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# Role of Prostaglandins in Controlling Plasma Fibronectin Levels[1] (42758)

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*Abstract.* Fibronectin is a normal glycoprotein component of plasma, interstitial fluid, and extracellular matrix which has binding sites for collagen, gelatin, actin, glycosaminoglycans, fibrin, *Staphylococcus aureus,* and some cells. Since it is a dimer, it can crosslink these substances to each other or to extracellular components of basement membrane, thereby affecting many physiological processes. The level of circulating fibronectin is markedly reduced following even moderate blunt or operative trauma, thermal injury, starvation, advanced cancers, hemorrhage, etc. Replacement therapy has been tried with some success in patients who become septic following multiple injuries. The reduction in plasma fibronectin has been attributed to several causes including consumption by binding to cell debris at the site of injury, binding to circulating cell debris and its subsequent removal by elements of the phagocytic system, and degradation by proteolytic cleavage. However, the amount of fibronectin removed from circulation raises some question about this. In this paper, we used indomethacin, ibuprofen, imadazole, and essential fatty acid deprivation to inhibit the synthesis of prostaglandins in young adult rats. Thirty minutes after ip administration of one of the inhibitors, the rats were subjected to a midline laparotomy and mild intestinal manipulation. Blood samples were taken at intervals following closure of the incision and analyzed for fibronectin. In all cases, the normal decline in plasma fibronectin seen in untreated rats was abrogated by inhibiting prostaglandin synthesis. Since imadazole specifically inhibits thromboxane A synthesis, this strongly suggests that thromboxanes directly or indirectly control the trauma-induced reduction in circulating fibronectin. This was confirmed by ip injection of thromboxane into the rats which resulted in a decline in plasma fibronectin levels. 

Fibronectin (Fn) is a normal plasma, interstitial fluid, and extracellular matrix glycoprotein with a molecular weight of 440,000 Da (1). It is a dimer of nearly identical peptides which have binding sites for collagen, glycosaminoglycans, fibrin, actin, some cells, and *Staphylococcus aureus* (2). Many of its biological properties can be attributed to its ability to promote adhesion of cells to other cells or to substrata such as the extracellular components of basement membrane and cell debris (3). Because of the fundamental importance of adhesion in many physiological systems, Fn is involved in a wide variety of different activities, e.g., fibroblast and macrophage adhesion to extracellular components of basement membranes (4), prevention of cancer growth (5), wound healing (6), and host defense against trauma (7). Rybski and his co-workers have shown that Fn inhibits antigen-induced lymphoproliferation in mice (8) and inhibits both lymphoproliferation and antibody synthesis in rats (submitted).

Interest in Fn has grown since it was shown that the plasma concentration is greatly reduced following most types of trauma (9, 10), and the extent of reduction is somewhat related to the extent of injury (11). This reduction has been attributed to consumption of Fn both at the site of an injury (since Fn does localize at the wound site (12)) and by the reticuloendothelial system as it phagocytizes tissue debris which has been opsonized with Fn (13–16). In addition, a variable amount of Fn may be degraded by proteases released during the inflammatory process since Fn is very sensitive to proteolytic degradation (17). Lack of plasma Fn results in hypofunction of the reticuloendothelial system (RES) (18, 19) which has a critical role in prevention of infection and in wound debridement following injury. This blockade results from Fn's role as an opsonin for denatured collagen (gelatin) (13), damaged membranes (14), actin (15), and fibrin (16). Since

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repletion of plasma Fn restores normal RES function (20), clinical attention has focused on the use of Fn in replacement therapy of traumatized patients, particularly those who give evidence of becoming or being septic (21, 22).

Although we had previously shown that Fn is actively transported to the site of an injury in at least some types of wounds, it seemed unlikely that this mechanism could account for all of the decrease (9). Glucocorticoids have been shown to stimulate Fn synthesis by both chick hepatocytes (23) (which contribute to plasma Fn levels) and human fibroblasts (24) (which secrete insoluble Fn), so plasma Fn levels may be, at least somewhat, under metabolic control. However, it does not seem likely that reducing the Fn synthetic rate would result in such quick changes following trauma. Since prostaglandin levels are elevated by the same kinds of traumas which lower Fn concentrations and prostaglandins are known to be important in controlling other physiological processes, it seemed reasonable to examine the role of prostaglandins in influencing plasma Fn levels following surgical trauma.

**Materials and Methods.** *Chemicals.* Ibuprofen, indomethacin, imidazole, alkaline phosphatase, *p*-nitrophenyl phosphate, polybrene, thromboxane $A_2$ ($TxA_2$), prostacyclin $I_2$ ($PgI_2$) and cyanogen bromide were all obtained from Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased from Fisher Chemical Co. (Fairlawn, NJ). Sepharose 4B was from Pharmacia Fine Chemicals (Upsula, Sweden). $TxA_2$ and $PgI_2$ were reconstituted in rat serum albumin (50 $\mu$g/ml DPBS) to help stabilize them. $TxA_2$ and $PgI_2$ were injected ip rather than iv to more nearly approximate the way they would be released *in vivo.*

*Fibronectin preparation.* Fn was isolated from citrated rat plasma by affinity chromatography on gelatin–Sepharose 4B using our (25) modification of the method of Engvall and Ruoslahti (26). Briefly, 90 ml of citrated rat plasma was incubated for 2 hr at room temperature with 75 g of gelatin–Sepharose (coupled by the method described by (27)). The slurry was poured into a column and washed thoroughly with Dulbecco's phosphate-buffered saline (DPBS), pH 7.4, followed by 1 *M* urea in 0.05 *M* Tris (trishydroxymethylaminomethane), pH 7.4. Fn was then eluted with 4 *M* urea in 0.05 *M* Tris buffer. The urea was removed by extensive dialysis against DPBS. The final concentration of Fn was determined by measuring the optical density at 280 nm and by converting to mg/ml using an extinction coefficient of 1.28 OD/mg/ml. When this material was run on polyacrylamide disc gel electrophoresis under reducing conditions and visualized with a silver stain, only the monomer and no smaller fragments were observed. This was confirmed by results from high-performance liquid chromatography (not shown).

*Animals.* The Sprague–Dawley rats (250–300 g young adults or retired breeders) used as the experimental animals in the following experiments were obtained from Harlan Laboratories. Rats used in the experiments to determine the effect of essential fatty acid (EFA) deficiency on trauma-induced Fn depression had previously been used in wound healing experiments (6). They were maintained on essential fatty acid-free diets for 30 days (young rats) or 60 days (retired breeders) prior to having 6 cm diameter skin plugs removed from their backs. The Fn levels in these animals had returned to normal within 3 days, and the wounds were all completely healed within 2 weeks. They were maintained on EFA-free diets for an additional 2–3 weeks following complete healing of the wounds and were then used in the experiments described herein. Therefore, plasma Fn had been at normal levels for a month before they were used in these experiments, and the rats had not been subjected to any trauma or other manipulation for a month or more.

*Surgical trauma.* Surgical trauma was induced by making a 2- to 3-cm midline incision, exteriorizing about 5 cm of the small intestine, kneeding the intestine gently for about 30 sec, and replacing the intestine. The muscle layer was closed with sutures of 4 O silk, and the skin was closed with wound clips. The entire procedure was done under deep ether anesthesia.

*Fn assay.* Fn concentrations were determined using the competitive inhibition assay of Doran *et al.* (28). Briefly, gelatin was coupled to wells of a 96-well microtitration plate

with carbodiimide, sealed with adhesive plate sealers, inverted 2–3 times to ensure mixing, and stored in the refrigerator until use (storage life of at least 1 month). Alkaline phosphatase was coupled to freshly prepared Fn (AP-Fn) with glutaraldehyde (26). Immediately prior to use, the 96-well plate was washed thoroughly with DPBS. To each well was added, in order, 25 μl Fn standard or unknown plasma, 25 μl polybrene (to inhibit the interfering heparin), and 25 μl AP-Fn. After a 90-min incubation at room temperature, the plates were washed 5 times with DPBS, and 200 μl of *p*-nitrophenyl phosphate (1 μg/ml) was added. The plates were incubated in the dark at room temperature until a bright yellow color had developed (5–10 min), then the reaction was stopped with 50 μl of NaOH. The optical densities were determined on a Titertek Multiskan spectrophotometer (Flow Laboratories). Concentrations of the unknown were determined by comparison with a standard curve prepared using freshly prepared Fn.

*Statistical treatment.* Significance of differences between pretreatment and post-treatment plasma Fn was calculated using a paired Student's *t* test.

**Results.** Surgical trauma has long been known to reduce circulating Fn levels (12). We chose to use this model because of its relative simplicity and its similarity to common clinical procedures (30).

EFA-deficient rats lack arachidonic acid which renders them unable to synthesize prostaglandins. These rats were subjected to the surgical procedure, and plasma Fn levels were determined as usual. Control EFA-deficient rats were anesthetized for approximately the same length of time as the experimental rats, but no incision was made. Blood samples (about 3 ml each) were collected via cardiac puncture, and the plasma was analyzed for the concentration of Fn. The results shown in Table I (expressed as the mean % of the initial Fn concentration) indicate that the inability to synthesize prostaglandins also abrogates the surgery-induced depression in Fn. Anesthesia itself had no effect on either EFA-deficient controls or normal rats (Table II, line 1). Identical results were obtained with young (4 months old) and middle aged (12–16 months old) rats. Thus, products of arachidonic acid metabolism appear to be involved either directly or indirectly in control of plasma Fn levels.

The results in Table II (lines 1 and 2) show that the relatively mild surgical trauma used in these experiments results in a lowering of circulating Fn within 1–2 hr, followed by a rebound to slightly above normal levels by 8 hr. Further efforts were directed at defining which product(s) of arachidonic acid medabolism is responsible for Fn's regulation. Indomethacin is a widely used inhibitor of cyclooxygenase and, thereby, of prostaglandin synthesis. Line 3 of Table II shows that indomethacin completely abrogated the trauma-induced dip in Fn levels at 1 and 2 hr post-surgery. This strongly suggests that prostaglandins are negative regulators of plasma Fn, although other explanations are possible,

TABLE I. EFFECT OF SURGICAL TRAUMA ON FIBRONECTIN LEVELS IN ESSENTIAL FATTY ACID-DEFICIENT RATS[a]

| | Time (hr) after incision and intestinal manipulation | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 0 (%) | 1 (%) | 2 (%) | 4 (%) | 6 (%) | 8 (%) |
| None | 100 (4) | 106 ± 12 (4) | 101 ± 3 (4) | 104 ± 12 (4) | 102 ± 14 (4) | 108 ± 17 (4) |
| Surgery | 100 (6) | 98 ± 2 (6) | 100 ± 2 (6) | 101 ± 2 (6) | 103 ± 3 (6) | 108 ± 6 (6) |

[a] Young adult rats were maintained on an essential fatty acid-free diet (EFAD) for 8 weeks prior to the experiment. Control rats were maintained on Purina Lab Blox *ad libitum*. A 2- to 3-cm midline laparatomy was performed. About 5 cm of small intestine was exteriorized, kneeded gently for about 30 sec, and replaced in the abdominal cavity. The muscle layer was sutured with 4 O silk, and the skin was closed with wound clips. Blood samples were taken at the times indicated, and Fn concentrations in the plasma determined using an ELISA-based competitive inhibition assay. Fn levels were converted to the percentage of the 0 hr value (Experimental/Control × 100%). Fn levels are shown as the mean percentage ± SEM of the number of rats shown in parentheses. There were no significant differences between pretrauma and post-trauma Fn levels as determined by a paired Student's *t* test.

TABLE II. EFFECT OF SURGICAL TRAUMA ON FIBRONECTIN LEVELS IN RATS TREATED WITH INDOMETHACIN, IBUPROFEN, OR IMIDAZOLE[a]

| Treatment | Time (hr) after incision and intestinal manipulation | | | | | |
|---|---|---|---|---|---|---|
| | 0 (%) | 1 (%) | 2 (%) | 4 (%) | 6 (%) | 8 (%) |
| None | 100 (4) | 106 ± 3 (4) | 102 ± 5 (4) | 99 ± 5 (4) | 102 ± 4 (4) | 103 ± 8 (4) |
| Surgery | 100 (14) | 83 ± 3 (14)[b] | 86 ± 3 (14)[b] | 94 ± 3 (14)[c] | 96 ± 3 (12) | 102 ± 2 (12) |
| Indomethacin | 100 (4) | 101 ± 1 (4) | 104 ± 6 (4) | 102 ± 3 (4) | 102 ± 5 (4) | 102 ± 4 (4) |
| Ibuprofen | 100 (5) | 104 ± 2 (5) | 102 ± 2 (5) | 104 ± 7 (5) | 107 ± 7 (5) | 109 ± 6 (5) |
| Imidazole | 100 (4) | 96 ± 2 (3) | 96 ± 2 (3) | 98 ± 2 (3) | 100 ± 1 (3) | 97 ± 9 (2) |

[a] Surgical trauma was induced as in Table I in young adult rats which had been injected ip 30 min previously with 30 mg indomethacin, ibuprofen, or imidazole/kg body. Control animals were injected with a similar volume of phosphate-buffered saline (PBS). Blood samples were collected, analyzed, and Fn levels converted to percentages as described in Table I. Values shown are the means ± SEM of the numbers of rats shown in parentheses. Significance of differences between Fn levels pre- and post-treatment was determined using a paired Student's $t$ test. ([b]$P = 0.001$, [c]$P = 0.05$).

e.g., if prostaglandins interfered with the Fn assay in some way.

Since most drugs act on more than one system, we repeated the indomethacin experiment using ibuprofen as the cyclooxygenase inhibitor. The results (Table II, line 4) again show the postsurgical decline in plasma Fn was eliminated, confirming the results from the indomethacin studies. Possible prostaglandin(s) involvment in abrogation of the surgical trauma-induced depression in plasma was further restricted by repeating the above experiments with imadazole which specifically inhibits thromboxane synthetase (29). As can be seen in Table II, line 5, this treatment also abolished the surgery-induced dip in Fn levels, indicating that it is the thromboxanes which are directly or indirectly involved in regulating circulating Fn levels.

Since the inhibitors used above may have effects other than on prostaglandins, $TxA_2$ and $PgI_2$ were injected ip into rats, and Fn levels were measured periodically thereafter. As can be seen in Table III, $TxA_2$ caused an immediate, significant decline in plasma Fn, whereas injections of an equal amount of $PgI_2$ (line 3) or of rat serum albumin–PBS alone (line 1) had virtually no effect. The slight decline in Fn levels in the control animals at later times in these experiments was probably due to the trauma induced by repeated cardiac puncture.

**Discussion.** The surgical trauma used in this study results in a brief depression in circulating Fn levels (Table II) similar to that seen in other systems (12) including humans (30). However, when all prostaglandin synthesis was prevented by absence of arachidonic acid (Table I) or in the absence of cy-

TABLE III. EFFECT OF THROMBOXANE $A_2$ AND PROSTACYCLIN $I_2$ ON FIBRONECTIN LEVELS IN RATS[a]

| Treatment | Time (hr) after injection | | | | | |
|---|---|---|---|---|---|---|
| | 0 (%) | 1 (%) | 2 (%) | 4 (%) | 6 (%) | 8 (%) |
| Control | 100 (8) | 102 ± 4 (8) | 98 ± 4 (7) | 94 ± 4 (8) | 92 ± 4 (8) | 93 ± 5 (7) |
| $TxA_2$ | 100 (8) | 83 ± 4 (8)[b] | 85 ± 5 (8)[c] | 89 ± 8 (8) | 85 ± 5 (6)[d] | 101 ± 13 (6) |
| $PgI_2$ | 100 (8) | 96 ± 8 (8) | 91 ± 8 (8) | 94 ± 9 (6) | 104 ± 19 (6) | 96 ± 7 (6) |

[a] Young adult rats were placed under light ether anesthesia and injected ip with PBS, thromboxane $A_2$ ($TxA_2$), or prostacyclin $I_2$ ($PgI_2$). Blood samples were collected and analyzed, and Fn concentrations were converted to percentages as for Table I. Values shown are the means ± SEM of the numbers of rats shown in parentheses. Significance of differences between Fn levels pre- and post-treatment were determined using a paired Student's $t$ test. ([b]$P = 0.005$, [c]$P = 0.025$, [d]$P = 0.05$).

clooxygenase products (Table II, lines 3 and 4), the dip in plasma Fn concentrations was abrogated. The particular prostaglandins responsible were further defined by using imidazole to specifically inhibit thromboxane synthesis (29). The results were identical to those obtained by the more general inhibition (line 5).

Results obtained using inhibitors of syntheses of various prostaglandins suggest that prostaglandins may be one of the systems which regulates circulating levels of Fn. The regulatory role of prostaglandins in this system was confirmed by direct ip injection of $TxA_2$ which induced a decline in plasma Fn levels (Table III) similar to that induced by surgery (Table II, Line 2). (Intraperitoneal injection of $TxA_2$ and $PgI_2$ rather than iv injection was used because their entry into circulation from the peritoneal cavity is more nearly like the way they would be taken up by the blood stream following an injury; i.e., they are not taken up as a bolus.) It is interesting, but probably coincidental, that the depression induced by ip injection of $TxA_2$ is almost identical to that induced by surgical trauma and intestinal manipulation.

The effect of $PgI_2$ on plasma Fn levels is more variable than that of $TxA_2$, but no consistent pattern was found. In those instances in which it depressed Fn levels, it may have been due to $PgI_2$'s anti-clotting effects which may have sometimes led to excessive bleeding following cardiac puncture to obtain blood samples for testing. (One control rat and two each of $TxA_2$ and $PgI_2$-treated rats died during the experiments. However, there was no difference in Fn levels of those which died and those which did not.)

Fn's *in vitro* synthesis and its secretion into the medium by both chick hepatocytes (23) and mouse fibroblasts (23) are under positive control by glucocorticoids. Amrani *et al.* (22) also showed that hepatocyte stimulation factor acts in an additive manner with dexamethasone to increase Fn secretion. Although Fn synthesized by fibroblasts *in vivo* probably does not contribute significantly to plasma Fn levels since it is usually incorporated into the extracellular matrix, Fn synthesized by hepatocytes probably is an important component of circulating Fn. Since two quite different cell types respond to glucocorticoid stimulation by increased synthesis of Fn, it is quite possible that other cell types respond in the same way. If glucocorticoids act similarly *in vivo,* the observed surgery-induced decline in plasma Fn occurs despite the release of glucocorticoids during trauma (31) which should increase the Fn levels. However, the increased synthesis of Fn *in vitro* did not reach its peak until 24 hr after addition of the glucocorticoids (23). Interestingly, this is the time when we normally observe a "rebound" to above pretrauma Fn levels; thus, glucocorticoids released along with prostaglandins following trauma are the prime candidate for the cause of the rebound.

Malnutrition has been reported to result in reduced Fn plasma levels and impaired RES function (32, 33). However, the EFA-deficient diet had no effect on preoperative Fn levels (Table I). EFA-deficient animals have been reported (34) to be resistant to the lethal effects of shock. Abrogation of the trauma-induced Fn decline in EFA deficient rats (Table I) may contribute to the shock resistance.

One of the most interesting regulatory effects of Fn is its ability to stimulate macrophages to secrete IL-1 (35). Since one of the effects of IL-1 is to induce release of various prostaglandins from fibroblasts (36), it is possible that a regulatory loop exists which acts to maintain plasma Fn levels.

Fn is important in the normal function of several systems (1, 37). Its decline following trauma may contribute to pathogenesis of a number of clinically important conditions including RES blockade (14), prolonged wound healing (37), shock (reviewed in (38)), and sepsis (30). Schirmer *et al.* (39) have reported that indomethacin, ibuprofen, and imidazole administration favorably affect survival in septic and/or endotoxic shock models. This effect was associated with improved hepatic perfusion (40). However, it is possible that maintenance of normal Fn levels may also contribute to improved survival. Elucidation of the role of thromboxanes and prostacyclins in control of circulating Fn levels may eventually lead to a method of manipulating plasma Fn concentrations for therapeutic benefits.

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