Activation of Human Valve Interstitial Cells by a Viridians Streptococci Modulin Induces Chemotaxis of Mononuclear Cells

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Infective endocarditis is characterized by inflammatory infiltrates of mononuclear cells in infected cardiac valve leaflets. To delineate the role of valve interstitial cells (VICs) in leukocyte recruitment, we stimulated human VICs with glucosyltransferase, a modulin from viridians streptococci. Interstitial cells were activated directly by glucosyltransferase in a dose-dependent manner through concerted mitogen-activated protein kinase and nuclear factor–κB signaling pathways; activation resulted in up-regulation of synthesis and release of interleukin-6, interleukin-8, or monocyte chemoattractant protein–1 and enhanced transwell migration of U937 monocytic cells or primary mononuclear cells. The expression of glucosyltransferases and activation of VICs (nuclear localization of RelA) were detected in a rat model of experimental endocarditis. Proinflammatory cytokines also were detected in VICs from diseased human autopsy specimens but not in VICs from normal specimens. These results indicate that interstitial cells in the cardiac valve can be activated directly by bacterial modulins to recruit and retain mononuclear cells, likely contributing to the persistent inflammation characteristic of infective endocarditis.

Heart valve interstitial cells (VICs) constitute the major stromal population in valve leaflets, and they exhibit diverse and dynamic phenotypes ranging from fibroblast-like cells to myofibroblasts [1, 2]. Similar to the fibroblast-like stromal cells found in many tissues, VICs maintain a functional extracellular matrix in valve leaflets [3]. Cardiac valves are avascular, and in pathological conditions such as rheumatic valvular heart disease or infective endocarditis (IE) they are populated with infiltrating leukocytes, which leads to persistent inflammation [4, 5]. Whether VICs contribute to persistent inflammation by releasing chemotactic mediators and recruiting inflammatory infiltrates is unknown.

Viridans streptococci are prevalent opportunistic pathogens that cause endocarditis [6]; the most common blood isolates are Streptococcus sanguinis, Streptococcus bovis, Streptococcus mutans, and Streptococcus oralis [7]. The pathogenesis of IE is characterized by persistent inflammation induced by septic vegetations in which the causative microorganism forms biofilms that are refractory to antibiotic therapy [8, 9]. The persistent nature of biofilms also contributes directly to chronic bacteremia and thromboembolic events—serious complications associated with IE [10]. Metabolically active bacteria in biofilms are believed to constantly release pathogen-associated molecular patterns (PAMPs) or modulins that are capable of triggering thromboembolism through the aberrant expression and shedding of adhesion molecules [11]. Glucosyltransferases (GTFs) are cell wall–associated or secreted enzymes responsible for exopolysaccharide synthesis, which stimulates biofilm formation by viridians streptococci, thereby promoting, for example, colonization of tooth surfaces [12]. S. mutans expresses 3 GTFs (GTFB, GTFC, and GTFD) with an amino acid sequence identity >50%; isozymes have also been identified in other viridans streptococci, such as S. sanguinis, S. gordonii, and S. salivarius [13]. To
host cells, GTFs are potent modulins that directly activate up-regulation of the expression and release of cytokines by endothelial cells through mitogen-activated protein kinase (MAPK) or nuclear factor (NF)–κB signaling pathways [14]. A direct and specific role for GTFs in modulating inflammatory cytokine production in vivo and in situ has also been demonstrated in an experimental rat model of endocarditis [15, 16]. Therefore, modulins from viridians streptococci are indispensable for sustaining a microenvironment favorable for persistent local inflammation of cardiac valves.

Stromal cells in tissue microenvironments might play important roles in perpetuating the inflammatory process by sustaining cytokine or chemokine gradients to direct leukocyte migration in a site-specific manner. Although VICs are thought to secrete chemokines and cytokines, direct experimental evidence is lacking. The aim of this study was to determine the role of VICs in leukocyte recruitment after exposure to bacterial modulins derived from IE-inducing viridians streptococci. We used primary cultured human VICs to examine whether streptococcal GTFs directly activate secretion of chemokines by stromal cells and induce migration of leukocytes in vitro. Furthermore, we analyzed the expression of GTFs inside septic vegetations and the activation of VICs after bacterial infection in vivo.

METHODS

Human specimens. Heart valves affected by IE were obtained from the heart of a 12-year-old boy with congenital heart disease who received surgical intervention for recurrent endocarditis caused by S. gordonii. Normal heart valves were obtained as forensic biopsy specimens. For the culture of VICs, valve tissue specimens were obtained from patients who received heart transplants for either ischemic heart disease or cardiomyopathy. A written statement of informed consent was obtained from patients, in accordance with the regulations of the National Taiwan University Hospital Committee, and approval for the use of human specimens followed the regulations of the National Taiwan University Hospital Committee for Regulation of Human Specimens and Volunteers.

Cell detection and immunohistochemical analysis. PJS-3, a rabbit anti-serum, recognizes all 3 GTFs expressed by S. mutans [17]. PJS-3 was used to detect bacteria and GTFs in situ by immunostaining. Preimmune rabbit serum was used as a negative control.

Leukocytes were initially identified by hematoxylin and eosin staining. Macrophages in tissue sections were identified using anti-CD68 monoclonal antibody (mAb) MCA341R (Serotech) or sc-20060 (Santa Cruz Biotechnology) for rat and human specimens, respectively. For human specimens, formalin-fixed sections were prepared to preserve the morphology of intracellularly accumulated cytokines [18]. Human VICs were stained with anti-prolyl 4-hydroxylase mAb (clone 5B5; GeneTex); interleukin (IL)–6 was detected using the polyclonal antibody sc-7920 (Santa Cruz Biotechnology), and NF-κB was detected using anti-p65 (sc-8008; Santa Cruz Biotechnology). Immunostaining was performed with Avidin-Biotin-Complex kits (Dako) followed by colorization with diaminobenzidine.

Cell isolation and culture. Primary VICs were prepared from trimmed valve leaflets and cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, as described elsewhere [19]. All experiments were performed on subcultures of confluent cells between passages 2 and 7. Mononuclear cells (MNCs) were isolated by layering on Histopaque (Sigma) and conclusively identified by immunostaining with anti-CD14 mAb (clone 61D3, eBioscience). U937, a human monocytic leukemia cell line, and MNCs were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum.

Characterization of human VICs. Human VICs were characterized by indirect fluorescence immunostaining by use of mAbs against the following antigens: von Willebrand factor (clone AWV-3; GTI), desmin (clone DE-U-10; Sigma), vimentin (clone V9; Sigma), prolyl 4-hydroxylase (clone 5B5; GeneTex), and smooth muscle (SM) α-actin (clone 1A4; R&D Systems). Cells with positive immunostaining results for SM α-actin, prolyl 4-hydroxylase, and vimentin and negative immunostaining results for von Willebrand factor and desmin were considered VICs, as reported elsewhere [20].

Activation of human VICs with recombinant GTFC (rGTFC). His-tagged rGTFC was purified and conclusively identified as described elsewhere [14]. Possible endotoxin content was removed using polymyxin B agarose beads (Sterogene Bioseparations). Confluent human VICs were treated with varying concentrations of rGTFC, and cultured cells were collected at different times for cytokine and mRNA analyses. All experiments included polymyxin B (40 μg/mL; Sigma) to exclude possible endotoxin contamination.

rGTFC-binding assay. Confluent human VICs were treated with purified rGTFC or fluorescein isothiocyanate–conjugated rGTFC and rIDG60 [21] at 37°C for 1 h. The adherence of rGTFC to cells was detected by immunofluorescence microscopy or Western blotting analysis, as described elsewhere [14].

Determination of cytokine and chemokine concentrations by ELISA. The concentrations of IL-6, IL-8, and monocyte chemoattractant protein–1 (MCP-1) in culture supernatants were assayed by sandwich immunoassays that used ELISA kits (R&D Systems).

RNA isolation and reverse-transcriptase polymerase chain reaction. After stimulation with rGTFC, total RNA was isolated from VICs (5 × 10⁶ cells) by guanidine isothiocyanate–phenol-chloroform extraction. Primers specific for IL-6, IL-8, MCP-1, and glyceraldehyde 3-phosphate dehydrogenase and reverse-transcriptase polymerase chain reaction procedures were as described elsewhere [14].

Leukocyte migration assay. Leukocyte chemotaxis was analyzed using Transwell inserts (Corning) in 24-well plates, as...
described elsewhere [22]. MNCs or U937 cells that migrated and adhered to the VICs on the cover slips in the bottom chambers were fixed and then stained with 10% crystal violet or identified using anti-CD14 mAb. The mean (±SD) numbers of transmigrated cells were determined from examination of 10 high-resolution fields.

**Analysis of cellular signaling pathways.** For the inhibition of cellular signaling, human VICs were preincubated with specific inhibitors of phosphatidylinositol 3-kinase (LY294002; Sigma), extracellular signal-regulated kinases 1 and 2 (ERK1/2) (PD98059; Cashmere Biotech), p38 MAPK (SB203580; Cashmere Biotech), c-Jun-N-terminal kinase (JNK) (SP600125; Sigma), or NF-κB (Bay 11–7082; Sigma). Experiments were conducted in triplicate, and the results are reported as mean ng/mL or as the percentage of inhibition (±SD). Pretreatment with inhibitors alone did not affect cell viability or the release of IL-6, IL-8, or MCP-1.

Cell lysates were prepared as described elsewhere [14], and Western blot analyses of MAPKs or IκB were performed using polyclonal antibodies specific for the phosphorylated forms of ERK1/2 (Thr202/Tyr204), p38 MAPK (Thr180/Tyr182), JNK (Thr183/Tyr185), and inhibitory protein IκB-α (Ser32) or using antibodies against total ERK1/2, total p38 MAPK, and actin (Cell Signaling Technology). Nuclear translocation of NF-κB in VICs after rGTFC activation was detected by immunostaining, as described elsewhere [14].

**Rat model of experimental endocarditis.** A rat model of left-sided endocarditis has been established [15]. The number of *S. mutans* GS-5 strain bacteria [23] necessary to induce endocarditis in 95% of the exposed animals is 1 × 10⁷ colony-forming units [15]. The aortics and the associated vegetations that formed 6–24 h after infection, including the surrounding tissues at the base of the vegetations, were left intact after animals were euthanized and frozen immediately under liquid nitrogen. Animal experiments followed the guidelines of the National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee.

**Statistical analysis.** Data are presented as means (±SDs) and were analyzed by use of the 2-sided Student’s *t* test to compare the mean levels of cytokine secretion after a particular treatment. Differences with *P* values <.05 were considered statistically significant.

**RESULTS**

**Leukocyte infiltration and cytokine production by VICs in human endocarditis.** To investigate the role of VICs during inflammation, we initially examined clinical specimens from a patient with IE due to *S. gordonii*, a viridans *Streptococcus* species that expresses GTFs. Mononuclear phagocytes (CD68⁺) were the predominant cell type (mean [±SD] percentage, 46% ± 6% of total cells/mm², calculated from examination of 10 high-resolution fields) recruited to the inflamed regions inside the valve leaflet or endocardium (figure 1a–1c). Vascular proliferation was observed and leukocyte infiltrations were prominent around neocapillaries (figure 1b). Other infiltrates were primarily lymphocytes (CD4⁺ or CD8⁺; data not shown). The identity of VICs was confirmed by positive immunostaining results for prolyl 4-hydroxylase (figure 1d). Both mononuclear phagocytes and VICs from diseased tissue showed positive immunostaining results for the cytokines IL-1β, tumor necrosis factor (TNF)–α, and IL-6 (representative results for IL-6 are shown in figure 1e and 1f). Immunoreactivity in phagocytes or VICs was abolished completely by preincubation of the antibodies with the relevant cytokine prior to staining. In contrast, immunostaining for IL-1β, IL-6, or TNF-α was absent in tissue sections of normal mitral valves taken from forensic autopsy specimens (representative results for IL-6 are shown in figure 2, which appears only in the electronic version of the *Journal*). These results indicated that during the inflammation of IE in-
duced by viridians streptococci, VICs may play an important role in secreting cytokines to sustain inflammatory infiltrates.

**Binding of streptococcal modulin GTFs to VICs.** To test whether VICs are activated directly to recruit leukocytes, human VICs were cultured and stimulated by rGTFC, one of the *S. mutans* GTF isozymes purified from *Escherichia coli* [14]. Cardiac VICs exhibited an elongated, cube-like shape with multiple filopodia (figure 3a); they also expressed highly uniform levels of SM α-actin (figure 3b), which was suggestive of myofibroblasts [20, 24]. The identity and homogeneity of the VIC cultures were confirmed by positive immunostaining results for vimentin and prolyl 4-hydroxylase and by negative immunostaining results for von Willebrand factor and desmin, specific markers for endothelial and cardiac myocytes, respectively (figure 3c–3f). Similar to the results observed in endothelial cells [14], rGTFC bound rapidly to VICs in a dose-dependent manner (figure 4a [concentration per 1 × 10⁵ VICs] and 4b), and this binding could be blocked by PJS-3 preincubation (data not shown). In contrast, rDG60, a cell wall–associated immunodominant antigen of viridians streptococci [21], failed to bind to VICs even when added at a significantly higher concentration than rGTFC (100 μg/mL per 1 × 10⁵ VICs), which suggests specific and selective binding of rGTFC to VICs (figure 4c–4f).

**Bound rGTFC and activation of VICs to release cytokines and a chemokine.** Direct, dose-dependent activation by rGTFC was demonstrated by upregulated transcription and secretion of IL-6, IL-8, and MCP-1 by stimulated VICs (figure 5a and 5b). Induction of IL-6 and IL-8 in VICs was prominent at 2 μg/mL GTF and steadily increased to a maximum at 50 μg/mL rGTFC (figure 5a). Coculture of the primary VICs with 50 μg/mL rGTFC for 24 h did not induce cytotoxic changes in morphology or cell death (data not shown). Transcription of the cytokines or chemokine investigated peaked ∼3–6 h after stimulation and persisted ≥24 h (figure 5b). Although a time-dependent increase in IL-8 and MCP-1 was observed in unstimulated control VICs, the addition of rGTFC increased the amount of chemokines at 6, 9, and 24 h by 2.2-fold, 2.9-fold, and 12.3-fold for IL-8 and by 3-fold, 2.7-fold, and 4.3-fold for MCP-1, compared with unstimulated cells (figure 5c–5e). In addition, the induction of either IL-6 or IL-8 in VICs was blocked by preincubation of rGTFC with anti-GTF PJS-3 IgG (data not shown), which suggests that specific binding of rGTFC to VICs is essential. The enhancement in cytokine or chemokine production...
secretion by human VICs stimulated with 25 chain reaction. Glyceraldehyde 3-phosphate dehydrogenase mRNA was adherence of U937 cells stimulated and rGTFC-stimulated cells are indicated by white and black bars, respectively. The numbers of migrated cells were calculated by examination of 10 high-resolution fields (HRF) obtained from 3 independent experiments; bar graph data are presented as means ± SDs. *P < .05, by Student’s t test; **P < .01, by Student’s t test.

in VICs by rGTFC was specific and not attributable to the his-tagged fusion because another cell-wall associated protein, IDG60, carrying the identical fusion-tag, could not stimulate the release of these mediators. These results indicated that rGTFC was able to directly activate production of IL-6, IL-8, and MCP-1 by VICs.

Activated VICs and induction of migration and adhesion of MNCs. To test whether rGTFC-induced chemokines from VICs are biologically active and stimulate leukocytes to migrate, a chemotaxis assay was performed (figure 5f). After 6 h of rGTFC stimulation of VICs in the lower chamber of a 2-chamber unit (with U937 cells or MNCs in the upper chamber), the mean (± SD) number of MNCs and U937 cells that had transmigrated to the bottom chamber and adhered to DMEM-treated control VICs was 7.4 ± 10.2 and 0.67 ± 0.82, respectively, and the mean (± SD) number that had adhered to rGTFC-activated VICs was 42.67 ± 16.37 and 33.71 ± 5.47, respectively (figure 5g), demonstrating that the chemokines produced by rGTFC-induced VICs recruited U937 cells and MNCs.

Signaling pathways involved in activation of VICs. Production of IL-6, IL-8, or MCP-1 in rGTFC-activated VICs was inhibited to different degrees by various inhibitors of MAPK or NF-κB (figure 6a–6c). Consistent with these results, a rapid and persistent increase in the phosphorylation of ERK1/2 and JNK and a transient increase in phosphorylation of p38 MAPK were readily detected 5–15 min after stimulation by rGTFC (figure 6d). Similarly, enhanced phosphorylation of IκB was detected at 5 min and persisted for ≥60 min after rGTFC stimulation. In addition, nuclear translocation of NF-κB p65 was detected in rGTFC-activated VICs (figure 6f; 60 min after rGTFC stimulation), whereas the p65 subunit of NF-κB was retained in the cytoplasm of unstimulated VICs (figure 6e). Together, these results suggested that rGTFC directly activates MAPK or NF-κB and triggers expression of downstream genes that encode cytokines and chemokines in human VICs.

Expression of GTFs and activation of VICs in vivo. IE-inducing streptococci form biofilms on infected valves [10]. To confirm the expression of GTFs inside septic vegetations and the in vivo activation of VICs after bacterial infection, immunohistochemical analysis was performed using a rat model of left-sided endocarditis. Figure 7 shows a typical feature of S. mutans biofilms, the formation of multiple clusters of bacterial cells embedded in a fibrin matrix. Interestingly, leukocytes were rarely identified adjacent to bacterial clusters, which suggests that the movement of white blood cells was hindered by fibrin [25]. Immunostaining with anti-GTF PJS-3 IgG confirmed the presence of S. mutans inside the biofilm and also indicated that these bacteria actively expressed GTFs (figure 7a–7c). No positive reaction was obtained with rabbit preimmune IgG. In addition, small particles that were reactive to the PJS-3 antibody were detected around the S. mutans clusters, which suggests that either bacteria or secreted GTFs were diffusing from the biofilm. Septic vegetations were detected on catheter-injured aortic valves 6 h after intravenous challenge with bacteria, and infiltration of leukocytes in the aortic valve was clearly demonstrated; mononuclear phagocytes (CD68+) were the predominant cell type (figure 7d and 7e). In the catheter-injured valves of uninfected control rats, the infiltration of mononuclear phagocytes was limited, which suggests that the infiltration observed was a response induced primarily by the infecting bacteria or their components (figure 7f). VICs inside infected aortic valves were identified by their typical morphology and positive immunostaining for prolyl 4-hydroxylase (figure 8a and 8b). Intracoronary translocation of
RelA (p65) was also detected in some of the VICs (figure 8c and 8d), which suggests that these VICs were activated. These results suggested that cardiac VICs were activated after vegetation formation during IE. Such interactions might provide a microenvironment for retaining activated macrophages and for continuous recruitment of monocytes by maintaining cytokine or chemokine gradients within interstitial stromas.

**DISCUSSION**

The pathogenesis of IE is characterized by the formation of septic vegetation and inflammatory reaction in the infected valves or adjacent endocardium. The intensity of inflammation and tissue destruction may vary, depending on the bacterial species and the distribution of inflammatory infiltrate [26]. Persistent or recurrent inflammation induced by residual septic vegetations after antibiotic therapy may lead to valve damage, one of the most severe complications in endocarditis. The contribution of VICs, conceivably exposed on damaged valves, to the recruitment of inflammatory infiltrates within the interstitial stroma of heart valves is an important issue to be resolved. We demonstrated previously that streptococcal modulins, such as GTFs, in either their cell-bound or free forms, could directly activate in vitro recruitment of monocytes by endothelial cells [14]. In this study, we demonstrated further that *S. mutans* embedded inside fibrin-platelet clots are active in secreting GTFs and that VICs at infected valves could be activated in an experimental rat model of IE. It should be noted that other streptococcal proteins are likely to induce cytokines, so that it will be very difficult to detect in vivo a contribution by GTF alone.

It is also interesting that recognition of PAMPs or modulins by VICs appeared to be selective and specific, because GTF, but not rIDG-60, from bacterial cell walls bound to VICs in a...
dose-dependent manner. We hypothesized that these modulins, secreted in their free form, might easily gain egress from the embedded vegetations into the surrounding endothelium or valvular stroma, where the interaction with VICs would take place directly. Alternatively, GTFs could activate cytokine production in VICs indirectly, by interacting first with the leukocytes infiltrating vegetations. Both interactions achieved by bacterial modulins could result in the activation of VICs, as demonstrated in our experimental rat model of IE. The chronic persistent nature of septic vegetations facilitates the continuous release of these modulins and thereby provides a microenvironment favorable for persistence, rather than resolution of, inflammation during IE.

Cardiac VICs are a heterogeneous and dynamic population of mesenchymal cells with distinct characteristics. VICs maintain normal valve structure and function and, in diseased valves VICs are activated to regulate repair and remodeling [27]. Two cell morphologies have been observed in cultured VICs, namely small islands of cube-like cells and spindle-shaped elongated cells (figure 3). Immunostaining with a panel of mAbs directed against specific cellular components indicated that cultured VICs exhibit the characteristics of myofibroblasts [28]. Myofibroblasts have characteristics of both fibroblasts and SM cells and are characterized by the expression of contractile proteins with exquisite sensitivity to mechanical perturbations [29–31]. Cultured human VICs, whether they are from the aortic and pulmonary outflow valves or the mitral and tricuspid atrioventricular valves, similarly express SM/H9251-actin [24]. In normal mitral valves, interstitial cells express vimentin in situ, but not SM α-actin, desmin, or SM1, which suggests that VICs are fibroblast like [1]. Similarly, the majority of VICs in healthy adult humans are “quiescent” VICs that are fibroblast-like cells immunoreactive for vimentin but not SM α-actin, SMemb (a nonmuscle myosin expressed in activated mesenchymal cells), or matrix metalloproteinase–13 [32]. The higher percentage of active VICs

Figure 7. Glucosyltransferase expression and monocytic cell recruitment in experimentally induced endocarditis due to Streptococcus mutans. Typical vegetations stained with hematoxylin and eosin (HE) (a); panel b shows an enlarged view of the boxed region in panel a (arrowhead, biofilm clusters). S. mutans biofilm was immunostained with anti-GTF PJS-3 rabbit IgG after 24 h infection (c). Recruitment of mononuclear cells (MNCs) was detected 6 h after infection (d; HE staining); CD68+ MNCs detected 6 h after infection (e) are marked by arrows. CD68+MNCs in an uninfected, catheter-injured aortic valve 24 h after catheter insertion (f) are marked by arrows. Sections were stained with the anti–rat CD-68 mAb MCA341R and were counterstained with Mayer’s hematoxylin. Scale bar, 1 µm.

Figure 8. Nuclear translocation of p65/RelA in valve interstitial cells (VICs) in an experimental rat model of endocarditis—photomicrographs of an infected valve (a and c). Panels b and d show enlarged views of the boxed regions in panels a and c, respectively. VICs were identified by positive staining results for anti-prolyl 4-hydroxylase (a and b) or anti-p65 subunit of nuclear factor–κB (c and d) and counterstained with Mayer’s hematoxylin. Nuclear translocation of p65/RelA detected in VICs is marked by arrows in panel d. Scale bar, 1 µm.
with positive staining results for SM α-actin observed during culture and increasing passage in vitro suggests that manipulation stimulates VIC activation [32].

Under conditions of pathological injury or abnormal hemodynamic and/or mechanical stress, quiescent VICs become active VICs. Furthermore, active VICs increase secretion of the cytokine transforming growth factor (TGF)–β, which stimulates expression of SM α-actin, SM myosin, and calponin, which are key autocrine effectors [33]. The binding of TGF-β to TGF-β receptor type I and II initiates signaling through SMAD proteins to regulate the cell cycle as well as the proliferation, migration, extracellular matrix synthesis, and cytokine secretion of VICs [34]. In our experimental system, unstimulated VICs cultured in vitro, unlike endothelial cells [14], secreted higher levels of cytokines or chemokines (2–5 ng/mL) in culture supernatants. Whether cytokine or chemokine expression is also attributable to signaling through TGF-β awaits further investigation. Nevertheless, stimulation of cytokine and chemokine production in conjunction with rGTFC-enhanced activation of NF-κB and MAPK signaling molecules indicates that rGTFC further induces active VICs to enhance expression and secretion of cytokines and chemokines.

Fibroblasts, in addition to macrophages and leukocytes, are major sources of the chemokines and cytokines specialized for leukocyte recruitment. Primary cultured human fibroblasts isolated from different tissues universally express the chemokines stromal cell–derived factor-1, IL-8, and MCP-1, but some also selectively express MCP-4, RANTES, and macrophage inflammatory protein-1α [35]. In addition to tissue-specific diversity, fibroblast-like stromal cells may define a stromal “address code” that directs leukocyte development or infiltration [36]. Overexpression or aberrant expression of chemokines caused by injury or activation of PAMPs might direct leukocyte migration to specific locations and contribute directly to pathogenesis in chronic inflammatory diseases such as rheumatoid arthritis [37]. In contrast to other tissues, normal adult heart valves are avascular; however, in all pathological valve conditions, including IE, there are numerous neocapillaries (figure 1). The mechanisms of valve angiogenesis and leukocyte infiltration into injured valves remain unclear. Immunohistochemical analysis of diseased valve specimens indicated that mononuclear phagocytes, probably macrophages (CD68+), are the predominant population inside valve leaflets (figures 1 and 7). In accordance with this finding, our in vitro leukocyte migration assay indicated that significantly more U937 monocyteic cells and primary MNCs were recruited by rGTFC-stimulated VICs. Enhanced chemotaxis was also observed when the 2-chamber migration assay was conducted using a Jurkat T cell line or primary CD3+ T cells purified from peripheral blood (data not shown), which suggests that VICs, after being activated by a bacterial PAMP or modulin, secrete chemokines capable of recruiting different subsets of leukocytes.

References


