Activation of Peripheral Blood CD3\(^+\) T-lymphocytes in Patients With Atrial Fibrillation

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**Summary**

Atrial fibrillation (AF) is a common disease with a poorly understood pathophysiological mechanism. Increasing evidence indicates that AF may be associated with immunologic inflammation responses, but it remains unclear whether activation of peripheral blood CD3\(^+\) T-lymphocytes plays a role in the pathogenesis of AF. The aim of this study was to evaluate this phenomenon. Fifty paroxysmal AF patients and 56 persistent AF patients who underwent successful electrical cardioversion were enrolled. The percentages of CD69 and human leukocyte antigen DR (HLA-DR) positive peripheral blood CD3\(^+\) T-lymphocytes, which indicate T-lymphocyte activation, were examined by flow cytometric analysis in the patients and 51 healthy controls. The patient groups had higher levels of CD69 and HLA-DR than the healthy controls. During the 3-month follow-up, 37 patients had recurrence of AF (recurrence group) and 50 patients remained in sinus (sinus group). The CD69 and HLA-DR levels in the sinus group were all significantly down-regulated at follow-up compared with before cardioversion. However, there were no statistically significant differences between the CD69 and HLA-DR levels in the recurrence group at follow-up and before cardioversion. Our findings suggest that activation of peripheral blood CD3\(^+\) T-lymphocytes was associated with AF, and might be a diagnostic or therapeutic marker. (Int Heart J 2012; 53: 221-224)

**Key words:** Human leukocyte antigen DR, CD69, CD3\(^+\) T-lymphocyte, Atrial fibrillation

Atrial fibrillation (AF) has received much attention since it is one of the most common arrhythmia diseases. It has high morbidity and mortality rates due to hemodynamic impairment and thromboembolic events.\(^1\)\(^2\) Although many investigations have proposed many theories, including atrial pathology and atrial electrical remodeling,\(^3\) the pathophysiological mechanism of AF remains largely unknown.

Recently, the link between inflammation and AF has been receiving increasing attention. Many studies have demonstrated that serum or plasma inflammation biomarkers are linked with the development of AF, which suggests chronic inflammatory responses might participate in the development of AF.\(^4\)\(^-\)\(^7\) Meanwhile, other studies have reported that activated T-lymphocytes and macrophages infiltrated the endomyocardium of patients with AF, suggesting the activation of local T-lymphocytes plays a role in the pathogenesis of AF.\(^8\)\(^-\)\(^10\) However, there is still no evidence supporting a link between the activation of peripheral blood T-lymphocytes and AF.

CD69 and HLA-DR are markers of activated T-lymphocytes. In other words, they are both specifically expressed on the surface of activated T-lymphocytes.\(^11\)\(^-\)\(^12\) CD69 is known as an early activation marker of T-lymphocytes\(^8\)\(^-\)\(^10\) and HLA-DR is known as a late activation marker of T-lymphocytes.\(^14\) They both play a role in the specific immune response to inflammation.\(^13\)\(^-\)\(^20\)

To our knowledge, there is little information available about the expression of CD69 and HLA-DR on peripheral blood CD3\(^+\) T-lymphocytes in patients with AF. In this study, we investigated the relationship between the activation of peripheral blood CD3\(^+\) T-lymphocytes and AF by flow cytometric analysis with the aim of providing more evidence to support this phenomenon.

**Methods**

**Patients:** Fifty paroxysmal AF patients and 56 persistent AF patients who underwent successful electrical cardioversion from February 2010 to September 2011 at the Department of Cardiology and outpatient clinic of Tangdu Hospital, Xi’an, PR China, were enrolled in this study. A diagnosis of AF was made based on the ACC/AHA/ESC 2006 guidelines on atrial fibrillation.\(^3\) Exclusion criteria included: infectious diseases, immunological diseases, anti-inflammatory or immunosuppression treatment, recent trauma and surgery, coronary heart disease, myocardopathy, rheumatic heart disease, valvular heart disease, heart failure, chronic lung diseases, hepatic and renal diseases, and cancer. In all patients, anticoagulant treatment was used routinely in accordance with current guidelines.

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Sample preparation and flow cytometric analysis:

Blood samples were withdrawn from the antecubital vein before cardioversion and at follow-up 3 months after cardioversion. Recurrence of AF was defined as AF documented by ECG at any time after the cardioversion.

Another 51 healthy volunteers with no history of arrhythmias who were undergoing a regular routine clinical examination served as the control group. Informed consent was obtained from the patients and control subjects, and the study was approved by the Ethical Committee of the Fourth Military Medical University.

### Results

**Baseline clinical characteristics:** The characteristics of the subjects are shown in the Table. There were no statistically significant differences in age, gender, hypertension, hyperlipidemia, or diabetes among the 3 groups (all $P > 0.05$).

We also determined the types of drugs being used. The results showed that there were no significant differences in medications, including angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blocking agents (ARB), calcium channel blockers (CCBs), and statins (all $P > 0.05$).

β-Blockers as heart rate-slowing agents were used significantly more often in the AF patients than in the control group ($P < 0.001$). Other parameters, including white blood cells (WBC), lymphocytes, and monocytes showed no significant differences among the 3 groups (all $P > 0.05$). As expected, AF patients had higher levels of C-reactive protein (CRP) compared with the control group and the CRP level in the persistent AF group had higher levels of C-reactive protein (CRP) compared with healthy individuals (26.6% ± 8.41; all $P = 0.001$). The difference between the paroxysmal group and persistent group was not statistically significant ($P = 0.193$) (Figure 2).

**Comparision between AF group and control group:** The expression of CD69 and HLA-DR on peripheral blood CD3+ T-lymphocytes (CD3+CD69+ T-lymphocytes and CD3+HLA-DR+ T-lymphocytes) was investigated by flow cytometric analysis. They were all evaluated as a percentage of total CD3+ T-lymphocytes (Figure 1). In order to evaluate whether there were differences in CD69 and HLA-DR levels between the AF patients and healthy individuals, we tested for statistical differences in the CD69 and HLA-DR levels in the paroxysmal and persistent AF groups and the control group before cardioversion. The results showed that the mean value of CD69 was significantly up-regulated in patients with paroxysmal (1.48% ± 0.42) and persistent AF (1.55% ± 0.38) compared with healthy individuals (1.07% ± 0.37; all $P < 0.001$). The difference between the paroxysmal group and persistent group was not statistically significant ($P = 0.333$). The mean value of HLA-DR was also significantly up-regulated in patients with paroxysmal (35.16% ± 9.97) and persistent AF (37.73% ± 10.78) compared with the healthy individuals (31.5% ± 4.1; all $P < 0.001$). The difference between the paroxysmal group and persistent group was not statistically significant ($P = 0.193$) (Figure 2).

**Comparison between before cardioversion and at follow-up:** In the subsequent follow-up, we lost 19 patients. Among the

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**Table. Characteristics of the Study Population**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 51)</th>
<th>Paroxysmal AF (n = 30)</th>
<th>Persistent AF (n = 56)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.4 ± 8.5</td>
<td>63.2 ± 9.5</td>
<td>67.2 ± 9.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Men</td>
<td>31 (61%)</td>
<td>32 (64%)</td>
<td>34 (61%)</td>
<td>0.927</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (25%)</td>
<td>20 (40%)</td>
<td>25 (45%)</td>
<td>0.106</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>11 (22%)</td>
<td>8 (16%)</td>
<td>7 (13%)</td>
<td>0.448</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td>5 (9%)</td>
<td>0.566</td>
</tr>
<tr>
<td>Drugs:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I/ARB</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
<td>21 (38%)</td>
<td>0.074</td>
</tr>
<tr>
<td>Statins</td>
<td>8 (16%)</td>
<td>7 (14%)</td>
<td>6 (11%)</td>
<td>0.743</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>8 (16%)</td>
<td>26 (52%)</td>
<td>30 (54%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CCBs</td>
<td>5 (10%)</td>
<td>12 (24%)</td>
<td>7 (13%)</td>
<td>0.108</td>
</tr>
<tr>
<td>WBC count (per ul.)</td>
<td>6349 ± 1891</td>
<td>6768 ± 1859</td>
<td>6375 ± 1663</td>
<td>0.819</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>30.9 ± 3.8</td>
<td>32.5 ± 4.3</td>
<td>31.5 ± 4.1</td>
<td>0.147</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>5.5 ± 1.2</td>
<td>5.8 ± 1.1</td>
<td>5.4 ± 1.1</td>
<td>0.123</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.24 ± 0.12</td>
<td>0.48 ± 0.25</td>
<td>0.60 ± 0.223</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocking agents; CCBs, calcium channel blockers; WBC, white blood cells; CRP, C-reactive protein; $P$, probability of significance (difference among 3 groups); and $^*$, $P < 0.05$ persistent AF versus paroxysmal AF.

### Considered statistically significant.
remaining 87 patients, AF recurrence (recurrence group) occurred in 37 (42.5%) and 50 (57.5%) patients remained in sinus (sinus group). In order to determine whether the mean levels of CD69 and HLA-DR changed after cardioversion, we tested for statistical differences in the CD69 and HLA-DR levels in the sinus group before cardioversion and at the follow-up, as well as in the recurrence group. The results showed that the mean values of CD69 and HLA-DR in the sinus group at follow-up (1.17% ± 0.38, 28.71% ± 8.70) were all significantly down-regulated compared with before cardioversion (1.45% ± 0.44, 34.71% ± 9.75; all \( P < 0.05 \)). However, there were no statistically significant differences between the recurrence group at follow-up (1.57% ± 0.39, 36.40% ± 9.32) and before cardioversion (1.60% ± 0.35, 37.72% ± 11.11; \( P = 0.721, P = 0.544 \)) (Figure 3).

**DISCUSSION**

In this study, we found that the respective expressions of CD69 and HLA-DR on peripheral blood CD3+ T-lymphocytes in AF patients were significantly higher than the control group, which might suggest that high expression of CD69 and HLA-DR was associated with AF. In the subsequent follow-up, we further found that the expression of CD69 and HLA-DR in the sinus group at follow-up was significantly down-regulated compared with before cardioversion. However, the expression of CD69 and HLA-DR in the recurrence group at follow-up was not significantly down-regulated. This might further support that the CD69 and HLA-DR levels were related to the state of AF.

As demonstrated by the present study, there was a link between high levels of expression of CD69 and HLA-DR and AF. CD69, known as an early activation marker of lymphocytes, is a type II transmembrane glucoprotein and may enhance activation and proliferation/differentiation of T-lymphocytes.\(^{9,20,22}\) HLA-DR belongs to the MHC class II system and is known as a late activation marker of lymphocytes. It is required for antigen presentation and activation of helper T-lymphocytes.\(^{14,19,23}\) They respectively expressed in some inflammatory infiltrates and played important roles in the pathogenesis of some inflammatory diseases such as allergic airway inflammation,\(^{18,24}\) rheumatoid arthritis,\(^{15}\) psoriasis vulgaris lesional skin,\(^{17}\) and active inflammatory bowel disease.\(^{15,16}\) Thus, the increases in CD69 and HLA-DR on CD3+ T-lymphocytes implied there is an activation of peripheral blood CD3+ T-lymphocytes in AF patients.

In fact, the activation of peripheral blood CD3+ T-lymphocytes may play an important role in the pathogenesis of AF. A few studies have reported that some inflammatory lymphocytes, such as CD45+ cells, CD3+ T cells, and CD68+ macrophages, infiltrated into the endomyocardium in patients with AF, which supports the hypothesis that local activation of T-lymphocytes plays a role in the pathogenesis of AF.\(^{6-10}\) On the other hand, many studies have reported that serum or plasma inflammation biomarkers, such as C-reactive protein (CRP), interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor (TNF)-\(\alpha\), monocyte chemotactic protein (MCP)-1, and vascular endothelial growth factor (VEGF), are increased in AF patients, which suggests that T-cell-associated chronic inflammatory responses might be involved in the pathogenesis of AF.\(^{4-7}\) In our study, we provided further evidence to support that there is activation of peripheral blood CD3+ T-lymphocytes in AF patients, as demonstrated by the upregulation of CD69 and HLA-DR by flow cytometric analysis.

The present results further emphasize that activation of T cells is involved in AF. T-lymphocytes are widely known to be the main cells that participate in cell-mediated immunity, which is one of the primary methods of immunity in humans. It can be suppressed by various immunosuppressants such as cyclosporine and rapamycin. If the activation of peripheral blood T-lymphocytes does participate in the pathogenesis of AF, perhaps we can prevent AF recurrence through the suppression of activation of peripheral blood T-lymphocytes.

As for the underlying mechanisms of how activated peripheral blood CD3+ T-lymphocytes participate in the progression of AF, we believe there are 3 possibilities, as follows. First, the activation of peripheral blood CD3+ T-lymphocytes might cause the upregulation of IL-6 and MCP-1, which could affect the contractility and electrical stability of myocytes inhomogeneously and induce fibroblast activation leading to de-
posts of extracellular matrix fibrosis. Second, activation of peripheral blood CD3+ T-lymphocytes might promote local immunologic inflammatory responses in endomyocardial cells, and may promote the infiltration of inflammatory lymphomononuclear cells into the endomyocardium in patients with AF. Third, the activation of peripheral blood CD3+ T-lymphocytes could activate calcineurin-nuclear factor, which is involved in the T-lymphocyte signal transduction pathway. It is important to note that there are several limitations to this study. First, the results cannot be taken as evidence that supports roles for CD69 and HLA-DR in the pathogenesis of AF; they only indicate the possible association of CD69 and HLA-DR with AF. Second, how CD69 and HLA-DR contribute to the pathogenesis of AF and the exact details of the underlying mechanism need to be further investigated. Third, we did not study the influence of other immune activation-associated molecules such as CD25, CD71, and CD122 and co-stimulatory molecules such as CD28, CTLA-4, CD80, and CD86 during the progression of AF.

Conclusions: High levels of expression of CD69 and HLA-DR were associated with AF. These results indicate that the activation of peripheral blood CD3+ T-lymphocytes is associated with AF. The activation of peripheral blood CD3+ T-lymphocytes and immunologic inflammatory responses may play a role in the pathogenesis of AF. Our findings may provide a novel insight into the pathogenesis of AF. However, there are several limitations to this study, which need to be further investigated.

REFERENCES