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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Recruitment of Immune Cells Across Atrial Endocardium in Human Atrial Fibrillation

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Background: Although clinical studies have suggested a link between inflammation markers and atrial fibrillation (AF), it is still unclear whether local immunologic responses actually exist in human atria during AF.

Methods and Results: To address this point, human left appendages were obtained from 16 patients who underwent cardiac surgery (5 with sinus rhythm (SR) and 11 with AF) and subjected to immunohistochemical analysis. In all the AF specimens, adhesion and migration of CD45-reactive cells were consistently observed predominantly in the atrial endo- and subendomyocardium and more prominently than in SR. Most of them were immunologically active CD68-positive macrophages, whereas CD3-positive T cells infiltrated to a lesser extent. Scavenger-receptor A staining revealed maturation of macrophages not in the endocardium but in the midmyocardium, a gradient from endo- to midmyocardium. In the endocardium, along with adhesion molecules (intracellular adhesion molecule-1 and vascular cell adhesion molecule-1), a chemotactic protein-1, which facilitates the recruitment, was more abundantly expressed in AF than in SR. Cytokines including transforming growth factor-β and interleukin-6 were frequently expressed by these macrophages.

Conclusions: These observations collectively imply active adhesion and recruitment of macrophages across the endocardium in human fibrillating atria, thereby supporting the concept of local immunologic inflammatory responses around the atrial endocardium of AF. (*Circ J* 2010; **74:** 262–270)

Key Words: Atrial fibrillation; Endocardium; Inflammation

Atrial fibrillation (AF) is the most common type of sustained tachyarrhythmia affecting approximately 0.9% of the population and it is well known to be associated with significant morbidity and mortality.[1–4] Although there is an increasing need for improved management of AF to reduce the health burden, the currently available pharmacotherapy has been shown to be relatively inefficient.[5–7]

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In the broad spectrum of cardiovascular conditions, including coronary artery disease, diabetes mellitus and hypertension, there is now well-known evidence linking inflammation to their pathophysiology.[8–11] Similarly, the hypothesis that inflammatory processes are involved in AF pathogenesis has attracted renewed focus because of combined clinical, epidemiological and pharmacological observations.[12,13] In fact, concentrations of serum hs-CRP and interleukin (IL)-6 have been noted to be higher among patients with AF compared with controls in sinus rhythm (SR).[14–16] Treatment with glucocorticoids, statins and blockers of the renin-angiotensin system seems to reduce AF development or recurrence, for which anti-inflammatory effects of these agents may contribute to some extent.[17–22]

However, there is limited evidence demonstrating a direct link between inflammation and AF compared with vascular inflammation.[11] In AF, precise mechanisms of increased circulating inflammation markers are still uncertain. Therefore, we examined whether active inflammation and immune responses actually exist in the human atrium with or without AF.

Methods

Patients

Left atrial appendages were obtained as surgical specimens from patients undergoing the Maze procedure and repair of nonrheumatic mitral valve regurgitation or atrial septal defect (mean age, 60.9±10.1 years). The study included 5 patients with SR with a history of paroxysmal AF and 11 patients with persistent AF. The heart rhythm was ascertained from electrocardiograms just before the operation. Table 1 describes the detailed clinical

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Table 1. Patient Characteristics

	Sex	Age	AF history (months)	HD	EF (%)	LAD (mm)	Drug therapy
SR							
	F	57		ASD	77	41	B,L,W
	M	69		MR	60	47	A,B,L,W
	M	63		ASD	76	48	B,L,W
	M	64		MR	61	45	A,B,L,W
	M	36		MR	55	68	A,B,L,W,AD
Total	4M/1F	57.8±12.9			65.8±10.0	49.8±10.5	
AF							
	M	56	12	MR	65	43	A,B,L,W
	F	49	12	MR	67	44	L,N,W
	M	63	26	MR	62	57	A,B,L,W
	M	59	28	MR	76	58	A,C,L,W,AD
	M	56	31	MR	60	45	A,B,C,L,W
	F	59	48	MR	64	56	A,B,L,W,AD
	F	73	71	MR	57	62	A,L,W
	F	73	117	MR/TR	67	58	B,C,L,W
	M	66	135	MR	72	61	A,B,W
	M	55	180	MR	51	60	A,C,L,W
	M	77	180	MR/AR/TR	59	66	A,C,D,L,W
Total	7M/4F	62.3±8.9	76.4±65.3		63.6±7.0	55.5±7.9	

There were no significant differences in age and LAD between SR and AF, although they were both tended to be greater in AF than in SR.
AF, atrial fibrillation; HD, heart disease; EF, ejection fraction; LAD, left atrial dimension; SR, sinus rhythm; A, ACE or ARB inhibitors; B, β-blockers; C, calcium-channel blockers; L, loop diuretics; N, nitrates; W, warfarin; AD, antiarrhythmic drug.

Table 2. Antibodies Used in This Study

Antibody	Cat#	Manufacturer
CD45	422721	Nichirei Biosciences
HLA-DR β	ab20184	Abcam
CD68	KT013	Trans Genic
Macrophage scavenger receptor	KT022	Trans Genic
CD3	413591	Nichirei Biosciences
CD8	413201	Nichirei Biosciences
MCP-1	sc-1785	Santa Cruz Biotechnology
ICAM-1 (CD54)	205-020	Ancell Corporation
VCAM-1 (CD106)	BBA5	R&D Systems
IL-6	RB206	Dako Cytomation
TGF-β	sc-146	Santa Cruz Biotechnology

IL, interleukin.

characteristics of the patients. All patients did not have previous myocardial infarction, febrile disorders, systemic inflammatory diseases, malignancy or chronic renal failure. This investigation conforms to the principles outlined in the Declaration of Helsinki. All patients gave written informed consent and the study was approved by an institutional review board.

Histology and Immunohistochemistry

Blocks of tissues were optimal cutting temperature compound-embedded and immediately frozen in liquid nitrogen after resection. Frozen cryostat sections (8-μm thick) were cut, air-dried, fixed in acetone and then evaluated with standard protocols for staining with H&E and Masson's trichrome. Immunostaining was carried out in sequential sections by Dako EnVision+ Systems (Dako) with primary antibodies listed in Table 2.

Infiltration of immune cells in the atrium was examined by light microscopy with immunostained images at a magnification of 100×captured with a digital camera (Nikon). Using Image Pro-plus software (Mediacybernetics, Carlsbad, CA, USA), the percentage of positive staining representing immunoreactivity was recorded and corrected by the total section areas. The mean value was obtained from 10 blindly selected different fields for each patient.

Immunofluorescence labeling for microscopy was carried out by treatment with Alexia Flour 488 or 568– conjugated goat anti-rabbit antibodies or goat anti-mouse antibodies (Molecular Probes, 1:300 dilution). Immunofluorescence-labeled samples were examined with a Pascal Zeiss laser scanning microscope. The green channel had an excitation of 488 nm and an emission of 525 nm. The red channel had an excitation of 594 nm and an emission 620 nm. A lack of any cross-talk between the channels was established. Control

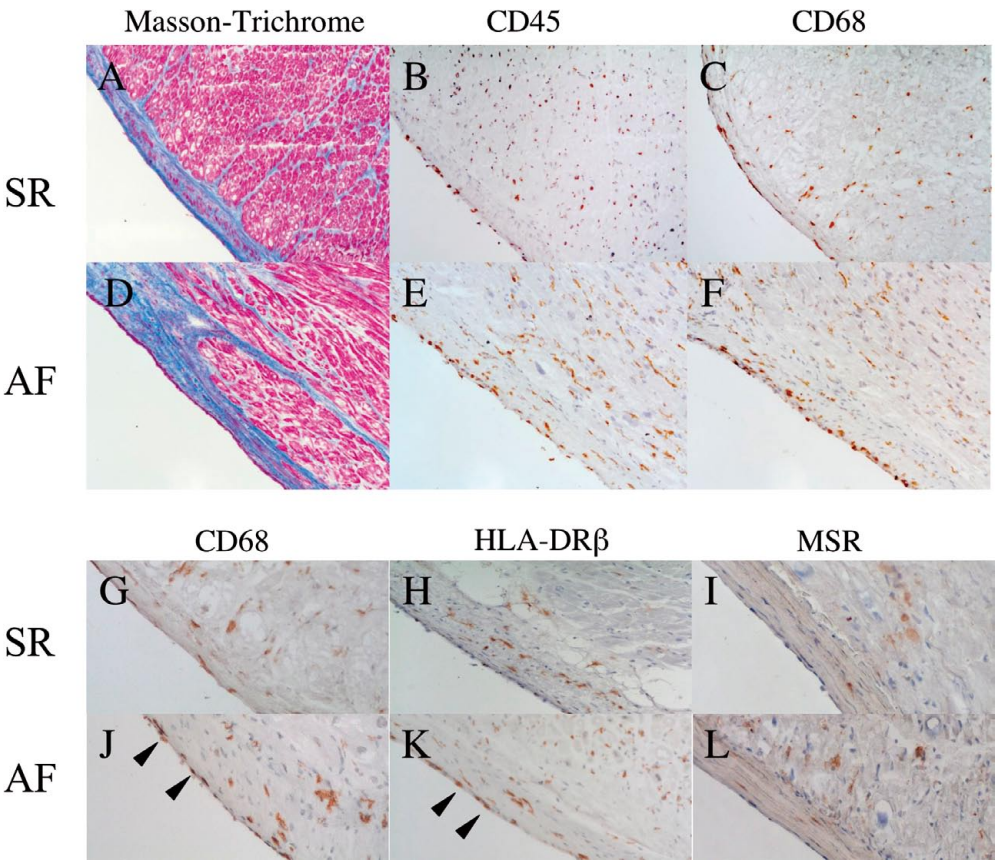

Figure 1. Masson's trichrome staining (**A**, **D**) of left atrial tissue from patients with sinus rhythm (SR: **A**) and with atrial fibrillation (AF: **D**). Fibrosis and intercellular space were more remarkable in AF than in SR. Immunohistological results in left atrial tissue from patients with SR (**B**, **C**, **G**–**I**) and patients with AF (**E**, **F**, **J**–**L**). CD45 staining identified gathering leukocytes around the atrial endo- and subendocardium, which was more evident in AF (**B**, **E**). The distribution of leukocytes was quite similar to that of CD68-positive macrophages (**C**, **F**). Magnified images identified the adhesion of macrophages (**G**, **J**) and immunologically active leukocytes (**H**, **K**) to the atrial endocardium, more prominently in AF than in SR. In AF, apparent endocardial cells were positive for CD68 and HLA-DRβ (arrow in **J**, **K**). The endocardium-attached cells were negative for a scavenger-receptor A antibody (MSR), which identified matured macrophages in the midmyocardium (**I**, **L**). (**B**, **E**) for CD45; (**C**, **F**, **G**, **J**) for CD68; (**H**, **K**) for HLA-DRβ; (**I**, **L**) for scavenger-receptor A. Original magnification ×100 in (**A**–**F**), and ×400 in (**G**–**L**).

experiments carried out by incubation with secondary antibodies only did not show positive staining under the same experimental conditions.

ELISA Assay

Snap frozen blocks of tissues were homogenized in TE buffer and centrifuged. The aqueous extracts were subjected to a specific ELISA assay. All of the tissue samples were run in parallel for total protein content (Pierce, Rockford, IL, USA), and the results were standardized by expressing values as ng or pg of a particular protein per mg total protein.

Statistical Analysis

Values were expressed as mean±SD and compared using the unpaired t-test. Statistical significance was set at a P-value of <0.05.

Results

Light Microscopy of Human Atria With SR and AF

Light microscopic analysis showed a loss of contractile elements in some atrial myocytes and interstitial fibrosis to a varying degree in all specimens (**Figures 1A**, **D**). Patients with AF showed a greater extent of sarcomere loss and fibrosis than those with SR (fibrotic area: 9.9±2.2 vs 4.0±0.8 %, $P<0.05$). In addition, the intercellular space was more hyperexpanded in AF than in SR, as shown in **Figure 1**. These observations were all consistent with previous studies.[23–25]

Immune Cells in Human Fibrillating Atria

Immunohistochemistry revealed the inflammatory infiltration of immune cells, leukocytes positive for CD45, in human atria with AF in all specimens examined (**Figure 1E**), while it was also observed in the atria with SR to a lesser extent (**Figure 1B**). Their distribution was proved inhomogeneous, and the endo- and subendomyocardium of the atrium were more subjected to their infiltration than the midmyocardium (**Figures 1B**, **E**). Sequential sections suggested that the distribution of these CD45-positive leucocytes resembled that of cells positive for the macrophage-specific CD68 antibody (**Figures 1C**, **F**). To quantify the infiltration of immune cells, the acquired images for CD45 and CD68 antibodies were

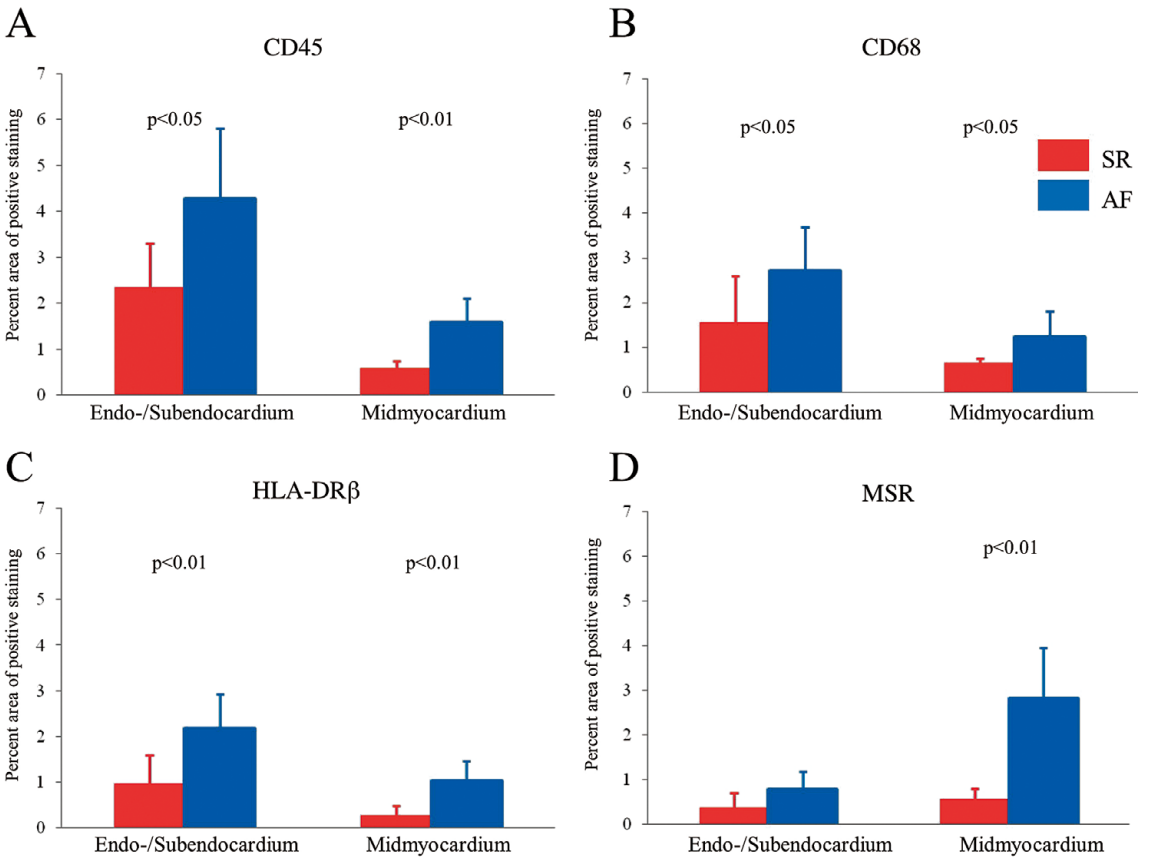

Figure 2. The percentage area of positive staining representing immunoreactivity for immune cells. (**A**) CD45-positive leukocytes were observed more extensively in the endo-/subendocardium than in the midmyocardium both in sinus rhythm (SR) and atrial fibrillation (AF). The percentage area of positive staining was significantly greater in AF than in SR. (**B**) CD68 staining revealed that the distribution of CD68-positive macrophages was quite similar to that of CD45-positive cells. (**C**) HLA-DRβ-positive cells showed a similar distribution and infiltration in the atrium. (**D**) In contrast, scavenger-receptor A (MSR) positive cells showed quite a different distribution, more prominent in the midmyocardium than in the endo-/subendocardium. In the midmyocardium, MSR-positive cells were more observed in AF than in SR.

analyzed, and the results revealed that the infiltration of leucocytes and macrophages resembled each other in their distribution (more prominent in the endo/subendomyocardium than in the midmyocardium) and were also significantly greater in AF than in SR (**Figures 2A,B**).

Recruitment of Immune Cells Across Atrial Endocardium

Magnified images of the specimens identified the adhesion of many monocytes/macrophages to the atrial endocardium and showed their staged infiltration at several layers of the endocardium (**Figure 1J**). And, interestingly, HLA-DRβ staining showed that some of the apparent endocardial cells were also positive for this antibody, suggesting that they were immunologically active leukocytes (arrow in **Figure 1K**). These findings were more evident in AF (**Figure 2C**) and could be well explained by the fact that immunologically active monocytes/macrophages attached to and migrated into the atrium across the endocardium more in AF than in SR.

Immunofluorescence images supported this hypothesis, showing that most of the CD45-positive cells were positive also for CD68 (**Figure 3A**) and that the CD68-positive macrophages were positive also for the HLA-DRβ antibody (**Figure 3B**). Scavenger-receptor A (MSR) is known to be one of the maturation markers of macrophages.[26] The antibody labeling of the specimens showed another piece of evidence for the recruitment of monocytes/macrophages across the atrial endocardium. The receptor labeling was remarkably inhomogeneous in the atrium and scarce in the endo- and subendocardium, suggesting a site-specific existence of immature and mature macrophages (**Figures 1I,L**). In the midmyocardium of the atrium, almost all macrophages were positive for scavenger-receptor A (matured macrophages, **Figure 3C**). In contrast, in the endo- and subendocardium, many macrophages did not express scavenger-receptor A (immature macrophages, **Figure 3D**), implying that immature macrophages migrated from the endocardium to the midmyocardium through the processes of maturation. The inhomogeneous distribution of MSR-positive cells was also confirmed quantitatively using immunohistochemical images stained with scavenger-receptor A (**Figure 2D**). The receptor-positive macrophages were observed much more in the midmyocardium than in the endo- and subendocardium, and more in AF than in SR.

Innate and Acquired Immune Responses in Human Fibrillating Atria

To identify other cell types infiltrating the atrium, CD3 and

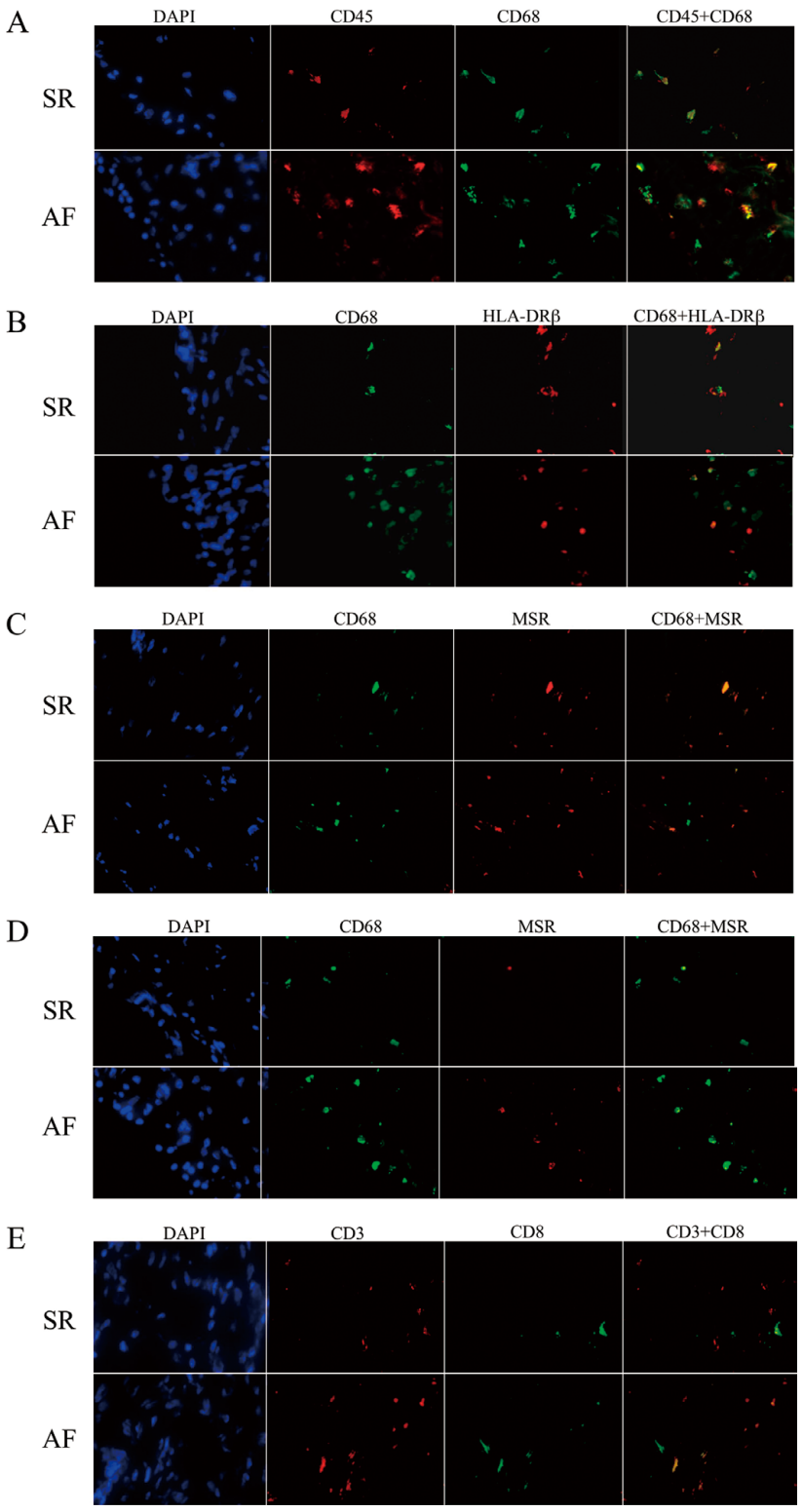

Figure 3. Immunofluorescent stainings of human atrial samples. The DNA binding dye (DAPI, blue) enables visualization of the cellular nucleus, and thereby the cell distribution in each field. (**A**–**E**) Upper and lower panels show pictures from patients with sinus rhythm (SR) and atrial fibrillation (AF), respectively. (**A**) Colocalization of CD45 (red) and CD68 (green) with overlapping expressions (yellow). (**B**) Colocalization of CD68 (green) and HLA-DRβ (red). Most of the macrophages had the HLA-DRβ antigen, indicating that they were immunologically active. (**C**) Colocalization of CD68 (green) and scavenger-receptor A (MSR, red) with overlapping expressions (yellow) in the atrial midmyocardium. Most of the macrophages were matured and expressed MSR. (**D**) Discordant expression of CD68 (green) and scavenger-receptor A (MSR, red) in the endo- and subendocardium. Few macrophages expressed MSR in these areas. (**E**) Colocalization of CD3 (red) and CD8 (green) with overlapping expressions (yellow).

CD8 staining was carried out and showed that CD3-positive T cells also infiltrated into the atrium (**Figure 3E**), although the degree was much smaller than that of the macrophages. This finding suggests that not only do innate immune responses operate in human atria with AF, but acquired immune responses also, at least in part, operate in human atria with AF. The infiltration was more evident in AF than SR, as was with that of macrophages. Some of the CD3-positive T cells were also positive for CD8 antibody (**Figure 3E**).

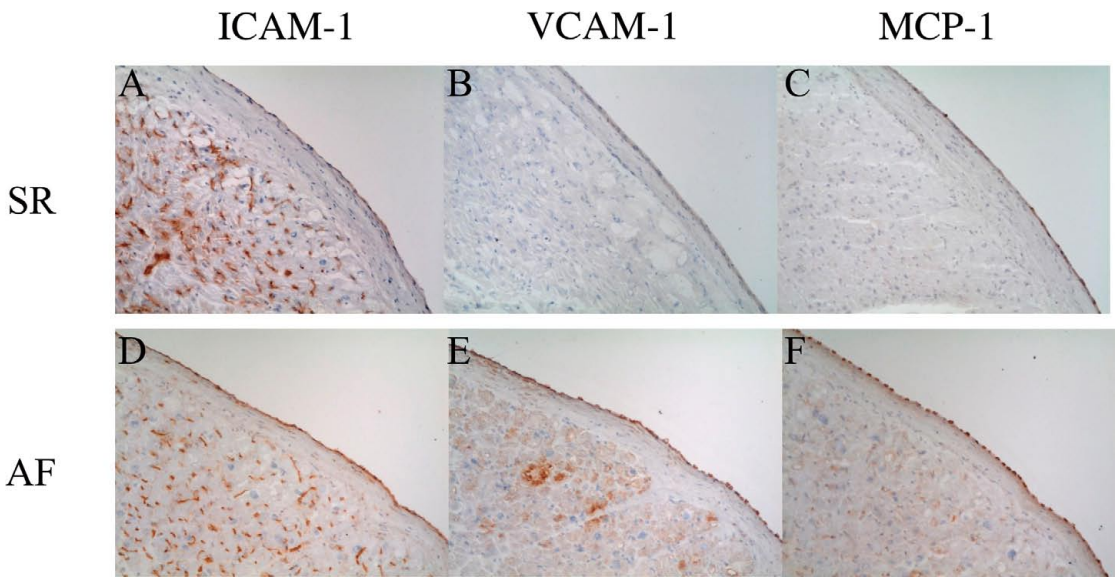

Figure 4. Immunostaining of human atrial samples with (**A**,**D**) intracellular adhesion molecule-1 (ICAM-1), (**B**,**E**) vascular adhesion molecule-1 (VCAM-1) and (**C**,**F**) monocyte chemotactic protein-1 (MCP-1) antibodies. The upper and lower panels show pictures from patients with sinus rhythm (SR) and atrial fibrillation (AF), respectively. (**A**,**D**) ICAM-1 was observed in the vasculatures and atrial endocardium. While the signals were similar in the vasculatures in SR and AF, endocardial expression was more evident in AF. (**B**,**E**) VCAM-1 expression was observed more prominently in the vasculature and endocardium in AF. (**C**,**F**) MCP-1 was predominantly expressed in the atrial endocardium both in SR and AF.

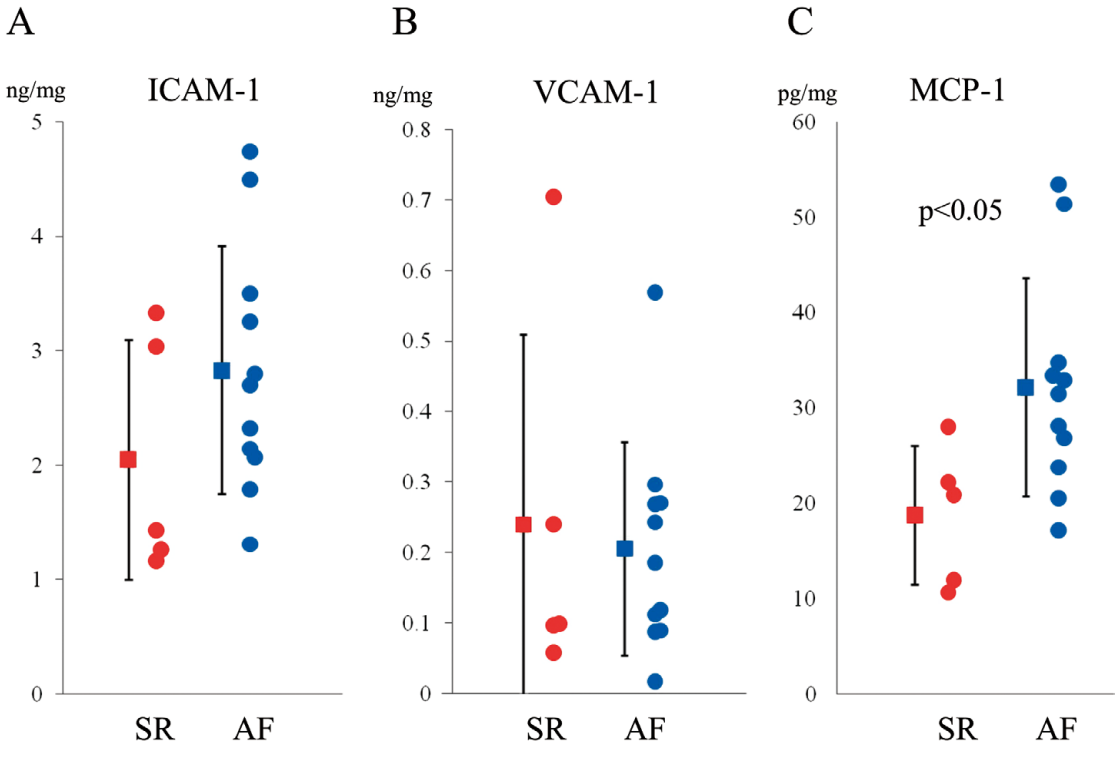

Figure 5. Content of (**A**) intracellular adhesion molecule-1 (ICAM-1), (**B**) vascular adhesion molecule-1 (VCAM-1) and (**C**) monocyte chemotactic protein-1 (MCP-1) in tissue homogenates by the ELISA assay. Although ICAM-1 and VCAM-1 contents were not significantly different between SR and AF, MCP-1 content was significantly greater in AF than in SR ($P<0.05$).

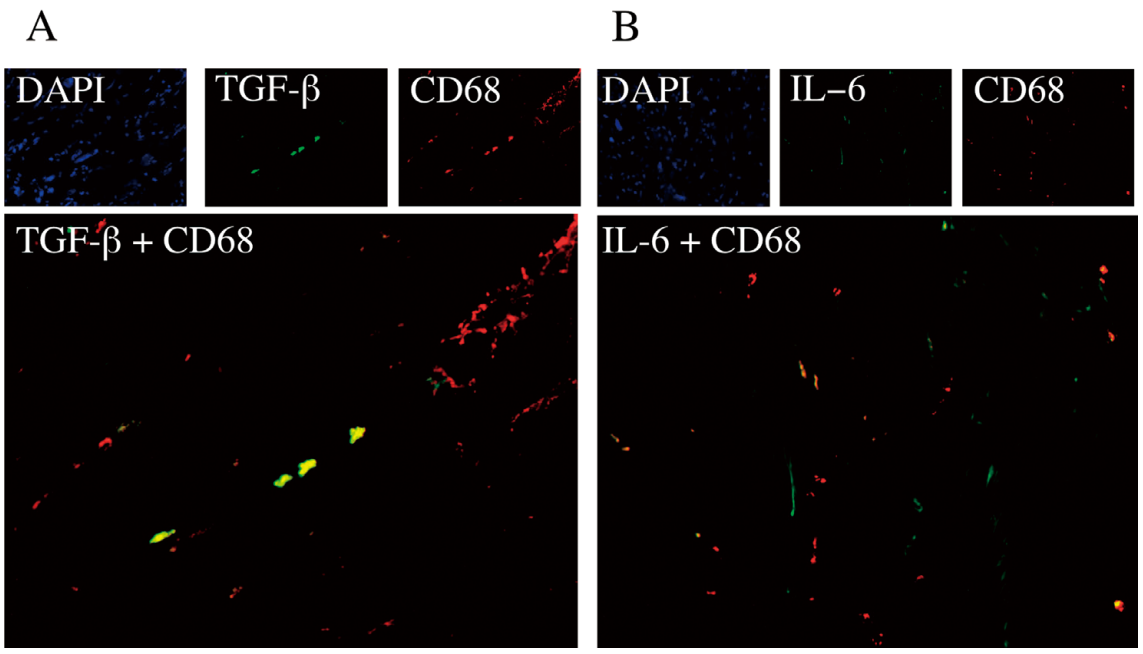

Figure 6. Expression of (**A**) transforming growth factor (TGF)-β and (**B**) interleukin (IL)-6 in atria with atrial fibrillation by immunofluorescent staining. The DNA binding dye (DAPI, blue) enables visualization of the cellular nucleus. (**A**,**B**) The green signal shows the expression of TGF-β and IL-6, respectively. The red signal shows the distribution of CD68-positive macrophages. (**A**) Colocalization of TGF-β and macrophages (yellow signal in the lower panel). (**B**) Most of IL-6 was detected colocalized with macrophages (yellow signal).

Adhesion and Chemotactic Molecules Expressed in Atrial Endocardium

In the vasculatures, recruitment of immune cells to the vessel wall across an endothelium requires activation of the endothelium, including expression of adhesion and chemotactic molecules.[27–29] We sought to examine whether similar processes occurred in the endocardium of human atria. Immunohistochemical analysis showed that ICAM-1 expression was observed not only in the vasculature of the atrium, but also in the atrial endocardium (**Figures 4A**,**D**). Moreover, VCAM-1 expression was more abundant in the endocardium than in the atrial vessels in AF (**Figure 4E**), although its expression was scarce in SR. These observations would suggest that activation of the endocardium interacts actively with immature macrophages, leading to dominant localization of immature macrophages in the subendocardium. However, quantitative analysis using an ELISA assay with tissue homogenates could not detect any significant differences in the expression between SR and AF (**Figures 5A**,**B**) and it is partly because these adhesion molecules were expressed both in the vasculature and the atrial endocardium of the specimens.

After adhesion of immune cells, their transmigration into the endothelial layer is known to be governed by chemotactic proteins, the most characterized of which is monocyte chemotactic protein-1 (MCP-1).[29,30] MCP-1 was more abundantly expressed in the atrial endocardium than in the vasculature both in SR and AF (**Figures 4C**,**F**). Comparing its expression between them showed that AF was more associated with the abundant expression of MCP-1 than SR, which was also confirmed by the ELISA assay with tissue homogenates (**Figure 5C**).

Expression of Transforming Growth Factor (TGF)-β and IL-6 in Atria With AF

In human atherosclerosis, many cytokines secreted from immune cells are known to be involved in lesion formation or thrombus formation. Therefore, we sought to determine whether the prominent recruitment of immune cells in atria with AF is associated with the local expression of cytokines including TGF-β and IL-6. **Figures 5A**,**B** shows the location of macrophages and expression of TGF-β and IL-6 by immunofluorescence images, respectively. These figures clearly showed that some of the macrophages that migrated into the atria highly expressed these cytokines. While TGF-β positive macrophages were detected in only 1 of 5 patients with SR, they were detected in 7 of 11 patients with AF. Similarly, IL-6 positive macrophages were detected in only 1 patient with SR, and they were significantly more detected in the 9 patients with AF (both $P<0.05$).

Discussion

The major findings of the present study were as follows: (1) In human left atrial appendages with AF, immune cells infiltrated atria predominantly in the endo- and subendocardium, more evidently in AF than in SR; (2) the cells were mostly composed of immunologically active monocytes/macrophages in addition to a smaller number of CD3+ T cells; (3) the atrial endocardium expressed abundant ICAM-1, VCAM-1 and MCP-1, possibly providing feasible states for adhesion and transmigration of the immune cells; (4) in accordance with these alterations of the atrial endocardium, many immature macrophages were observed attached to the surface of, as well as buried in the layers of, the atrial endocardium,

with their maturation occurring in the midmyocardium; and (5) the migrated macrophages expressed TGF-β or IL-6 in atria more frequently in AF. These observations, all taken together, suggest that recruitment of immune cells, mostly monocytes/macrophages, occurred predominantly across the atrial endocardium during AF.

AF and Inflammation

There is mounting clinical evidence to support the influence of inflammation in the pathogenesis of AF. Numerous studies have demonstrated increases in serum or plasma inflammation markers in AF patients.[22] Serum concentration of hs-CRP, one of the most sensitive markers for inflammation, has been known to be higher in AF patients than in controls and also to be higher in persistent AF than in paroxysmal AF.[14] Levels of serum IL-6 concentrations, a cytokine that is produced by immune cells and endothelial cells, are also noted to be increased in AF patients compared with healthy participants.[16] Although the precise mechanism for the increased circulating markers is uncertain, the results might reflect active participation of local inflammatory responses during AF and/or systemic inflammation caused by patient comobidities.

Irrespective of these results, there have been few histological studies that investigate the association between AF and inflammation, which would provide direct evidence for the link. Results of atrial biopsies from patients with AF have demonstrated inflammatory infiltrates and oxidative damage within the atrial tissue.[31] Other studies have also demonstrated that inflammatory CD45-positive cells infiltrated the right and left atrium with AF,[32,33] as is consistent with the present results. In fact, both in SR and AF, the percentage area of CD45-positive staining in the endo/subendocardium in the patients of the present study was almost identical to that reported recently.[33] These previous studies have emphasized the role of occult myocarditis in AF, and therefore data is limited about the distribution or cell types of the leukocytes in atria.

In the present study, we examined whether active and local inflammatory responses occur in human atria with or without AF, using immunohistochemical analysis. Our observations not only supported the presence of immunologically active immune cells in human atria with AF, but further identified the immune cell types and their distribution. The immune cells were mostly composed of active monocytes/macrophages, which were abundantly attached to the surface of the endocardium and thereafter apparently transmigrated into the myocardium. Moreover, the infiltration of the immunocytes seemed to be affected by AF duration in the AF group, suggesting that the inflammation occurs slowly and progressively as the rhythm disorder continues. The gradient distribution of the maturation marker (scavenger-receptor A) supported the recruitment of macrophages across the atrial endocardium. This novel mode of recruitment in AF requires more attention because the finding implies 2 different sources of macrophages infiltrating into the atria: well-known intraatrial vasculatures and unnoticed atrial endocardium.

Atrial Endocardial Dysfunction in AF

Recently, atrial endocardial dysfunction during AF has gained considerable attention. Previous studies, including ours, have demonstrated the upregulation of prothrombotic molecules (eNOS and PAI-1) and downregulation of antithrombotic molecules (tissue factor pathway inhibitor and thrombomodulin) in the endocardium of fibrillating atria in animals.[34,35] Also, a recent study has reported the upregulation of VCAM-1 in human AF.[36] These alterations of the endocardial function in AF are attributed to oxidant stress produced by AF itself.[37] Until now, atrial endocardial dysfunction has been believed to contribute to thrombus formation.

Furthermore, the present study advances the role of endocardial dysfunction in AF. In addition to providing feasible states for thrombus formation, the atrial endocardium of human AF facilitates the recruitment of macrophages into the atrium by expressing adhesion molecules and chemotactic proteins. Two imunoglobulin-like adhesion molecules, ICAM-1 and VCAM-1, which are abundantly expressed in the endocardium, cause a firm adhesion by the interaction between adhesion molecules and the surface of immune cells. Thereafter, abundant MCP-1 in the endocardium guides the adherent immune cells across the endocardium.

Roles of Immune Cell Infiltration

Proinflammatory macrophages are well known to contribute importantly to the progression of various cardiovascular diseases. They include atherosclerosis, myocardial infarction and congestive heart failure. In atherosclerosis, activated macrophages transmigrate across the endothelium and participate critically in every stage of lesion formation by secreting matrix-degrading enzymes, prothrombotic molecules and also a variety of proinflammatory cytokines.

It would be reasonable to consider that similar mechanisms by immune cells could also operate in AF progression because the degree of the recruitment of immune cells increased in AF with the increment of MCP-1, TGF-β and IL-6 expression in fibrillating atria. These cytokines are well known to affect the contractility and electrical stability of myocytes inhomogeneously and to induce fibroblast activation leading to deposits of extracellular matrix fibrosis.[38] These effects provide substrates for reentrant arrhythmias. In contrast, stretching of the myocardium is reported to induce activation of the endocardium, which initiates and promotes inflammation processes.[39] Therefore, inflammation facilitates AF occurrence and atrial stretching by AF induces atrial inflammation (a vicious cycle between AF and inflammation). However, whether the local inflammation is a cause or a result of AF could not be determined and should be investigated in future studies. Even though the inflammation may be a consequence of AF in combination with underlying heart disease, it might subsequently perpetuate AF by triggering inflammatory cascade with secreted cytokines.

Study Limitations

A potential limitation of the present study is the lack of information regarding patients without structural heart diseases because of ethical issues, and our data focused on the differences between SR with a history of paroxysmal AF and persistent AF patients undergoing surgery. Therefore, it may be argued that surgical intervention or the valvular disease itself might induce local inflammation in the atrium. However, the different infiltration pattern of immune cells between SR and AF could not be explained only by these factors, provided that no comment can be made about the impact of immune cells in other patient populations with AF. Second, only left atrial appendage samples were available; therefore, our present findings may not represent other areas of the atrium. However, a recent study has demonstrated a good correlation in the number of inflammatory cells between the right and left atrial myocardium.[33] Also, from a clinical point of view, thrombus formation in the left atrial appendage is more common and more important than other atrial tissues and, therefore, our findings in the left atrial append-

age are significant. Lastly, the number of patients examined was relatively small. Although limited for these reasons, the present study, by demonstrating the recruitment of immune cells, mostly active macrophages, across the atrial endocardium with abundant chemotactic protein in human AF, provides an aid for constructing an idea that AF is associated with chronic inflammation disorders. However, the time sequence and interrelationships among inflammation and AF perpetuation remain to be determined by future studies.

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