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Inflammation and Wound Repair in Oysters[1]

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When cells are injured or destroyed in vertebrates and many invertebrates, an immediate protective response is initiated. The inflammatory response is an attempt to destroy, dilute, or isolate the injurious agent and the dead or damaged cells (Sparks, 1972). The successful conclusion of the inflammatory response is wound repair. Major features of the reparative process are removal of the inflammatory exudate and restoration of the architectural integrity through regeneration or formation of scar tissue by progressive proliferation of fibroblasts and deposition of collagen.

The pioneering study in oyster inflammation was the classic study by Stauber (1950) of the role of the leukocyte in the defense mechanism of *Crassostrea virginica*. Intravascular injection of india ink results in virtual embolism in much of the arterial system followed by massive aggregation of leukocytes in and around the emboli. Within 2 to 4 hours post injection, phagocytosis is initiated. Subsequently, the ink-laden leukocytes migrate, both by passage to blood sinuses and through the walls of blocked arteries, through the Leydig tissue of the oyster and are eliminated from the oyster by migration across the epithelial borders (diapedesis), primarily of the digestive system. Diapedesis occurs rarely through the pericardial wall and through the epithelium of the mantle and palps, but almost never across the epithelia of the external shell-secreting mantle, excretory tubules, or gonoducts.

Tripp (1958) demonstrated, by intracardial injection of malarial-parasitized duck erythrocytes that intracellular digestion of digestible foreign materials occurs in addition to diapedesis. Tripp summarized the response of leukocytes to particulate matter as engulfment of the particle followed by migration. If the particle is not metabolizable, it is eliminated by diapedesis, but metabolizable materials are eliminated by a combination of intracellular digestion and diapedesis.

Pauley and Sparks (1965, 1966) studied the acute inflammatory response of the Pacific oyster (*Crassostrea gigas*) to injections of turpentine and talc. Turpentine is an irritant that elicits a typical inflammatory response in vertebrates and talc characteristically invokes a post-operative inflammation resulting in granulomatous scar tissue formation in vertebrates and has been used in a study of the wound repair process in cockroaches (Schlumberger, 1952). The use of these two substances, therefore, provided a comparison of inflammation and wound repair in vertebrates, insects, and oysters.

Grossly, injections of talc into the connective tissue result in a greenish discoloration around the wound within 16 hours which persists for 72 hours then gradually fades to a normal appearance by 96 hours. Adductor muscle injections develop an identical discoloration along the line of injection that persists, but gradually fades, through 168 hours. Turpentine injections into the connective tissue are unremarkable for approximately 32 hours at which time the adductor muscle becomes swollen and undergoes loss of function (one of the cardinal signs of vertebrate inflammation). Injection of turpentine directly into the adductor muscle elicits a similar, but earlier and more marked, gross response. The muscle becomes obviously swollen and loses its functional capability within 16 hours and frequently becomes detached from the shell after 32 hours. Abnormally large amounts of silt accumulate on the gills and mantle; pus develops in the wound within 48 hours, and grossly identifiable cellulitis is apparent by 176 hours and persists for long periods.

The first histological manifestation of the inflammatory response to talc injection is leukocytic infiltration, accompanied by edema, after about 16 hours. Blood sinuses adjacent to the injected talc become congested and contain talc after 24 hours, with congestion of large vessels some distance from the wound. Granulomatous tissue, consisting of elongate cells with round nuclei, begins forming around the talc in the larger blood vessels at about 24 hours. The nuclei of these cells gradually become elongated (by 160 hours) and the granulomatous tissue becomes well organized into thrombi that partially occlude the vessels.

Lesions begin to form in the connective tissue as early as 24 hours post injection, consisting initially of talc particles and loosely aggregated leukocytes surrounded by a compacted, peripheral band of leukocytes. The talc particles cause extensive local mechanical damage to the connective tis-

[1]Most of the material in this paper was excerpted from A. K. Sparks (1972). Reaction to injury and wound repair in invertebrates. *In* Invertebrate pathology, noncommunicable diseases. Academic Press, N.Y.

sue (Leydig) cells. After 40 hours, the peripheral leukocytes elongate and become arranged parallel to one another. Somewhat later (88 hours) leukocytes in the center of the lesion undergo karyolysis with aggregations of compact, apparently viable leukocytes present between the necrotic center of the lesion and the peripheral band of elongated leukocytes. The nuclei in the peripheral band begin to elongate between 128 and 200 hours. The central area becomes infiltrated with elongate cells by about 300 hours, and well-formed granulomas develop, consisting of a band of elongated cells 25-100 cells wide surrounding a mass of talc granules and necrotic leukocytes heavily infiltrated by elongated leukocytes. The lesions also contain large numbers of brown pigment cells after 56 hours, the function of which is unknown. Adjacent normal tissue lack such cells.

Talc injection into the adductor muscle elicits a similar response with granulomas forming in the same manner, but containing necrotic muscle fibers in the center of the lesion. Additionally, infarcts, characterized by coagulation and necrosis, are common, probably resulting from anoxia caused by partial thrombic occlusion of blood vessels.

It is clear that oysters have an effective defense mechanism against solid, nontoxic particles; they localize the particles by forming granulomas similar to those observed in insects and vertebrates. As will be shown subsequently, however, oysters do not possess the ability successfully to combat a caustic liquid such as turpentine. Such substances may spread throughout the body causing systemic necrosis of vital tissues.

Turpentine injected into the Leydig tissue causes edema and leukocytic infiltration in the area of injury within 8 hours accompanied by congestion of the adjacent smaller blood vessels and sinusoids. Marked vascular dilation develops by 16 hours with apparent leukocytic pavementing of large vessel walls in the vicinity of the injury. Increased numbers of leukocytes appear in the blood vessels and sinuses and begin to move from the blood channels towards the injury site.

By 24 hours post injection, digestive tubules and Leydig cells in the area of injection undergo massive necrosis, with the cells characterized by faded cytoplasm and pycnotic nuclei. Heavy leukocytic infiltration of the wound occurs by 40 hours and 8 hours later a conspicuous band of leukocytes surround the necrotic area. Multinucleate giant cells, normal products of post-mortem change, appear at about 64 hours and are common thereafter.

A section through the injection site at this time reveals a central mass of liquified material containing fragments of destroyed cells surrounded by necrotic, but still recognizable, Leydig cells, digestive tubules, and massive numbers of infiltrated leukocytes. The architecture appears more normal and the cells more viable progressively from the center of the abcess outward. Finally a thick band (several hundred cells thick) of apparently normal leukocytes forms the periphery of the lesion. Although histologically the oyster at this point appears to have succeeded in encapsulating and thus isolating the turpentine, subsequent events demonstrate that this is not correct. Since these events are not part of the acute inflammatory response, they will not be described.

Pauley and Sparks (1967) made some rather casual observations on the repair of surface wounds caused by the needles during the injection of seawater, talc, or turpentine in their experiments on the acute inflammatory response. These led to a more detailed investigation of wound repair in *C. gigas* by DesVoigne and Sparks (1968).

Grossly, the area surrounding a surface wound (with a cataract knife) in an oyster begins to darken at approximately 16 hours after injury; this is probably analogous to the redness of vertebrate inflammation. The lesion becomes yellow-green within 24 hours and subsequently (48-96 hours) dark green. This dark coloration surrounds the wound and persists for approximately 9 days then gradually fades. However, at 28 days post wounding, when the experiment was terminated, much of the discoloration was retained.

Histologically, the first recognizable response to wounding occurs after about 4 hours. Small blood vessels in the vicinity of the lesion become pavemented by leukocytes, and infiltrating leukocytes begin to form a band underlying the mantle epithelium adjacent to the wound. Small numbers of leukocytes begin infiltrating the injured area and by 24-48 hours post injury a thick band of normal, round leukocytes surrounds the entire lesion. The blood vessels adjacent to the wound become heavily packed with leukocytes, the band of leukocytes underlying the adjacent mantle epithelium thickens, and a heavy infiltration into the region of the wound is well established.

Healing proceeds from the interior of the lesion toward the surface. Leukocytes, after delineating the margin of the wound, become fusiform and line up parallel to the wound channel. After about 160 hours the nuclei of these leukocytes also assume a fusiform appearance. As the band of fusiform leukocytes thickens around the periphery of the wound, the wound channel is heavily infiltrated by round leukocytes and fibroblasts and the wound channel is effectively plugged by 144 hours post wounding.

Subsequently the leukocytes plugging the wound channel elongate and align along the axis of the lesion. Collagen deposition becomes marked in the wound channel and normal, round leukocytes and fibroblasts continue to infiltrate along the periphery of the wound until 120-160 hours post injury. The fusiform leukocytes filling the wound channel and arranged along the axis of the lesion form randomly arranged whorls. At this point, varying in time from 88 to 488 hours post wounding, the tissue remarkably resembles a vertebrate scar. However, the original architecture is eventually restored; the whorls of leukocytes and collagen deposits are replaced by Leydig cells that are indistinguishable from normal tissue. It is not yet known whether this is accomplished by invasion of adjacent Leydig cells or by differentiation of the infiltrated, fusiform leukocytes into Leydig cells. Nor is it known how the heavy deposits of collagen are removed.

At the body surface, the band of fusiform leukocytes underlying the mantle epithelium closes the wound within 24-32 hours. The band of leukocytes gradually thickens and forms a laminated border between the underlying tissue and the exterior. The external lamina consists of fusiform leukocytes

arranged parallel to the surface; the middle lamina consists of fusiform cells perpendicular to the surface but infiltrated with round leukocytes, and the inner layer contains normal leukocytes and randomly directly fusiform leukocytes.

DesVoigne and Sparks (1968) believed that the fusiform cells in the outer lamina were totipotent, differentiating into a pseudoepithelium, then into a cuboidal epithelium and finally into a columnar ciliated epithelium identical to adjacent mantle epithelium. Ruddell (1969), however, clearly demonstrated mitotic activity and epithelial migration, beginning at 120 hours post injury and continuing for up to 720 hours. The migrating epithelial cells completely cover the wound between 144 and 200 hours after wounding. The inability of DesVoigne and Sparks to detect mitotic activity and epithelial migration despite extensive search for evidence of both these phenomena can perhaps be explained by the higher magnifications with which Ruddell worked and the incorporation of large numbers of leukocytes into the newly formed wound epithelium.

Ruddell (1969) used histochemical and electron microscopic techniques to further elucidate the wound repair process and, in addition to correcting the misconception of leukocyte differentiation into eipthelium, corroborated most of DesVoigne and Sparks' findings. He showed that copper was released in the wound by the swelling and bursting of one type of leukocyte (basophilic granular amoebocyte), that another type (agranular amoebocyte) invades the wound and phagocytizes cellular debris, and that the agranular amoebocytes differentiate into fibroblasts. Both Ruddell and DesVoigne and Sparks noted that the newly formed epithelium covering the wound is frequently hyperplastic, however, the ultimate outcome of the hyperplastic response remains unknown.

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