# Immune cells and *vasa vasorum* in the tunica media of atherosclerotic coronary arteries

Ruda Zorc-Pleskovič[1], Aleš Pleskovič[2], Olga Vraspir-Porenta[1,3], Metka Zorc[1,3], Aleksandra Milutinović[1*]

[1]Institute of Histology and Embryology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [2]Department of Internal Medicine, University Medical Centre of Ljubljana, Ljubljana, Slovenia, [3]International Center for Cardiovascular Diseases MC Medicor d.d., Izola, Slovenia

## ABSTRACT

In coronary artery disease (CAD), the disruption of the tunica media immune privilege manifests as increased leukocyte infiltration and the formation of *vasa vasorum*. We aimed to characterize the immune privilege status of the tunica media in human coronary arteries (CAs) with atherosclerotic plaques, by comparing the abundance and composition of immune-cell infiltrates within the individual arterial-wall layers, and by evaluating *vasa vasorum* neovascularization of the tunica media. The tissue samples were obtained from 36 symptomatic patients with diffuse CAD (aged 60–72 years) who underwent coronary endarterectomy. T and B cells, macrophages and endothelial cells in the CAs were detected by immunohistochemistry. Morphological analysis of CAs showed significant atherosclerotic changes in all specimens. In the media, we observed damage and loss of smooth muscle cells, destruction of the extracellular matrix architecture, and fibrosis. There were 43.3% of immune cells in the intima, 50% in the adventitia, and 6.7% in the media. In the media, 51.1% of the immune cells were T cells ($p < 0.001$ compared to B cells and macrophages; ANOVA, Scheffe *post hoc* analysis), 23.5% were B cells, and 25.4% were macrophages. The number of *vasa vasorum* in the media was 1 in 38.9% of CAs, 2–3 in 36.1%, and ≥4 in 25% of CAs. Our results indicate that, in atherosclerotic CAs, the immune privilege of the media is disrupted by the infiltration of T and B cells, macrophages, and the presence of *vasa vasorum*.

KEY WORDS: Atherosclerosis; coronary arteries; tunica media; T cells; B cells; macrophages; immune privilege; vasa vasorum
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## INTRODUCTION

Coronary artery disease (CAD) is characterized by atherosclerotic changes in the arterial wall [1], and different components of the vascular, metabolic, and immune systems are involved in this process. Most of the previous studies investigating morphology of CAD have been focused on changes in the innermost layer of the arterial wall, namely the tunica intima, however over the last few years, there is a growing interest in the role of the other layers in CAD development [2,3].

The arterial wall consists of three, structurally different, layers: tunica intima (innermost layer), tunica media (medial layer), and tunica adventitia or externa (outer layer). The tunica media is made up of multiple layers of vascular smooth muscle cells (VSMCs), supported by connective tissue. The media is normally avascular, and VSMCs in this compartment have contractile as well as synthetic functions (i.e., maintaining the extracellular matrix [ECM]) [4]. Coronary arteries (CAs) are muscular arteries which means they have a thicker tunica media with more smooth muscle cells, compared to elastic arteries which have a large amount of elastin and collagen fibers in the media. The main role of CAs is to supply the heart muscle with blood.

Inflammatory processes compromise the integrity of the arterial wall, affecting the functions of all three layers. It is assumed that the immune privilege of the media is based on both passive (mechanical) and active (biological) mechanisms. The passive mechanisms include the absence of lymphatic and blood vessels which prevents immune responses, and the presence of elastic lamina as a barrier to leukocyte trafficking [5,6]. The active mechanisms of the tunica media immune privilege involve the synthesis of indoleamine 2,3-dioxygenase (IDO) in VSMCs [7,8] and transforming growth factor beta (TGF-β) [9], low expression of major histocompatibility complex (MHC) molecules [10], absence of costimulatory molecules (e.g., OX40 ligand and inducible T-cell costimulator [ICOS] or CD278) [11], and presence of coinhibitory molecules (e.g., programmed death-ligand [PD-L] 1 and 2) [11]. The

*Corresponding author: Aleksandra Milutinović, Institute of Histology and Embryology, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia. Phone: +386 1 543 73 60; Fax: +386 1 543 73 61. E-mail: sandramilutinovic@yahoo.com

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failure of the tunica media immune privilege manifests as an intense infiltration of leukocytes, damage and loss of VSMCs, and the destruction of the ECM architecture [4].

In normal arteries, the media does not contain small blood vessels (*vasa vasorum*) with endothelial cells that could attract immune cells [4,12]. However, in some circumstances neovascularization of the media may occur, for example, when the intima is abnormally thickened [13]. Studies have shown that, in atherosclerotic CAs, the degree of neovascularization of the intima and media correlates with the intensity of inflammation [14]. In other cases, VSMCs can excrete factors that promote angiogenesis [15,16], affect the function of macrophages and T cells, and enhance/amplify the innate and adaptive immune response [4]. In the adaptive immune response, which plays an important role in atherogenesis, T cells recognize self-antigens and induce a humoral immune response that involves the activation of B cells [17].

Different immune cells have distinct functions in the development of inflammation, remodeling of the ECM and weakening of the tunica media. The aim of our study was to assess the immune privilege status of the media in atherosclerotic CAs by determining the abundance and proportion of macrophages, T and B cells in the tunica media in relation to the other layers of the arterial wall, as well as by evaluating *vasa vasorum* neovascularization of the media.

## MATERIALS AND METHODS

### Tissue samples

We obtained tissue samples of 36 symptomatic patients with diffuse CAD (aged 60–72 years), who underwent coronary endarterectomy. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (NMEC 170/07/13, NMEC 110/03/16). All participants provided their written informed consent to participate in this study. The study was conducted according to the Declaration of Helsinki, 2013.

Immediately after the surgery, the samples of CAs were fixed for 24 hours in formaldehyde and embedded in paraffin. The tissue specimens were cut transversely with a microtome into 5 μm-thick serial sections and stained with hematoxylin and eosin (HE). The severity of atherosclerosis was graded according to the modified American Heart Association (AHA) classification [18,19]. Among the 36 samples of CAs, fibrous-cap atheroma was found in 14 CAs (38.9%), thin-cap fibroatheroma in 16 CAs (44.4%), and lesions with healed thrombi in 6 CAs (16.7%).

### Detection of immune and endothelial cells

Immunohistochemistry was used for the detection of T cells (anti-CD3, pan-T-cell antigen, Dako, Denmark, 1:400), B cells (anti-CD79, B cell antigen, Dako, Denmark 1:50), macrophages (anti-CD68, macrophage antigen, Dako, Denmark, 1:100), and endothelial cells (anti-human von Willebrand factor [vWF], Dako, Denmark, 1:800).

### Morphological analysis and statistics

The morphological analysis was performed on three transverse histological sections for each CA.

The number of immune cells was counted in every CA section and expressed as the average ± standard deviation (SD) and percentage of T and B cells, and macrophages in the tunica intima, media and adventitia. A statistically significant difference in the number of immune cells between the intima, media and adventitia was determined by one-way analysis of variance (ANOVA) followed by Scheffe *post hoc* analysis ($p < 0.05$).

In the media of the CAs stained with anti-human vWF, the density of *vasa vasorum* was evaluated as follows: absence of vessels (degree 0), 1 vessel in the section (degree 1), 2 or 3 vessels in the section (degree 2), and 4 or more vessels in the section (degree 3).

We calculated the frequencies and percentages of CAs in relation to the degrees of vascular density.

## RESULTS

### Morphological analysis

The morphological analysis of HE-stained CAs revealed significant atherosclerotic changes in all specimens. Diffuse fibroproliferation was observed in the intima (Figure 1A). The elastic laminae were interrupted, and in some cases they were completely absent. Focal or diffuse infiltrations of immune cells in the tunica media were localized mainly around *vasa vasorum* (Figure 1A-F). The disarrangement, damage and loss of VSMCs was observed, as well as the destruction of the ECM architecture leading to fibrosis.

### Immune cells in the intima, media, and adventitia

Immune cells were found in the three arterial-wall layers in all CAs. The immunohistochemistry staining showed 43.3% of immune cells in the tunica intima (average 51.9 cells ± 12.7), 50% in the tunica adventitia (60.3 cells ± 19.0), and 6.7% in the tunica media (8.2 cells ± 3.1).

The most abundant immune cells in the media were T cells (51.1%, average 4.2 cells ± 2.8) [$p < 0.001$ compared to B cells and macrophages], followed by B cells (23.5%, average 1.9 cells ± 1.5), and macrophages (25.4 %, average 2.1 cells ± 1.4). The difference between the numbers of B cells and macrophages in the media was not significant (Figure 1C-E; Figure 2A).

In the tunica adventitia and tunica intima macrophages were significantly more numerous. In the adventitia, there were 44.3%

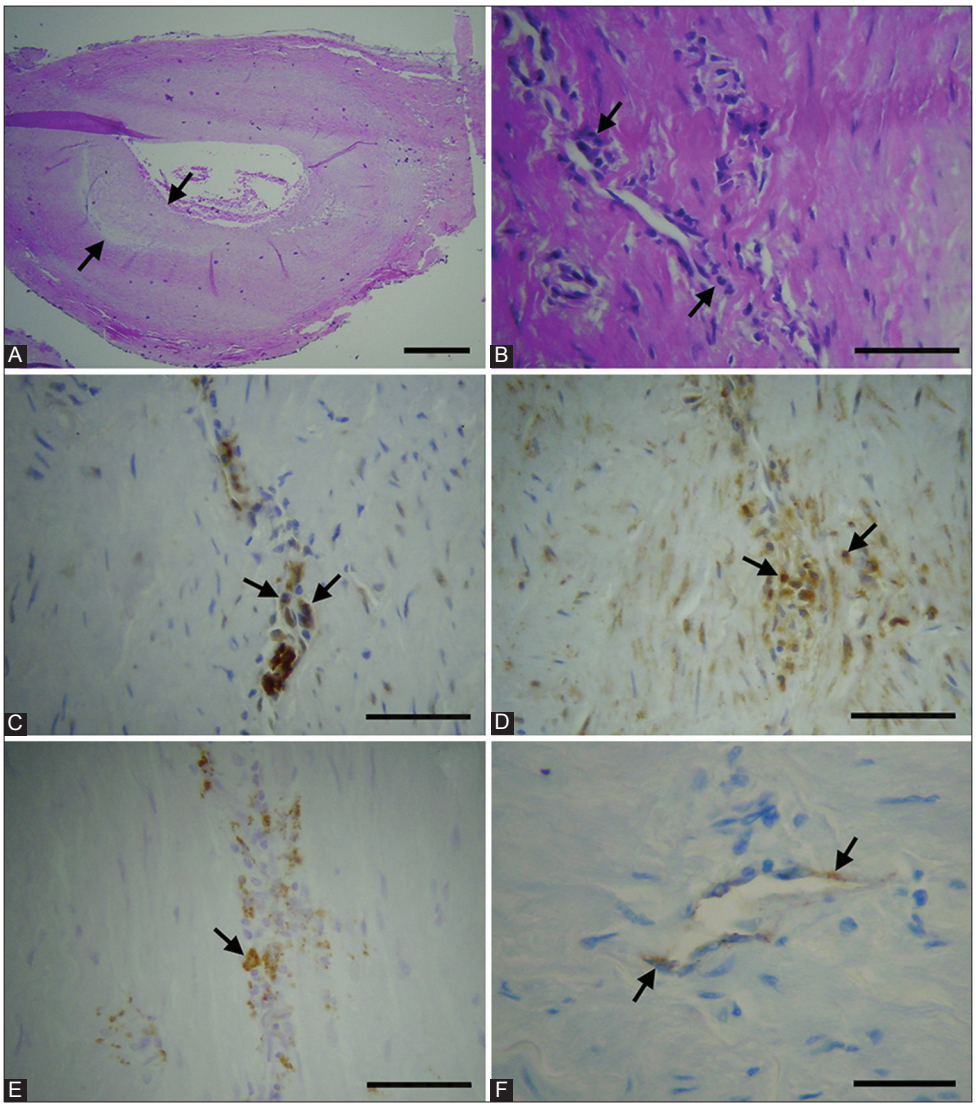


**FIGURE 1.** (A) Fibroproliferation in the tunica intima of human atherosclerotic coronary arteries (the thickened tunica intima is between the two arrows, HE, 2.5×, bar = 500 µm); (B) Perivascular infiltration (arrows) around *vasa vasorum* in the tunica media (HE, 40×, bar = 50 µm); (C) B cells (arrows) and *vasa vasorum* in the tunica media (anti CD79, 40×, bar = 50 µm); (D) T cells (arrows) and *vasa vasorum* in the tunica media (anti CD3, 40×, bar = 50 µm); (E) Macrophages (arrows) and vasa vasorum in the tunica media (anti CD68, 40×); (F) Endothelial cells (arrows) of *vasa vasorum* in the tunica media (anti human von Willebrand factor, 63×, bar = 30 µm).

macrophages (average 27.2 cells ± 11.5) [$p < 0.001$ compared to T and B cells]; 27.6% T cells (16.7 cells ± 6.8); and 27.3% B cells (16.5 cells ± 7.9) with a nonsignificant difference between the numbers of T and B cells (Figure 2A). In the intima, there were 47.6% macrophages (24.7 cells ± 8.5), 30.8% T cells (16.0 cells ± 7.2), and 21.6% B cells (11.2 cells ± 6.7), with a significant difference between the numbers of all three cell types [$p < 0.05$] (Figure 2A).

### *Vasa vasorum* in the tunica media

The density of *vasa vasorum* in the tunica media was estimated by examining the vWF-stained sections (Figure 1F, Figure 3A-C). All CAs had one or more *vasa vasorum* in the media. Most of the *vasa vasorum* were observed in the outer part and only a few in the inner part of the tunica media. In the media, there were 14 CAs (38.9%) with 1 blood vessel (degree 1), 13 CAs (36.1%) with 2 or 3 blood vessels (degree 2), and 9 CAs (25.0%) with more than 4 blood vessels [degree 3] (Figure 2B, Figure 3A-C).

## DISCUSSION

The main role of the tunica media is maintaining the stability of the vessel wall. Due to the increased secretion of cytokines and chemokines by VSMCs in disease state, immune cells infiltrate the media leading to the remodeling of the ECM and weakening of the media. Moreover, the occurrence of *vasa vasorum* with endothelial cells in the media amplifies this effect [4].

The aim of our study was to assess the immune privilege status of the tunica media by determining the abundance and proportion of macrophages, T, and B cells within the individual wall layers of atherosclerotic CAs, and by estimating the degree

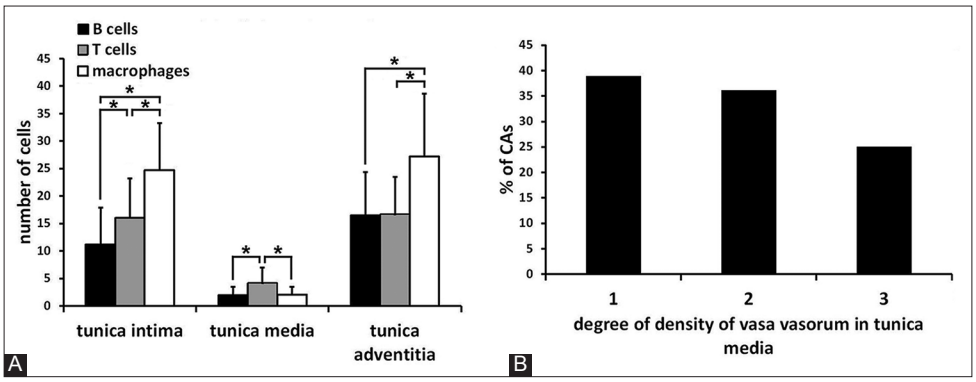


**FIGURE 2.** (A) Average number ± SD of B cells, T cells and macrophages in the tunica intima, tunica media and tunica adventitia of human atherosclerotic coronary arteries (CAs). *Significantly different number of immune cells (ANOVA, Scheffe *post hoc* test, $p < 0.05$ for all); (B) The percentage of CAs with different degrees of vascular density in the media; note that all CAs had one or more *vasa vasorum* in the tunica media.

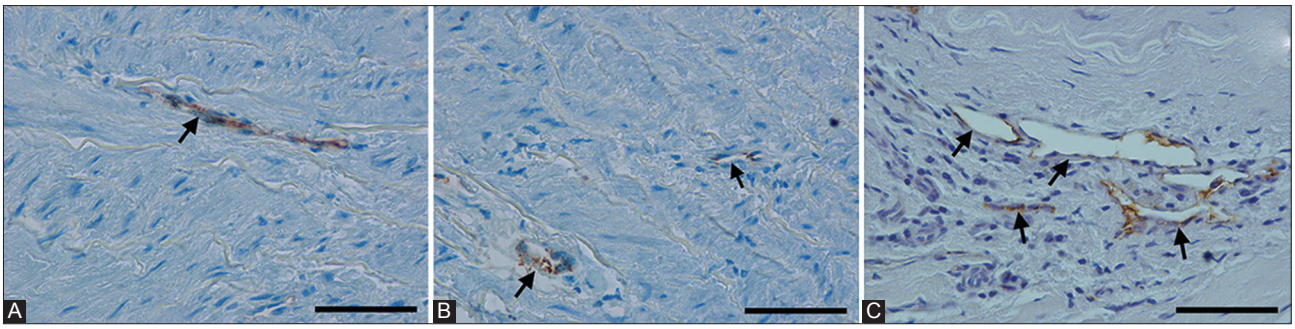


**FIGURE 3.** Endothelial cells of *vasa vasorum* in the tunica media of human atherosclerotic coronary arteries [CAs] (anti-human von Willebrand factor, 40×, bar = 50 μm). The estimation of *vasa vasorum* density: degree 1 - one vessel in the section (A, arrow); degree 2 - two or three vessels in the section (B, arrows); and degree 3 - four or more vessels in section (C, arrows). Note that all CAs have one or more *vasa vasorum* in the tunica media.

of *vasa vasorum* neovascularization of the tunica media. The histological analysis of atherosclerotic CAs revealed 52% of the immune cells in the intima, 60% in the adventitia, and 8% in the media. In the media, the immune cells were mainly localized around the *vasa vasorum*. In the intima and adventitia, 40% to 50% of the immune cells were macrophages. In the intima, there were approximately 30% of T cells and slightly above 20% of B cells, while in the adventitia, there were 27% of both T and B cells. More than half of the immune cells in the media were T cells, one quarter of the immune cells were B cells and another quarter were macrophages. Our results are similar to those reported by Hagemeijer et al. [20] who investigated the markers of mononuclear cells (MNC), cytokines and chemokine receptors in human CAs with cardiac allograft vasculopathy (CAV). They detected 5- to 10-fold fewer immune cells in the media compared to the intima or adventitia. In the tunica media, they observed 41% of T cells, 7% of B cells and 8% of macrophages [20].

Passive and active mechanisms of immune privilege have been assumed and/or demonstrated in the media, but not in the intima and adventitia [4]. Those mechanisms protect the media from inflammation and their disruption is typically seen in arteriosclerotic diseases, such as atherosclerosis and transplant vasculopathy [4]. The media of normal and atherosclerotic arteries is generally spared of infiltrating immune cells such as leukocytes, T cells and macrophages, and has only scattered resident leukocytes [4,21-23]. However, in response to inflammation, the resident leukocytes as well as infiltrates of other immune cells in the media may stimulate the production of inflammatory cytokines and chemokines in VSMCs and endothelial cells which in turn recruit additional leukocytes [4]. Moreover, in response to diverse stimuli, microbial infection and cellular injury, VSMCs are able to initiate the transcription of both anti- and pro-inflammatory genes. The produced pro-inflammatory molecules, among others, include cytokines that may activate and recruit macrophages, and adhesive molecules that support leukocyte trafficking [4,24-30]. Our analysis showed that a quarter of immune cells in the media of atherosclerotic CAs were macrophages.

Another study showed that VSMCs treated with interferon (IFN)-γ express very high amounts (perhaps 100-fold that of endothelial cells) of IDO, which is an enzyme involved in the catabolism of tryptophan. As the result of tryptophan degradation, T-cell activation and proliferation is inhibited [7,30]. In the intima and adventitia, IFN-γ is produced by activated T cells; the cytokine diffuses into the media and activates synthesis of IDO in VSMCs [4].

The infiltration of T cells in the intima and adventitia prevents their infiltration in the media [4]. In our study, more than half of immune cells in the media were T cells, but the number of T cells in the intima and adventitia was four times higher than in the media. Nevertheless, the T cells in the intima and adventitia represented only about 30% of the total immune cell population in those layers. We assumed that the number of T cells in the intima and adventitia was too small for the production of sufficient quantities of IFN-γ that could activate IDO synthesis in VSMC. Moreover, we observed the disarrangement, damage and loss of VSMCs. Overall, we speculate that due to the damage and loss of VSMCs in the atherosclerotic CAs, the VSMCs could not produce sufficient quantities of IDO, resulting in an increased infiltration of the media with T cells. Thus, the decreased amount of IDO contributed to the disruption of the active immune privilege of the media [4].

To the best of our knowledge, this is the first study to describe the presence of B cells in the media in non-transplant vasculopathy. Macrophages and T cells have been observed in the intima at all stages of atherosclerosis, whereas B cells were only occasionally found within intimal plaques [17,31,32]. In contrast, a larger number of B cells was found in the adventitia [32-34]. In this study, we demonstrated the presence of B cells as well as a high number of T cells in the media. In the atherosclerotic process, T cells recognize self-antigens and induce humoral immunity driven by B cells [17] which contributes to the breakdown of the active immune privilege in the media.

The CAs in our study had a thickened intima, immune cells in all three layers, and one or more *vasa vasorum* in the media, mainly arising from the adventitia. Similarly, it was shown that in arteries with wall thickening or other pathological changes, *vasa vasorum* in the media may arise from the adventitial layer or even from the central lumen [35]. Furthermore, in the inner media of aneurysmal and atherosclerotic thoracic aortas, the neovascularization correlated with increased leukocyte infiltration [36,37]. The same result was shown in atherosclerotic CAs where the neovascularization correlated with increased leukocyte infiltration in the intima-media layer [14]. A pro-inflammatory role for the neovascularization of the media has been indicated in murine models [38,39]. Endothelial cells in the *vasa vasorum* of the media could contribute to the inflammatory process by attracting immune cells, presenting antigens, and demonstrating immunoregulatory properties [12]. Overall, the presence of *vasa vasorum* in the media contributes to the breakdown of the passive immune privilege in that compartment [4].

It is generally assumed that the ratio of T and B cells, and macrophages in the media of the vascular wall plays an important role in the development of atherosclerotic processes. Further research is needed to better understand the specific events during atherosclerotic changes of the vascular wall.

## CONCLUSION

Our results suggest that, in the progressive state of atherosclerotic CAs that contain lesions with healed thrombi, the immune privilege of the media is disrupted by the infiltration of T and B cells, macrophages and the presence of *vasa vasorum*.

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## DECLARATION OF INTERESTS

The authors declare no conflict of interests.

## REFERENCES

[1] Goodarzynejad H, Boroumand M, Behmanesh M, Ziaee S, Jalali A. Cholesteryl ester transfer protein gene polymorphism (I405V) and premature coronary artery disease in an Iranian population. Bosn J Basic Med Sci 2016;16(2):114-20. https://doi.org/10.17305/bjbms.2016.942.

[2] Tosheska K, Labudovic D, Jovanova S, Jaglikovski B, Alabakovska S. Cholesteryl ester transfer protein, low density lipoprotein particle size and intima media thickness in patients with coronary heart disease. Bosn J Basic Med Sci 2011;1(3):169-73. https://doi.org/10.17305/bjbms.2011.2569.

[3] Satilmis S, Celik O, Biyik I, Ozturk D, Celik K, Akın F, et al. Association between serum vitamin D levels and subclinical coronary atherosclerosis and plaque burden/composition in young adult population. Bosn J Basic Med Sci 2015;15(1):67-72. https://doi.org/10.17305/bjbms.2015.238.

[4] Tellides G, Pober JS. Inflammatory and immune responses in the arterial media. Circ Res 2015;116(2):312-22. https://doi.org/10.1161/CIRCRESAHA.116.301312.

[5] Wilens SL, McCluskey RT. The comparative filtration properties of excised arteries and veins. Am J Med Sci 1952;224(5):540-7. https://doi.org/10.1097/00000441-195211000-00009.

[6] Dal Canto AJ, Swanson PE, O'Guin AK, Speck SH, Virgin HW. IFN-gamma action in the media of the great elastic arteries, a novel immunoprivileged site. J Clin Invest 2001;107(2):R15-22. https://doi.org/10.1172/JCI11540.

[7] Cuffy MC, Silverio AM, Qin L, Wang Y, Eid R, Brandacher G, et al. Induction of indoleamine 2,3-dioxygenase in vascular smooth muscle cells by interferon-gamma contributes to medial immunoprivilege. J Immunol 2007;179(8):5246-54. https://doi.org/10.4049/jimmunol.179.8.5246.

[8] Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol 2013;34(3):137-43. https://doi.org/10.1016/j.it.2012.10.001.

[9] Lebastchi AH, Khan SF, Qin L, Li W, Zhou J, Hibino N, et al. Transforming growth factor beta expression by human vascular cells inhibits interferon gamma production and arterial media injury by alloreactive memory T cells. Am J Transplant 2011;11(11):2332-41. https://doi.org/10.1111/j.1600-6143.2011.03676.x.

[10] Tellides G, Tereb DA, Kirkiles, Smith NC, Kim RW, Wilson JH, et al. Interferon-gamma elicits arteriosclerosis in the absence of

leukocytes. Nature 2000;403(6766):207-11.
https://doi.org/10.1038/35003221.

[11] Zhang P, Manes TD, Pober JS, Tellides G. Human vascular smooth muscle cells lack essential costimulatory molecules to activate allogeneic memory T cells. Arterioscler Thromb Vasc Biol 2010;30(9):1795-1801.
https://doi.org/10.1161/ATVBAHA.109.200758.

[12] Al-Soudi A, Kaaij MH, Tas SW. Endothelial cells: From innocent bystanders to active participants in immune responses. Autoimmun Rev 2017;16(9):951-62.
https://doi.org/10.1016/j.autrev.2017.07.008.

[13] Geiringer E. The mural coronary. Am Heart J 1951;41(3):359-68.
https://doi.org/10.1016/0002-8703(51)90036-1.

[14] Zhang Y, Cliff WJ, Schoefl GI, Higgins G. Immunohistochemical study of intimal microvessels in coronary atherosclerosis. Am J Pathol 1993;143(1):164-72.

[15] Ho-Tin-Noe B, Le Dall J, Gomez D, Louedec L, Vranckx R, El-Bouchtaoui M, et al. Early atheroma-derived agonists of peroxisome proliferator-activated receptor-gamma trigger intramedial angiogenesis in a smooth muscle cell dependent manner. Circ Res 2011;109(9):1003-14.
https://doi.org/10.1161/CIRCRESAHA.110.235390.

[16] Nicoletti A, Khallou-Laschet J, Guedj K, Clement M, Gaston AT, Morvan M, et al. L19. Lymphoid neogenesis in vascular chronic inflammation. Presse Med 2013;42(4 Pt 2):558-60.
https://doi.org/10.1016/j.lpm.2013.01.018.

[17] Chistiakov DA, Orekhov AN, Bobryshev YV. Immune-inflammatory responses in atherosclerosis: Role of an adaptive immunity mainly driven by T and B cells. Immunobiology 2016;221(9):1014-2033.
https://doi.org/10.1016/j.imbio.2016.05.010.

[18] Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: A comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20(5):1262-75.
https://doi.org/10.1161/01.ATV.20.5.1262.

[19] Medscape [Internet]. Ladich ER, Virmani R, Kolodgie F, Otsuka F: Atherosclerosis Pathology; 2016 [2017 September 12]. Available from: https://reference.medscape.com/article/1612610-overview.

[20] Hagemeijer MC, van Oosterhout MF, van Wichen DF, van Kuik J, Siera-de Koning E, Gmelig Meyling FH, et al. T cells in cardiac allograft vasculopathy are skewed to memory Th-1 cells in the presence of a distinct Th-2 population. Am J Transplant 2008;8(5):1040-50.
https://doi.org/10.1111/j.1600-6143.2008.02198.x.

[21] Billingham ME. Cardiac transplant atherosclerosis. Transplant Proc 1987;19(4 Suppl 5):19-25.

[22] van der Wal AC, Das PK, Bentz van de Berg D, van der Loos CM, Becker AE. Atherosclerotic lesions in humans. In situ immunophenotypic analysis suggesting an immune mediated response. Lab Invest 1989;61(2):166-70.

[23] Emeson EE, Robertson AL Jr. T lymphocytes in aortic and coronary intimas. Their potential role in atherogenesis. Am J Pathol 1988;130(2):369-76.

[24] Yang X, Coriolan D, Murthy V, Schultz K, Golenbock DT, Beasley D. Proinflammatory phenotype of vascular smooth muscle cells: Role of efficient Toll-like receptor 4 signaling. Am J Physiol Heart Circ Physiol 2005;289(3):H1069-76.
https://doi.org/10.1152/ajpheart.00143.2005.

[25] Yang X, Murthy V, Schultz K, Tatro JB, Fitzgerald KA, Beasley D. Toll-like receptor 3 signaling evokes a proinflammatory and proliferative phenotype in human vascular smooth muscle cells. Am J Physiol Heart Circ Physiol 2006;291(5):H2334-43.
https://doi.org/10.1152/ajpheart.00252.2006.

[26] Ahmad U, Ali R, Lebastchi AH, Qin L, Lo SF, Yakimov AO, et al. IFN-gamma primes intact human coronary arteries and cultured coronary smooth muscle cells to double-stranded RNA- and self-RNA-induced inflammatory responses by upregulating TLR3 and melanoma differentiation-associated gene 5. J Immunol 2010;185(2):1283-94.
https://doi.org/10.4049/jimmunol.0902283.

[27] Cole JE, Navin TJ, Cross AJ, Goddard ME, Alexopoulou L, Mitra AT, et al. Unexpected protective role for Toll-like receptor 3 in the arterial wall. Proc Natl Acad Sci U S A 2011;108(6):2372-7.
https://doi.org/10.1073/pnas.1018515108.

[28] Yang X, Coriolan D, Schultz K, Golenbock DT, Beasley D. Toll-like receptor 2 mediates persistent chemokine release by Chlamydia pneumoniae-infected vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2005;25(11):2308-14.
https://doi.org/10.1161/01.ATV.0000187468.00675.a3.

[29] Sun J, Ding Y. NOD2 agonist promotes the production of inflammatory cytokines in VSMC in synergy with TLR2 and TLR4 agonists. ScientificWorldJournal 2012;2012:607157.
https://doi.org/10.1100/2012/607157.

[30] Pober JS, Tellides G. Participation of blood vessel cells in human adaptive immune responses. Trends Immunol 2012;33(1):49-57.
https://doi.org/10.1016/j.it.2011.09.006.

[31] Canducci F, Saita D, Foglieni C, Piscopiello MR, Chiesa R, Colombo A, et al. Cross-reacting antibacterial auto-antibodies are produced within coronary atherosclerotic plaques of acutecoronary syndrome patients. PLoS One 2012;7(8):e42283.
https://doi.org/10.1371/journal.pone.0042283.

[32] Ketelhuth DF, Hansson GK. Adaptive response of T and B cells in atherosclerosis. Circ Res 2016;118(4):668-78.
https://doi.org/10.1161/CIRCRESAHA.115.306427.

[33] Hansson GK, Libby P. The immune response in atherosclerosis: A double-edged sword. Nat Rev Immunol 2006;6(7):508-19.
https://doi.org/10.1038/nri1882.

[34] Mohanta SK, Yin C, Peng L, Srikakulapu P, Bontha V, Hu D, et al. Artery tertiary lymphoid organs contribute to innate and adaptive immune responses in advanced mouse atherosclerosis. Circ Res 2014;114(11):1772-87.
https://doi.org/10.1161/CIRCRESAHA.114.301137.

[35] Gössl M, Rosol M, Malyar NM, Fitzpatrick LA, Beighley PE, Zamir M, et al. Functional anatomy and hemodynamic characteristics of vasa vasorum in the walls of porcine coronary arteries. Anat Rec A Discov Mol Cell Evol Biol 2003;272(2):526-37.
https://doi.org/10.1002/ar.a.10060.

[36] Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: Implications for plaque vulnerability. Circulation 2004;110(14):2032-8
https://doi.org/10.1161/01.CIR.0000143233.87854.23.

[37] Tang PC, Yakimov AO, Teesdale MA, Coady MA, Dardik A, Elefteriades JA, et al. Transmural inflammation by interferon-gamma-producing T cells correlates with outward vascular remodeling and intimal expansion of ascending thoracic aortic aneurysms. FASEB J 2005;19(11):1528-30.
https://doi.org/10.1096/fj.05-3671fje.

[38] Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvin E, et al. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad Sci U S A 2003;100(8):4736-41.
https://doi.org/10.1073/pnas.0730843100.

[39] Eliska O, Eliskova M, Miller AJ. The absence of lymphatics in normal and atherosclerotic coronary arteries in man: A morphologic study. Lymphology 2006;39(2):76-83.