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SEPTIC PENETRATING SETAE/BACTERIA/BACTERIAL EMBOLI/SEPTIC PENETRATING SETAL EMBOLI: A HYPOTHESIS TO EXPLAIN THE PATHOGENESIS OF THE MARE REPRODUCTIVE LOSS SYNDROME

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ABSTRACT

In the summer 2002 one of the authors (TT) hypothesised that the fundamental patho-physiological mechanism of mare reproductive loss syndrome (MRLS), (early fetal loss [EFL], late fetal loss [LFL], uveitis, pericarditis and encephalitis) is the penetration of septic, barbed eastern tent caterpillar (ETC) setae (septic penetrating setae, SPS) through moving tissues, including blood vessels, with haematogenous spread of bacteria, bacterial emboli (BE), or, perhaps more consistent with the clinical syndromes and a companion mathematical analysis, septic penetrating setal emboli (SPSE). MRLS would therefore be due to: 1) the ability of barbed ETC setal fragments to penetrate moving tissues; 2) the probability of such fragments penetrating blood vessels, releasing bacteria, BE or SPSE, each or all of which distribute haematogenously; 3) the high sensitivity of the pregnant mare to bacteria introduced into her uterus or fetal membranes from, by, or with ETC setal fragments or related bacterial materials; and 4) the less effective antibacterial responses in susceptible tissues, ie fetal fluids and the eye. In most tissues, septic entities or septic setal fragments are handled by antibacterial defences and yield no detectable damage. MRLS lesions in the heart, brain, eye and fetal membranes are haematogenous, and the driving force for the abortions is bacterial proliferation in the fetal membranes and fluids, which provides a critical amplification step, enabling 1.0 g caterpillars to cause abortions in 1,500 lb mares within 32 h.

The sequence of events starts with caterpillar ingestion and barbed setal fragments randomly penetrating intestinal tissues. This is followed by penetration of blood vessels, releasing bacteria, or bacterial emboli or the septic setal fragment itself into the blood. These materials distribute haematogenously to all points in the body, the distribution following cardiac output. The late term fetus represents a large capture area for these materials, the early term fetus a smaller area, but both are highly sensitive to bacterial insult. The rapid onset of late fetal loss in experimental ETC administration requires rapid delivery of the abortifacient material to the fetus. A single eye represents the smallest capture area associated with clinical signs of MRLS; the unilateral eye lesions are unique and may be explained by haematogenous distribution of discrete infective quanta with penetrating capabilities such as septic emboli (SE), or, perhaps more satisfactorily, by septic penetrating setal emboli (SPSE). Entry of the septic factor accounts for the acute onset exudative, treatment-resistant ophthalmitis, followed by loss of the eye. The pericarditis is also caused by these circulating materials which enter the coronary circulation and penetrate the cardiac musculature; a fraction of these migrate through the heart and into the pericardial space, yielding the pericarditis. Bacteria cultured from the pericarditis cases are similar to those associated with MRLS; however, no bacteria were cultured from some pericarditis cases, suggesting loss of septic contaminants during passage of the septic material (SM) through the cardiac musculature. The pericarditis cases may be best explained by the SPSE variant of this hypothesis.

Identification of the ETC abortigenic activity with the integument of the caterpillar and the finding of large numbers of granulomatous lesions

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containing setal fragments in the intestines of ETC-dosed pigs strongly supports the septic penetrating setal portions of this hypothesis. Review of the specifics of the clinical syndromes, as set forth above, and a recently developed mathematical analysis of MRLS may suggest that haematogenous events associated with the setally mediated entry into blood vessels of bacterial pathogens (BP), bacterial emboli (BE) or SPSE are key to understanding the pathogenesis of the unusual constellation of syndromes that constitute MRLS.

Background

Considering MRLS we visualised setal fragments from orally ingested eastern tent caterpillars (ETC) penetrating the intestinal wall, entering small blood vessels and facilitating entry of bacteria into the blood stream (Tobin *et al*. 2003a,b; 2004). We then considered the consequences if these setal fragments or related materials redistributed haematogenously. If their distribution followed cardiac output, the unusual bacteriological and other characteristics of the various discrete clinical syndromes that constitute MRLS, and the lack of clinical signs in affected mares, despite rapid onset of experimental MRLS, became more understandable.

Observations demonstrating the presence of large numbers of setal fragments in the intestinal walls of ETC-dosed pigs (McDowell *et al*. 2003a), rats (Fitzgerald 2003) and one horse (McDowell *et al*. 2003b) support the first step of this hypothesis. Based on this, and a unique toxicokinetic/statistical analysis of MRLS (Sebastian *et al*. 2003a), we present the septic penetrating setal hypothesis of MRLS.

Hypothesis

The hypothesis is specifically written to include penetration of moving tissues by septic penetrating setae, including setal fragments, followed by haematogenous distribution of each or all of bacteria, (BE) or SPSE, together 'septic materials (SM)', following initial tissue penetration by septic setal fragments from ETC, which events ultimately yield the differing clinical syndromes comprising MRLS. The pivotal assumption in this hypothesis is that, in seeking to understand MRLS, we previously underestimated the combined effects of 4 key steps:

Step 1: The penetration and migration of barbed caterpillar setal fragments in moving tissues. This hypothesis proposes that exposure to ETC barbed setal fragments results in penetration of the oral and/or intestinal mucosa or other tissues by setal fragments and the non-specific introduction of local commensal bacteria.

Step 2: The migration of setal fragments, by virtue of their barbed structure, in moving tissues. A proportion of these septic fragments penetrates blood vessels and releases SM, which rapidly spread haematogenously in the horse.

Step 3: The haematogenous relocation of SM to points distant from the point of entry, their retained ability to penetrate moving tissues, and the apparently high sensitivity of the fetoplacental unit to bacterial insult, especially as compared with the sensitivity of the non-pregnant horse.

Step 4: The poor anti-bacterial responses in clinically affected tissues. Rapid bacterial growth follows bacterial contamination of fetal and other extracellular fluids, which results in fetal loss (Hong *et al*. 1993). The eye is immunologically privileged, and its extracellular fluids are less well protected than many tissues (Rocha *et al*. 1992). The brain and cerebrospinal fluid also have immunological deficits. The pericardial lesions may relate in part to the motility and central location of the heart in the circulatory system and its resultant high level of exposure to blood-borne SM, especially SPSE.

In most tissues, small numbers of 'lodged' SM are handled readily by the specific and non-specific immune systems and cause no clinically significant or apparent long-term damage. However, the lack of effective anti-bacterial systems in fetal fluids appears to leave the fetus largely unprotected once bacterial contamination of fetal fluids occurs, leading rapidly to the early and late fetal loss manifestations of MRLS.

The SPSE portion of this hypothesis is apparently without precedent in biology or medicine. In its support, the hypothesis well fits the unique toxicokinetics of MRLS set forth by Sebastian *et al*. (2003a) and the unique grouping of clinical syndromes that constitutes MRLS. Additionally, the hypothesis is well supported by evidence concerning multiple setal fragment penetrations in the intestines of ETC-dosed pigs, rats and horses, the unusual speed of onset of the abortions, calculations concerning the likely numbers of circulating SM quanta, the difficulty in culturing bacterial pathogens from the blood of affected horses and the unusual general lack of systemic clinical signs in MRLS affected horses.

Haematogenous spread of SM and MRLS syndromes

From clinical cases and experimental work, it appears that the initiating event in each of the MRLS-associated syndromes is local bacterial infection of haematogenous origin, with the bacteria initially entering the blood stream at the site of exposure to the caterpillar.

The unilateral ophthalmitis cases are consistent with and best explained by a haematogenous source. In particular, a number of these cases apparently started with haemorrhage deep in the eye, and the difficulty in treating these cases is also consistent with a septic haematogenous insult originating deep in the eye.

Preliminary supporting experimental evidence

Analysis of experimental work (Sebastian *et al.* 2003b) suggested that bacterial proliferation was the driving force in late fetal loss, and the source of the fetal bacterial pathogens was haematogenous and not ascending (ie, via the cervix) in origin.

This hypothesis was further supported by the work of Bernard (reported by Herbert 2003) who first reproduced early fetal loss in mares administered ETC integuments by stomach tube.

Bacteria/bacterial emboli/septic penetrating setal emboli

The second step in this hypothesis is that the setal fragments penetrate blood vessels, especially thin-walled veins, and release SM, a proportion of which move rapidly in the blood to new and more distant locations in the body. All such SM movements are passive, secondary to tissue movement and/or blood flow (cardiac output), and all events are statistically determined.

For this mechanism of MRLS to be operative, and especially for the SPSE portion of this hypothesis to be effective, the starting number of setal fragments should optimally be large, since all penetration and distribution events are statistically determined. This requirement is not inconsistent with recent estimates suggesting that aborting mares were exposed to the equivalent of 5 to 30 g/day of ETC (Sebastian *et al.* 2003a). Beyond this, recent reports that the intestinal tracts of pigs necropsied following exposure to broadly equivalent doses of orally administered ETC contain very large numbers of small setal fragments encased in microgranulomatous masses (Hong *et al.* 1993; Fitzgerald 2003; Sebastian *et al.* 2003a) are entirely consistent with the SPSE portion of this hypothesis.

This hypothesis is specifically written to cover 3 distinct possibilities. It assumes the primary entry of the pathogenic bacteria is associated with the penetrating setal fragments. The second step, haematogenous spread of SM, may involve distribution of either B, BE, or SPSE.

The SPSE portion of this hypothesis requires that haematogenously spread setal fragments either pass through or bypass the lung capillary beds to enter the systemic circulation. While the ETC setal fragments seen in pig intestinal tissues are small barbed cylinders of about 15–25 microns in diameter and 100 microns in length, the question arises as to how such fragments could pass through the lung capillary beds. We suggest 3 mechanisms that may allow movement of SPSE past or through the lung capillary beds and into the systemic circulation.

An obvious mechanism is that SPSE move through the lungs in the same way as they enter into and move through the intestine, ie they are driven or 'ratcheted' through lung tissues by respiratory movements, which, sooner or later, allow passage of a fraction of SPSE through the lung.

SPSE may also bypass lung capillary beds through well-characterised anatomical and pathological shunts. Gillespie and Tyler (1969) estimated the percent venous admixture (anatomical shunts) in the pulmonary circulation to be about 5% in normal horses. Anatomical shunts therefore provide a mechanism for SPSE to rapidly bypass lung capillary beds and enter the systemic circulation.

Finally, it may also be that some SM/SPSE fragments are themselves small enough to pass relatively unhindered through capillary beds, as suggested by the small diameter of some terminal setal fragments compared with the diameter of equine lung capillaries.

Unusual susceptibility of the pregnant mare to SM

Bacteria, BE or especially SPSE lodged in a uterine blood vessel of a pregnant mare will again migrate through these tissues when the mare moves. Myometrial movement, either due to the musculature of the myometrium or the physical activity of the mare and fetus, will drive migration of bacteria, BE, or a lodged SPSE. Eventually, the SM will penetrate the fetoplacental unit.

Well established clinical experience suggests that modest bacterial contamination of amniotic fluid can result in rapid bacterial overgrowth, followed by death and expulsion of the fetus within days. Based on this, penetration of the fetal membranes by a modest quantity of SM would presumably be sufficient to produce early or late fetal loss. Sebastian *et al.* (2003b) suggested that bacterial proliferation was a primary or driving event in late fetal loss, apparently occurring prior to signs of fetal distress and fetal death.

A striking characteristic of experimental MRLS is the speed (within 32 h) with which experimental late fetal loss can occur (Sebastian *et al.* 2003a). We suggest that the late fetus presents a large 'capture area' for randomly distributing SM; as such, a late fetus is statistically more likely to be 'hit' by randomly

distributing SM within a given period than a much smaller early fetus. Additionally, uterine movements are greater in a mare carrying a late fetus, driving the tissue migration of SM lodged in uterine tissue, and ensuring their rapid penetration through a fetal membrane. Together, these steps may explain the apparently more rapid onset of late than early fetal loss, and especially the extremely rapid onset of high dose experimental late fetal loss.

This hypothesis also readily explains cases of early or late fetal loss occurring some time after exposure to caterpillars has ceased. Inopportune location of SM, especially SPSE, in a less mobile area of the myometrium would delay the entry of SM into the fetal membranes.

The SPSE and to some extent the BE models are consistent with the lack of positive blood cultures and virtual lack of systemic clinical signs of bacteraemia from cases of early and late fetal loss. This is because the blood-borne bacterial contamination is carried in small numbers of discrete quantal packets on or in individual setal fragments or bacterial emboli and not diffused throughout the bloodstream.

Uveitis, pericarditis and encephalitis cases

We consider it well established that the cases of uveitis are of haematogenous origin. We propose that the primary haematogenous event is delivery of a quantum of SM to the eye. The very low incidence of uveitis observed is presumably related to the relatively small target size and the equivalently small fraction of cardiac output supplying an individual eye.

A significant argument in favor of the SPSE portion of this hypothesis is the fact that all incidents of uveitis were unilateral. This observation well fits a quantal and entirely random haematogenous distribution of SPSE, as compared with haematogenous distribution of BE, and especially bacteria, delivery of which are less likely to be so clearly quantal in nature.

With reference to Step 4, the eye is also an immunologically privileged area; as such, it may be particularly susceptible to damage by penetrating SM. Our ability to observe eye pathology associated with MRLS is also most likely due to the ease of observing events occurring in the eye, and the highly significant consequences of eye damage compared with limited local damage in other areas of the body, and the possible therapeutic and immunological difficulty of controlling a septic focus that has entered deep in the eye.

The pericarditis cases

The pericarditis cases presumably represent SM that enter the coronary blood supply, lodge in blood vessels, and then migrate through the moving/contracting cardiac vessels and tissue. Of all tissues in the body, the contracting heart is one through which one might expect bacteria, BE or, especially, SPSE to migrate fastest.

A problem with this part of the hypothesis is that pathologists have reported no evidence of setal tracks in cardiac tissues. A careful search should be made for signs of sub-clinical pericarditis associated with intestinal exposure to ETC. The central role of the heart in the circulatory system and its ongoing contractile activity may suggest a considerable probability of transient positive histological and bacteriological culture findings in pericardial fluid associated with ETC exposure. On the other hand, analysis of recent pig data suggests that the penetrating setal fragments are relatively small, and the eye data suggest that the actual numbers distributing may be very small. In this circumstance, it may be challenging to visually detect setal tracks in cardiac tissue. In this regard, it is worth noting that the microgranulomas associated with lodged setae in the intestinal tract of pigs and rats are themselves very small, and not grossly observable (Fitzgerald 2003).

The encephalitis cases

Since MRLS was first described, 3 specific cases of Actinobacillus encephalitis have been recognised as occurring in the same period. Like the pericarditis cases, these cases are unquestionably haematogenous in origin and occurred in about the same time period as MRLS (Sebastian *et al*. 2003b).

Intellectual economy of the hypothesis

We initially focused on ETC setae because of their well established overall role in caterpillar defence mechanisms. This hypothesis is a modification and simplification of the hypothesis that drove our first mouse setal experiments, which assumed that the setae were introducing a protein or other toxin or factor that was the primary pathogen. Further reflection, along with identification of bacterial proliferation as the driving force in MRLS, suggested that a setal toxin is not necessarily required, and the speed of onset of late fetal loss in laboratory experiments also does not suggest a classic catalytic toxic mechanism.

This hypothesis does not require the presence of any extra toxins in the ETC, or viruses, or microsporidia, or unusual weather patterns, or

cyanide precursors, or cyanide, or plant toxins, or frass, or fungal overgrowth on frass; it simply requires ETC.

This hypothesis may also explain why no significant hormonal patterns or other clinical chemistry changes have yet been identified in the aborting mares. Early and late fetal loss are dependent on the direct and relatively non-specific seeding of small amounts of bacterial contaminants into the fetal membranes, followed by bacterial proliferation and abortion.

Although MRLS is clearly associated with the haematogenous distribution of different bacterial species, it has not, to our knowledge, been possible to demonstrate a bacteraemia associated with this condition. The SPSE portion of this hypothesis, suggesting that the invading bacteria are carried in discrete quanta on small numbers of setal fragments, may offer an explanation for the lack of apparent evidence of bacteraemia in natural and experimental MRLS mares.

This hypothesis is grounded in the well established physics and mechanics of the movement of barbed fragments through motile soft tissues, and the likelihood of bacterial contamination of such barbed fragments. These then distribute throughout the horse as bacteria, BE or SPSE. We propose that this biologically unique hypothesis accounts for all of the unique mathematical, epidemiological, clinical, pathological and bacteriological characteristics of the 5 simultaneously occurring MRLS syndromes associated with exposure to ETC.

If this setal hypothesis is correct, similar exposure to mechanical and bacteriologically equivalent setae from other caterpillar species, or possibly any other mechanically equivalent structure, may also have the potential to produce syndromes akin to MRLS.

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