

(super-secondary structures) commonly found in globular proteins (Sibanda & Thornton, 1985; Taylor *et al.*, in preparation). In these structures it is found that residue variation is restrained at particular locations in the motifs for general structural reasons. The observed patterns of conservation can then be used to predict the occurrence of the structural motif in a sequence of unknown structure using pattern recognition techniques such as the template matching method of Taylor & Thornton (1983, 1984) and Taylor (1986).

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A Model for the Role of Hyaluronic Acid and Fibrin in the Early Events during the Inflammatory Response and Wound Healing

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A model is presented outlining the molecular and cellular events that occur during the early stages of the wound healing process. The underlying theme is that there is a specific binding interaction between fibrin, the major clot protein, and hyaluronic acid (HA), a constituent of the wound extracellular matrix. This binding interaction, which could also be stabilized by other cross-linking components, provides the driving force to organize a three-dimensional HA matrix attached to and interdigitated with the initial fibrin matrix. The HA-fibrin matrix plays a major role in the subsequent tissue reconstruction processes. We suggest that HA and fibrin have both structural and regulatory functions at different times during the wound healing process. The concentration of HA in blood and in the initial clot is very low. This is consistent with the proposed interaction between HA and fibrin (ogen), which could interfere with either fibrinogen activation or fibrin assembly and cross-linking. We propose that an activator (e.g. derived from a plasma precursor, platelets or surrounding cells) is produced during the clotting reaction and then stimulates one or more blood cell types to synthesize and secrete HA into the fibrin matrix of the clot. We predict that HA controls the stability of the matrix by regulating the degradation of fibrin. The new HA-fibrin matrix increases or stabilizes the volume and porosity of the clot and then serves as a physical support, a scaffold through which cells trapped in the clot or cells infiltrating from the peripheral edge of the wound can migrate. The HA-fibrin matrix also actively stimulates or induces cell motility and activates and regulates many functions of blood cells, which are involved in the inflammatory response, including phagocytosis and chemotaxis. The secondary HA-fibrin matrix itself is then modified as cells continue to migrate into the wound, secreting hyaluronidase and plasminogen activator to degrade the HA and fibrin. At the same time these cells secrete collagen and glycosaminoglycans to make a more differentiated matrix. The degradation products derived from both fibrin and HA are, in turn, important regulatory molecules which control cellular functions involved in the inflammatory response and new blood vessel formation in the healing wound. The proposed model generates a number of testable experimental predictions.

1. Introduction

Wound healing is a complicated multi-step process, which is crucial to the survival of the organism, and which parallels many of the complex events that occur during embryonic development. During the repair process the extracellular matrix is

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sequentially remodeled and rebuilt by the concerted action of different cell types. The wound itself becomes a transitory organ or structure whose function is to remodel, successively, a series of increasingly complex and ordered extracellular matrices. The matrix develops sequentially from a lesion (a void; the wound), to a platelet plug, to a fibrin (blood) clot, to a relatively loose matrix of glycosaminoglycans and collagen, to a denser granulation tissue and then to the final repaired tissue (Bentley, 1968; Irvin, 1981). The importance of extracellular matrix molecules during wound healing was underscored by the ability of a bilayer polymeric membrane containing glycosaminoglycans and collagen, to serve as a template for the formation of new skin (Yannas *et al.*, 1982).

The latter stages of wound healing have been studied to a greater extent and are better understood than the earlier stages in this process. There is evidence, however, that one of the early events shortly after injury is an increase in hyaluronic acid (HA) content of the wound (Bentley, 1967, 1968). The origin of this large polysaccharide at the wound site is uncertain, since normally there is very little HA present in the blood; 150–550 ng/ml (Laurent & Laurent, 1981). In fact some tissues, particularly the liver, have a large capacity (i.e. on the order of 3–15 mg/24 h/kg) to remove circulating HA from the blood very rapidly (Eriksson *et al.*, 1983; Fraser *et al.*, 1984), which suggests that elevated levels of HA in the blood may be deleterious to the organism.

Wound healing shares certain common features with the early events during embryonic development of the chick cornea or neural crest and the complete regeneration of a limb in amphibians (Toole, 1976; Hay, 1980). The latter process can be viewed as an extremely efficient type of wound healing. There appears to be a well defined sequence of events in these cases: (i) First, a matrix rich in HA is laid down in a cell-poor space. (ii) Second, mesenchymal cell migration is stimulated and the HA matrix is infiltrated by cells migrating from the adjacent tissues. (iii) Finally, cells within the HA matrix secrete both hyaluronidase, which degrades the HA, and sulfated glycosaminoglycans and collagen, which concomitantly replace the HA as the matrix is remodeled. In each of these three developmental systems the HA matrix is first synthesized and then degraded. It is, therefore, very likely that this transitory cell-poor HA matrix is required before the complicated series of cell-mediated events that follows its destruction can proceed (Toole, 1976). The increased content of HA in the early wound matrix (Bentley, 1967, 1968) and the demonstration of hyaluronidase activity in wounds at an early stage (Bertolani & Donoff, 1978; Alexander & Donoff, 1979; Thet *et al.*, 1983) supports the idea that this general scheme also occurs during wound healing.

Here we present a model describing the mechanisms controlling the early reorganization of the extracellular matrix and the progression of the inflammatory response during wound healing. In this model HA plays a major role and is a multifunctional macromolecule which interacts with fibrin and a variety of cell types and facilitates the sequence of events leading to successful wound healing. We propose that HA is required for a variety of organizational, structural and regulatory functions at both the molecular and cellular level. We also suggest a rationale for why the concentration of HA in blood is low.

2. The Model

We propose that very soon (0–2 days) after the formation of the initial fibrin-based matrix, the blood clot, it is remodeled into a matrix which contains HA bound to the fibrin. This protein and polysaccharide complex then provides an organized scaffold through which the peripheral neutrophils, monocytes, macrophages and fibroblasts, which are needed to remodel the wound matrix and to initiate granulation tissue synthesis, will migrate into the wound. There are two premises which are necessary for this working hypothesis. First, the HA matrix should be directly and/or indirectly anchored or in some way bound specifically to the fibrin matrix. A direct interaction between fibrin and HA would mean that fibrin, and probably fibrinogen as well, has one or more specific binding sites for HA. An indirect interaction would be mediated by a third molecule which binds to both fibrin and to HA and functions as a crosslink between these two macromolecules. An example of such a crosslinking protein would be the link protein from cartilage which binds to both HA and to the core protein of a proteoglycan monomer and stabilizes their interaction (Baker & Caterson, 1979). In this regard plasma fibronectin could serve to crosslink HA and fibrin in the clot (Mosher, 1982). Second, since the level of HA in a newly formed blood clot is very low (Laurent & Laurent, 1981), it is reasonable to suggest that new HA is made or released at the site of the wound. We propose that one or more blood cell types, either present during the formation of the initial fibrin matrix or entering the matrix shortly after its formation, is stimulated to synthesize and secrete HA. This stimulation could be readily achieved by factors generated or released during the clotting process. For example, an inactive plasma precursor protein could be activated by a protease generated during the clotting cascade. Alternatively, the activator molecule could be a protein or low molecular weight mediator (e.g. a prostaglandin) released from platelets or from other cells in or immediately adjacent to the wound. In either case, both the activator and the target cell type(s) would be present at the same location, the clot, and the stimulation of HA synthesis and secretion would be efficient and rapid. These two postulates form the basis of the proposed Model.

Figures 1–5 schematically depict the proposed temporal sequence of molecular and cellular processes that occur at the wound site. The key to the symbols used to represent various cell types and molecules is shown in Fig. 1. The proposed order of events for the early stages of wound healing are the following:

- (i) *During the blood clotting process a three dimensional matrix of crosslinked fibrin is produced.* Blood cells of various types (e.g. platelets, neutrophils, monocytes and lymphocytes) are trapped within this clot (Fig. 2(a)) and an activator is generated or released within the clot. At the molecular level, fibrin polymers form a porous gel-like meshwork containing trapped or adsorbed plasma proteins such as plasminogen and fibronectin (Fig. 2(b)). Fibronectin is incorporated into the clot and distributed along the fibrin strands (Grinnell *et al.*, 1981). We suggest this is also true for plasminogen.
- (ii) *The activator molecule stimulates certain blood cell types lodged throughout the clot to increase the rate of HA synthesis and secretion into the milieu of the clot.* Within

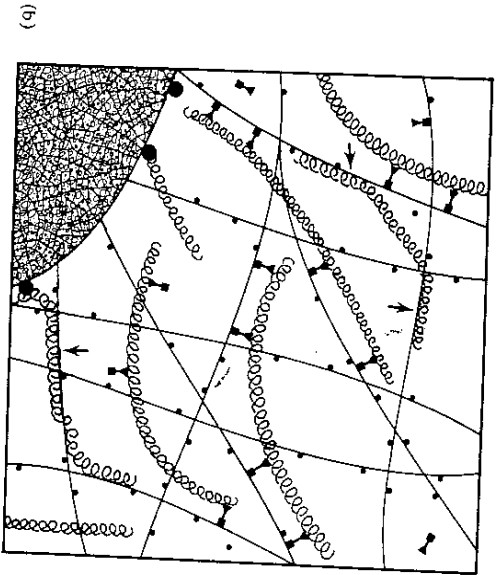
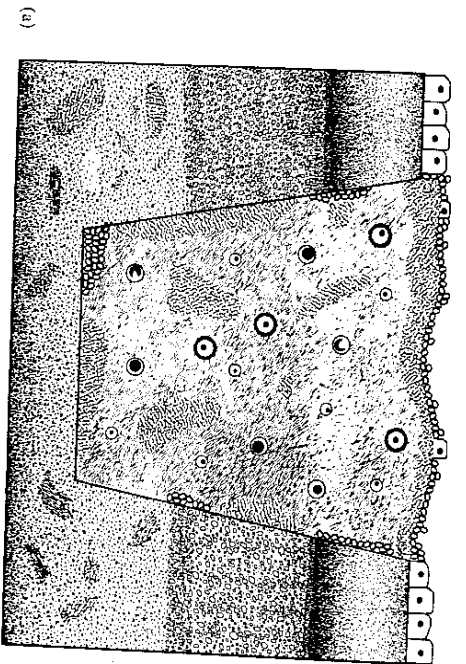


Fig. 3. Development of the HA-fibrin matrix. (a) The fibrin matrix is modified by the presence of HA produced by one or more blood cell types. The wavy lines represent the new HA-fibrin matrix. Islands of the original fibrin matrix gradually disappear as they are infiltrated by HA. (b) At the molecular level HA penetrates into the porous fibrin network and is specifically bound to the fibrin directly (indicated by the arrows) or indirectly by a crosslinker protein. The HA can also be attached to cell surfaces, as in the lower left corner of the figure, by specific HA receptors.

(iv) *The newly constructed HA-fibrin matrix within the wound site serves both a structural and a regulatory function for subsequent cell migration.* We propose that HA is chemotactic and/or chemokinetic for many of the cells around the margin of the wound and thereby promotes and directs a steady influx of peripheral cells

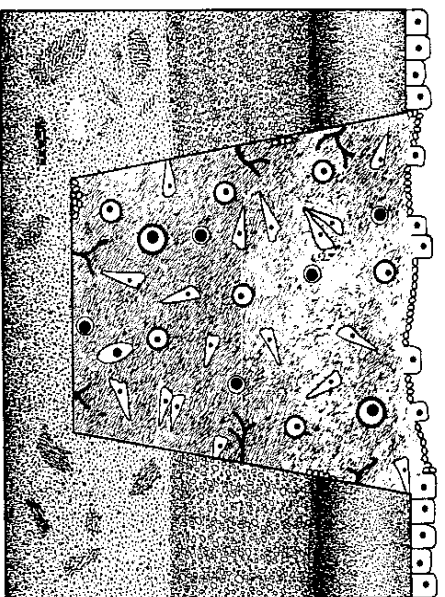


Fig. 4. Cellular infiltration and proliferation in the wound. Neutrophils, monocytes and then lymphocytes sequentially infiltrate the wound matrix. Migration of fibroblasts from the adjacent tissues into the HA-fibrin matrix is very active. Monocyte activation to macrophages and the budding of capillaries into the matrix is also occurring.

into the clot (Fig. 4). In addition, the viscoelastic and other physical properties of HA provide a strong yet mechanically deformable scaffold through which cells can migrate. These two related functions of HA result in the stimulation of locomotion of cells, such as neutrophils, trapped within the clot as well as the active infiltration of cells from the margin of the wound into the clot. The biochemical changes in the wound matrix parallel the migration into the wound of fibroblasts and cells mediating the inflammatory response. These cells direct the synthetic and degradative events that occur in the changing wound matrix. During this early period of wound healing there is a serial migration of leucocytes into the clot; neutrophils appear first, followed by monocytes and then lymphocytes (Ross & Benditt, 1961). The biological significance of this sequential arrival of different inflammatory cells is not known but the recruitment of cells into the wound is critically important for successful healing and is very active from about day 3 through 8 (Irvin, 1981). We propose that this serial migration of leucocytes is dependent on the formation of the HA-fibrin matrix. As one cell type migrates through the clot it could secrete molecules which modify the HA-fibrin matrix (e.g. fibronectin, proteases, etc.) so that another cell type is then able to enter or is stimulated to enter this matrix. In this way a serial progression of cell types would be marshalled to infiltrate the wound in an organized way.

The present view of embryonic cell migration is that HA is an important molecule which regulates this activity (Le Douarin, 1984). HA has been shown to stimulate the migration of embryonic cardiac cushion endothelial cells (Bermanke & Markwald, 1984) and neural crest cells (Tucker & Erickson, 1984) through collagen gels. Decreases in HA correlate with the arrest and aggregation of previously migrating

cells in the embryo and neural crest cell migration ceases after the introduction of hyaluronidase into the space through which the cells are moving. The formation and remodeling of tissues, particularly the interactions between mesenchymal and epithelial cells, is coordinated with changes in the synthesis and extracellular accumulation of HA and the degradation of HA mediated by hyaluronidases. The temporal and spatial regulation of the biosynthesis and secretion of HA and hyaluronidases in different regions of the embryo can therefore control the timing and direction of cell migration and tissue development (Stern, 1984). We suggest that HA also plays this role in wound healing.

(v) *In addition to a structural role, HA also serves an important regulatory role at the molecular level by stabilizing and controlling the degradation of the fibrin matrix.* Recent studies have shown that plasminogen binds to fibrin and fibrinogen to form stable complexes (Lucas *et al.*, 1983; Lewis *et al.*, 1984) and that bound plasmin is less susceptible to inactivation by alpha-2-antiplasmin (Thorsen, 1975). Plasminogen will therefore be associated with and incorporated into the fibrin matrix of the wound during clot formation. Plasminogen can subsequently be converted to the protease plasmin by the action of plasminogen activator which is released from blood cells either trapped within the clot during its formation or later migrating into the clot. Monocytes/macrophages probably function in this way since they are activated during clot formation and activation causes an increased secretion of plasminogen activator (Unkless *et al.*, 1974). We suggest that the HA bound to fibrin regulates its degradation by plasmin. This protective effect could be due to a direct interaction between HA and plasmin, to the blocking of plasmin cleavage sites in fibrin by the bound HA or to the inhibition by HA of the activation of plasminogen. HA will inhibit fibrin matrix (clot) degradation until a sufficient number of cells have infiltrated the wound site and produced enough hyaluronidase to efficiently and coordinately remodel the matrix to the next level of complexity.

(vi) *The inflammatory response, which is initiated very soon after the trauma or wound event, is the first phase of wound healing.* During this response the wound and surrounding tissues become inflamed and cells, particularly neutrophils and monocytes, are mobilized to infiltrate the clot and start the processes involved in granulation tissue synthesis (Irwin, 1981). We believe that HA is an important regulatory molecule for cells involved in the inflammatory response (Fig. 4). HA has been shown to increase intracellular ATP levels and to stimulate a variety of functions in leucocytes including phagocytosis, adherence, migration and chemotaxis (Hakansson *et al.*, 1980). HA enhances the phagocytic activity of monocytes (Ahlgren & Jarstrand, 1984) and also participates in the disappearance reaction displayed by peritoneal macrophages (Shannon *et al.*, 1980). High molecular weight, but not low molecular weight fragments of HA inhibit phagocytosis in these cells (Forrester & Balazs, 1980). These studies demonstrate that HA can regulate a variety of cellular functions which are important during the inflammatory response and during wound healing. The degradation of HA will therefore be recognized by macrophages in the wound as a signal to increase their phagocytic activity and begin the debridement process. The timing of this signal is clearly important because if

the matrix is removed too early, not enough cells will have infiltrated the clot to sustain the inflammatory response and begin the healing process.

(vii) *As the wound becomes a cell-rich space the HA-fibrin matrix is modified.* Cells secrete hyaluronidase and proteases, such as plasminogen activator, which degrade the HA and fibrin (Fig. 5(a)). We propose that the plasminogen which is bound to and present throughout the HA-fibrin matrix provides a primed system ready to achieve the rapid and synchronous degradation of fibrin. Furthermore, the bound HA regulates the stability of the HA-fibrin matrix (see (v) above) by controlling either the ability of plasminogen to be activated or of plasmin to cleave fibrin. Therefore cells which secrete hyaluronidase will cause the local degradation of HA, relieve this inhibition and allow the fibrin to be degraded. Low molecular weight HA fragment will also stimulate the phagocytic activity of the macrophages responsible for clearing away the breakdown products and debris from the initial clot and the secondary HA-fibrin matrix (see (vi)). Cells in the wound not only degrade the HA-fibrin matrix, they also synthesize and secrete large amounts of fibronectin, collagen and sulfated glycosaminoglycans which are then organized into the extracellular matrix of the newly forming granulation tissue (Fig. 5(b)). Fibronectin is deposited along the newly synthesized collagen in this tissue (Grinnell *et al.*, 1981; Mosher, 1982). The older HA-fibrin matrix serves a structural and organizational role for the assembly of this next transitory matrix.

(viii) *HA and fibrin degradation products stimulate vascularization of the wound.* Vascularization of the wound occurs during the period of active matrix remodeling and cell proliferation when these degradation products are produced (Figs 4 and 5). Hyaluronidase secreted into the wound matrix generates small breakdown products from the large polymeric HA. In the developing embryo HA plays a role in the formation of new blood vessels. High concentrations of intact HA inhibit the formation of vascular tissue in the peripheral mesoderm of the chick embryo limb bud (Feinberg & Beebe, 1983). In contrast, small HA oligosaccharides but not intact HA stimulate angiogenesis in chick chorioallantoic membranes (West *et al.*, 1985). We suggest that in a similar manner the small degradation products of HA are very important at the wound site and stimulate the formation of new blood vessels.

Plasmin systematically cleaves fibrin into well-defined fragments (Doolittle, 1984) some of which have potent biological activity. For example, fragment D greatly stimulates the production of monokines and is an important regulator of the acute phase response (Ritchie *et al.*, 1982; Nham & Fuller, 1984). In addition, fragment D specifically causes disorganization of cultured vascular endothelial cells (Dang *et al.*, 1985). Although the way that this is brought about is not clear, this cellular response could also be necessary for vascularization of the wound (Fig. 4). The development of new blood vessels requires reorganization of existing vessels and their endothelial cells. These latter studies indicate that fibrin peptides generated in a clot can regulate and influence the behavior of various cell types associated with the wound healing process. We predict that HA, due to its association with fibrin, plays an important role in controlling the rate of formation of these biologically important fibrin degradation products.

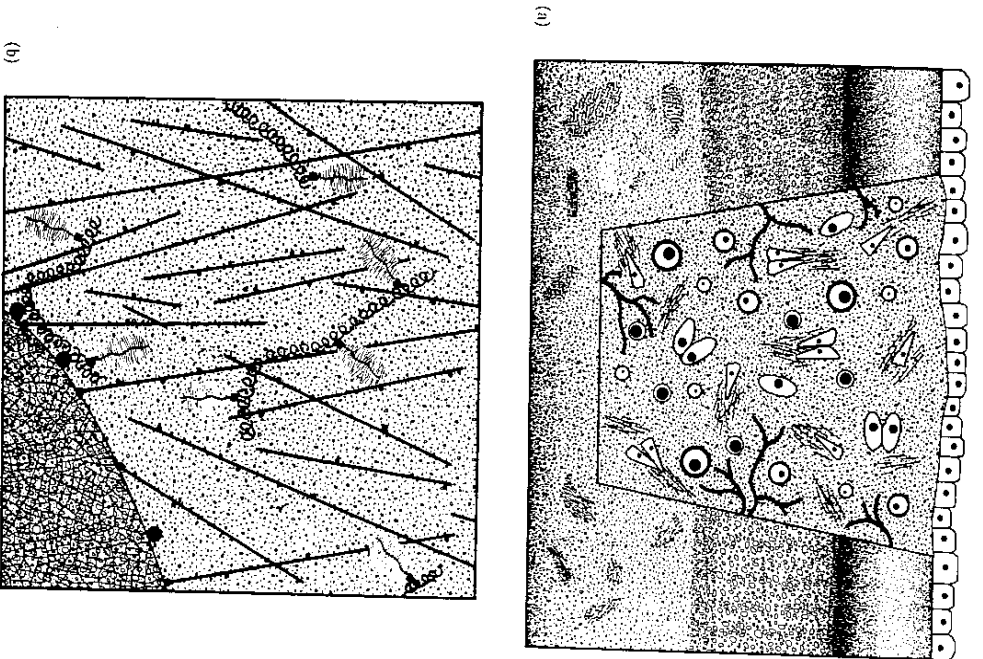


FIG. 5. Transformation of the HA-fibrin matrix. (a) The wound site becomes more densely populated as fibroblasts, endothelial cells, lymphocytes and later mast cells continue to infiltrate and proliferate within the matrix. These cells together with macrophages and monocytes act coordinately to degrade and remodel the HA-fibrin matrix, replacing it with collagen and proteoglycans, as the granulation tissue is constructed. At this stage in the wound healing process, vascularization, specifically stimulated by HA and fibrin degradation products, is very active and the epithelial cell layer covering the wound is more extensive. (b) At the molecular level the extracellular matrix contains collagen fibrils, various interstitial glycoproteins and proteoglycan monomers (containing glycosaminoglycans) attached to HA to form proteoglycan aggregates. A link protein stabilizes this latter interaction. Many cells also contain surface proteoglycans and receptors for HA and collagen, which attaches the cell to the external matrix (lower right).

(ix) *Cell and tissue differentiation increase as the HA content of the wound decreases.* Generally, in developing systems cell migration, proliferation and plasticity occur when the extracellular matrix is rich in HA, whereas cell quiescence and differentiation occur in an HA-poor matrix (Toole, 1976; Hay, 1980). For example, in the developing chick limb bud, there is a gradient of HA concentration. The distal tip, which contains the apical ectodermal ridge, is temporally at the earliest stage of development and has the highest HA content (Kosher *et al.*, 1981). The HA concentration decreases toward the proximal region of the limb. The decreasing HA gradient correlates with an increasing gradient of cell and tissue differentiation. This spatial correlation between a decreased HA content and increased tissue differentiation can also be viewed as a temporal change that occurs like a wave at each location in the developing limb. We propose that this same temporal sequence occurs during wound healing.

3. Specific Experimental Predictions of the Model

There are several testable predictions which arise from the scenario of cellular and molecular events proposed in the above Model.

A. *Fibrin (and probably fibrinogen) should bind specifically to HA.* We have tested this prediction in several ways using purified human fibrinogen and HA. For these studies we prepared and used unique derivatives of HA oligosaccharides, modified only at the reducing end to contain an alkylamine group (Raia *et al.*, 1984). The resulting HA-amine can be used to make ¹²⁵I-labelled derivatives by preparation of the 3(4-hydroxyphenyl)-propionyl adduct. The alkylamine derivative of HA can also be used to make affinity chromatography media (e.g. by coupling to CNBr-activated Sepharose). These novel experimental tools have enabled us to perform studies from which we conclude that HA and fibrinogen specifically bind one another (LeBoeuf *et al.*, 1984). Current experiments indicate that fibrin also binds HA. This first prediction, that fibrin(ogen) and HA specifically interact, is therefore confirmed. It remains to be determined whether any other plasma proteins or serum proteins generated during the clotting cascade are able to stabilize the interaction between HA and fibrin by serving as a crosslink.

B. *After the formation of the clot, one or more specific blood cell types will be stimulated to synthesize and secrete large quantities of HA.* Stimulation of HA synthesis is mediated by a factor or factors produced during the clotting reaction. This prediction can be tested, although it may be difficult to determine the basal, unstimulated level of HA synthesis by blood cells. Since the isolation procedures may perturb (and activate) the cells, they could already have elevated levels of HA synthesis. Unfractionated blood cells can be cultured in a real clot or in a variety of control matrices (e.g. agarose, collagen or purified fibrin) and the rate of HA synthesis determined. Confirmation of a stimulation of HA synthesis by blood cells in a clot would then indicate the presence of an activator molecule mediating this response. Such an activator molecule could be generated, as for the possible cross-linking protein mentioned above, by release from platelets or other cells during clotting or by activation of an inactive plasma precursor. The source of the activator

and the responsive cell type can be determined by studying various purified blood cell types reconstituted with defined matrices ranging in complexity from pure fibrin alone to a cell free plasma clot.

C. *The presence of HA will alter the ability of the fibrin matrix to be formed and to be degraded.* We predict that HA will interfere with the formation of a normal fibrin matrix if added to blood or plasma prior to clotting. HA could inhibit the interactions between thrombin and fibrinogen, fibrin and Factor XIII and/or plasmin and fibrin. Evidence for any of these interactions would substantiate the rationale presented below for why the HA content in blood must be kept low. Initial experiments with thrombin and purified human fibrinogen have recently shown that both HA and chondroitin sulfate greatly increase the kinetics and extent of fibrin polymer formation (LeBoeuf *et al.*, 1985). We also predict that, if high molecular weight HA and plasminogen are incorporated into a fibrin matrix, that the presence of HA will inhibit the ability of subsequently added plasminogen activator to cause breakdown of the fibrin. Hyaluronidase activity will degrade the HA, alleviate this inhibition and accelerate the degradation of fibrin. These hypotheses can also be tested *in vitro*.

D. *Cell infiltration and migration into and within the HA-fibrin matrix will be stimulated by HA.* We predict that HA in the transient HA-fibrin matrix can stimulate general cell locomotion (chemokinesis) and/or directed cell migration (chemotaxis) along an HA concentration gradient. These predictions can be tested using cultured established cell lines or primary cells seeded in or on fibrin matrices made by the action of thrombin on purified fibrinogen. The effects of HA concentration and size on the rate of cell migration and the extent of cell infiltration into the matrix can be determined. Likewise, chemotactic chambers can be used to test whether cells are responsive to concentration gradients of different size HA molecules. Various control matrices, as indicated above, can be used to assess the role of the fibrin matrix in the cellular responses to HA.

4. Discussion

A number of extracellular proteins have been demonstrated to bind specifically to HA including the link protein of cartilage (Baker & Caterson, 1979), plasma fibronectin (Yamada *et al.*, 1980), naturally occurring antibodies (Underhill, 1982), a glycine-rich gelatin-binding protein from plasma (Isemura *et al.*, 1982) and hyaluronectin, a brain glycoprotein (Delpach & Halvett, 1981). In addition, cell surface binding sites for HA have been reported on lymphocytes (Chevrier *et al.*, 1982), liver endothelial cells (Eriksson *et al.*, 1983; Raja & Weigel, 1985), rat hepatocytes (Truppe *et al.*, 1977; Raja & Weigel, 1985), SV3T3 mouse embryo fibroblasts (Underhill & Toole, 1979) and human synovial cells (Truppe *et al.*, 1977). In short, HA is capable of specific interactions with several cell surface and extracellular matrix molecules. Fibrin or fibrinogen have been shown to interact specifically with platelets (Marguerie *et al.*, 1979), fibroblasts (Dejana *et al.*, 1984), plasminogen (Lucas *et al.*, 1983; Lewis *et al.*, 1984) and plasma fibronectin (Seidl & Hormann, 1983). Based on these precedents it is reasonable to propose that HA and fibrin(ogen)

specifically interact. To our knowledge this has not been systematically examined nor previously reported by others.

It is probably significant that the circulating level of HA in blood is very low. Assuming our proposed model is correct and since, as our recent experiments demonstrate, HA binds specifically to fibrinogen, then a high HA content in blood could be potentially deleterious for at least two reasons. First, the interaction of two macromolecules which can themselves form three-dimensional crosslinked hydrated gels could adversely increase the viscosity of blood and create problems related to blood pressure regulation, capillary obstruction, etc. Second, the binding of HA to fibrinogen might dramatically inhibit the ability of this molecule to be activated by thrombin to fibrin or of the resulting fibrin to polymerize, be assembled to form a clot, or be cross-linked by the transglutaminase activity of Factor XIII. Since clotting ability is crucial for survival of the organism (and the species), there would have to be an effective way to obviate these problems. If there was a physiological need, as we propose, to preserve the molecular interaction between fibrin and HA in the wound matrix, then an effective solution would be to keep the HA content in blood low and then to raise it specifically at the wound site where it is needed, immediately after clot formation. The liver, which is well known for the function of clearing various molecules from plasma, has recently been shown to remove HA efficiently from the circulation. The experimental half life of an injected dose of HA in the rabbit or human is 2.5-5.5 min (Fraser *et al.*, 1984). The liver removes approximately 90% of the circulating HA and the remainder is removed by the spleen. This function of the liver may be very important in ensuring that certain blood cells, which may be activated or regulated by HA, only encounter HA at the wound site itself.

A core premise of the Model presented here is that the interaction between the cell surface/cytoskeleton and the extracellular matrix is interdependent and dynamic. This notion has been developed to various extents by others (e.g. Toole, 1976; Grinnell, 1978; Hay, 1982) and can briefly be summarized as follows. Cells, in response to the matrix or to an external signal (e.g. a hormone), can change the extracellular matrix in specific ways, for example by secreting matrix components or enzymes that degrade or modify the matrix. The altered matrix can in turn feed back to change the way the same cell or a different cell type interacts with the matrix or other cells. The cellular response to the external signal by generating soluble specific receptors, which can transduce the external signal by generating soluble cytoplasmic second messengers, or by transmembrane proteins, which directly link the matrix to the cell cytoskeleton and affect, for example, cell shape or migration. The proposed interaction between HA and fibrin may be fundamental and general in biology. We believe that these two types of molecules are the major components of evolutionarily primitive and embryonic extracellular matrices, such as the systems cited in the Introduction. We anticipate that fibrin will also be found, along with HA, as a normal component of the extracellular matrix during embryonic development and in regenerating tissues and that fibrin is also a normal component of the differentiated extracellular matrix. We propose in fact that the series of matrices produced during wound healing in an organism reflect those generated during its

embryogenesis. In both situations the generation of a cell poor space containing HA and fibrin could initiate the processes of cell migration, infiltration and extracellular matrix remodeling which are necessary early events for the development of the final differentiated tissue whether for the first time in the embryo or a second time in the wound. Fibrinogen and fibrin are ideally suited as a self-assembling gel forming molecular system which cells can use for the rapid generation of an external three-dimensional matrix. The clotting/hemostasis function may represent a specialized, amplified utilization of this primitive matrix generating machinery for the benefit of the organism. After developing the Model presented here we became aware of evidence that supports this latter premise. Ghilala and his co-workers (personal communication) have recently demonstrated the presence of material in the extracellular matrix of a variety of differentiated tissues that reacted specifically with antibodies to all three polypeptide chains of fibrinogen. Subsequent work will probably support the conclusion that fibrin is a common component of the normal extracellular matrix.

In summary, we propose that the early accumulation of intact HA in the wound clot is necessary to physically stabilize the matrix, stimulate cell infiltration and migration and control the degradation of fibrin. We also suggest that the degradation products of the HA-fibrin matrix are necessary regulator molecules of the wound healing process. Small HA oligosaccharides stimulate angiogenesis, the production of new blood vessels, which are critically important for providing oxygen and nutrients and removing waste products in the rapidly developing, actively metabolizing wound tissue. The small HA fragments also stimulate the phagocytic activity of macrophages, which remove dead cells and older matrix components. This function is important because it allows cell proliferation and the deposition of new matrix components to proceed efficiently. The early appearance of high molecular weight HA and the later appearance of low molecular-weight HA at the wound site as it is degraded provides a mechanism to regulate and integrate the timing of the cellular activities needed to initiate and sustain the inflammatory response and the wound healing process. Degradation products derived from fibrin act both at and away from the wound site to stimulate monocytes and macrophages to produce hormones, which in turn stimulate the liver to synthesize acute phase proteins. Additionally, fibrin degradation products at the wound also stimulate the reorganization of vascular endothelial cells and the very important vascularization process.

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Theoretical Characterization of Ion Channel Blockade: Ligand Binding to Periodically Accessible Receptors†

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With repetitive stimulation, the time course of use-dependent blockade as assessed by peak membrane ion currents can be described by a sequence of blocking relationships that have the form of recurrence equations. The equations of the sequence describe blockade acquired during each interval of a stimulus where the possibly different binding and unbinding rates are assumed constant during each interval. The solution predicts that use-dependent uptake follows an exponential time course. Furthermore, the exponential uptake rate is a linear function of uptake rates associated with the stimulus time intervals. Similarly, the fraction of blocked channels at steady state is a linear function of the interval dependent blockade equilibria. Several novel tests of consistency between the model and observations are derived from these theoretical results. It is also shown that as the stimulus interval increases to infinity, steady state dissociation constants measured by peak membrane currents are theoretically equivalent to those measured with true equilibrium methods such as radioligand binding studies.

Introduction

With the modulated receptor hypothesis, Hille (1977) introduced the formal notion of a state-dependent ion channel affinity for blocking agents. Recently, we investigated the case where the state-dependent variation in channel binding affinity was considered to be the result of channel gate control of receptor access (Starmar *et al.*, 1984; Starmar & Hollett, 1985). Using the Hodgkin-Huxley gate formalism (Hodgkin & Huxley, 1952) in conjunction with a bimolecular first order binding process, we were able to numerically integrate the differential equations describing use-dependent ion channel blockade. However, due to the complexity of the H-H gating coefficients, we were unable to derive a closed form solution.

Recent observations of single channel events using the patch clamp technique show transitions between open and closed conformation to be rapid, and for the most part, channel open times follow an exponential distribution (Grant *et al.*, 1983; Cachein *et al.*, 1983). Assuming that binding sites become simultaneously accessible and remain accessible for a time equal to the mean conformation dwell time, we have shown that channel blockade associated with simple pulse train stimulation can be described by a sequence of simple algebraic recurrence relations (Starmar & Grant, 1985). Here we will extend and generalize the description to cover more

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