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Review article

The Wound Environment, Microbial Virulence and Postoperative Infection: Practical Lessons for the Surgeon☆

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ABSTRACT

For the practicing surgeon, the development of a postoperative wound infection represents a major complication that can be both costly and disabling. As a result, surgeons apply multiple methods of prevention including skin decontamination, use of antibiotics, irrigation with or without antiseptics and meticulous use of technique. In elective surgery, however, most wound infections cannot be predicted.

In this review we discuss emerging concepts in wound infection pathogenesis and include a discussion on how the wound environment may directly activate bacteria to express a more harmful or virulent phenotype. Based on these emerging concepts, we provide the practicing surgeon with molecular level evidence to explain why some methods of wound infection protection may be useful while others are not.

El ambiente de la herida, la virulencia microbiana y la infección postoperatoria: lecciones prácticas para el cirujano

Palabras clave:
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RESUMEN

El desarrollo de infecciones postoperatorias representa una grave complicación que puede tener un alto precio y llegar a ser muy frustrante para los profesionales de la cirugía. Por ello, los cirujanos han adoptado diversos métodos para prevenirlas, como el uso de antibióticos, métodos de esterilización de la piel, soluciones para la irrigación con o sin antisépticos, así como técnicas que minimicen el trauma en los tejidos. Sin embargo, en la cirugía electiva la gran mayoría de las infecciones de herida son imposibles de predecir.

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En esta revisión discutimos los conceptos emergentes sobre la patogénesis en las infecciones de herida y analizamos la influencia que tiene el medio ambiente de la herida en la activación de las bacterias que expresan un fenotipo nocivo o virulento. Basándonos en estos conceptos emergentes, buscamos ofrecer al cirujano la evidencia a nivel molecular que explique la razón por la cual algunos métodos de protección de la herida quirúrgica son efectivos, mientras que otros no.

Introduction

In every environment within which a surgeon's works, bacteria are present and have the potential to compromise healing and impair recovery. Surgeons are keenly aware of this and despite their best efforts, contamination of the wound, the peritoneum and other sterile tissues regularly occurs during surgery. Yet remarkably, infection is rare following surgery and surprisingly does not necessarily correlate with the degree of intraoperative contamination.[1–3] For example, it is now well established that even when surgical wounds are culture negative at the end of an operation, a wound infection can occur postoperatively.[1–3] Conversely it is also well established that in most cases when a surgical wound culture is positive at the end of an operation, rarely if ever, does a clinical infection develop.[1–3] How then can we reconcile these opposing observations when most surgeons consider it to be axiomatic that wound infections develop as a result of intraoperative contamination? In this review we will introduce the idea of the surgical wound environment as a major factor that can control the state of virulence of contaminating bacteria such that they can either silently become eliminated or cause a clinical wound infection. Here we will argue that a wound infection is not simply a function of excess pathogenic bacterial contamination in an immune compromised host, but rather it is a function of how bacteria are activated to express virulence in response to a particular wound environment that enables them to then overwhelm host immune mechanisms. The main factors within the wound that drive bacteria virulence expression are local activating ''cues'' released as a result of ischemia, injury and compensatory host factors produced from the physiologic and traumatic aspects of surgical injury. Implicit in this discussion will be to offer practical aspects to the surgeon to minimize these factors by considering approaches that can mitigate the release, and hence the presence of, local environmental factors that drive wound infection pathogenesis.

What Are Environmental ''Cues'' and How Are They Released?

It is now well established that bacteria have evolved complex information processing mechanisms to ''sense'' changes in the local environment and ''respond'' with enhanced virulence.[4] In order for bacteria to be able to both grow and thrive in harsh environments, such as when nutrients are limited, information processing provides them a unique advantage to be able to adhere to and invade host cells in order to obtain nutrients.[5] Environmental signals that activate this response in bacteria include both physico-chemical ''cues'' such as iron, phosphate, amino acids, redox potential, pH, etc., and host derived factors such as cytokines, opioids and products released during ischemia.[6] The receptors and pathways that mediate these ''sense and respond'' circuits are integrated into the quorum sensing signaling system, a system of virulence activation whereby bacteria can sense their population density and respond with enhanced virulence, ostensibly that amount needed to overcome a host.[7] Thus the ability to sense both the environment, their host and their population density has equipped bacteria with a unique set of mechanisms to respond to harsh and complex circumstances and enhance their fitness (reproductive capacity).[8] The host on the other hand is similarly equipped to sense the presence of bacteria on its cells through pathogen recognition receptors (PPRs) such as the well described TLR4 system.[9] In this manner when bacteria get too close to host cells and become activated to invade, host cells can mount a counter-response by producing mucus, antimicrobial peptides and recruiting immune cells such as neutrophils and macrophages.[10] Thus microbes and host cells continually co-exist in a state of ''trust but verify'' whereby they can sense each other's presence, understand each other's biologic activity and maintain a state of molecular détente. The host knows it needs to feed its microbiota and the microbiota know that they need to

metabolize nutrients to maintain the host's health status, upon whom their survival depends. As a result of this ongoing chemical dialog, the host and its microbiota continually develop a molecular understanding of each other's needs, even in time of distress, such as during periods of starvation or physiologic stress. In this state of mutualism, it is in each party's best interest, to maintain a state of molecular détente as a long term strategy to protect each other. Applying behavioral economics, investigators have recently conceptualized the host-microbial interaction as a biological marketplace where there are public goods to be exchanged where reciprocal exchange is rewarded and hoarding of public goods is punished.[11] In this manner each party keeps the other ''in check'' to maintain fair trade of public goods. However there are some pathogens that are simply short-sighted and who cheat.[12] Such pathogens, with minimal provocation, can express lethal virulence traits that lead to fatal infections, thus killing the very host upon whom their survival depends. There is a worrisome trend that such strains, many of which can be multi-drug resistant, are more frequently colonizing our most at-risk patients as they are exposed to multiple levels of health care encounters such as when they undergo biopsies, radiation treatment, exposure to antibiotics etc. In such cases when the normal microbiome is depleted by the prolonged use of antibiotics, these highly pathogenic strains can cause life-threatening infections.[13,14] When such high risk patients undergo a prolonged and high risk surgery, it would be useful to know whether such strains are present as well as the status both of their microbiome. Efforts are now underway to decolonize patients of these highly lethal strains using fecal microbiota transplant (FMT).[14]

A surgeon might imagine that during a typical operation on the gastrointestinal tract, there can be a significant disruption of this fragile biologic marketplace when we impose a period of fasting prior to surgery, administer purgatives and antibiotics, make patients ''non per os'' postoperatively and then allow them only to eat processed foods for the next several days during recovery. Although in this scenario, the extent to which molecular détente is maintained in unknown, it is important to keep in mind that the majority of patients recover from major surgery without infections. It is assumed that their microbiomes return to normal and once again participate in maintaining the health of their host. Yet in other circumstances, pathogenic bacteria may outcompete the normal microbiota for nutrients, especially when antibiotic use is prolonged and oral nutrition is delayed. If the host remains physiologically stressed and continues to release host factors (i.e. ''cues'') into the local environment, these pathogenic bacteria can become signaled to invade host tissues to obtain nutrients. In order to do so, they must also express virulence factors to suppress and subvert immune clearance mechanism. As a counter response, host cells then express inflammatory mediators resulting in a situation where bacterial virulence and host inflammation confront one another and become pathoadaptive to the normal process of healing.[15]

Our laboratory has identified several key elements within the host–microbial interchange that govern whether a microbiome and host exist in a state of molecular détente versus molecular confrontation. We discovered three major classes of host derived factors that are released during surgical injury that directly activate bacteria to express enhanced virulence.[16] Using the model organism *Pseudomonas aeruginosa* we identified that cytokines (interferon gamma), opioids (dynorphin, morphine, fentanyl), and products of ischemia (adenosine) can induce certain pathogens to express lethal virulence traits against its host. Interestingly, conditions of low extracellular phosphate was observed to activate virulence in *P. aeruginosa* and other pathogens through the well described PsTs membrane sensor. When extracellular phosphate is depleted, *P. aeruginosa* virulence is further enhanced in the presence of the host-derived signaling molecules, yet when phosphate is abundant, *P. aeruginosa* does not respond to these host factors. We established a connection between bacterial signaling by phosphate and the quorum sensing signaling system of virulence activation.[17] The simple lesson here is that when bacteria get the nutrients they need, they do not respond to host compensatory cues and do not activate a virulence response.[18] This line of investigation may have practical applications to our patients when we consider how enhanced recovery programs after surgery (ERAS) function to improve outcomes. One mechanism by which ERAS might reduce infection is simply by promoting the concept of early feeding.[19] Surgeons have long recognized that when patient are fed early in the postoperative course, their length of stay is reduced. Given that phosphate signaling is a universal cue to which most bacteria respond similar to *P. aeruginosa*, maintaining intestinal phosphate levels during surgical injury by initiating early feeding may be a logical approach to improve overall outcome.

Minimally Invasive Surgery to Suppress Microbial Virulence and Prevent Infections

Having now established that bacteria can sense and respond to host stress/injury via their ability to gather, process and transduce signals released by host tissues, surgeons must make every effort to minimize physiologic stress during surgery. Minimally invasive surgery (MIS) is a clear example of an innovation that has been proven to reduce infections and complications following surgery. Yet the mechanisms remain unknown. Surgery using the MIS approach is known to reduce cytokine release, reduce pain (i.e. endogenous opioid release), reduce bleeding (i.e. ischemia) and have many other benefits, that at the molecular level, could minimize the total exposure of colonizing bacteria to the elements that activate their virulence.[20,21] Also the MIS approach, in most cases, results in a lower length of stay and earlier feeding

allowing intestinal bacteria to obtain the nutrients they need. The unanticipated influence of MIS on the intestinal microbiome may be a major advantage of this approach and may be an unaccounted for mechanisms of its overall success.

It is presumed that MIS surgery reduces surgical site infections (SSIs) by minimizing the size of a wound and hence its exposure to environmental contaminants. However, it is important to realize that MIS surgery also minimizing the local trauma to a wound. Less trauma to the wound will necessarily minimize the release of host factors and therefore even if bacteria enter this space, they are less likely to become activated. This same mechanism may be working when surgeons apply wound edge protectors to the wound during open surgery.[22,23] Certain wound protectors distribute pressure more evenly across the wound and thus minimize the trauma of metal wound retractors that can cause sustained ischemia.

In support of this theory is the curious observation that wound cultures at the end of an operation do not correlate with wound infection rates. Across a variety of observational studies, when wound cultures are positive following major open surgery, they are not predictive of a wound infection.[1] In most cases (i.e. ~100%) where the wound culture is positive prior to closure of the surgical wound, no infection develops. In the rare case that an infection does develops, most often it is due to a different organism than the one present at the closure of the wound. How is this possible?

Despite the universal claim that all wound infections following surgery are due to intraoperative contamination, the evidence to support this claim is weak to non-existent. Surgeons have a major problem of ''confirmation bias'' in this regard. For example, it is common to dismiss and forget all the cases where there was gross contamination and no SSI occurred (i.e. the majority of cases) and recall and emphasize only those patients in whom gross contamination was associated with an SSI (i.e. the minority of cases). An additional issue is that of ''attribution bias'' where when gross contamination occurred and no SSI resulted, it is attributed to use of irrigation, antibiotics and good technique. In contrast when an SSI occurred after gross contamination, it is often attributed patient related factors (poor nutritional status, obesity, smoking, etc.). Lack of objective concordance in such observational studies confirms the bias that all wound infections occur as a result of intraoperative contamination continues.

Our laboratory has studied the mechanisms of wound infection as it relates to local trauma and ischemia and the release of host factors into the wound. Using *P. aeruginosa*, *Acinetobacter baumannii* and methicillin resistant *Staphyolococcus aureus*, we demonstrated that without local ischemia and injury, direct inoculation of these pathogen into an abdominal wound in mice does not cause infection.[24–26] Yet an infection can be produced in these mice when local trauma and ischemia is created in the wound resulting in the release of host factors that activate bacterial virulence. Interestingly, when key nutrients (i.e. iron or phosphate) are placed directly into the wound, wound infections do not occur as their virulence mechanisms are molecularly silenced. Taken together these data suggest that not all wound infection occur as a result of direct intraoperative bacterial exposure, it takes more and the environmental context is likely to be much more important than previously recognized.

We recently introduced the idea of the Trojan Horse hypothesis of SSI.[26] In this study, we tested the hypothesis that bacteria that asymptomatically colonize the intestinal mucosa such as methicillin resistant *Staphylococcus aureus* (MRSA), can be taken up by neutrophils, enter the bloodstream and silently travel to a surgical wound site releasing their infectious payload. In this study, we observed that, in the process of healing, a surgical wound is neutrophil chemoattractant. If neutrophils carrying bacteria such as MRSA end up traveling to the wound, the can infect it by releasing the MRSA into the wound site. The extent to which the wound is injured, with the attendant release of bacterial activating host factors, will increase it attraction by neutrophils. Thus it is possible that bacteria hiding in intestinal crypts, teeth, lungs, etc. can be taken up by a local neutrophil, enter the bloodstream and then silently travel along a chemical trail and land onto a traumatized wound remote from the initial site in which the neutrophil ingested the bacteria. Depending on the environmental context, neutrophils can adhere to and release their microbial payload onto the tissues where they grow and cause an infection. This mechanism might explain why certain SSIs seem to occur late in the recovery phase of surgery and often are caused by bacteria such as *S. aureus* or *Enterococcus faecalis*, now the two most common causes of SSIs following gastrointestinal surgery.[27]

Hyperoxygenation as a Method to Prevent Wound Infections: Unexplained Success and Unexplained Failure

Multiple lines of evidence suggest that wounds that sustain long periods of ischemia are more vulnerable to infection.[28] Under elective circumstances, both surgeons and anesthesiologist do all they can to prevent wound ischemia by providing fluids, maintaining perfusion and avoiding hypoxemia. Consistent with this practice is the observation that risk factors for SSIs include hypoxia, hypotension and inadequate perfusion. Using the logic that if ''some is good, more must be better,'' the practice of hyperoxygenation has emerged as a mechanism to further reduce SSIs. Yet in the era of adequate skin decontamination, maintenance of perfusion and use of antibiotics, it may be difficult to demonstrate its benefit. Dismissive of the knowledge that too much oxygen may be just as bad as too little, investigators have developed the logic that hyperoxygenation will decrease SSI rates despite having little to no molecular understanding of its effect either at the level of the wound or the microbiome.

Intriguingly, results of hyperoxygenation trials (i.e. 80% oxygen during and 2 hour following major surgery) have been mixed since its onset. Early major trials were positive,[29] latter trials were negative.[30] Recently a group has independently published what appeared to be highly promising results, which were then later disputed forcing retraction of the article.[31] It is perhaps time to analyze, not only the foundational science upon which this hypothesis was formulated, but also why investigators chose 80% oxygen as a therapeutic target. Although beyond the scope of this discussion, a review of the studies leading up to the practice of supplemental oxygen to reduce SSIs provide evidence that the practice of supplemental oxygen was not only ill-conceived, but was designed based on empirical logic.[32] As mentioned above, investigators reasoned that if wound hypoxia is a risk factor for infection, hyperoxygenation at 80% should prevent and even reduce infection risk. What investigators failed to consider was the precise dose of oxygen needed to achieve this endpoint and the potential harmful effect of hyperoxia on the wound environment and the microbiome.

In response to growing concern that the mechanisms by which supplemental oxygen reduces infection remain unknown, Qadan and others demonstrated that 80% oxygen administered to human volunteers, while exerting a positive effect on immune cell function, also resulted in an 87% increase in reactive oxygen species (ROS).[33] Perhaps not considered by investigators promoting the use of 80% oxygen during surgery is the well-known effect of ROSs on bacterial virulence.[34,35] Bacteria are equipped with redox-response regulators that ''sense'' the presence of ROS and ''respond'' by secreting superoxide dismutase, an ROS deactivating enzyme, and expressing virulence as a counter response.[36] What effect this might have in the overall balance in a given tissue between bacterial virulence and immune responsiveness remains unknown. However this mechanism could contribute to the failure of 80% oxygen supplementation to reduce infection rates and may be responsible for the results of clinical trials where an increase infection rate is observed with supplemental oxygen. Unfortunately all these trials lack both species identification of infections as well as any molecular analysis of the effect of 80% on ROS release and bacterial virulence expression. As a result, until the mechanism and potentially harmful effects of supplemental oxygen during surgery are more properly studied, this practice remains controversial and lacks sufficient evidence to be recommended.

Avoidance of Opioids as a Method to Suppress Microbial Virulence Expression and Prevent Wound Infections

It has long been recognized that opioids, both when administered as drugs or when endogenously released during stress, can suppress the immune system.[37] Traditionally the mechanisms of infection risk has been attributed to the effect of opioids on the immune system.[38] For surgeons, opioid use can have an adverse effect on intestinal motility, prolong hospitalization and thus increase the risk of a nosocomial infection. Our laboratory has demonstrated that opioids, such as the endogenously released opioid dynorphin, as well as exogenously administered agents such as morphine and fentanyl, can directly activate bacteria to express a more virulent and lethal phenotype.[39,40] Primarily as a result of clinical observations, ERAS programs have recommended limitation of opioid use in patients during the postoperative period.[41,42] Yet significant evidence now exists at the molecular level, to justify avoidance of opioids during and after surgery when possible. For example, direct molecular-level evidence that opioids exert their unwanted motility effects via intestinal bacteria has been recently published and involves the intestinal microbiome.[43] Indirect evidence for this effect can be observed in patients preoperatively treated with oral non-absorbable antibiotics demonstrating a low incidence of ileus.[44] Finally, studies stratifying burn patients by age and percent surface area burned have demonstrated that when opioids are withheld, lower infection rates in the wounds are observed.[45] Thus even at the level of the wound, opioid use may promote an infection vulnerable environment. As such, the rationale to avoid opioids when possible during and after surgery is well supported by both mechanistic level and clinical studies and is generally recommended.

Lack of Evidence to Support the Use of Wound Irrigation, Antibiotic Powders and Antibiotic Collagen Sponges

Given the growing body of evidence that the wound environment plays a major role in the predisposition to infection, either from hypoxia, the presence of host tissues factors that can induce bacterial virulence or bacterial contamination occurring from the Trojan Horse mechanism, surgeon continue to be attracted to practices with the potential to durably sterilize the wound. These approaches have included wound irrigation with antibiotics, chlorhexidine gluconate, jet lavage with antimicrobials and the use continuous delivery systems (i.e. powders, collagen sponges). Surprisingly not only has none of these approaches been efficacious in preventing wound infections, many actually result in increased wound infection rates.[46] While it seems counterintuitive that these approaches would lead to an increase wound infection rate, when considering the wound environment's effect on bacterial virulence expression, molecular plausibility exists. For example a large clinical trial in patients undergoing open colorectal surgery had a collagen sponge with gentamicin placed in the wound compared to a no treatment group.[27] Wound infections

were higher in the collagen gentamicin sponge group with a higher incidence of antibiotic resistant pathogens. A study of patients undergoing elective spine surgery had vancomycin powder placed into the wound with a higher than expected (2.83%) wound infection rate mostly due to gram negative and polymicrobial infections.[47] Jet lavage is associated with a higher wound infection rate in some studies and antibiotic wound irrigation appears to have a weak or null effect in reducing wound infection rates.[46]

While antibiotic application to an at-risk wound seems logical, failure of these trials beg further explanation. It is possible that the above measures alter the wound environment in some way, either by disturbing its microbiome, causing the release of bacterial activating host factors or promoting the Trojan Horse mechanism by further sterilizing the wound and reducing competitive exclusion of transient microbes entering the wound. Given the current state of technology to measure both wound factors and microbial genes and metabolites, many of the above hypotheses can now be formally tested in clinical trails using genetic and metabolic detection and identification techniques. The assembly of teams of microbiologist, geneticist, clinical trialist, surgeon-investigators and patients at risk for serious postoperative infection will be required. In this manner a more molecular approach to the problem of wound infections could inform novel anti-virulence approaches to infection prophylaxis across many surgical procedures.

Conclusions

Traditionally surgeons aspire to adhere to the founding principles of good practice that includes careful dissection, economy of motion, meticulous attention to technique, minimizing bleeding, use of minimally invasive techniques, early enteral feeding and limitation of opioids. As a result of these efforts, worldwide, infection rates within centers of excellence appear to be at a historic low. Understanding the mechanisms by which these practices are effective at the molecular level has the potential to push infection rates even lower, especially as surgeons advance their techniques and take on the most challenging cases. Consideration of bacterial virulence expression as a key unrecognized factor in the pathogenesis of SSIs will allow for the discovery of non-antibiotic approaches to prevention. Such novel approaches could limit the emergence of antibiotic resistance, a growing problem facing surgeon who operate on the highest risk patients. The technology now exists to improve our understanding of the molecular pathogenesis of SSI and minimize its risk to our patients.

Conflict of Interest

The author declares no conflict of interest.

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