To clarify immune-mediated mechanisms in rheumatic heart disease caused by group A streptococcal infection, valve tissues from rheumatic patients with valvular heart disease who required valve replacement were studied for reactivity with monoclonal anti-CD4 or anti-CD8 monoclonal antibodies or anti-vascular cell adhesion molecule-1 (VCAM-1). At the valve surface, CD4+ and CD8+ T lymphocytes were adherent to valve endothelium and penetrated through the subendothelial layer. T cell extravasation into the valve through the surface valvular endothelium appeared to be an important event in the development of rheumatic heart disease. VCAM-1 was expressed on the valvular endothelium in rheumatic valves. Evidence suggested that the pathogenesis of rheumatic heart disease involved the activation of surface valvular endothelium with the expression of VCAM-1 and the extravasation of CD4+ and CD8+ lymphocytes through the activated endothelium into the valve. Lymphocytic infiltration through the valve surface endothelium has not been appreciated as a potential initiating step in disease pathogenesis.
enough intact valvular endothelium for study inclusion. All presented with mitral regurgitation.

**Tissues.** Valve specimens were obtained during surgery or autopsy from patients with rheumatic heart disease at the University of Witwatersrand (Johannesburg, South Africa) and at the King Faisal Hospital (Riyadh, Saudi Arabia). The patients were 8–65 years old. Rheumatic valves were compared with 3 anatomically and functionally normal mitral valves from autopsy of middle-aged men after myocardial infarction or heart failure. Only 2 rheumatic valves, from children 8–11 years old, contained enough surface endothelium to yield information for our study. Normal tonsils and lymph nodes were used as positive controls for immunostaining of T lymphocyte subsets, and tonsillar tissue was used as a positive control for activated endothelial cells. Tissues were fixed in 10% neutral buffered formalin and were embedded in paraffin.

**Histology.** Examination of hematoxylin-eosin (HE)-stained sections were characterized according to the cellular infiltration, fibrosis (scarring), neovascularization, and mineralization in the valves. Valves had lymphocytic infiltrates with evidence of chronic valvulitis, defined by the presence of inflammatory cellular infiltrates (predominantly lymphocytes), with scarring, neovascularization, and absence of significant mineralization. Mineralization was not observed in patients <13 years old.

**MAbs.** All MAbs used are IgG1. Anti-CD4 MAb (clone DD4-2; Southern Biotech Associates) reacts with the 55–60-kDa protein found on 54% of peripheral blood lymphocytes and 50% of thymocytes, and anti-CD8 MAb (clone C8/144B; Southern Biotech) reacts with the 55±60-kDa protein of peripheral blood cells. Both anti-CD4 and anti-CD8 MAbs react with the rheumatic valve (figure 1B). CD8+ lymphocytes also adhered to the valvular endothelium and infiltrated the valvular lesion (figure 1C). The isotype control MAb did not react with the rheumatic valve (figure 1D). Thus, there was evidence of T lymphocyte adherence to valve endothelium with extravasation through the endothelium. Neovascularization of diseased valves was also accompanied by the extravasation of CD4+ and CD8+ lymphocytes through the newly formed vessel walls into surrounding scar tissue (data not shown).

**Valvular endothelium.** To investigate the role of the surface endothelium in the extravasation of T lymphocytes into the valve directly from the blood, anti–VCAM-1 antibody was reacted with the valve sections. Rheumatic valvular endothelium expressed VCAM-1 on the valve surface endothelium (figure 2A). Rheumatic valve tissue sections did not react with the isotype MAb control (figure 2B). Anti–VCAM-1 did not react with the endothelium of 3 normal heart valve controls (not shown).

**Discussion.** In our study of rheumatic valves, the valve surface endothelium was a prominent site of lymphocytic infiltration in rheumatic heart disease, and lymphocytes adhered to superficial valve surface endothelium that expressed VCAM-1. Endothelial transmigration of lymphocytes at the valve surface endothelium may be an important initial step in disease pathogenesis. Previous studies showed CD4+ and CD8+ lymphocytic infiltrates in neovascularized regions of the valve [7]. Rheumatic valves with intact endothelium were observed [5], but the significance of the valve surface endothelium (endocardium) in the disease pathogenesis was not recognized. Previous work focused on the identity of Anitschkow cells and Aschoff’s lesions [6, 13]. The pathology of Aschoff’s body was elegantly described by
Our work focused on the importance of the valve surface endothelium in the pathogenesis of rheumatic carditis. Lesions within ARF valves contain both CD4+ and CD8+ lymphocytes, and MHC antigens are expressed on macrophage-like cells in the lesional tissues [7, 9]. A macrophage cell type has also been described in ARF lesions [13]. The presence of activated macrophages in rheumatic lesions is consistent with granuloma formation and the development of a CD4+ Th1 lymphocyte response. Cytokine profile studies suggest the production of tumor necrosis factor-α and interleukin-1 by macrophages in Aschoff’s bodies [14]. Hypothetically, the production of interferon-γ as a product of a Th1 granulomatous response would enhance the cytotoxic activity of the CD8+ lymphocytes present in the lesions, but in fewer numbers than the CD4+ lymphocytes (not shown).

We found evidence of immune mechanisms in the pathogenesis of rheumatic carditis. Initial streptococcal infection with the activation of B and T lymphocytes by streptococcal antigens and superantigens in susceptible patients would lead to antibody and cytokine production [1]. The reaction of antistreptococcal/antimyosin antibodies from rheumatic carditis with the valve endocardium supports the hypothesis that cross-reactive antibodies may bind to the endothelium and lead to inflammation, cellular infiltration, and valve scarring [10]. The binding of cross-reactive antibodies to valvular endothelial cells in ARF may up-regulate VCAM-1 on the endothelium. Influential cytokines may also affect the endothelium and the expression of VCAM-1. In either case, the valve would become a localized microenvironment for continual cytokine production and lymphocyte infiltration.

Expression of VCAM-1 on the endothelium is a hallmark of inflammation and heralds cellular infiltration [15]. Although it may not be surprising to find VCAM-1 expressed on rheumatic valvular surface endothelium, it has not been recognized previously. VCAM-1 interacts with very late antigen-4 on activated lymphocytes and leads to the extravasation of activated CD4+ and CD8+ lymphocytes into the valve tissue.
Scarring is an important event in the progression of valvular disease, because it is accompanied by neovascularization of the otherwise avascular tissue of the valve. The fact that the valve is avascular underscores the importance of our findings that extravasation would initially occur through the endocardium. Since the valve is normally avascular, transendothelial migration of lymphocytes through the endothelial surface would play an important role in the pathogenesis of valvular heart disease. Once the leaflets become inflamed through the valvular surface endothelium and neovascularization occurs, lymphocytes can infiltrate the valve both through the valvular surface endothelium from without and through the neovascularization from within. Even in mineralized valve lesions of older persons, a lymphocytic infiltrate was present, indicating progress of persistent disease in the valve. The role of activated endothelium would obviously play a dramatic role in the initial development of rheumatic valvulitis and the progression of the disease throughout a lifetime.

In summary, our findings support the hypothesis that activated valve surface endothelium binds activated T cells, which extravasate into the valve and lead to the cycle of scarring, neovascularization, and infiltration of lymphocytes. The avascular valvular tissue is normally protected by the endothelium until some initiating factor, which could be antibody, inflammatory cytokines, or both, breaks the endothelial barrier to the valve and allows for the cycle of cellular infiltration and scarring to begin. The data suggest that the mechanism of pathogenesis in rheumatic carditis begins at the valve surface endothelium.

Acknowledgments

We thank John B. Barlow (University of Witwatersrand, Johannesburg Hospital, South Africa) for encouragement and Kum Cooper and Wendy Fraser (Departments of Anatomical Pathology and Immunology, School of Pathology, South African Institute for Medical Research, University of Witwatersrand) for providing fixed tissues.

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