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CELL-MATRIX INTERACTIONS IN SCHISTOSOMAL PORTAL FIBROSIS: A DYNAMIC EVENT

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In recent years, one of the most significant progress in the understanding of liver diseases was the demonstration that liver fibrosis is a dynamic process resulting from a balance between synthesis and degradation of several matrix components, collagen in particular. Thus, fibrosis has been found to be a very early event during liver diseases, be it of toxic, viral or parasitic origin, and to be spontaneously reversible, either partially or totally.

In liver fibrosis cell matrix interactions are dependent on the existence of the many factors (sometimes acting in combination) which produce the same events at the cellular and molecular levels. These events are: (i) the recruitment of fiber-producing cells, (ii) their proliferation, (iii) the secretion of matrix constituents of the extracellular matrix, and (iv) the remodeling and degradation of the newly formed matrix. All these events represent, at least in principle, a target for a therapeutic intervention aimed at influencing the experimentally induced hepatic fibrosis. In this context, hepatosplenic schistosomiasis is of particular interest, being an immune cell-mediated granulomatous disease and a model of liver fibrosis allowing extensive studies in human and animals as well as providing original in vitro models.

Liver fibrosis results from chronic damage to the liver which may be caused by a variety of agents ranging from chemical to viral or parasitic origin. All these agents have the common property of eliciting an inflammatory response at the site of damage (Popper & Udenfriend, 1970). In normal conditions tissular repair involves a *precise sequence of migration of specialized cell types* which secrete matricial components, and control its local deposition (Kurkinen et al., 1980). In fibrosis, an excessive accumulation of connective tissue occurs which traduces an inappropriate regulation – still under investigation – of one or several steps of the tissular repair (Popper & Udenfriend, 1970; Grotendorst et al., 1985). In recent years, one of the most significant progress in the knowledge of this process has been the demonstration that liver fibrosis is a dynamic process (Popper & Udenfriend, 1970; Rojkind & Kershenobich, 1983) resulting from a *balance between synthesis and degradation* of multiple matrix components, 2) occuring *since the early beginning of tissular damage* and 3) being *spontaneously reversible*, either partially or totally. Current evidence suggests that the "trigger" may correspond to different types of insults but with a common final pathway. This provides the possibility of interfering on the different etiologic types of fibrosis. All this makes fibrosis the possible target of a *therapeutic strategy in man* (Kershenobich et al., 1979).

In this chapter, we present the pathology of portal fibrosis in schistosomiasis as a model of the precise sequence of four dynamic events acting *in vivo* namely: 1) The *cellular recruitment* in the prefibrotic stage, 2) the intra and extracellular step in collagen synthesis during *matrix deposition*, 3) the collagen packaging or the secondary *matrix rearrangement*, 4) the collagen turnover and the *matrix degradation*.

I – PORTAL FIBROSIS IN SCHISTOSOMIASIS

Schistosomiasis is a worldwide disease affecting about 200 million people in tropical and developing countries (Warren, 1973, 1979). Schistosomiasis mansoni is a systemic disease mainly affecting the liver and leading to severe portal fibrosis in its chronic form. Its morbidity and mortality result from the combined effect of schistosome products and the mechanical damage caused by the eggs (Warren, 1973, 1979). The initial lesion is a *cell-mediated granulomatous inflammation* which develops

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around eggs (Warren et al., 1967). In the liver, the arrival of eggs continuously deposited by adult worms present in the mesenteric veins leads to extensive *damage of hepatoportal vascular system* (Bogliolo, 1957; Andrade & Cheever, 1971): in human or in animal models, the eggs carried by the blood flow are trapped in the portal radicles and induce a granulomatous reaction followed by a secondary tissular fibrosis (Carter & Schaldon, 1959; Andrade, 1965).

The permanent arrival of eggs in the liver makes *hepatosplenic schistosomiasis a good model* of studying the various and complex steps which, *from inflammation to fibrosis,* mark the evolution of this hepatic disease (Table I).

1. *Early pathological change:* the acute vasculitis

Mechanical occlusion of portal radicles by schistosome eggs acting as *microemboli* is probably one of the first event leading to the inflammatory response in the liver (von Lichtenberg, 1955). Although the precise mechanism leading to vasculitis is not clear, it can be speculated that the phenomenon begins with *endothelial injury* and *cell necrosis.* Transient attracted inflammatory cells (polymorphonuclear and monocyte) release specific factors for enhancement of local permeability and subendothelial matrix degradation. A parietal oedema develops immediately which facilitates the diffusion of secretory and excretory products from the eggs through the portal vein wall (Baki & Grimaud, 1985; Grimaud & Borojevic, 1986). Direct contact of eggs with connective matrix provokes the attraction of inflammatory cells; the egg shell induced initially a foreign body reaction made of giant cells.

2. *The periovular granuloma*

An immune cell-mediated phenomenon occurs around the egg (Warren et al., 1967). Diffusion of egg secretion through the shell (soluble egg antigen: SEA) attracts macrophage around the egg (Boros & Warren, 1970). Macrophages take up soluble egg antigens and present them in the context of Ia-Dr membrane antigens to the lymphocytes. They interact with clonally selected T lymphocytes in lymphoid tissue and facilitate the process of sensitization. Sensitized T lymphocytes are chemotactically attracted from the blood stream to the vicinity of eggs by interleukin I (I1-1) secreted by macrophages. These lymphocytes secrete lymphokines that mediate the recruitment and mobilization of blood-born monocytes, eosinophils and other cells which move towards the eggs and aggregate around them. Local secretion of lymphokines (I1-2, gamma interferon) maintains the inflammatory response by facilitating lymphocytes multiplication *and mediating the activation of macrophages* (Boros et al., 1975). At this early stage, connective tissue cells are recruited and activated by local release of macrophage and lymphocyte products (cytokines) and specific eggs secretion. Recruitment proliferation and activation of connective tissue cells coincide with the initiation of matricial deposition in the granuloma (Wyler et al., 1978, 1987; Wyler & Rosenwasser, 1982; Boros & Lande, 1983).

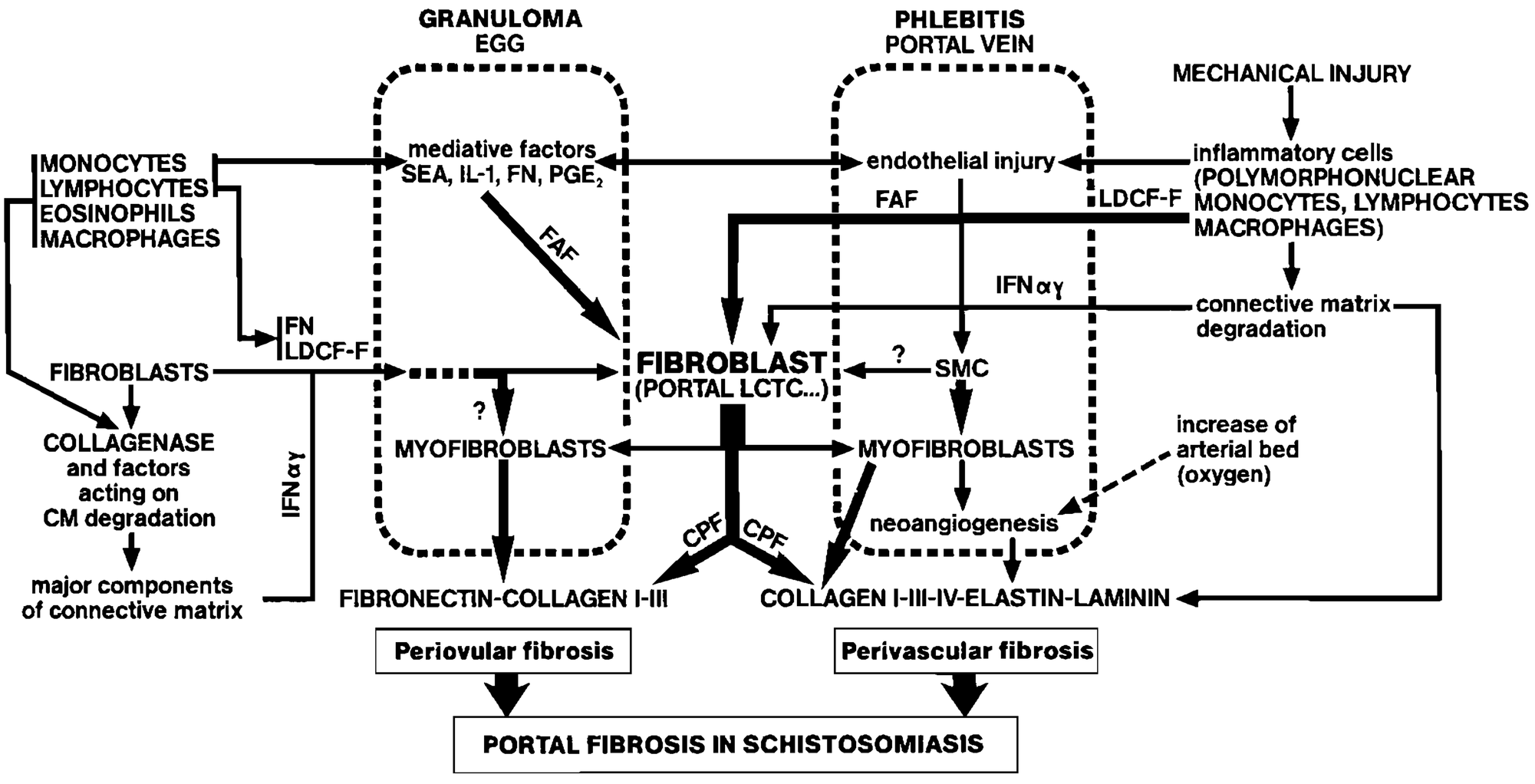
3. *The liver fibrosis*

a) Periovular fibrosis

The simultaneous secretion of cytokines and specific factors from the egg acts on connective tissue cells and induces the local secretion of multiple connective matrix components (glycosaminoglycans, glycoproteins such as fibronectin, laminin, collagen isotypes) (Nishimura et al., 1985; Grimaud et al., 1987). *The progressive deposition and organization of this macromolecular matrix* around the egg isolates the inflammatory focus from the pre-existing portal stroma. The precise regulatory mechanism of this sequential matricial deposition remains to be investigated. In the early beginning of the granuloma formation, fibronectin mainly produced by macrophages is present around the inflammatory cells as the major component of the matricial edifice concomitantly with heparan sulfate proteoglycan followed by deposition of interstitial collagens (type I and III). Type IV collagen and basement membrane related proteins (laminin) appear later, exclusively in the mature granulomas, restricted to neovessels (small capillaries) observed around and inside the granulomas (unpublished observations). *Connective tissue cell population* involved in periovular fibrosis are probably of *two distinct origins* namely (Grimaud & Borojevic, 1977b, 1986) 1) locally activated fibroblasts, and 2) smooth muscle cells (Voss et al., 1982; Borojevic et al., 1985) (myofibroblasts

TABLE I

POSTULATED MECHANISM FOR LIVER FIBROSIS IN SCHISTOSOMIASIS



FAF : Fibroblast Activating Factor
CPF : Collagen Production Factor
LDCF-F : Lymphocyte-derived Chemotactic Fibroblast Factor

IFNαγ : Interferon
FN : Fibronectin
SEA : Soluble Egg Antigen

IL-1 : Interleukin 1
PGE_2 : Prostaglandin E_2
LCTC : Liver Connective Tissue Cells

present in the granuloma probably result from dedifferentiated smooth muscle cells as evidenced by their desmin and α-actin smooth muscle content). Inside the granuloma, fibroblasts and myofibroblasts are in close association to inflammatory cells while around the granuloma they delineate the limiting cellular plate with the parenchyma.

b) Perivascular fibrosis

Since the early beginning (see above, vasculitis) the *wall of portal radicles* appears to be the primary site of events. Later on, in chronic schistosomiasis mansoni, the diffuse involvement of the intra-hepatic portal vasculature represents the most prominent change responsible for production of portal hypertension – the main clinical manifestation in this form of disease (Bogliolo, 1957; Andrade & Cheever, 1971). The portal vascular tree is simultaneously the target of the irritative action of eggs and worm products released in the mesenteric venous system, and also the site of the host's inflammatory reaction (namely portal hepatitis) (Baki & Grimaud, 1985). The importance of portal hepatitis in the production of portal fibrosis ("pipe-stem" fibrosis of Symmers) has been emphasized. The involvement of the intra-hepatic venous portal tree with *thrombophlebitis*, disorganization of the vascular muscle layer and phlebosclerosis act probably as the key factor for portal fibrosis in human schistosomiasis (Grimaud & Borojevic, 1986). The relationship between these changes and the known pathogenic factors identified in schistosomiasis mansoni have been already indicated but it is interesting to note that in man the vascular lesions begin at the inner sides of the vascular wall and extend progressively to its outer layers. It has been demonstrated that intact endothelium acts as a barrier to penetration of blood-born substances into the vascular wall, some of which upon exposure to smooth muscle cells are able to promote their proliferation and the secretion of extracellular matrix (Ross & Glomset, 1973). The thickening of portal vascular walls may represent a healing reaction initiated with the oedematous and lytic lesion of the subendothelial region. The concomitant *arterialization* of the local blood supply stimulates the subendothelial remodelling (Andrade & Cheever, 1971). Partially dedifferentiated smooth muscle cells and/or myofibroblasts are apparently responsible for the secretion and packaging of connective matrix components.

So, portal fibrosis in chronic schistosomiasis is probably the *result of both periovular and perivascular fibrosis.* Local inflammatory reaction and dedifferentiation of smooth muscle cells into myofibroblasts, neovascular proliferation and telangiectasia act synergically to promote and increase the extensive portal fibrosis of schistosomiasis. *In vitro* studies on human fibrotic livers demonstrate the considerable ability of vascular connective tissues and smooth muscle cell lines to proliferate (Voss et al., 1982; Borojevic et al., 1985). This fact may in part explain the self-perpetuating character of portal fibrosis in chronic human schistosomiasis, which still remains to be elucidated.

c) Lobular fibrosis

In chronic human schistosomiasis lobular fibrosis begins in *the Disse space* (Grimaud & Borojevic, 1977a). Both *interstitial and basement membrane collagens* are present along the sinusoids and tend to separate and isolate secondarly the hepatocytes. The lobular architecture can be disturbed and a considerable *decrease of contact between plasma constituents and hepatocytes.* A dramatic drop in the bidirectional exchange of nutrients occurs subsequently.

II – THE DYNAMIC OF CELL-MATRIX EVENTS IN FIBROSIS

From the early beginning (i. e. endothelial injury) to the late stage (i. e. large scar of the pipe-stem fibrosis), no relevant mechanisms are related to each cell-matrix event. In this way, *portal fibrosis in schistosomiasis* present the significant advantage to associate different focal lesions offering an exciting model to follow in a *limited space the natural history of fibrosis.* However it must be pointed out that the final result of the tissular changes is not different to other fibrotic conditions. The neo-formed connective tissue appears similar to those described in other fibrotic disorders. The myofibroblasts present in large amounts of newly deposited connective tissue characterizes most of the fibrotic lesions (Gabbiani & Rungger-Brandle, 1981). This suggests that a common mechanism may underlie many of the different fibrotic disorders.

In keloids or in hepatic fibrosis, myofibroblast is not a normal resident cell (Gabbiani & Rungger-Brandle, 1981; Grotendorst et al., 1985). This cell population derives probably from preexisting cell types under an adequate stimuli. Moreover, it is sometimes difficult or impossible to define precisely at the early beginning the differences between normal repair and fibrosis in term of cell types involved or quality of the deposited connective matrix, however the outcome is quite different. It must be assumed that this difference could be related to the timing or the dynamic of the process.

In the following paragraphs, the attention will be focalized on the fact that dynamic process will regulate the interactions between the cells and the matrix from the prefibrotic stage to the secondary remodeling events leading to the scar formation.

a) Prefibrotic stage: cellular recruitment and control

Tissular repair after focal injury involves a precise sequence of migration of specialized cell types which act to replace the damaged tissue.

Apart few interstitial fibroblasts, both *inflammatory and connective tissue cells*, mainly myofibroblasts, are not normally resident at the site of matrix deposition. It is generally assumed they *are recruited from surrounding tissues.* At the early beginning of fibrosis, a redistribution of cell types occurs from the periphery to the site of the injury. This migration occurs by chemotaxis of connective tissue cells and inflammatory or immunocompetent cell populations.

Chemotaxis: Attractant molecules represent specific appropriate signals which stimulate the target cell to migrate to site offering increasing release of these molecules. Various substances have been demonstrated patent chemoattractants. The chemotactic response of the inflammatory cells namely polymorphonuclear and macrophages have been widely demonstrated.

We will restrict in this paragraph to connective tissue cells chemotaxis (Table II).

Cytokines regulation of fibroblast functions: It has been assumed that fibrosis in schistosomiasis is related to deposition of extracellular matrix protein in excess under the control of granuloma cells derived factors (Wyler et al., 1987). Regulation of connective tissue cells functions (proliferation and collagen synthesis) depends on multiple factors derived from inflammatory cells (Tables III, IV).

TABLE II

Chemotaxis of connective tissue cells

Cells	Functions	Attractant	References
Human dermal fibroblasts	chemotaxis	peptides col. I, III, II	Postlethwaite et al. (1978)
Human dermal fibroblasts	chemotaxis	SDCF (80 000)	Postlethwaite et al. (1979)
Human dermal fibroblasts	chemotaxis	TGF β	Postlethwaite et al. (1987)
Human dermal fibroblasts	chemotaxis	FN (140 000)	Postlethwaite et al. (1981)
Human dermal fibroblasts	chemotaxis	PDGF (30 000)	Seppa et al. (1982)
Human pulmonary fibroblasts	migration inhibition	lymphokine FIF (15 000 et 34 000)	Rola-Pleszczynski et al. (1982) Hamel &Rola-Pleszczynski (1985)
Human dermal fibroblasts	chemotaxis	lymphokine human LCDF-F (22 000)	Postlethwaite et al. (1976)
		guinea-pig LCDF-F (80 000)	Postlethwaite & Kang (1980)

TABLE III

Regulation of connective tissue cells functions: fibroblast proliferation

Cytokines	Cells	Functions	References
Lymphokine FSF (10000-40000)	mouse newborn *fibroblasts*	proliferation stimulation	Lammie et al. (1986)
Lymphokine (16000 and 60000)	human dermal *fibroblasts*	proliferation stimulation	Postlethwaite & Kang (1983)
Lymphokine FAF (40000)	human and guinea pig dermal *fibroblasts*	proliferation stimulation	Wahl et al. (1978) Wahl & Gately (1983)
Monokine M-FSA	guinea-pig and mouse *fibroblasts*	proliferation stimulation	Leibovich & Ross (1976) Leibovich (1978)
Monokine IL-1 activity (13-16000)	human and guinea-pig dermal *fibroblasts*	proliferation stimulation	Postlethwaite & Kang (1982) Schmidt et al. (1982) Wyler & Rosenwasser (1982)
Monokine TNF	human foreskin *fibroblasts*	proliferation stimulation	Vilcek et al. (1986)
Lymphokines (50000)	guinea-pig (kidney) *fibroblasts*	proliferation stimulation or stimulation	Neilson et al. (1980, 1982)
Lymphokine (12000)	human pulmonary and dermal *fibroblasts*	proliferation inhibition	Elias et al. (1984)
Monokine (12-20000)	human dermal *fibroblasts*	proliferation inhibition	Korn et al. (1980)

TABLE IV

Regulation of connective tissue cells functions: collagen synthesis

Cytokines	Cells	Functions	References
FSA	human pulmonary *fibroblasts*	collagen synthesis stimulation	Johnson & Ziff (1976)
Monokine CEMF (23-71000)	rat granulomas *fibroblasts*	collagen synthesis stimulation	Jalkanen & Penttinen (1982)
(10000 and 33000) (22000)	chicken embryonic calveria and human foreskin fibroblasts	collagen synthesis and fibronectin stimulation	Wyler et al. (1987)
Lymphokines	guinea pig kidney *fibroblasts*	collagen synthesis stimulation or inhibition	Neilson et al. (1980, 1982)
Lymphokines (55000) (100-170000)	human dermal *fibroblasts*	collagen synthesis stimulation or inhibition	Postlethwaite et al. (1984)
Lymphokine (CSIF) (55000)	human dermal *fibroblasts*	collagen synthesis inhibition	Jimenez et al. (1979) Rosenbloom et al. (1983) Jimenez & Rosenbloom (1985)
Purified and recombinant-γ-IFN	human dermal *fibroblasts*	collagen synthesis inhibition	Jimenez et al. (1984) Rosenbloom et al. (1984, 1986)
IL-1	human dermal *fibroblasts*	collagen synthesis inhibition	Whiteside et al. (1984) Bhatnagar et al. (1986)

The first consequences of this process are 1) limitation of the extension of the injury, 2) restriction of the diffusion of the injurious agent, 3) preparation of the local microenvironment to matrix deposition by releasing patent factors active on collagen and associated protein secretion.

b) Matrix deposition

It is generally assumed that collagen synthesis and deposition which can occur in several cell types other than connective tissue cells (Clément et al., 1986) follow a *well defined and precise intra and extracellular steps* (Kivirikko & Majamaa, 1985; Trelstad & Birk, 1985). It is clear that in tissular fibrosis, the regulation of collagen synthesis (i. e. post-translational events) is of first importance (quantity of deposited matrix). The central role of collagen in fibrosis has stimulated attempts to characterize drugs that inhibit collagen accumulation (Kershenobich et al., 1979; Kivirikko et al., 1985).

Intracellular and extracellular events of collagen synthesis: Sequences of collagen synthesis within the cells and agents able to act naturally by modulating the synthesis of the procollagen polypeptide chains were actively investigated. Recent data suggest that the most suitable step for chemical regulation are 1) triple helix formation (for example inhibition by proline analogue or interference with proline-hydroxylation), 2) cleavage of propeptides from procollagen molecules (inhibition of the removal of the C-terminal propeptides which impairs fibrils formation, 3) inhibition of cross-link formation between the fibrils which leads to the formation of abnormally thin fibrils with reduced functional proteins and increased degradation role (Kivirikko & Majamaa, 1985).

The *spatial involvement of the fibroblast* in matrix deposition has been recently emphasized (Trelstad & Birk, 1985). Data from corneal and tendon fibroblasts suggests that different compartments are created and controlled by the cell during the process of fibril formation and matrix deposition. It has been speculated that the recess observed on the surface of the cell by the fusion of the secretory vacuoles are the site of procollagen processing. Specific membrane receptors would detect the amount of collagen present or procollagen to be cleaved for collagen fibrillogenesis.

Therefore, it seems that this extracellular compartment is the site for fibril-cell anchorage which supports mechanical transduction of the tension between the cell and the fibrils. The collagen fibrils assembly occurs in oriented way forming aggregates and bundles which represent the newly deposited matrix in the focus of tissular repair or fibrosis.

Biochemical and ultrastructural immunolabelling data have demonstrated the codistribution of collagen isotypes in schistosomal liver (Dunn et al., 1979; Grimaud et al., 1980; Biempica et al., 1983a). Moreover, two different patterns of organization of connective matrix have been reported evidenciating the molecular heterogeneity of the connective matrix in fibrosis (Grimaud et al., 1980).

	Loose connective matrix pattern	Dense connective matrix pattern
Predominant collagen isotypes	Type III – VI	Type I
Size of fibrils	$0 < 100$ nm	$0 \geqslant 100$ nm
Cross-banded periodicity fibrils	–	+ (110 to 180 nm)

c) Matrix rearrangement

A secondary remodelling process occurs on the newly deposited matrix. *In vitro* the consequence of the cell movement on adjacent matrix have been reported in the early 1940s (Stearns, 1940). Recently, Harris et al. (1984) have reported that on lattices, fibroblasts are able to *rearrange pre-existing fibrils on geometric patterns.* However detailed informations are lacking *in vivo* rearrangements of the matrix have been suggested in different situations. *In vivo* a morphological (and perhaps functional) continuity is observed at the interface of the old and new matrix like in skin wounds, in portal fibrosis between periovular and periportal fibrosis.

Compared to *in vitro* observations, a similar process occurs probably *in vivo.* Presence of myofibroblasts exhibiting an extensive cytosqueletton are the *candidate cell populations for the remodelling* and the rearrangement of deposited connective matrix (Grimaud & Borojevic, 1977b; Gabbiani & Rungger-Brandle, 1981). An unidirectional oriented disposition of fibrils in the collagen bundles is one of the pre-requisite for inter macromolecular and interfibrillar cross-links (Trelstad & Birk, 1985).

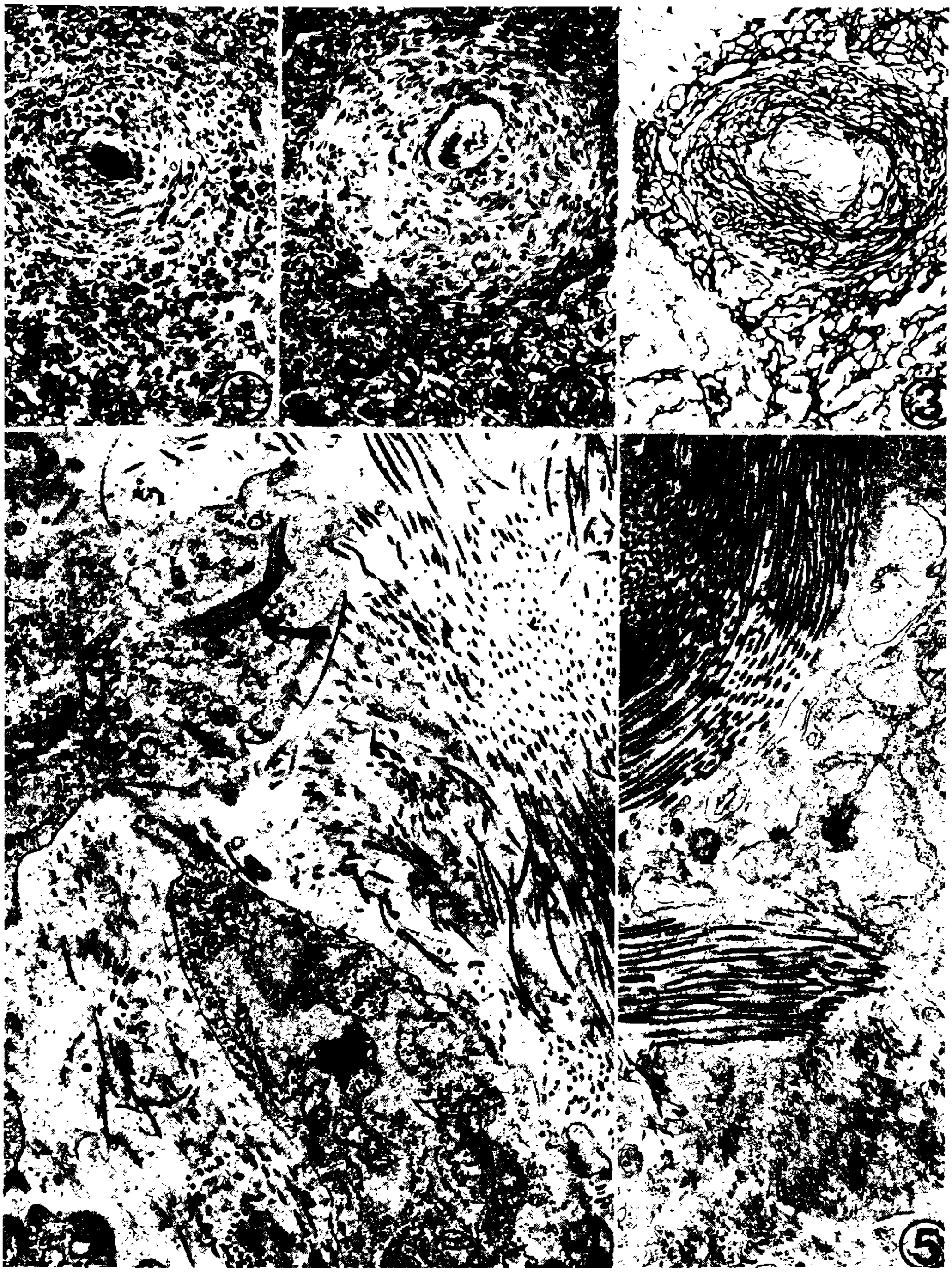


Fig. 1: florid phase of schistosomal granuloma. High amount of inflammatory cells around the egg. HE x 250. Fig. 2: periovular fibrosis in the mature granuloma. Recruited fibroblasts around a dense connective matrix. Masson's trichrome x 250. Fig. 3: reticulin framework of the fibrotic granuloma. Note the lobular fibrosis (perisinusoidal localization). Gordon Sweet's x 250. Fig. 4: synthesis ( ) and degradation ( ) of interstitial collagen in fibroblast (schistosomal portal fibrosis). x 15 000. Fig. 5: large and dense bundle of type I collagen fibers in close contact amongst a loose and poorly organized extracellular matrix. x 23 000.

Moreover, this *process of packaging* is responsible for an increasing *density of extracellular matrix* which becomes *less susceptible to cellular enzymatic attack.* The increased stability of the matrix is the main consequence of this process. It can be assumed that such matrix presents *low capacities to natural or therapeutic removal.*

d) Collagen turnover and matrix degradation

It has been largely demonstrated that under physiological conditions interstitial collagens are degraded extracellularly by *specific collagenase* by cleaving the helical region across the three chains at a similar locus (Krane, 1985). Fibroblasts and macrophages are the predominant sources of collagenases released in the extracellular compartments in inflammatory process (Biempica et al., 1983b). Synthesis and secretion seems to be controlled by specific stimulus-cytokines or non specific factor, like hormones. The collagenases are secreted in a latent form but until now the mechanism of its activation remains unclear.

Protein constituents of the matrix are susceptible to degradation during their maturation: 1) a portion of newly synthesized proteins can be degraded soon after synthesis and prior to secretion; 2) newly secreted proteins can be degraded before the incorporation into mature (highly cross-linked) forms. A very large fraction of the collagen is broken down very rapidly and fibroblasts degrade newly synthesized collagen within the cell before secretion (Bienkowski et al., 1978).

Several studies have demonstrated that *collagen fibrils can be internalized by fibroblasts and macrophages* and degraded within their lysosomes. In normal healing and in schistosomial granuloma phagocytosis of collagen has been observed both in fibroblast and macrophages (Andrade & Grimaud, 1986). The rapid and intense collagen synthesis and breakdown observed in murine schistosomiasis particularly after *chemotherapy* offers an exciting model for studying factors able to modulate the *stability or the degradability of matricial compounds.*

Extracellular and intracellular matrix degradation are probably associated processes, although subject to different mode of regulation, the two breakdown pathways involve fibroblasts and/or macrophages. In schistosomial granuloma phagocytosis of collagen has

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