress telomerase that ensures no significant telomere shortening during each division. Peripheral resting T cells (CD4+ and CD8+) as well as naive T cells have no active telomerase; it is activated following lymphocyte stimulation. Successive lymphocyte stimulations result in decrease in telomerase activity that restricts the number of subsequent cell divisions. The rate of telomere shortening is different for T CD4+ and T CD8+ T cells, being higher for CD4 (33 bp/year) than for CD8+ T cells (26 bp/year). Aged CD4 and CD8 T cells are low IL-2 producers so they poorly polarize into TH1/Th2 phenotype but retain their ability for Th17 differentiation (63).

Age-related thymus involution results in decreased generation of naive T cells in the thymus as well as in limited export of these cells into periphery. The process of thymus dependent T cell generation is called central T cell renewal which is especially high in infants and newborns and gradually decline with age, being replaced by peripheral renewal that takes place in peripheral lymphoid tissue and prevails in advancing age.

Central T cells renewal in children with limited thymus involution results in production of numerous population (about 90%) of “naive” T cells bearing CD45RA isoform of CD45 antigen (CD45Ag) and only small (about 10%) population of T cells expressing CD45RO isoform (“memory” T cells). These cells have a highly proliferative potential (have long telomers), represent a diverse specificity repertoir (are able to recognize virtually unlimited array of foreign antigens) and in general, give rise to rather protective immunity with no prolonged pro-inflammatory reactions (including autoimmunity phenomena).

In contrast, peripheral lymphocyte renewal generates T cell population with relatively lower numbers of “naive” T cells and increased “memory” T cell subset; these cells have rather low proliferative potential (have short telomers), limited specificity repertoir (recognize limited numbers of foreign specificities), and are engaged in less protective but more pro-inflammatory reactions, often with autoimmune background (63).

The aged T cells express certain functional and phenotype characteristics: 1) they produce low amount of IL-2 but increased amount of other pro-inflammatory cytokines (IFN-gamma, TNF-alfa, IL-6, etc.) following stimulation via their TCR receptor, 2) they poorly polarize towards Th1/Th2 but retain ability to differentiate to IL-17 producing cells which in turn may favour development of an inflammatory and autoimmune phenotype, 3) they showed increased abilities to differentiate towards T-regulatory cells bearing CD4/CD25/Foxp3 phenotype with retaining and gaining their functions, low IL-10 production and contributing to IL-17 bias (by production of IL-17, IL-21 and IL-22). In other words, these T-regs produce less IL-10 and part of them express IL-17, 4) they loss the CD28 surface marker especially in CD8 T cell subset and gain some other markers related to enhancement of cytotoxicity and inflammation (64, 65).

Adult hypertensive patients present increased numbers of peripheral CD8+ T cells bearing replicative senescence markers inluding loss of CD28 molecule and expression of CD57 protein together with enhanced production of inflammatory molecules including perforin, granzyme B interferon gamma, and TNF-alfa but with no increase in IL-17 expression (66). T cell immunosenescence is also associated with arterial stiffness; frequency of CD57 cells in the CD8 T cell subset independently correlates with the pulse wave velocity even after adjusting for age, sex, acute phase protein response and other confounders (67).

CD28 is an important costimulatory receptor that interacts with CD80 or CD86 expressed on monocytes, dendritic cells, macrophages providing second signal for T cell activation, that is necessary for their clonal expansion and differentiation into effectors. Loss of CD28 and gain of CD57 indicates on repeated antigenic stimulation. With each round of T cell division CD28 expression is progressively and irreversibly down regulated, that results in accumulation of CD28- T cells which express shortened telomers, suggesting their reduced replicative lifespan. This phenomenon occurs only in humans and nonhuman primates but not in mice. So this system underlines its importance in human studies. Loss of CD28 in CD8 T cells suggests their differentiation towards T cell effectors with

high expression of adhesion molecules and cytolytic molecules that may participate in target organ damage during response to vascular antigens.

The concept that repeated antigenic stimulation (possibly certain vascular neoantigens) may increase CD28- T cell pool is interesting (68). However, it still remains open the basic issue: which proposed mechanisms are secondary and which initiate immune response to vascular antigens and what conditions are necessary to break tolerance to these stimuli.

The studies described above were performed in adult patients with long-lasting hypertension. The known confounders (infections, non-infectious stimuli, obesity, etc.) may all induce T cell activation that results in increase of CD28- T cell pool which is elevated in many other inflammatory conditions of infectious and non-infectious nature, so precribing the results to hypertension is rather dubtless.

Our results indicate on subtle enhancement of CD45RO (characteristic for memory cells) isoforms expression in CD4 and CD8 T cells in hypeternsive children. Increase in their expression correlated with some indices of target organ damage. But again, this response may reflect just certain component of systemic immunity change in hypertension (42, and in preparation).

Some genetic factors associated with senescence certainly predispose to hypertension. With regard to this, it has recently been found that deficiency in aging suppressor gene *Klotho* (*KL*) caused salt-sensitive hypertension in mice via action of monocyte chemotactic protein-1 (MCP-1) and CC-chemokine receptor 2 (CCR-2) mediated inflammation. In mice, the *KL* gene extends the lifespan when overexpressed, and shortens it when disrupted.

Systolic blood pressure (SBP) in the *KL* deficient mice began to increase at the age of 15 weeks, and remained elevated thereafter, whereas systolic BP in the WT mice remained stable. High salt intake further increased SBP in KL deficient mice but not in WT mice. Blockade of CCR-2 that is involved in monocyte chemotaxis, abolished this effect. Salt loading substantially increased the expression of MCP-1, and infiltrations of macrophages and T cells in kidneys of the *KL* deficient mice; this effect was abolished by CCR-2 antagonist. Treatment of *KL* deficient mice with CCR-2 antagonist attenuated the increased renal expression of serum glucocorticoid-regulated kinase 1, thazide-sensitive NACL co-transporter, ATP-syntase beta as well as repaired renal structural damage and functional impairment induced by HS loading.

These observations indicate that accelerated aging (here deficiency in *KL* gene) may cause salt-sensitive hypertension and renal damage by CCR-2 mediated inflammation (innate immunity component) that depends on increasing infiltration of immune cells into kidney and other organs (69).

CONCLUSIONS

Concluding, there is increasing amount of data indicating that immune system plays significant role in pathogenesis of primary hypertension. Recent results indicate that it is not simply activation of innate and adaptive immune response what may be secondary to hemodynamic insult, but rather phenomenon secondary to accelerated aging, or in other words accelerated maturation of immune system.

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