

# Immune system and hypertension

## Wpływ układu immunologicznego na rozwój nadciśnienia tętniczego

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### Abstract

Hypertension is a very common disorder. It is a major risk factor of myocardial infarction, heart failure, stroke and renal failure. It is well known that the immune system also contributes to this disease. Numerous investigations have demonstrated that lymphocytes are important participants in the development of hypertension and consequent end-organ damage. They produce different cytokines such as tumor necrosis factor  $\alpha$ , interferon  $\gamma$ , and interleukin 6 that lead to the development of hypertension. On the other hand, they are also the source of anti-inflammatory interleukin 10. A better knowledge of immunology would lead to the discovery of new therapeutic interventions and more successful treatment of our patients.

Key words: hypertension, immune system, lymphocytes

Folia Cardiologica 2019; 14, 4: 363–367

### Introduction

Hypertension is a very common cardiovascular disease that has a significant impact on public health. It affects around 30% of patients in Europe and the United States. In Poland, according to the RYZYKO programme, 36.8% of patients suffer from the disease. The prevalence of hypertension increases with age, and with our ageing society will pose an ever-increasing challenge [1].

Although the diagnostics and treatment of hypertension are still developing, its pathomechanism remains unclear. The role of the kidneys, the central nervous system and the vascular system is widely acknowledged. It is also well known that low-grade inflammation contributes to numerous cardiovascular diseases, including hypertension.

The concept of immune system contribution to hypertension was first set out in the 1960s, when investigators demonstrated that immunosuppression decreased hypertension in a model of renal infarction. Moreover, the

transfer of lymphocytes from rats with renal infarction induced hypertension in animals with previously normal blood pressure [2]. Later, it was found that hypertension was not observed in thymectomised mice or in athymic nude mice with renal infarction [3]. Twenty years later, Ba et al. [4] observed that the transplantation of a thymus from a Wistar-Kyoto rat to a spontaneously hypertensive (SHR) rat resulted in a blood pressure decrease in the SHR animal.

More than a decade ago, Guzik et al. [5] demonstrated that hypertension induced by angiotensin II (Ang II) or deoxycorticosterone acetate (DOCA-salt) is blunted in mice without recombina-activating gene 1 (*Rag1*) lacking functional lymphocytes. The recombina-activating genes 1 and 2 are responsible for recombination of the genetic sequences encoding immunoglobulins and the T-cell receptor. The authors suggested that either T- or B-cells mediate hypertension. *Rag1*<sup>-/-</sup> mice did not exhibit elevated vascular superoxide production and endothelial

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dysfunction. The hypertensive response can be restored by adoptive transfer of lymphocytes T- but not B-cells [6]. Also, T-cells were shown to accumulate in the perivascular adipose tissue of the aorta. To support the role of T-cells in the development of hypertension, Crowley et al. [7] showed that mice with severe immunodeficiency were protected against hypertension and exhibited reduced renal damage and albuminuria.

Blood pressure elevation is moderated by different factors. The influence of the immune system on hypertension is a complex interplay between immune cells, oxidative stress, Ang II, low-grade inflammatory state, and the central nervous system.

### Central nervous system

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Vasculature, kidneys and the central nervous system are involved in the regulation of blood pressure. It's worth highlighting that T-cells can represent a link between these tissues because lymphoid tissue is rich in sympathetic nerves [8].

The circumventricular organs (CVO) can be influenced by circulating hormones such as Ang II due to its rich vasculature and incomplete blood-brain barrier. What's more, the CVO, and especially the subfornical organ (SFO), play an important role in both sending and receiving central signals that regulate cardiovascular function and electrolyte balance [9]. By the deletion of extracellular superoxide dismutase (ecSOD) it became possible to create a model to determine the role of central oxidative stress in hypertension. The absence of ecSOD resulted in elevation of reactive oxygen species (ROS) levels in the CVO, and hypertension. In addition, when animals with extracellular SOD depletion in the CVO were given Ang II at doses that do not cause hypertension in normal mice, significant blood pressure elevation was observed. This was also accompanied by T-cell infiltration around the aorta. When the investigation with ecSOD depletion was repeated using smooth muscles, no hypertension and no T-cell response were observed [10–12].

When discussing the role of the central nervous system, it's worth mentioning that lesions in the anteroventral third cerebral ventricle, a region where the SFO is situated, can prevent Ang II-induced hypertension. And the described lesions protect against T-cell activation and aortic infiltration in reaction to Ang II infusion. This demonstrates that Ang II infusion-dependent T-cell activation is caused by central signals, rather than by the direct actions of Ang II on T-cells [13, 14].

On the other hand, peripheral mechanisms seem to contribute to T-cell activation and vascular inflammation. T-cells have been also shown to contribute to stress-induced blood pressure elevation [9].

During the progression of hypertension, immune cells accumulate in the kidneys and vasculature. These tissues

produce multiple cytokines that affect vascular and renal function. Blood pressure elevation results in a significant change in the expression of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), IFN- $\gamma$ , interleukin 6 (IL-6), interleukin 17 (IL-17) and interleukin 10 (IL-10) [6].

### Adipose tissue

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Obesity is one of the strongest risk factors of hypertension, and is associated with low-grade inflammation state, vascular remodelling and endothelial dysfunction.

Adipose tissue is a compound of numerous cells, including adipocytes, endothelial cells, fibroblasts, pre-adipocytes, stem cells and immune cells. Immune cells are a source of multiple anti-inflammatory cytokines, for example IL-10 and transforming growth factor  $\beta$  (TGF- $\beta$ ). They increase insulin sensitivity and protect from inflammation and adipose tissue dysfunction.

In hypertension, perivascular adipose tissue (pVAT) inflammation is involved in the pathogenesis of vascular dysfunction. Inflammation results in a loss of the protective properties of pVAT, a loss of endothelium-dependent vasodilatation, and an increase in vasoconstriction. Functional changes within adipose tissue are associated with a change in its paracrine and endocrine properties. Decreased release of protective factors such as adiponectin, nitric acid and prostaglandins, and increased pathological adipokine release (resistin and visfatin), can be observed. Increased production of chemokines such as IP-10 (CXCL10) or RANTES results in the activation of T cells and macrophages. Hypertension is associated with immune cells infiltration which mediates endothelial dysfunction. Infiltrating immune cells release cytokines such as interleukin 17A (IL-17A), IFN- $\gamma$ , TNF- $\alpha$ , and IL-6 which modulate smooth muscle cell constriction, proliferation and migration [15].

### End-organ damage

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The cells of the innate and adaptive immune system contribute to end-organ damage in hypertension. An accumulation of T-cells and monocyte/macrophages in vessels and the kidneys can be observed. The vascular accumulation is predominantly in the adventitia and the perivascular adipose tissue. In the kidneys, T-cell concentration can be observed both in the medulla and the renal cortex. As mentioned before, these cells produce potent cytokines affecting vascular and renal function [16].

IL-17 contributes to numerous autoimmune diseases including psoriasis, asthma, Crohn's disease and rheumatoid arthritis. Recently, its role in the development of hypertension has been described. McMaster et al. [16] found that angiotensin II infusions increased IL-17A production in mouse T cells, and that plasma concentrations of IL-17A are increased in patients suffering from hypertension. Interestingly, animals

lacking IL-17A develop milder hypertension and do not develop endothelial dysfunction in response to Ang II infusion. The conclusions of the authors were that IL-17A might coordinate an inflammatory response in hypertension [17, 18].

In addition, it has been recently discovered that this cytokine plays an important role in aortic stiffness. In normal conditions, the aorta expands during systole and dilates during diastole. Ang II and DOCA-salt-induced hypertension lead to a significant deposition of collagen in the adventitia, and as a result to a marked loss of aortic compliance. This process does not affect Rag1<sup>-/-</sup> mice and is restored by the adoptive transfer of T-cells to the mice [19].

IL-6 is produced by numerous cells such as macrophages, monocytes, T cells and vascular cells. Elevated concentrations of IL-6 correlate with increased blood pressure. What is more, a reduction of IL-6 can be observed after treatment with Ang II blockade [16].

Increased levels of IFN- $\gamma$  have been found in hypertensive mice. However, its effect can be different in response to different hypertensive stimuli. Subcutaneous injections of IFN- $\gamma$  attenuate hypertension, proteinuria and glomerular damage in Dahl-salt-sensitive rats, but does not have any effect on spontaneously hypertensive rats. Moreover, mice lacking the IFN- $\gamma$  gene exhibit exaggerated left ventricular hypertrophy and worse diastolic dysfunction. Markó et al. [20] investigated the role of IFN- $\gamma$  using mice lacking IFN- $\gamma$  receptor 1. These animals developed less renal fibrosis and maintained glomerular rate. And they demonstrated reductions in cardiac fibronectin and collagen and less frequent arrhythmias than have been observed in wild-type mice undergoing Ang II infusion [20, 21].

TNF- $\alpha$  is produced by different cells such as T cells, macrophages, endothelial cells, neuronal cells, adipocytes and fibroblasts. Hypertension is associated with an elevation of TNF- $\alpha$  concentration. TNF- $\alpha$  activates reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nuclear factor  $\kappa$ B (NF- $\kappa$ B), which contribute to the induction of oxidative stress and as a result chemokine and adhesion molecule expression, vascular remodelling, and sodium retention by the kidneys. TNF- $\alpha$  also has an influence on endothelial nitric oxide production [22, 23].

## T regulatory cells

Although T cells are known to contribute to hypertension development, the role of specific subsets of T cells and their cytokines should be discussed.

The main role of Tregs is the maintenance of immunological tolerance and they have been found to be involved in the pathogenesis of numerous cardiovascular diseases. The impairment of Treg cells function or number might be involved in different autoimmune and inflammatory diseases, such as atherosclerosis, systemic lupus erythematosus, diabetes type 1 and many others [24].

T regulatory cells play an important protective role in vascular dysfunction induced by hypertension. They are thought to exert their anti-inflammatory function by influencing the production of IL-10. IL-10 is a potent anti-inflammatory factor, acting through IL-6 and TNF- $\alpha$  suppression and has a cardiovascular protective role in blood pressure elevation [25].

Radwan et al. [26] were the first to determine that the transfer of Treg cells before the development of hypertension prevented hypertension and vascular dysfunction in mice undergoing Ang II infusions. In their recent study, they found that established hypertension reduced the number of Treg cells by autophagy dysfunction and apoptosis mechanisms. Treg cells transferred into a mouse with induced hypertension rescued the microvascular function independently of arterial pressure-lowering effects. The inhibition of autophagy also improved the microvascular function independently of arterial blood pressure [26]. As described by many authors, Treg cells replacement by infusion could be an important new therapeutic strategy for the treatment of cardiovascular diseases [24–26].

## Gut microbiota

Many investigations have indicated that a reduction in gut microbiota is associated with systemic inflammation and hypertension [27]. However, the mechanism has not been clearly explained yet. Specific immunomodulatory effects have been demonstrated using the *Bacteroides fragilis*. Polysaccharides produced by this bacterium are able to restore the Th1/Th2 balance in a germ-free animal model and affect natural killer cells [28]. Lactobacilli, on the other hand, are able to produce peptides that inhibit angiotensin-converting enzyme (ACE) [29]. Interestingly, dysbiosis was found in patients suffering from hypertension compared to their healthy counterparts. The administration of probiotics resulted in mild blood pressure reduction [30, 31]. However, the definite interaction of gut microbiota and hypertension need to be further confirmed.

## Conclusions

Hypertension is a disease with a substantial impact on public health, resulting in stroke, heart failure and kidney disease. Defining the role of the immune system in hypertension could provide new insights into the pathomechanism of this disease, and could help to identify novel targets for the treatment of hypertension.

## Acknowledgments

This study was supported with funds from the Polish National Science Centre on the basis of Decision no.

DEC-2012/07/B/NZ5/00017. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Conflict(s) of interest

The authors report no conflict of interest.

## Streszczenie

Nadciśnienie tętnicze pozostaje w czołówce najczęstszych chorób. Jest głównym czynnikiem ryzyka zawału serca, niewydolności serca, udaru i niewydolności nerek. Od dawna wiadomo, że układ odpornościowy przyczynia się do rozwoju tego schorzenia. W wielu badaniach potwierdzono, że limfocyty pełnią kluczową rolę w rozwoju nadciśnienia tętniczego i w konsekwencji – powikłań narządowych. Komórki te stanowią źródło licznych cytokin. Wzrost ciśnienia tętniczego jest związany z podwyższeniem stężeń cytokin prozapalnych, takich jak: czynnik martwicy nowotworu  $\alpha$ , interferon  $\gamma$ , interleukina 6, interleukina 17, oraz obniżeniem stężeń cytokin przeciwzapalnych, na przykład interleukiny 10. Dokładne poznanie roli układu odpornościowego w nadciśnieniu tętniczym może zaowocować wprowadzeniem nowych strategii terapeutycznych i skuteczne leczenie pacjentów.

Słowa kluczowe: nadciśnienie tętnicze, układ odpornościowy, limfocyty

Folia Cardiologica 2019; 14, 4: 363–367

## References

- Trzeciak BG, Siebert J, Gutknecht P, et al. Cardiovascular risk factors determined via the Internet in 2 periods of time: 2004–2009 and 2010–2015 in Poland. *Int J Occup Med Environ Health*. 2017; 30(3): 499–510, doi: [10.13075/ijomeh.1896.00835](https://doi.org/10.13075/ijomeh.1896.00835), indexed in Pubmed: [28481381](https://pubmed.ncbi.nlm.nih.gov/28481381/).
- White FN, Grollman A. Autoimmune factors associated with infarction of the kidney. *Nephron*. 1964; 1: 93–102, doi: [10.1159/000179322](https://doi.org/10.1159/000179322), indexed in Pubmed: [14248727](https://pubmed.ncbi.nlm.nih.gov/14248727/).
- Svensden UG. The role of thymus for the development and prognosis of hypertension and hypertensive vascular disease in mice following renal infarction. *Acta Pathol Microbiol Scand A*. 1976; 84(3): 235–243, indexed in Pubmed: [1274588](https://pubmed.ncbi.nlm.nih.gov/1274588/).
- Ba D, Takeichi N, Kodama T, et al. Restoration of T cell depression and suppression of blood pressure in spontaneously hypertensive rats (SHR) by thymus grafts or thymus extracts. *J Immunol*. 1982; 128: 1211–1216.
- Guzik TJ, Hoch NE, Brown KA, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med*. 2007; 204(10): 2449–2460, doi: [10.1084/jem.20070657](https://doi.org/10.1084/jem.20070657), indexed in Pubmed: [17875676](https://pubmed.ncbi.nlm.nih.gov/17875676/).
- Nosalski R, McGinnigle E, Siedlinski M, et al. Novel immune mechanisms in hypertension and cardiovascular risk. *Curr Cardiovasc Risk Rep*. 2017; 11(4): 12, doi: [10.1007/s12170-017-0537-6](https://doi.org/10.1007/s12170-017-0537-6), indexed in Pubmed: [28360962](https://pubmed.ncbi.nlm.nih.gov/28360962/).
- Crowley SD, Song YS, Lin EE, et al. Lymphocyte responses exacerbate angiotensin II-dependent hypertension. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298(4): R1089–R1097, doi: [10.1152/ajp-regu.00373.2009](https://doi.org/10.1152/ajp-regu.00373.2009), indexed in Pubmed: [20147609](https://pubmed.ncbi.nlm.nih.gov/20147609/).
- Felten DL, Livnat S, Felten SY, et al. Sympathetic innervation of lymph nodes in mice. *Brain Res Bull*. 1984; 13(6): 693–699, indexed in Pubmed: [6532515](https://pubmed.ncbi.nlm.nih.gov/6532515/).
- Trott DW, Harrison DG. The immune system in hypertension. *Adv Physiol Educ*. 2014; 38(1): 20–24, doi: [10.1152/advan.00063.2013](https://doi.org/10.1152/advan.00063.2013), indexed in Pubmed: [24585465](https://pubmed.ncbi.nlm.nih.gov/24585465/).
- Lob HE, Marvar PJ, Guzik TJ, et al. Induction of hypertension and peripheral inflammation by reduction of extracellular superoxide dismutase in the central nervous system. *Hypertension*. 2010; 55(2): 277–283, doi: [10.1161/HYPERTENSIONAHA.109.142646](https://doi.org/10.1161/HYPERTENSIONAHA.109.142646), indexed in Pubmed: [20008675](https://pubmed.ncbi.nlm.nih.gov/20008675/).
- Shi P, Diez-Freire C, Jun JY, et al. Brain microglial cytokines in neurogenic hypertension. *Hypertension*. 2010; 56(2): 297–303, doi: [10.1161/HYPERTENSIONAHA.110.150409](https://doi.org/10.1161/HYPERTENSIONAHA.110.150409), indexed in Pubmed: [20547972](https://pubmed.ncbi.nlm.nih.gov/20547972/).
- Lob HE, Vinh A, Li Li, et al. Role of vascular extracellular superoxide dismutase in hypertension. *Hypertension*. 2011; 58(2): 232–239, doi: [10.1161/HYPERTENSIONAHA.111.172718](https://doi.org/10.1161/HYPERTENSIONAHA.111.172718), indexed in Pubmed: [21730294](https://pubmed.ncbi.nlm.nih.gov/21730294/).
- Marvar PJ, Thabet SR, Guzik TJ, et al. Central and peripheral mechanisms of T-lymphocyte activation and vascular inflammation produced by angiotensin II-induced hypertension. *Circ Res*. 2010; 107(2): 263–270, doi: [10.1161/CIRCRESAHA.110.217299](https://doi.org/10.1161/CIRCRESAHA.110.217299), indexed in Pubmed: [20558826](https://pubmed.ncbi.nlm.nih.gov/20558826/).
- Brody M, Fink G, Buggy J, et al. Critical role of the anteroventral third ventricle (AV3V) region in development and maintenance of experimental hypertension. *Perspect Nephrol Hypertens*. 1979; 6: 76–84.
- Guzik TJ, Skiba DS, Touyz RM, et al. The role of infiltrating immune cells in dysfunctional adipose tissue. *Cardiovasc Res*. 2017; 113(9): 1009–1023, doi: [10.1093/cvr/cvx108](https://doi.org/10.1093/cvr/cvx108), indexed in Pubmed: [28838042](https://pubmed.ncbi.nlm.nih.gov/28838042/).
- McMaster WG, Kirabo A, Madhur MS, et al. Inflammation, immunity, and hypertensive end-organ damage. *Circ Res*. 2015; 116(6): 1022–1033, doi: [10.1161/CIRCRESAHA.116.303697](https://doi.org/10.1161/CIRCRESAHA.116.303697), indexed in Pubmed: [25767287](https://pubmed.ncbi.nlm.nih.gov/25767287/).
- Gu C, Wu L, Li X. IL-17 family: cytokines, receptors and signaling. *Cytokine*. 2013; 64(2): 477–485, doi: [10.1016/j.cyto.2013.07.022](https://doi.org/10.1016/j.cyto.2013.07.022), indexed in Pubmed: [24011563](https://pubmed.ncbi.nlm.nih.gov/24011563/).
- Madhur MS, Lob HE, McCann LA, et al. Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. *Hy-*

- pertension. 2010; 55(2): 500–507, doi: [10.1161/HYPERTENSION-AHA.109.145094](https://doi.org/10.1161/HYPERTENSION-AHA.109.145094), indexed in Pubmed: 20038749.
19. Wu J, Thabet SR, Kirabo A, et al. Inflammation and mechanical stretch promote aortic stiffening in hypertension through activation of p38 mitogen-activated protein kinase. *Circ Res*. 2014; 114(4): 616–625, doi: [10.1161/CIRCRESAHA.114.302157](https://doi.org/10.1161/CIRCRESAHA.114.302157), indexed in Pubmed: 24347665.
  20. Markó L, Kvakan H, Park JK, et al. Interferon- $\gamma$  signaling inhibition ameliorates angiotensin II-induced cardiac damage. *Hypertension*. 2012; 60(6): 1430–1436, doi: [10.1161/HYPERTENSIONAHA.112.199265](https://doi.org/10.1161/HYPERTENSIONAHA.112.199265), indexed in Pubmed: 23108651.
  21. Ishimitsu T, Uehara Y, Numabe A, et al. Interferon gamma attenuates hypertensive renal injury in salt-sensitive Dahl rats. *Hypertension*. 1992; 19(6 Pt 2): 804–808, indexed in Pubmed: 1592485.
  22. Ramseyer VD, Garvin JL. Tumor necrosis factor- $\alpha$ : regulation of renal function and blood pressure. *Am J Physiol Renal Physiol*. 2013; 304(10): F1231–F1242, doi: [10.1152/ajprenal.00557.2012](https://doi.org/10.1152/ajprenal.00557.2012), indexed in Pubmed: 23515717.
  23. Garvin JL, Herrera M, Ortiz PA. Regulation of renal NaCl transport by nitric oxide, endothelin, and ATP: clinical implications. *Annu Rev Physiol*. 2011; 73: 359–376, doi: [10.1146/annurev-physiol-012110-142247](https://doi.org/10.1146/annurev-physiol-012110-142247), indexed in Pubmed: 20936940.
  24. Kassan M, Wecker A, Kadowitz P, et al. CD4+CD25+Foxp3 regulatory T cells and vascular dysfunction in hypertension. *J Hypertens*. 2013; 31(10): 1939–1943, doi: [10.1097/HJH.0b013e328362feb7](https://doi.org/10.1097/HJH.0b013e328362feb7), indexed in Pubmed: 23881298.
  25. Chen S, Agrawal DK. Dysregulation of T cell subsets in the pathogenesis of hypertension. *Curr Hypertens Rep*. 2015; 17(2): 8, doi: [10.1007/s11906-014-0521-1](https://doi.org/10.1007/s11906-014-0521-1), indexed in Pubmed: 25633669.
  26. Radwan E, Mali V, Haddox S, et al. Treg cells depletion is a mechanism that drives microvascular dysfunction in mice with established hypertension. *Biochim Biophys Acta Mol Basis Dis*. 2019; 1865(2): 403–412, doi: [10.1016/j.bbadis.2018.10.031](https://doi.org/10.1016/j.bbadis.2018.10.031), indexed in Pubmed: 30414897.
  27. Jose PA, Raj D. Gut microbiota in hypertension. *Curr Opin Nephrol Hypertens*. 2015; 24(5): 403–409, doi: [10.1097/MNH.000000000000149](https://doi.org/10.1097/MNH.000000000000149), indexed in Pubmed: 26125644.
  28. Rodríguez-Iturbe B, Pons H, Johnson RJ. Role of the Immune system in hypertension. *Physiol Rev*. 2017; 97(3): 1127–1164, doi: [10.1152/physrev.00031.2016](https://doi.org/10.1152/physrev.00031.2016), indexed in Pubmed: 28566539.
  29. Nakamura Y, Yamamoto N, Sakai K, et al. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. *J Dairy Sci*. 1995; 78(6): 1253–1257, doi: [10.3168/jds.S0022-0302\(95\)76745-5](https://doi.org/10.3168/jds.S0022-0302(95)76745-5), indexed in Pubmed: 7673515.
  30. Yang T, Santisteban MM, Rodríguez V, et al. Gut dysbiosis is linked to hypertension. *Hypertension*. 2015; 65(6): 1331–1340, doi: [10.1161/HYPERTENSIONAHA.115.05315](https://doi.org/10.1161/HYPERTENSIONAHA.115.05315), indexed in Pubmed: 25870193.
  31. Khalesi S, Sun J, Buys N, et al. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension*. 2014; 64(4): 897–903, doi: [10.1161/HYPERTENSIONAHA.114.03469](https://doi.org/10.1161/HYPERTENSIONAHA.114.03469), indexed in Pubmed: 25047574.