Valve Site-Specific Pathogenetic Differences Between Right-Sided and Left-Sided Bacterial Endocarditis*

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*Bacterial endocarditis is the most common serious and left-threatening cardiac infection, with an estimated 15,000 to 20,000 new cases each year in the United States. With a burgeoning geriatric population with advanced valvular heart disease now surviving long enough to be candidates for prosthetic valve placement, the number of BE cases is expected to increase dramatically over the next decade. Despite improved chemoprophylactic strategies, the yearly incidence of BE in prosthetic valve recipients remains at 1 to 4 percent.1,2 Moreover, despite development of potent new antibiotics and advances in cardiac surgical techniques in BE cases, the morbidity and mortality of this infection have stayed high, particularly in left-sided valve involvements (aortic and mitral). For example, tricuspid BE due to Staphylococcus aureus and Pseudomonas aeruginosa is curable with antimicrobial therapy alone in ~95% and ~75% of cases, respectively; in contrast, left-sided staphylococcal and pseudomonal BE have only ~60% and ~25% cure rates, respectively, with medical therapy, often requiring valvulectomy for definitive cure.3,4 We have previously confirmed the more salutary outcomes of right-sided vs left-sided BE using experimental animal models;5,6 in these studies, antibiotic-induced bacterial clearances from cardiac vegetations were less rapid and complete in animals with aortic (vs tricuspid) BE. The exact mechanisms underlying this distinct difference in therapeutic efficacy of BE depending on valve site are undoubtedly complex and remain largely un-delineated. It does appear from a number of experimental studies that the pathogenetic aspects of this valve-site-specific disparity in left-sided vs right-sided BE are multifactorial, involving selected organism factors, antimicrobial factors and host-defense factors.

THERAPEUTIC DIFFERENCES IN RIGHT-SIDED VS LEFT-SIDED EXPERIMENTAL BE

One of the first indications that there were basic pathogenetic differences between right-sided and left-sided vegetations came from treatment studies of experimental Pseudomonas BE. In animals with tricuspid BE given synergistic combination treatment with amikacin plus ceftazidime, the mortality rates and frequencies of hemorrhagic pulmonary infarctions were significantly less than for control animals and those receiving single-drug β-lactam (ceftazidime) or aminoglycoside (amikacin) regimens.5 Moreover, the amikacin-ceftazidime regimen was significantly better than the single-drug therapies with respect to lowering mean vegetation bacterial densities and rendering vegetations culture-negative. In contrast to these results in right-sided BE, animals with experimental aortic pseudomonal BE caused by the homologous strain and treated with the identical antimicrobial regimens had strikingly different outcomes.6 By the end of two weeks of antibiotic treatment, single-drug regimens of amikacin or ceftazidime had resulted in little significant decrease in intravascular bacterial densities, with mean counts being >log_{10} 6 CFUs/g of vegetation in each therapy limb. Moreover, even in the amikacin-ceftazidime combination therapy group, initially lowered intravascular bacterial densities at day 7 of treatment had risen to pretherapy levels by the 14th treatment day. Additionally, intravascular bacterial densities in all three treatment groups had risen to pretreatment levels by the fifth posttherapy day (the relapse period) in surviving animals. In a similar set of studies, treatment of experimental right-sided pseudomonal endocarditis was highly efficacious with a synergistic combination of amikacin and a ureidopenicillin (azlocillin), while a nearly identical regimen was ineffectual in aortic endocarditis caused by the same infecting strain.7,8
Pathogenetic Factors Important in Valve-Site-Specific Outcomes in Experimental BE

Differential in Vivo Inoculum

One of the readily identifiable distinctions between right-sided and left-sided cardiac vegetations in BE is the achieved bacterial density. It is clear from a number of studies that in vivo inoculum is considerably higher in aortic vs tricuspid vegetations. For example, with experimental P aeruginosa BE, aortic valve vegetations generally contain >log_{10}9-10 CFUs/g of tissue, whereas mean bacterial densities in tricuspid vegetations rarely exceed log_{10}8 CFUs/g.\textsuperscript{5,8} Perlman and Freedman,\textsuperscript{10} as well as Durack et al.,\textsuperscript{11} have confirmed similar findings in experimental staphylococcal and viridans streptococcal BE, respectively. The mechanisms underlying this valve-site-specific enhancement of in vivo bacterial growth are not fully characterized but may be related to the increased oxygen tensions on the left side of the heart.

Differences in Extent of Extracardiac Metastatic Infection

Most studies of experimental BE reveal that the infection tends to run a more virulent course in aortic than tricuspid valve involvements, irrespective of the etiologic organism. Also, the degree and frequency of extracardiac septic complications are substantially higher in left-sided vs right-sided experimental BE. For example, Archer and Fekety\textsuperscript{9} found that 100 percent of animals dying with aortic pseudo-mononal BE had renal abscesses, as compared with only 13 percent of those with tricuspid BE caused by the homologous strain. Similarly, Garrison and Freedman\textsuperscript{12} found that a minority of animals with staphylococcal tricuspid BE had frank renal abscess formation despite active bacteremia, septic pulmonary infarcts and ongoing vegetation infections. These data suggested that the lungs serve as an important filter for larger septic emboli for metastatic, systemic seeding in tricuspid BE.

The presence of extracardiac infection appears to be important pathogenetically in the maintenance of ongoing valvular infection in BE, especially with left-sided involvements. Francioli and Freedman\textsuperscript{13} showed that, following catheter removal in animals with tricuspid streptococcal endocarditis, the infection tended to self-heal. In contrast, in animals with concomitant aortic and tricuspid valve BE, the tricuspid infection tended to be maintained, suggesting that persistent bacteremia (in this example from the aortic valve) could perpetuate tricuspid valvar infection via vegetation reseeding by circulating intravascular organisms. In this setting of bivalvular BE, mean bacterial densities in tricuspid vegetations were several logs higher than those found in blood cultures, excluding simple blood contamination of the tricuspid valve cultures. Our own laboratory recently showed that, despite mitigating initial valvular attachment of S aureus to the aortic valves of rabbits, vaccine-induced antistaphylococcal antibodies could not prevent renal lodgment of the organism following intravenous challenge.\textsuperscript{14} Thus, the eventual frequencies of staphylococcal BE were not different in immunized versus unimmunized animals with aortic catheters, again suggesting that the presence of extracardiac infection (in this case renal) plays an important role in maintaining valvular infection.

Differential Frequencies of Antimicrobial Resistance

There have been substantial data in both human and experimental BE to confirm that the intravetation bacterial acquisition of antibiotic resistances is almost exclusively a province of left-sided BE. For example, in one study of experimental aortic pseudomonal BE,\textsuperscript{6} by the second week of therapy between 22 and 64 percent of sampled vegetations contained variants highly resistant to amikacin or ceftazidime or to both despite animals receiving synergistic combination chemotherapy with these agents. In contrast, in animals with pseudomonal tricuspid BE caused by the same strain and receiving the same antimicrobial regimen, no antibiotic-resistant variants were isolated from culture-positive vegetations.\textsuperscript{5} Similar findings have ensued in animals with aortic BE receiving fluoroquinolone therapy\textsuperscript{15} or amikacin-ureidopenicillin synergistic regimens,\textsuperscript{8} as well as in humans with left-sided pseudomonal BE receiving tobramycin-ureidopenicillin (piperacillin)\textsuperscript{16,17} or fluoroquinolone (ciprofloxacin) treatment.\textsuperscript{18} The aminoglycoside resistance in the experimental BE studies was due to defective intracellular accumulation of the drug by the variants, related to inability to generate adequate transcytoplasmic membrane energy potential.\textsuperscript{18,20} β-lactam resistance was due to intravetation selection of mutants that were stably derepressed for constitutive β-lactamase overproduction which was inducible in the parental strain.\textsuperscript{21} This β-lactamase conferred resistance by the dual mechanisms of non-hydrolytic trapping of the β-lactam, as well as slow enzymatic drug hydrolysis at low substrate concentrations. Quinolone resistance was related to selection of intravetation mutants with alterations of target DNA gyrase enzymes.\textsuperscript{22} The experiences in human BE have confirmed similar mechanisms of in vivo development of antibiotic resistances.\textsuperscript{16,17} It is of considerable interest that drug-resistant variants exhibiting these same mechanisms of antibiotic resistance are readily produced in vitro by exposure of the bacterial cells to drug concentrations above the minimal inhibitory or bactericidal concentration (MICs/MBCs) of the particular organism in serial passage.\textsuperscript{23-25} The pathogenetic...
pathways involved presumably relate to the selection of spontaneously derived, antibiotic-resistant subpopulations contained within the large in vivo inoculum of left-sided cardiac vegetations, following suppression of the drug-sensitive, quantitatively dominant bacterial population.⁵,⁶,¹⁶ In addition, sub-MIC/MBC antibiotic concentrations achieved in certain vegetation loci may serve to perpetuate this situation by suppressing more drug-sensitive intravertebral organisms in the overall population, while allowing for the more drug-resistant subpopulations to flourish.

**Differential Antimicrobial Penetration**

There is considerable indirect data to support the concept of ineffectual antibiotic penetration or drug maldistribution in left-sided cardiac vegetations. For instance, scanning and transmission EM of bacteria in the center of aortic vegetations from animals with pseudomonal BE treated with amikacin show none of the frequently observed morphologic changes expected when such bacteria are exposed in vitro to ≥1/₂ MICs of aminoglycosides.¹⁹ Studies have examined the overall penetration of various antibiotics into experimental aortic valve vegetations; in general, these show that the intravertebral PK profile of β-lactams roughly parallels that of serum PK.²⁶,²⁷ McColm and Ryan²⁸ compared the PK characteristics of the β-lactam ceftazidime into experimental aortic vegetations and subcutaneous fibrin clots. As compared with aortic vegetations in which PK profiles paralleled serum (rapid entrance and exit phases), the PK profile into fibrin clots featured a relatively slower penetration rate but a higher area under the time-concentration curve. The slow penetration rate into subcutaneous fibrin clots was attributed to the low surface area-volume ratio of this compartment as well as to exposure to slow capillary blood flow near the clot. In contrast, the PK profile of intra-aortic valve penetration was typical of a high surface area-volume compartment in contact with constant high-pressure arterial flow (ie, large peak-trough concentration fluctuations). We recently compared the overall PK and pharmacodynamic profiles of ceftazidime and amikacin within infected aortic vs tricuspid vegetations in animals with pseudomonal BE.²⁹ As in the study of McColm and Ryan,²⁸ the drug PK profiles into aortic vegetations paralleled those of serum, while time-concentration curves for tricuspid vegetations resembled those of subcutaneous fibrin clots. The times-above-MBC of the infecting strain within tricuspid vegetations were significantly longer than those achieved within aortic vegetations for ceftazidime and amikacin. The properties of tricuspid vegetations that allow them to behave like fibrin clots in relation to antimicrobial penetration are unknown. It is possible that lower intracardiac flow rates or pressures generated within the right ventricle, or both, as opposed to the left ventricle contribute to slower drug penetrances into tricuspid vegetations.

As noted before, EM evaluation had suggested a geographic maldistribution of aminoglycosides within aortic vegetations with a lesser penetration into the central interstices of the lesion in comparison with more peripheral loci.¹⁹ Cremieux et al²⁹ evaluated the single-dose intravertebral distribution of various antibiotics into streptococcal-infected aortic vegetations of rabbits utilizing radioactively labelled drugs and autoradiographic techniques. They showed that penicillin distributed more extensively to the vegetation periphery and less so to the vegetation center. In contrast, the aminoglycoside tobramycin distributed relatively evenly throughout the lesion, while the complex glycopeptide teichoplanin distributed only to the periphery of the vegetations. It should be noted that the study of Cremieux et al²⁹ was semiquantitative and not performed at drug steady-state. We have recently characterized the estimated steady-state aminoglycoside distribution within various geographic sectors of aortic vegetations of rabbits and humans, utilizing an integrative computer-generated model.³⁰ With standard dose-strategies, aminoglycosides did, in fact, penetrate throughout rabbit and human vegetations. However, drug levels substantially below the MBC of the infecting pseudomonal organism were calculated as achievable in the center of the vegetation, despite supra-MBC levels being attained in plasma and more peripheral vegetation loci. Computer simulations also confirmed that aminoglycoside daily doses at least two to four times higher than those ordinarily recommended to treat severe infections are necessary to consistently achieve uniform supra-MBC levels at the vegetation center at steady state for an entire dosing interval.

**Pathogenetic Differences in Host Defenses**

As mentioned before, a number of investigators have shown that mean bacterial intravertebral densities in aortic vegetations significantly exceed those observed in tricuspid vegetations in experimental BE. Also, in experimental aortic BE, bacteraemia tends to be high-grade (>100 CFUs/ml of blood) and long-lasting, while in tricuspid BE bacteraemia tends to be short-lived and intermittent.⁹ Moreover, in experimental aortic BE, mean intravertebral bacterial densities tend to remain at a high, and often increasing, log count over a one to three-week post-infection course; in contrast, tricuspid valve BE tends to have a disease course characterized by trends toward spontaneous self-sterilization of intravertebral bacteria.⁶,⁷,¹² Several studies have examined both the influence of the transvalvular catheter in maintaining active BE,
as well as delineating the infection-site specific trends in spontaneous sterilization of endovascular infection. Perlman and Freedman\textsuperscript{10} were the first to confirm the consequences of catheter removal in experimental BE. They showed that removal of transtricuspid catheters in animals with established staphylococcal right-sided BE led to prompt sterilization of tricuspid vegetations; on the other hand, removal of transaortic catheters led to self-sterilization of aortic vegetations in only \textasciitilde{}50 percent of animals. Similar findings emanated from Durack et al\textsuperscript{11} and Archer and Fekety.\textsuperscript{9} Of interest, Francioli and Freedman\textsuperscript{13} showed that if experimental endovascular infection was catheter-induced only 5 to 10 mm above the aortic valve in the thoracic aortic, self-sterilization similar to the tricuspid valve ensued. Yersin et al\textsuperscript{20} have performed the most convincing study on the role of the transvalvular catheter in inducing and maintaining ongoing BE. The initiation of BE depended on the length of time the catheter had been in place and whether the catheter was present at the time of initial bacterial challenge. The time necessary for onset of self-sterilization trends in aortic valve BE was directly proportional to the length of time the catheter remained in place following bacterial challenge with \textit{Escherichia coli}.

The mechanisms responsible for more prominent self-sterilization trends in right-sided vs left-sided BE remain only partially defined. It does appear, however, that the host cellular defense system, particularly the PMN, plays an important role in this regard. Cellular host defenses had previously been assumed to be minimally operative within left-sided cardiac vegetations since postmortem studies in human and experimental BE cases showed the lesion to be a relatively acellular platelet-fibrin matrix surrounding the bacterial colonies.\textsuperscript{32,33} However, autopsy evaluations of patients and experimental animals with right-sided BE documented the presence of a more intense PMN inflammatory response within tricuspid vegetations than seen in left-sided lesions.\textsuperscript{34,35} Yersin et al\textsuperscript{35} and Francioli and Freedman\textsuperscript{13} examined this phenomenon in detail in experimental tricuspid streptococcal BE. They showed that induction of severe granulocytopenia and monocytopenia resulted in intravascular depletion of PMNs, as well as in an abrogation of the self-sterilization of tricuspid valve lesions. It was shown that systemic immunosuppression with dexamethasone also mitigated the tendency of streptococcal-infected experimental tricuspid valve vegetations to undergo spontaneous sterilization; this latter study did not distinguish, however, between a direct effect of the steroid on PMN phagocytic function and a more global drug effect on removal of circulating bacteria by the reticuloendothelial system. The previously noted studies also did not distinguish between the role of the monocyte and PMN in mediating ongoing right-sided BE. We recently confirmed the primary role of PMN in this regard.\textsuperscript{30} We rendered animals with tricuspid pseudomonal BE either granulocytopenic and monocytopenic with nitrogen mustard or selectively monocytopenic with etoposide. Only treatment with nitrogen mustard was associated with a concordant decrease in intravascular inflammatory influx and a significant increase in intravascular bacterial densities over time in tricuspid valve lesions; in contrast, untreated control animals and etoposide-treated animals underwent the customary spontaneous intravascular sterilization trends.

Meddons et al\textsuperscript{37-39} have published several recent reports also confirming the role of the PMN in the course of experimental BE. Of note, their studies have implicated a modulating role for PMNs even in aortic BE. In streptococcal and \textit{E coli}, granulocytopenia induced by the nitrogen mustards was associated with a more prolonged bacteremia and a higher intravascular mean bacterial density than was observed in untreated control animals with normal PMN counts. Their results in experimental staphylococcal BE\textsuperscript{39} were somewhat different in that it appeared that both monocytes and PMNs had a role in keeping aortic endocardial infection in check.

It thus appears cogent that the PMN plays a significant role in eradicating intravascular bacteria in tricuspid vegetations (presumably via opsonophagocytic mechanisms) while also serving to modulate aortic valve BE by preventing bacterial densities from undergoing unbridled growth and reaching supranormal concentrations.

\textbf{Oxygen-Dependent Differences in Right-Sided vs Left-Sided BE}

There have been few investigations into the influence of local intracardiac factors on the course of BE. One obvious microenvironmental difference between right-sided and left-sided vegetations is the oxygen tension to which intravascular bacteria are exposed \textit{in vivo}. We studied the effects of various oxygen tensions on bacterial exopolysaccharide production as well as bacterial growth inhibition and killing kinetics \textit{in vitro} and \textit{ex vivo}.

Using two oxygen tensions simulating those found in the right and left ventricles \textit{in vivo} (PO\textsubscript{2} = \textasciitilde{}40 and 80 mm Hg, respectively) we found aminoglycoside-induced \textit{in vitro} and \textit{ex vivo} growth inhibition and killing of \textit{P aeruginosa} was significantly less at the higher PO\textsubscript{2}, despite no overall differences in the rate of bacterial replication at the two oxygen tensions. Polysaccharide stains showed significantly more extracellular glycocalyx surrounding cells exposed to the higher PO\textsubscript{2}; monoclonal antibody staining confirmed this exopolysaccharide to be pseudomonal-specific mucoid glycocalyx.\textsuperscript{41} Also, by scanning EM, aortic vegetations from animals with
pseudomonal BE contained more exopolysaccharide surrounding bacterial cells than was found in tricuspid vegetations. These studies defined that various oxygen tensions may be involved in upregulation or downregulation of exopolysaccharide production both \textit{in vitro} and \textit{in vivo}. These data also suggested that excess exopolysaccharide production at higher oxygen tensions may adversely affect antibiotic-induced bacterial killing, possibly via barrier mechanisms. There have been no similar studies looking at the influence of various oxygen tensions on the virulence factors of other bacterial species known to cause human BE (eg, streptococci). It should be noted, however, that Pfaller et al\textsuperscript{42} and Dall et al\textsuperscript{42} have recently confirmed the importance of the viridans streptococcal glycolalxy (dextran) in blocking penicillin-induced clearance of intravascular organisms in experimental aortic BE.

References