Long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT) are primary electric diseases characterized by catecholamine-induced ventricular arrhythmias. Unbalanced autonomic innervation of the heart may trigger arrhythmic events and stellectomy is a treatment option for patients who are resistant to pharmacological drugs. We analyzed left stellectomy specimens of LQTS and CPVT patients for signs of inflammatory activity.

Methods and Results—Stellate ganglia were retrieved from 12 consecutive patients (8F; 4 mol/L; mean age, 23.4±17 years) with either LQTS (n=8) or CPVT (n=4) and serious arrhythmias. Control stellate ganglia were obtained from 10 accidently deceased patients (6F; 4 mol/L; mean age, 35±17.6 years). Sections were immunostained with antibodies against T cells (CD3, CD4, CD8, CD20, Granzyme B), CD68 (macrophages), and HLA-DR (human leukocyte antigen-DR) antigens (activation marker). Immunopositive cells were quantified as cells/mm². Polymerase chain reaction (PCR) and reverse transcription PCR were performed to screen for herpes virus DNA. Stellate ganglia of all 12 LQTS/CPVT patients revealed mild but distinct inflammatory infiltrates composed of T lymphocytes and macrophages, which were diffusely spread, but also clustered in small foci opposed to ganglion cells, interpreted as T-cell–mediated ganglionitis. Morphometric analysis showed that CD3+ and CD8+ T cells/mm² were significantly higher in the ganglia of LQTS/CPVT cases than in healthy controls (P=0.0018 and P=0.0009, respectively). Molecular analyses were negative for neurotropic viruses.

Conclusions—T-cell–mediated cytotoxicity toward ganglion cells may boost adrenergic activity as to trigger or enhance electric instability in LQTS/CPVT patients who are already genetically predisposed to arrhythmias. (Circ Arrhythm Electrophysiol. 2014;7:224-229.)
refractoriness, thus reducing propensity to ventricular fibrillation because of an increase of ventricular fibrillation threshold.15,16 The mechanisms by which autonomic tone influences the arrhythmic risk and the pathology of cardiac innervation, both intrinsic and external to the heart, are not known. Because pathological investigations on the sympathetic innervation of the hearts of these patients are scarce, we evaluated the stellate ganglia of LQTS/CPVT patients with resistant arrhythmias for the occurrence of structural abnormalities. Specifically, we investigated the occurrence of inflammatory activity and the type of immune response with the use of immunohistochemical techniques. In addition, PCR and reverse transcription PCR were used to study the presence of genomic sequences of neurotropic viruses within the stellate ganglia of these patients.

Methods

Study Population

Surgically excised left stellate ganglia and thoracic ganglia T2 to T4 were retrieved from 12 consecutive patients (8F; 4 mol/L; age range, 2–54 years; mean age, 23.4±17 years) with either LQTS (n=8) or CPVT (n=4) who underwent LCSD at 1 referral center (Academic Medical Center, Amsterdam, The Netherlands). The diagnosis was based on conventionally reported criteria.17,18 Details of the patients are summarized in Table 1. All LQTS and CPVT patients were under maximum β-blockers therapy and had persistent serious arrhythmia events. Genotype of the patients was available in all cases, except 1. Control stellate ganglia were obtained from 10 patients who died in accidents (6F; 4 mol/L; age range, 20–64 years; mean age, 35±17.6 years). The study was approved by the local ethics committee, and informed consent was obtained from each patient.

LCSD Procedure

The LCSD was performed as previously described in detail.13 Briefly, LCSD involves ablation of the lower half of the left stellate ganglion, together with the thoracic ganglia T2 to T4 through a videoscopic transthoracic approach. It provides adequate cardiac denervation leaving the upper stellate ganglion intact to minimize the risk of Horner syndrome.

Histology/Immunohistochemistry

Tissue samples were fixed in 10% buffered formalin and paraffin embedded; 5-μm-thick sections were stained with hematoxylin–eosin and Masson trichrome to evaluate the presence and extension of fibrosis. For immunohistochemical studies, the following antibodies were used: polyclonal anti-S100 (general neuronal marker, DAKO, Glostrup, Denmark) to visualize the neural network, monoclonal anti-CD3 (Pan-T, clone SP7, Thermo Scientific/LabVision, Fremont, CA), monoclonal anti-CD4 (T-helper lymphocytes, clone 4b12, Thermo Scientific/LabVision, Fremont, CA), monoclonal anti-CD8 (Cytotoxic T cells, Cytotoxic T-lymphocytes [CTLs], clone SP16, Thermo Scientific/LabVision, Fremont, CA); monoclonal

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Sex</th>
<th>Age, y</th>
<th>Clinical Picture</th>
<th>ICD</th>
<th>Mutation</th>
<th>Histomorphometry</th>
<th>Outcome</th>
<th>Follow-Up, mo</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>20</td>
<td>CPVT</td>
<td></td>
<td></td>
<td>CD3 38/mm²</td>
<td>Alive, free of symptoms</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>2</td>
<td>LQTS8</td>
<td>+</td>
<td>CACNA1C: G406R</td>
<td>CD3 180/mm²</td>
<td>Dead†, 1 wk post surgery</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>4</td>
<td>LQTS3</td>
<td>+</td>
<td>SCN5a: R1623Q</td>
<td>CD3 34/mm²</td>
<td>Alive, rare recurrence of appropriate ICD shock</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>17</td>
<td>CPVT</td>
<td>–</td>
<td>RyR2: Glu4076Lys</td>
<td>CD3 60/mm²</td>
<td>Alive, free of symptoms</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>21</td>
<td>LQTS2</td>
<td>+</td>
<td>KCNHA2: E691X</td>
<td>CD3 9/mm²</td>
<td>Alive, rare recurrence of appropriate ICD shock</td>
<td>90</td>
</tr>
<tr>
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<td>25</td>
<td>LQTS2</td>
<td>+</td>
<td>KCNHA2: G785V</td>
<td>CD3 35.6/mm²</td>
<td>Alive, free of symptoms</td>
<td>83</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>47</td>
<td>LQTS1</td>
<td>–</td>
<td>KCNQ1: Arg595Gln</td>
<td>CD3 19/mm²</td>
<td>Alive, free of symptoms</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>19</td>
<td>LQTS2</td>
<td>+</td>
<td>KCNHA2: Thr163Met</td>
<td>CD3 27/mm²</td>
<td>Alive, free of symptoms</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>6</td>
<td>CPVT (not RyR)</td>
<td>+</td>
<td>Not detected</td>
<td>CD3 42/mm²</td>
<td>Alive, free of symptoms</td>
<td>37</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>45</td>
<td>LQTS1</td>
<td>–</td>
<td>KCNQ1: Ala341Val</td>
<td>CD3 49/mm²</td>
<td>Alive, free of symptoms</td>
<td>36</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>21</td>
<td>CPVT</td>
<td>–</td>
<td>RyR2: 3959G-&gt;A (VUS 2)</td>
<td>CD3 52/mm²</td>
<td>Alive, significantly less ventricular arrhythmias</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>54</td>
<td>LQTS2</td>
<td>+</td>
<td>KCNHA2: Arg582Cys</td>
<td>CD3 110/mm²</td>
<td>Alive, free of symptoms</td>
<td>9</td>
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</tbody>
</table>

Table 1. Clinical Data, Genetic and Histomorphometric Data of the Study Population

CPVT indicates catecholaminergic polymorphic ventricular tachycardia; ICD, implantable cardioverter defibrillator; LQT, long QT syndrome; NSVT, nonsustained ventricular tachycardia; PVB, premature ventricular beats; RyR2, ryanodine receptor R2; and VT, ventricular tachycardia.

*Many stands for >10.
†Patient 2 died of severe brain damage because of intractable arrhythmias that occurred during surgery.
PCR and reverse transcription PCR analysis for detection of herpetic viral genomes was performed on frozen tissue. Total RNA and DNA were extracted from homogenates of entire sections using phenol chloroform methods. Briefly, reduced frozen specimens were resuspended in 2 different digestion solutions: 1 for RNA and 1 for DNA extraction. The nucleic acids were measured using spectrophotometer. The oligonucleotides used to ascertain the quality of extracted RNA or DNA were complementary to the mRNA glyceraldehyde-3-phosphate dehydrogenase (3GPDH) and α-antitrypsin genes, respectively. PCR was used to evaluate DNA viruses (cytomegalovirus, herpes simplex virus [HSV], Epstein-Barr virus, human herpes virus 6, and varicella zoster virus).22

Slides were examined independently by 2 histopathologists (A.C.W. and S.R.), blinded to group allocation and clinical characteristics. All morphology studies (light microscopy and immunohistochemistry) were performed on stellate ganglia and thoracic ganglia T2 to T4.

Results

Histological Findings

Histological examination with standard hematoxylin–eosin staining revealed inflammatory cells infiltration and degenerative changes of ganglion cells with vacuoles within the cytoplasm in all LQTS/CPVT cases (prevalence=1.00; 95% confidence interval, 0.74–1.00).

Mononuclear inflammatory cells were diffusely spread, but also clustered in small foci in close apposition to ganglion cells (Figure 1). There were no neutrophils or eosinophils present. In 2 controls, only scattered lymphocytes were observed, in the absence of clusters in close apposition to ganglion cells. There were no inflammatory changes in the peri-ganglionic fat tissues of LQTS/CPVT cases and controls. Inflammatory changes were accompanied by mild fibrosis in both LQTS/CPVT cases.

Immunohistochemistry

Immunohistochemical analysis demonstrated a diffuse infiltration with CD68+ macrophages and CD3+ T cells in all LQTS/CPVT cases. The largest fraction of CD3+ T cells was CD8+ CTLs, of which many contained GranzymeB+ granules (a marker of activation) and HLA-DR expression, and clustered around some of the ganglion cells (Figures 2 and 3). These clusters also contained macrophages, showing abundant expression of HLA-DR antigens. At these sites, aberrant expression of HLA-DR was also noticed in the adjacent affected ganglion cells.

Quantitative analysis of inflammatory cells in stellactomy specimens and surrounding tissues revealed that the density (cells/mm²) of CD3+ and CD8+ T cells in the LQTS/CPVT ganglionic samples was significantly higher than in the adjacent fat tissue and also significantly higher than in control ganglia of the 10 accidentally deceased persons.

Specifically, the median number/mm² of CD3+ T cells was 40 (9–180) in cases versus 13.95 (7–20) in control ganglia (P=0.0003) and of CD8+ CTLs was 32.5 (8.7–147.0) in cases versus 12.2 (6–16) in controls (P=0.0001; ie, 81% and 87% of CD3+ T cells, respectively). In contrast, the number of CD4+ T-helper cells and CD20+ B cells was low: 0.25 (0.00–8.10) and 0.73 (0.00–3.57), respectively, similar to that of controls (Table 2).
No difference was found when comparing ganglion-related T cells of LQTS and CPVT patients: CD3+ 34.8 (9–180) versus 47 (38–60; \(P=0.3677\)), CD8+ 27 (8.7–147) versus 36.3 (32–48; \(P=0.4828\)).

**Molecular Analysis**

Ganglion tissue was screened for the presence of herpes viral DNA to determine the potential trigger for the immune cell infiltration. The ganglia were assessed for the occurrence of cytomegalovirus, HSV-1 and HSV-2, Epstein-Barr virus, human herpes virus 6, and varicella zoster virus. No viral DNA was present in homogenates of ganglion entire sections of LQTS/CPVT cases and controls.

**Discussion**

LCSD raises the ventricular fibrillation threshold and this represents the rationale for the use of stelllctomy to reduce or prevent malignant arrhythmias typical of LQTS and CPVT. In our series of 12 consecutive patients with either LQTS or CPVT who underwent LCSD, the resected stellate ganglia revealed signs of a chronic ganglionitis, characterized by an elevated number of activated T lymphocytes and degeneration of adjacent ganglion cells. These inflammatory changes were found in all patients and independently of sex, age, onset of the disease, and genetic substrate. All patients had persistent serious arrhythmia events, despite maximum \(\beta\)-blockers therapy. Normal ganglia also

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**Figure 3.** Immunophenotypical characterization of the inflammatory infiltrate in a long QT syndrome patient (pt #12). A, Panoramic view (CD3; scale bar, 50 \(\mu\)m); B–F, close-up of a cluster around the ganglion cells (B, CD3; C, CD20; D, CD8; E, HLA-DR [human leukocyte antigen-DR]; and F, Granzyme B). Lymphocytes mostly consisted of CD3+ CD8+ T cells.

**Figure 2.** Immunophenotypical characterization of the inflammatory infiltrate in a catecholaminergic polymorphic ventricular tachycardia patient (pt #1). A, Panoramic view (CD3; scale bar, 50 \(\mu\)m); B–F, close-up of a cluster around the ganglion cells (B, CD3; C, CD20; D, CD8; E, HLA-DR [human leukocyte antigen-DR]; and F, Granzyme B). The majority of lymphocytes were CD3+ CD8+ T cells.
displayed some inflammatory activity, but to a significantly lower extent.

Intracardiac ganglionitis and its potential effect on arrhythmic risk have been previously described in LQTS subjects who died suddenly.25–27 Ganglionitis and neuritis in the sinus node area were found also in liquid-protein–modified fast diet dieters who developed prolongation of the QT interval and ventricular arrhythmias after dieting.28 In these studies, diffuse infiltration of mononuclear cells associated with neural degeneration was found at histological examination, lacking however of an immune characterization of the cellular composition and activity of the inflammatory population. By means of immunohistochemistry, we demonstrated a predominant CD3+ CD8+ CTL’s infiltrate in close proximity of macrophages and high expression of class II major histo compatibility (MHC) antigens in the ganglionic specimens. Such an inflammatory pattern can be considered as the immunohistological footprint of the involvement of an (auto) immune-mediated disorder or viral diseases and makes a nonspecific inflammatory reaction in the ganglia unlikely. Moreover, several of the lymphocytes surrounding ganglion cells were stained for Granzyme B, a serine protease expressed in the cytotoxic granules of lymphocytes implicated in triggering apoptosis in target cells.29 The recruitment of CD8+ CTLs may be because of the aberrant expression of HLA-DR by ganglion cells, but also immune reaction itself can induce MHC class II antigen expression in the nervous system.30

The most frequently proposed theories suggest either a viral31 and autoimmune pathogenesis, but also paraneoplastic, toxic, or neurological disorders may underlie the onset of an inflammatory process in the ganglia via neurotransmitters, growth factors, and cytokines.

The similarity of the histological abnormalities in all our patients suggested an infectious disease as the most probable cause.24–26 However, as the diagnostic test for the detection of viral antigens is limited, and since the diagnosis of viral or paraneoplastic disorders are not possible by typical histological examination, the presence of acute ganglionic inflammation could not be assessed in our series.32 In our LCSD specimens, many ganglion cells were indeed surrounded by CD8+ CTLs, but none of the stellate ganglia contained HSV-1, HSV-2, or varicella zoster virus DNA. Therefore, it remains unresolved why T cells surround apparently not infected ganglion cells in our series. It cannot be excluded that these neurons host other viruses or minimal amounts of viral antigen, which are not detectable with the techniques applied in the present study. Finally, many ion channels also express in neurological tissue; as a speculation, it could be that aberrant ion-channel function dysregulates the homeostasis in the ganglia leading to a secondary sterile inflammation.

However, it is fair to state that at present, the origin of the inflammatory infiltrates in ganglia, and more specifically, whether they might be causally related to the primary electric diseases of the heart remains unsettled. Despite this, we suppose that the presence of active ganglionic inflammation, albeit low grade, can be of importance for the symptomatology of such patients. From our observations it can be speculated that ganglionic inflammation might contribute to the cardiac electric instability in subjects with LQTS/CPVT and particularly in those who are heavily symptomatic.

In conclusion, small foci of active chronic inflammation with affinity for the ganglion cells in the excised stellate ganglia of all 12 patients with LQTS/CPVT who underwent LCSD support the hypothesis that T-cell–mediated cytotoxicity toward ganglion cells may boost adrenergic activity and thus may trigger or enhance electric instability in these patients who are already genetically predisposed to arrhythmias. Ganglia removed from not affected individuals also displayed a mild inflammation. This could imply that ganglionic inflammation is functionally important only in symptomatic patients who are genetically predisposed to arrhythmias and remains unnoticed in most other individuals.

Limitations

The study population is a small number (12 patients), but relatively large in a genotyped juvenile population. Within the limitations of small numbers, the data show differential features between LQTS/CPVT patients and controls, suggesting a triggering role of inflammation for the sympathetic nervous system.

Sources of Funding

Stefania Rizzo was a visiting fellow at the Academic Medical Center, Amsterdam, The Netherlands, supported by the Registry of Cardio-Cerebro-Vascular Pathology, Veneto Region, Venice, Italy.

Disclosures

None.

References


Table 2. Morphometric Analysis of Immunoresponse in the Stellsectomy Specimens of Arrhythmia Patients (LQTS/CPVT) and Controls

<table>
<thead>
<tr>
<th>T-Cell Subsets/mm²</th>
<th>LQTS/CPVT (n=12)</th>
<th>Controls (n=10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>40 (9–180)</td>
<td>13.95 (7–20)</td>
<td>0.0003</td>
</tr>
<tr>
<td>CD8</td>
<td>32.5 (8.7–147.0)</td>
<td>12.2 (6–16)</td>
<td>0.0001</td>
</tr>
<tr>
<td>CD4</td>
<td>0.25 (0.00–8.10)</td>
<td>1.27 (0.11–3.59)</td>
<td>0.3871</td>
</tr>
<tr>
<td>CD20</td>
<td>0.73 (0.00–3.57)</td>
<td>1.27 (0.12–2.35)</td>
<td>0.2604</td>
</tr>
</tbody>
</table>

CPVT indicates catecholaminergic polymorphic ventricular tachycardia; and LQTS, long QT syndrome.

**CLINICAL PERSPECTIVE**

A significant number of patients with long QT syndrome and catecholaminergic polymorphic ventricular tachycardia will develop symptomatic and potentially lethal ventricular arrhythmias during periods of physical or emotional stress, when neural influence on the heart is especially active. The arrhythmias associate with the level of sympathetic activation, and this may reflect an epiphemomenon, which evokes symptoms in persons who are already electrically unstable because of the ion-channel disease. The mechanisms by which autonomic tone influences the arrhythmic risk are not clear indeed. In 12 patients with either long QT syndrome or catecholaminergic polymorphic ventricular tachycardia who underwent left cardiac sympathetic denervation, we found the unmistakable histological signs of a low-grade chronic T-lymphocyte–mediated ganglionitis in their resected stellate ganglia. At present, the origin of inflammatory infiltrates in ganglia, and whether they are causally related to the ion-channel disease, remains unsettled. Nevertheless, we think that the mere presence of active ganglionic inflammation, albeit low grade, can be of importance for the symptomatology of such patients. We speculate that a T-cell–mediated cytotoxicity toward ganglion cells may boost adrenergic activity through release of inflammatory mediators in ganglia, and in this manner triggers or enhances electric instability in patients who are genetically predisposed to arrhythmias. Our observation that ganglia from unaffected individuals, who were used as control tissues in this study, also displayed a mild inflammation could implicate that ganglionic inflammation leads to symptoms only in patients who are genetically prone to develop arrhythmias, but remains unnoticed in most other individuals.
T-Cell–Mediated Inflammatory Activity in the Stellate Ganglia of Patients With Ion-Channel Disease and Severe Ventricular Arrhythmias

Stefania Rizzo, Cristina Basso, Dirk Troost, Eleonora Aronica, Anna Chiara Frigo, Antoine H.G. Driessen, Gaetano Thiene, Arthur A.M. Wilde and Allard C. van der Wal

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