Cardiovascular Pathology 43 (2019) 107148

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Immune checkpoint inhibition alters the inflammatory cell composition of human coronary artery atherosclerosis

Justine L. Newman, James R. Stone*

Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114, USA

ARTICLE INFO

Article history:
Received 19 June 2019
Received in revised form
19 July 2019
Accepted 9 August 2019

Key words:
Immune checkpoint inhibition
Atherosclerotic plaques
Inflammatory cell composition

ABSTRACT

Background: Immune checkpoint inhibition (ICI) has emerged as a promising new approach to treat malignancy. Such therapies can result in autoimmune-related complications such as myocarditis and hepatitis. The impact of ICI on sites of preexisting chronic inflammation has been less clear. Atherosclerosis is a chronic vascular disease with a significant inflammatory component.
Methods: To determine the effect of ICI on the inflammatory infiltrate in coronary artery atherosclerotic plaques, 11 patients who had recently been treated with ICI and subsequently underwent autopsy were matched with 11 cancer patients who had not received ICI treatment. The amount of $CD3^+$ T-lymphocytes, $CD8^+$ cytotoxic T-lymphocytes, and $CD68^+$ macrophages and the ratios of the various cell types in the coronary artery atherosclerotic plaques were compared.
Results: There was no significant difference in the absolute numbers of $CD3^+$, $CD8^+$, or $CD68^+$ cells in the atherosclerotic plaques. In the plaques of the ICI-treated patients, there was a significant increase in the ratio of $CD3^+$ cells to $CD68^+$ cells (CD3/CD68) (P=.002) and a trend towards an increased CD8/CD68 ratio. The increased CD3/CD68 ratio in the ICI-treated patients resulted in 6 of the 11 patients having lymphocyte-predominate inflammation in contrast to the macrophage-predominate inflammation typically found in atherosclerotic plaques.
Conclusions: These findings indicate that ICI alters the inflammatory composition of human atherosclerotic plaque and, thus, may influence plaque progression and/or clinical coronary events.
Summary: In cancer patients, treatment with immune checkpoint inhibition is associated with an altered inflammatory cell composition in coronary artery atherosclerotic plaques with an increased ratio of $CD3^+$ T cells to $CD68^+$ macrophages. Thus, immune checkpoint inhibition may influence plaque progression and/or clinical coronary events.

1. Introduction

Blockade of immune regulatory checkpoints has emerged as an important new approach to treat malignancy [1–3]. This immune checkpoint inhibition (ICI) often involves administration of antibodies that block cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and/or the programmed cell death 1 (PD-1) pathway. In some patients, the activity of these coinhibitory pathways appears to inhibit an effective immune response to the cancer. In the case of the PD-1 pathway, expression of the PD-1 ligand PD-L1 by the tumor cells suppresses an immune response to neoantigens expressed by these malignant cells. Patients are thought to respond to antibodies targeting either PD-1 or PD-L1 because of a preexisting, but inhibited, antitumor T cell response. While the PD-1 pathway is primarily functional in the tumor microenvironment, CTLA-4 primarily functions as an immune inhibitory pathway in draining lymph nodes, where it inhibits costimulatory signaling by outcompeting CD28 for binding costimulatory ligands. Lymphocyte activation gene 3 (LAG-3) is an additional immune inhibitory receptor that is being targeted with antibodies in clinical trials of ICI treatment for malignancy. In some patients, combination therapy with blockade of multiple inhibitory pathways results in a synergistic response to the tumor.

ICI treatment not only enhances immune reactions to the tumor cells but also frequently is associated with inflammatory reactions to nontumor cells. Although often mild, immune-related adverse events occur in 70%–90% of patients receiving ICI treatment, most often involving the skin, lungs, gastrointestinal tract, and endocrine organs [4,5]. ICI treatment is less commonly associated with clinically evident cardiovascular adverse events, including myocarditis and vasculitis [6–8]. Pathologically, immune-related adverse events in the setting of ICI treatment are largely characterized by the infiltration of the tissue by lymphocytes and macrophages with injury to the tissue [9–11].

* Corresponding author at: Massachusetts General Hospital, Department of Pathology, Room 7368, 149 13th Street, Charlestown, MA, 02129. Tel.: +1 617 726 8303; fax: +1 617 643 3566.
E-mail address: jrstone@partners.org (J.R. Stone).

https://doi.org/10.1016/j.carpath.2019.107148
1054-8807/

Persistent exposure to antigens, whether it be in the setting of cancer or chronic infections, is associated with the phenomenon known as T cell exhaustion, in which T cells are reversibly inhibited in part due to the upregulation of inhibitory receptors such as PD-1 and LAG-3 [12]. It is becoming increasingly accepted that human atherosclerosis is a chronic inflammatory disorder in which there is chronic exposure to antigens derived from lipoproteins retained within the vascular wall [13–16]. Thus, it is possible that, in human atherosclerosis, there is a state of T cell exhaustion such that ICI treatment might alter the immune response in the atherosclerotic plaque. To address this question, we examined the inflammatory cell composition of human coronary artery plaques in cancer patients who had either received or not received ICI treatment.

2. Materials and methods

2.1. Case selection and study criteria

Patients with cancer who had been treated with ICI and subsequently underwent autopsy at the Massachusetts General Hospital from 2013 to 2017 were retrospectively studied. The primary inclusion criteria were an autopsy with examination of the heart and prior treatment with an ICI agent, with the first dose administered more than 3 weeks prior to death and the final dose administered within 8 weeks of death. Patients who had received immune-depleting therapies, such as mycophenolate mofetil or anti-thymocyte globulin, were excluded. Each ICI-treated cancer patient was matched with a control cancer patient, who had not received ICI therapy, in which there was an autopsy heart evaluation. Patients were matched based on age, gender, year of autopsy, and cancer type. Patients without coronary artery tissue available for immunohistochemical analysis were excluded.

To identify potential confounders, demographical characteristics, clinical history, and medication history of the patients were obtained from the medical records. Demographical characteristics and clinical history compared included age, gender, race, smoking status, body mass index (BMI), cause of death, postmortem interval, and clinical history of hypercholesterolemia, hypertension, diabetes, autoimmune diseases, chest radiation, percutaneous coronary intervention, and myocardial infarction. Medication usage compared included 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor (statin), angiotensin-converting enzyme (ACE) inhibitor, β-blocker, calcium channel blocker, aspirin, immunosuppressant medications, and non-ICI cancer therapies.

The degree of coronary artery atherosclerosis at autopsy was graded as described previously using a semiquantitative scale based on the assessment in the pathology report: grade 1, 0%–30% estimated stenosis (mild stenosis); grade 2, 31%–74% estimated stenosis (moderate stenosis); and grade 3, 75%–100% estimated stenosis (severe stenosis) [17]. The highest score from the individual coronary arteries was used as the overall atherosclerosis score for each patient.

2.2. Immunohistochemistry

One coronary artery cross section with atherosclerosis from each ICI-treated patient was matched to a coronary artery cross section from the corresponding control patient based on the degree of atherosclerosis on the histologic slides and type of artery, i.e., the left main coronary artery (LM), left anterior descending coronary artery (LAD), left circumflex coronary artery (LCx), and right coronary artery (RCA). These matched crossed sections were utilized for immunohistochemical analysis.

Immunohistochemistry for the general T-lymphocyte marker CD3, the cytotoxic T-lymphocyte marker CD8, and the macrophage marker CD68 was performed on formalin-fixed, paraffin-embedded tissue using routine automated clinical immunohistochemical staining devices. All of the macrophages and lymphocytes throughout the entirety of the adventitia and atherosclerotic plaque of the selected cross sections were counted manually using an Olympus BX53 microscope at 200× magnification. Atherosclerotic plaque and adventitia areas were delineated using a Masson trichrome stain, and the areas were quantified using ImageJ. The data were expressed as cells per mm^2.

Table 1
Patient characteristics and cardiac pathology findings

	ICI	No ICI	*P*
Number of patients	11	11	
Biological sex, male/female	4/7	4/7	1.00
Age (years), median (IQR)	58 (45–69)	67 (48–76)	.17
Smoking (active or former), *n* (%)	5 (45)	3 (27)	.66
Hypercholesterolemia, *n* (%)	4 (36)	3 (27)	1.00
Hypertension, *n* (%)	6 (55)	4 (36)	.67
Diabetes, *n* (%)	3 (27)	0 (0)	.21
BMI (kg/m^2), median (IQR)	21 (20–24)	22 (18–28)	1.00
History of PCI/stent or CABG, *n* (%)	0 (0)	2 (18)	.48
Statin, *n* (%)	1 (9)	2 (18)	1.00
ACE inhibitor, *n* (%)	3 (27)	1 (9)	.59
Calcium channel blocker, *n* (%)	0 (0)	1 (9)	1.00
β-Blocker, *n* (%)	2 (18)	2 (18)	1.00
Aspirin, *n* (%)	3 (27)	3 (27)	1.00
Immunosuppression, *n* (%)	9 (82)	9 (82)	1.00
Non-ICI cancer therapy, *n* (%)	3 (27)	4 (36)	1.00
Type of cancer, *n* (%)			.98
Melanoma	5 (45)	4 (36)	
Gastrointestinal carcinoma	2 (18)	2 (18)	
Endometrial carcinoma	1 (9)	1 (9)	
Cholangiocarcinoma	1 (9)	1 (9)	
Lung carcinoma	1 (9)	1 (9)	
Hodgkin's lymphoma	1 (9)	1 (9)	
Sarcoma	0 (0)	1 (9)	
Cause of death, *n* (%)			.55
Cancer	9 (82)	8 (73)	
Pneumonia	1 (9)	1 (9)	
Myocarditis	1 (9)	0 (0)	
Diffuse alveolar damage	0 (0)	1 (9)	
Intracranial bleed	0 (0)	1 (9)	
Year of autopsy, median (IQR)	2017 (2016–2017)	2013 (2011–2017)	.02
Postmortem interval (h), median (IQR)	20 (17–39)	21 (16–33)	.51
Coronary artery analyzed, LAD/RCA/LM	6/4/1	8/3/0	.49
Coronary atherosclerosis grade, mean±SE	1.8±0.3	1.6±0.2	.59
Plaque/media area ratio, median (IQR)	1.8 (1.4–3.9)	2.2 (1.7–3.7)	1.00

2.3. Statistical analyses

The numbers of $CD3^+$, $CD8^+$, and $CD68^+$ cells and the ratios of the individual cell types (i.e., CD3/CD68, CD8/CD68, and CD8/CD3) in the adventitia and plaque of the ICI-treated patients were compared to the corresponding measurements in the control patients using Wilcoxon test. Potential confounders between the two groups were

Table 2
Inflammatory cells in coronary artery atherosclerotic plaque and adventitia

	ICI	No ICI	*P*
Plaque CD68 (cells/mm^2)	22 (7–123)	36 (13–115)	.41
Plaque CD3 (cells/ mm^2)	24 (10–74)	11 (5–24)	.21
Plaque CD8 (cells/ mm^2)	13 (7–23)	9 (2–17)	.11
Plaque CD3/CD68	1.16 (0.33–1.91)	0.25 (0.11–0.40)	.002
Plaque CD8/CD68	0.56 (0.33–1.00)	0.14 (0.059–0.27)	.042
Plaque CD8/CD3	0.49 (0.30–0.87)	0.68 (0.28–0.92)	.64
Adventitia CD68 (cells/ mm^2)	241 (174–354)	278 (226–372)	.21
Adventitia CD3 (cells/ mm^2)	25 (18–118)	52 (31–136)	.32
Adventitia CD8 (cells/ mm^2)	11 (6–50)	23 (12–60)	.21
Adventitia CD3/CD68	0.13 (0.051–0.48)	0.18 (0.083–0.44)	.90
Adventitia CD8/CD68	0.05 (0.020–0.24)	0.10 (0.035–0.24)	.52
Adventitia CD8/CD3	0.43 (0.23–0.61)	0.51 (0.44–0.84)	.07

Data are expressed as median (interquartile range).

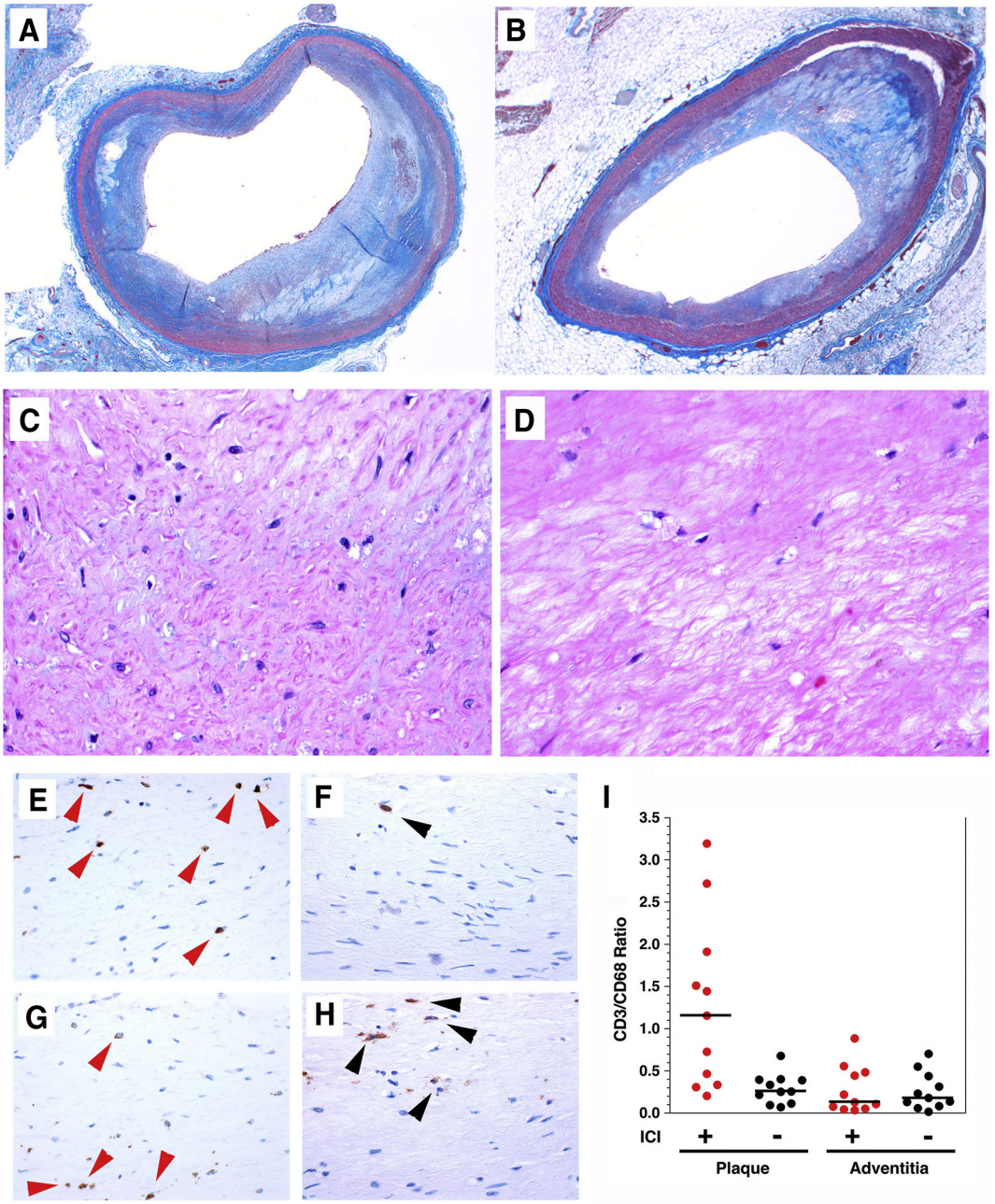

Fig. 1. Immune checkpoint inhibition alters the CD3/CD68 ratio in human atherosclerotic plaques. (A–B) Depicted are trichrome-stained sections of coronary arteries from an ICI-treated cancer patient (A) and a matched control patient (B) at 20× magnification. Within the media (red) is the atherosclerotic plaque (heterogeneously staining), and immediately outside the media is the adventitia (dark blue). Histologic images of the hematoxylin & eosin-stained slides at 400× magnification for the ICI-treated cancer patient (C) and the matched control patient (D) show scattered inflammatory cells in the atherosclerotic plaques. (E–H) Depicted are immunohistochemical stained sections of the coronary artery plaques from the ICI-treated patient (E, G) and the control patient (F, H) stained for the T-lymphocyte marker CD3 (E, F) and the macrophage marker CD68 (G, H). Representative positively stained cells (brown) are indicated with arrowheads. (I) The ratio of the number of $CD3^+$ cells to the number of $CD68^+$ cells was greater in the ICI-treated patients compared with the control patients. There was no difference between the two groups in the CD3/CD68 ratio in the adventitia.

compared using Fisher's Exact Test, χ^2 test, or Wilcoxon test as appropriate. Where indicated in the text, the *P* values were corrected for multiple assessments using the Benjamini–Hochberg method (P_{BH}). In secondary analyses, the relationship of the density of $CD3^+$ cells to the density of $CD68^+$ cells was assessed by linear regression after log transformation. *P* values less than .05, after any necessary adjustment for multiple comparisons, were considered significant.

3. Results

Eleven ICI-treated cancer patients met the study criteria. Four patients were treated with ICI monotherapy, three targeting PD-1 and one targeting PD-L1. The other seven patients received combination ICI therapy, five targeting PD-1 and CTLA-4, one targeting PD-1 and PD-L1, and one targeting PD-1 and LAG-3. These 11 ICI-treated cancer patients were matched with 11 cancer patients who had not received ICI treatment. The patient characteristics are listed in Table 1. There was no significant difference in demographical characteristics, coronary artery disease risk factors, coronary artery disease related medications, or prior PCI. All 22 patients were Caucasian, and one patient in each group had pre-existing rheumatoid arthritis. There was no significant difference between the two groups in the type of cancer or the frequency of non-ICI cancer therapy. One patient in each group had received

chest radiation. There was also no difference between the two groups in the frequency of immunosuppression within 8 weeks of death, which was largely glucocorticoids in both groups. Three of the ICI-treated patients had also received an anti-TNF agent. One of the control patients was treated with both methotrexate and an anti-TNF agent. Seven of the ICI-treated patients were felt clinically to have a complication related to ICI treatment, specifically myocarditis, colitis, gastritis, pneumonitis, nephritis, elevated hepatic enzymes, and/or fluid retention.

There was no significant difference between the two groups in regard to the cause of death (Table 1). There was a slight difference in the year of death between the two groups due to the current widespread use of ICI therapy. There was no significant difference in postmortem interval between the two groups or in the specific coronary artery segment analyzed by immunohistochemistry. There was no significant difference in the overall atherosclerosis grade or in the plaque/media area ratio of the matched coronary artery segments. For each group, the 11 plaques consisted of 2 type III intermediate lesions, 1 type IV atheroma, 5 type V fibroatheromas, 2 type VII predominantly calcified plaques, and 1 type VIII predominantly fibrous plaque as defined previously [18]. None of the plaques in either group showed features of acute or recent disruption.

There was no significant difference between the two groups in the absolute numbers of $CD3^+$, $CD8^+$, or $CD68^+$ cells in the atherosclerotic plaques (Table 2, Fig. 1). There was, however, a significant increase in the ratio of $CD3^+$ cells to $CD68^+$ cells (CD3/CD68) in the plaques of the ICI-treated cancer patients compared with the plaques of the control cancer patients (P=.002). This difference remained significant after adjusting for multiple assessments (P_{BH}=.02). Correspondingly, there was a trend toward an increased CD8/CD68 ratio in the plaques of the ICI-treated cancer patients compared with the plaques of the control cancer patients (P=.042). However, this difference was not significant after adjusting for multiple assessments (P_{BH}=.25). There was no significant difference in the CD8/CD3 ratio in the plaques between the two groups. There was also no difference between the two groups in the absolute cell numbers or ratios in the adventitia. The increased CD3/CD68 ratio in the ICI-treated patients was such that the inflammation in the plaques of 6 of the 11 patients was lymphocyte predominate in contrast to the macrophage-predominate inflammation typically found in atherosclerotic plaques (Fig. 1).

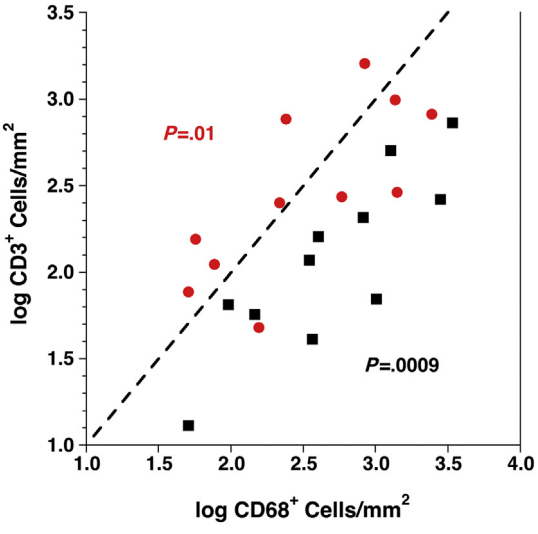

Fig. 2. Relationship of $CD3^+$ lymphocyte infiltration to $CD68^+$ macrophage infiltration. Shown are plots of the $CD3^+$ T-cell density after log transformation versus the $CD68^+$ macrophage density after log transformation for the patients treated with ICI (red circles) and the control patients (black squares). For both sets of patients, the density of $CD3^+$ T cells correlates with the density of $CD68^+$ macrophages. The P values were obtained from linear regression. The dashed line represents the unity line, above which the inflammatory infiltrate is lymphocyte predominant.

To gain insight into why the CD3/CD68 ratio was significantly different between the groups and the absolute density of $CD3^+$ T cells was not, the relationship between the density of $CD3^+$ T Cells and the density of $CD68^+$ macrophages was assessed by linear regression (Fig. 2). For both groups, the density of $CD3^+$ T cells was correlated with the density of $CD68^+$ macrophages. However, the density of macrophages was highly variable in each group, extending two orders of magnitude.

4. Discussion

The key observation in this study is that checkpoint inhibition alters the inflammatory composition of human atherosclerosis, increasing the ratio of T-lymphocytes to macrophages. Several studies have shown that, in stable atherosclerotic plaques, lymphocytes are present but are normally less frequent than macrophages, with lymphocyte/macrophage ratios typically ranging from 0.1 to 0.6 [19–21]. However, in unstable plaques, the lymphocyte/macrophage ratio increases such that there are roughly equal numbers of the two cell types [21,22]. Thus, the increased CD3/CD68 ratio observed here, in the setting of therapeutic ICI, may indicate a shift in the plaque inflammatory cell composition to one that promotes atherosclerosis and/or plaque instability. None of the plaques in either group showed features of acute or recent disruption. One of the fibroatheromas in the ICI group had a focally thin fibrous cap, but this particular plaque did not show an increased CD3/CD68 ratio. ICI did not significantly alter the proportion of $CD3^+$ T cells expressing CD8. The CD8/CD3 ratios observed here in coronary artery atherosclerotic plaques are similar to those reported previously in aortic and carotid plaques [19,22–28].

The role of lymphocytes in atherosclerosis has been extensively studied in murine models, which have shown that lymphocytes of the Th1 lineage promote atherosclerotic lesion development [29–31]. However, regulatory T cells are antiatherogenic in murine models, and the roles of lymphocytes of the Th2 and Th17 lineages are less clear. In murine models, interfering with immune coinhibitory pathways has been demonstrated to promote atherosclerotic lesion development. In $Ldlr^{-/-}$ mice, blocking PD-1 or deficiency of PD-1 or its ligands accelerated atherosclerosis and increased lymphocyte infiltration into the plaques [32,33]. In hypercholesterolemic mice, administration of antibodies that block CTLA-4 also resulted in increased atherosclerosis [34]. Conversely, $Apoe^{-/-}$ mice with transgenic constitutive CTLA-4 expression on T-lymphocytes had reduced atherosclerosis [35]. Lymphocytes of the Th1 lineage also have the ability to decrease plaque stability by secreting interferon-γ [14]. Overall, these prior mouse and human studies suggest that the increased CD3/CD68 ratio observed here in the setting of ICI treatment may be promoting atherosclerotic lesion development and/or plaque instability. However, clarification of the definitive role of this altered plaque inflammatory infiltrate in the setting of ICI treatment will require more extensive cellular analysis and long-term clinical follow-up.

This study has several limitations. This was an autopsy study, and the patients analyzed here may not be representative of all cancer patients. The number of patients analyzed was relatively small, and the influence of potential confounders cannot be entirely excluded. There is a clear but nonsignificant trend towards increasing $CD3^+$ T cells in the atherosclerotic plaques with ICI treatment; however, given the relationship of the $CD3^+$ T cell density to the macrophage density and given the high variability of

the macrophage density, a larger sample size will be required to establish the statistical significance of the absolute $CD3^+$ T cell density in this setting. Also, due to the nature of the pathologic material available for analysis, additional studies to more fully characterize the inflammatory cells, such as flow cytometry and FoxP3 immunohistochemistry, could not be performed.

In this series of cancer patients, ICI treatment was associated with an altered coronary artery atherosclerotic plaque inflammatory cell composition characterized by an increased ratio of $CD3^+$ T cells to the $CD68^+$ macrophages. Further studies will be required to delineate the clinical significance of this pathologic change.

Acknowledgments

There were no sources of external funding.

Declaration of competing interest

There are no conflicts of interest to report.

References

[1] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252–64.
[2] Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for cancer. Ann Rev Immunol 2016;34:539–73.
[3] Antoni Ribas, Wolcho Jedd D. Cancer immunotherapy using checkpoint blockade. Science 2018;359:1350–5.
[4] Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer 2016;54:139–48.
[5] Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med 2018;378:158–68.
[6] Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med 2016;375:1749–55.
[7] Mahmood SS, Fradley MG, Cohen JV, Nohria A, Reynolds KL, Heinzerling LM, et al. Myocarditis in patients treated with immune checkpoint inhibitors. J Am Col Cardiol 2018;71:1755–64.
[8] Salem JE, Manouchehri A, Moey M, Lebrun-Vignes B, Bastarache L, Pariente A, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study. Lancet Oncol 2018;19:1579–89.
[9] Glass CK, Mitchell RN. Winning the battle, but losing the war: mechanisms and morphology of cancer-therapy-associated cardiovascular toxicity. Cardiovasc Pathol 2017;30:55–63.
[10] Touat M, Maisonobe T, Knauss S, Ben Hadj Salem O, Hervier B, Auré K, et al. Immune checkpoint inhibitor-related myositis and myocarditis in patients with cancer. Neurology 2018;91:e985–94.
[11] De Martin E, Michot JM, Papouin B, Champiat S, Mateus C, Lambotte O, et al. Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. J Hepatol 2018;68:1181–90.
[12] Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 2015;15:486–99.
[13] Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. Immunity 2017;47:621–34.
[14] Gistera A, Hansson GK. The immunology of atherosclerosis. Nat Rev Nephrol 2017;13:368–80.
[15] Libby P, Loscalzo J, Ridker PM, Farkouh ME, Hsue PY, Fuster V, et al. Inflammation, immunity, and infection in atherothrombosis, JACC review topic of the week. J Am Col Cardiol 2018;72:2071–81.
[16] Stone JR, Bruneval P, Angelini A, Bartoloni G, Basso C, Batoroeva L, et al. Consensus statement on surgical pathology of the aorta from the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology: I. Inflammatory diseases. Cardiovasc Pathol 2015;24:267–78.
[17] Li Y, Stone JR. The impact of splenectomy on human coronary artery atherosclerosis and vascular macrophage distribution. Cardiovasc Pathol 2016;25:453–60.
[18] Stary HC. Natural history and histological classification of atherosclerotic lesions, an update. Arterioscler Thromb Vasc Biol 2000;20:1177–8.
[19] Xu Q, Oberhuber G, Gruschwitz M, Wick G. Immunology of atherosclerosis: cellular composition and MHC class II antigen expression in aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens. Clin Immunol Immunopathol 1990;56:344–59.
[20] Vince DG, Demirkaya O, Cothren RM, Cornhill JF. Quantitative analysis of inflammatory cells in aortic atherosclerosis of young adults. Cardiovasc Pathol 1996;5:3–10.
[21] Rohm I, Atiskova Y, Drobnik S, Fritzenwanger M, Kretzschmar D, Pistulli R, et al. Decreased regulatory T cells in vulnerable atherosclerotic lesions: imbalance between pro- and anti-inflammatory cells in atherosclerosis. Mediators Inflamm 2015;2015:364710.
[22] Jonasson L, Holm J, Skalli O, Bondjers G, Hansson K. Regional accumulation of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis 1986;6:131–8.
[23] Stemme S, Holm J, Hansson GK. T lymphocytes in human atherosclerotic plaques are memory cells expressing CD45RO and the integrin VLA-1. Arterioscler Thromb 1992;12:206–11.
[24] Kleindienst R, Xu Q, Willeit J, Waldenberger FR, Weimann S, Wick G. Immunology of atherosclerosis. Demonstration of heat shock protein 60 expression and T lymphocytes bearing alpha/beta or gamma/delta receptor in human atherosclerotic lesions. Am J Pathol 1993;142:1927–37.
[25] Curry AJ, Portig I, Goodall JC, Kirkpatrick PJ, Gaston JS. T lymphocyte lines isolated from atheromatous plaque contain cells capable of responding to Chlamydia antigens. Clin Exp Immunol 2000;121:261–9.
[26] Millonig G, Malcom GT, Wick G. Early inflammatory-immunological lesions in juvenile atherosclerosis from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY)-Study. Atherosclerosis 2002;160:441–8.
[27] Rossmann A, Henderson B, Heidecker B, Seiler R, Fraedrich G, Singh M, et al. T-cells from advanced atherosclerotic lesions recognize hHSP60 and have a restricted T-cell receptor repertoire. Exp Gerontol 2008;43:229–37.
[28] van Dijk RA, Duinisveld AJ, Schaapherder AF, Mulder-Stapel A, Hamming JF, Kuiper J, et al. A change in inflammatory footprint precedes plaque instability: a systematic evaluation of cellular aspects of the adaptive immune response in human atherosclerosis. J Am Heart Assoc 2015;4:e001403.
[29] Buono C, Binder CJ, Stavrakis G, Witztum JL, Glimcher LH, Lichtman AH. T-bet deficiency reduces atherosclerosis and alters plaque antigenspecific immune responses. Proc Natl Acad Sci U S A 2005;102:1596–601.
[30] Spitz C, Winkels H, Bürger C, Weber C, Lutgens E, Hansson GK, et al. Regulatory T cells in atherosclerosis: critical immune regulatory function and therapeutic potential. Cell Mol Life Sci 2016;73:901–22.
[31] Ley K, Gerdes N, Winkels H. ATVB distinguished scientist award: how costimulatory and coinhibitory pathways shape atherosclerosis. Arterioscler Thromb Vasc Biol 2017;37:764–77.
[32] Gotsman I, Grabie N, Dacosta R, Sukhova G, Sharpe A, Lichtman AH. Proatherogenic immune responses are regulated by the PD-1/PD-L pathway in mice. J Clin Invest 2007;117:2974–82.
[33] Bu DX, Tarrio M, Maganto-Garcia E, Stavrakis G, Tajima G, Lederer J, et al. Impairment of the programmed cell death-1 pathway increases atherosclerotic lesion development and inflammation. Arterioscler Thromb Vasc Biol 2011;31:1100–7.
[34] Ewing MM, Karper JC, Abdul S, de Jong RC, Peters HA, de Vries MR, et al. T-cell co-stimulation by CD28-CD80/86 and its negative regulator CTLA-4 strongly influence accelerated atherosclerosis development. Int J Cardiol 2013;168:1965–74.
[35] Matsumoto T, Sasaki N, Yamashita T, Emoto T, Kasahara K, Mizoguchi T, et al. Overexpression of cytotoxic T-lymphocyte-associated antigen-4 prevents atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2016;36:1141–51.