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MMP responses in the MRL mouse:

The MRL Mouse and the Relationship between Inflammation and Regeneration

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Abstract

The matrix metalloproteinases (MMPs) have been implicated the regenerative response in amphibians and various mammalian models of regeneration. The neutrophil response is known to bring MMPs and other proteases to the wound to promote bacterial elimination and tissue remodeling. These issues in relation to what is occurring in the MRL mouse model of regeneration/wound healing is discussed.

Keywords

Basement Membrane; Blastema; Inflammation; MRL mouse; Matrix metalloproteinases; Neutrophils; Regeneration

The response to injury ranges from a regenerative response seen in sponges, planaria, and newts to name a few organisms, to a scar-forming response seen most often in mammals. Recent findings in mice, however, clearly indicate that the regenerative response is not lost in mammals.

The ability to regenerate has been maintained in one particular strain of mouse, the MRL mouse (1) as well as its ancestral parent, the LG mouse. The MRL mouse has long been a model of SLE or lupus erythematosis (2-4). A serendipitous finding upon numbering the mice using ear hole punching led to the observation that the ear holes remain open for less than one month.

This ear hole closure was not a normal wound healing response but was rather accompanied by the production of new cartilage and perfect healing. A similar finding has been seen in the cryoinjury of the heart (5). After 2-3 months the heart heals perfectly, the wound site fills with new cardiomyocytes, little scarring accumulates, and functional recovery is seen. Most recently, this healing process has been found after digit amputation, where some growth occurs though full recovery and structure is never achieved (Gourevitch, ms in prep). This is unlike the digit tip studies previously carried out (6,7).

In all of these cases, new growth is seen with the formation of a blastema, a cellular structure of “de-differentiated” cells that grow and form new tissue, replicating what was there previously (8,9). Before this can form, the injured tissue undergoes a wound-repair type of response with infiltrating inflammatory cells, and matrix remodeling of both pre-existing and newly laid down extracellular matrix (ECM) containing collagens and other components.

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One key event that occurs and can be clearly seen in the MRL mouse ear hole is the remodeling and breakdown of the basement membrane that initially forms between the new epidermis that covers the wound after injury and the dermis beneath it (10). In mammals, the basement membrane is generally maintained throughout the wound healing process and supports tissue specificity and integrity, with scarring the usual result. By contrast, after amphibian limb amputation, a basement membrane never forms during the limb regeneration process and only re-appears when healing is complete (11,12). If one experimentally induces the formation of a basement membrane in the healing amphibian limb, then scar formation occurs and the regenerative response is halted (13). The MRL mouse represents a compromise in that a basement membrane is generated early after injury in the ear hole but it is then removed (10). The most likely explanation for the importance of these events is that there are molecular interactions important to the regenerative response (11, 12), similar to what is seen during development where this barrier is not present.

How does this important biological process occur? A likely set of candidate molecules are the matrix metalloproteinases or MMPs and their inhibitors, TIMPS or tissue inhibitors of matrix metalloproteinases (14-17). These molecules have been implicated for many years as one of the keys to the regenerative response and have been found in hydra (18), sea cucumbers (19), and in amphibians (20-25). MMPs and TIMPs have been localized to the margin between the epidermis and dermis, exactly where the basement membrane would be located, and appear to be produced by epithelial as well as dermal cells.

In the regenerative response mounted by the MRL mouse in the ear after injury, however, while there are stromal cells that are making MMPs, the majority of the MMPs are brought into the wound site by the cells of the inflammatory response. These include both neutrophils and monocytes that circulate in the blood and can rapidly reach the wound site (10).

The MMP responses shown to be involved with regenerative healing in amphibians include the gelatinases MMP 2 and 9 (24,25), the collagenases including a novel molecule nCol (25), and the stromelysins MMP 3/10a and 3/10b (25) which are found in the regenerating limb and the growth zone, the blastema. Timp1 (NvTimp1) which regulates the MMP response has also been identified in the salamander regenerate (26).

Using zymography, activated MMP 2 and 9 were initially found in the MRL earhole blastema at higher levels than in the nonhealing C57BL/6 earhole tissue (10). Histological analysis of the ear showed that the majority of the MMPs were found in cells migrating into the wound site, the inflammatory cells including neutrophils and monocytes. There were more MMP-positive inflammatory cells in the injured MRL ears when compared to the nonregenerating injured C57BL/6 ears.

Analysis of neutrophils in the circulation from normal and injured mice have shown that MRL neutrophils contain significantly more gelatinases (MMP2 and 9) and more stromelysin (MMP3) than C57BL/6 neutrophils (Gourevitch, data not shown). MMP3 is particularly significant because it is specific for collagen type IV found in the basement membrane and has activity similar to the MMP 3/10a and 10b seen in the amphibian (25).

From this data, we would predict that a low level of neutrophil infiltrates in a wound would lead to a non-regenerative response.

Neutrophils are generally considered to be involved with scavenging of dead tissue and complement-mediated bacterial opsonization and bacterial destruction using an oxidative response with superoxide and hydrogen peroxide production. The neutrophils thus decontaminate the wound site. It has been proposed that such cells are detrimental to

regeneration and that this is a primary reason for a scar wound repair response rather than a regenerative response (27-29). This case is made by examining fetal wound healing, but it is important to note that this phenomenon may not actually be regeneration.

In the central nervous system, the situation may be quite different with the possibility that MMPs and perhaps inflammation as well, are rapidly down-regulated. In a study examining cortical brain stab wounds in MRL and control Swiss Webster “nonhealing” mice (30), it was shown that early increased levels of MMPs and enhanced proliferation occurred after injury. However after one week, the MMP levels in the MRL dropped and concomitant scarring occurred. Other studies have shown the importance of MMPs in CNS regeneration (31-32). A recent study examining alkali burn wounds to the cornea of MRL mice (33) showed reduced inflammatory responses in MRL mice compared to B6 mice. In this particular case, the MRL wounds fared much better than the C57BL/6 wounds. Thus, we may expect that the role of inflammation and its impact on regeneration or other types of wound healing responses and organ systems will vary, depending on those various issues.

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