AD-A143 383 CORRELATION BETWEEN PLASMA FIBRONECTIN LEVEL AND MORTALITY FOLLOWING EXPE..(U) ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA D A DUBOSE ET AL. 1/1

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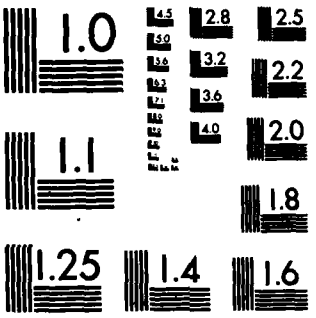

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Reticuloendothelial system (RES) clearance function correlates with the mortality rate associated with stresses which can induce shock. Likewise, mortality rate after experimental rat heat stress (ERHS) is altered by modulation of RES function. Since plasma fibronectin (PF) mediates in vivo phagocytosis by the RES, the relationship between mean plasma fibronectin level (MPFL) and mortality after ERHS was examined. A comparison of MPFLs prior to ERHS revealed that rats which ultimately comprised the survival group had a MPFL (269.0 + 11.2 µg/ml) which exceeded the value determined

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for the non-survivors (252.9 ± 11.9 µg/ml). Both groups had elevated MPFLs, up to 12 h following ERHS. However, after this time, MPFL began to decline. The decline was more severe for the non-survivors, with MPFLs at 15, 18, and 24 h, significantly ($p<0.01$) lower than the values for the survival group. Even the lowest MPFL (256.0 ± 30.7 µg/ml) noted for the survival group was still significantly ($p<0.01$) higher than the value (159.3 ± 13.3 µg/ml) determined for agonal samples collected from non-survivors. Furthermore grouping rats according to their pre heat PF level demonstrated that rats with levels exceeding 300 µg/ml had significantly ($p<0.05$) reduced mortality rates (13% vs 51%) as compared to rats with levels below this value. It was concluded that elevated PF levels correlated with tolerance to ERHS.

Correlation Between Plasma Fibronectin Level and Mortality Following Experimental Rat Heat Stress

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Key Words: Plasma fibronectin; heat stress; thermotolerance

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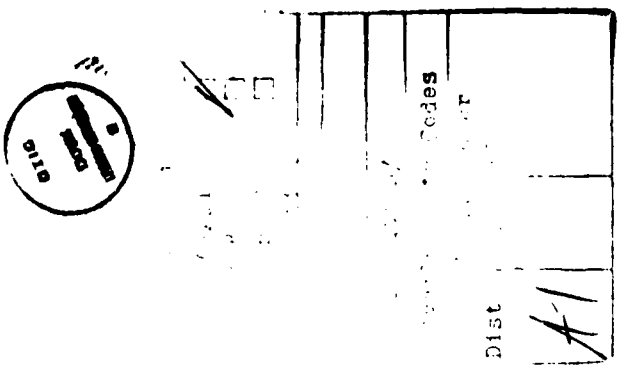
ABSTRACT

Reticuloendothelial system (RES) clearance function correlates with the mortality rate associated with stresses which can induce shock. Likewise, mortality rate after experimental rat heat stress (ERHS) is altered by modulation of RES function. Since plasma fibronectin (PF) mediates *in vivo* phagocytosis by the RES, the relationship between mean plasma fibronectin level (MPFL) and mortality after ERHS was examined. A comparison of MPFLs prior to ERHS revealed that rats which ultimately comprised the survival group had a MPFL (269.0 $\pm$ 11.2 µg/ml) which exceeded the value determined for the non-survivors (252.9 $\pm$ 11.9 µg/ml). Both groups had elevated MPFLs, up to 12 h following ERHS. However, after this time, MPFL began to decline. The decline was more severe for the non-survivors, with MPFLs at 15, 18, and 24 h, significantly ($p<0.01$) lower than the values for the survival group. Even the lowest MPFL (256.0 $\pm$ 30.7 µg/ml) noted for the survival group was still significantly ($p<0.01$) higher than the value (159.3 $\pm$ 13.3 µg/ml) determined for agonal samples collected from non-survivors. Furthermore, grouping rats according to their pre heat PF level demonstrated that rats with levels exceeding 300 µg/ml had significantly ($p<0.05$) reduced mortality rates (13% vs 51%) as compared to rats with levels below this value. It was concluded that elevated PF levels correlated with tolerance to ERHS.



INTRODUCTION

Since the reticuloendothelial system (RES) plays an important role in the removal of both exogenous and endogenous particulate material from the vascular space, function of this organ system is of particular relevance to survival following sepsis, shock, or trauma. Thus, elevated RES clearance function is associated with tolerance or resistance to a variety of forms of stress which can lead to an increase in ischemic injury, microaggregation and collagenous debris in the vascular fluids.

Stimulation of RES phagocytic function results in increased survival after hemorrhagic shock, epinephrine shock (13) and shock induced by drum trauma (1,10) or acceleration (24). Furthermore, an increase in susceptibility to shock occurs if there is an impairment or blockade of the RES (10,25,26). These findings indicate that the RES may serve as the common pathway for both the pathogenesis of and host resistance to shock.

The finding that plasma fibronectin levels correlate with blood clearance rates and Kupffer cell function indicates that this plasma protein modulates RES phagocytic function (2,14,15,17,19). Levels of plasma fibronectin are suppressed after shock and trauma (4,5,17,23). There is also a close relationship between reduced plasma fibronectin level and organ failure after trauma (17,21,22). In addition, non-surviving trauma patients have persistent suppression of plasma fibronectin levels, whereas survivors rapidly improve their levels of this plasma factor. Furthermore, treatment with antiserum to

fibronectin results in depression of phagocytosis and decrease resistance to trauma (11).

Heat stress injury can induce a shock-like state (8,12). Recent studies evaluating the effects of endotoxin tolerance (6) and alterations in RES function (7) indicate that as with mortality induced by other forms of shock, heat stress mortality correlates with the level of RES function. Since fibronectin modulates RES function, it was of interest to determine if level of this plasma factor also correlated with heat stress mortality rate.

Materials and Methods:

Heat Stress Model: As previously described (6,7), the experimental heat stress model employed male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 500 ± 30 g. Rats were individually caged and given food (Charles River Chow) and water ad libitum. Seven days prior to heat stress, cannulas were placed in the superior vena cava of anesthetized (0.4 ml I.V., sodium pentobarbital, Abbot. Labs., North Chicago, IL) rats via the right jugular vein. This cannula was exteriorized by passing the cannula through a small puncture made in the skin located between the shoulder blades. Such a procedure allowed for the drawing of central venous blood samples for plasma fibronectin testing both prior to and after heat exposure. During the heat exposure, rats were placed in small individual restrainer cages. A copper/constantan thermocouple was inserted 6.5 cm into the rectum to measure rectal temperature using a multipoint temperature scanning system (Leeds and Northrup, North Wales, PA). Rats were then placed in a heat stress chamber (Napco incubator, Portland, OR) maintained at 41.5 ± 1°C. Rectal temperature measurements were taken at 1-min intervals. When rectal temperature exceeded 40.4°C, the thermal area was calculated using the following formula (9): Thermal area (deg-min) = time interval (min) x 0.5 (°C above 40.4°C at the start of the interval + °C above 40.4°C at the end of the interval). After reaching the desired rectal temperature and thermal area, rats were removed from the heat stress chamber and allowed to

cool passively at a temperature of 26°C. Both maximum rectal temperature (°C) and total thermal area (deg-min) were used to evaluate the degree of heat exposure experienced by each rat. After heat stress, rats were observed for mortality over a 24-hour period. At this time, rats were grouped as either survivors or non-survivors. In addition, rats were grouped according to their pre-heat stress plasma fibronectin value and the 24-hour post heat stress mortality rates determined.

Measurement of Plasma Fibronectin: An immunoturbidometric assay for rat plasma fibronectin was developed following previously described procedures (16). Rat fibronectin antiserum was obtained from Calbiochem-Behring (San Diego, CA). A 0.2 ml rat plasma sample was added to 0.5 ml of a 1:5 dilution of the antiserum and a timer started. This solution was mixed for 45 sec. At one minute, level of absorbence was determined in a spectrophotometer at 365 μm . A second reading of the mixture was made once 10 mins had elapsed. The first min value was subtracted from the 10 min value to determine change in absorbence (ΔA). These values were compared to the ΔA obtained from a known level of rat fibronectin (Calbiochem-Behring) such that ΔA could be reported as μg of fibronectin per ml.

Citrated blood samples were collected for plasma fibronectin testing prior to and at various time intervals following heat exposure. All samples were tested for plasma fibronectin within 3 hours after collection.

Statistics: Statistical analysis employed chi-square, the student t-test, and analysis of variance. Where appropriate, Yates correction factor was applied. All values are reported as means; ± standard error.

Results:

Testing indicated that PF level returned to normal (261.7 ± 7.7 μg/ml) within 7 days following surgical implantation of the venous cannulas. Prior to heat stress, mean plasma fibronectin level (MPFL) for rats which ultimately comprised the survival group (269.0 ± 11.2 μg/ml) was greater than the value (252.9 ± 11.9 μg/ml) determined for non-survivors, but was not significantly different ($p<0.05$) (Figure 1). Up to 12 hours following ERHS, both groups experienced an elevation in their MPFL. After this time, MPFL began to decline. The decline was more severe for the non-survivors, with MPFLs at 15, 18, and 24 hours significantly ($p<0.01$) lower than the values for the survival group (Figure 1). A MPFL of 159.3 ± 13.3 μg/ml was determined for the agonal samples obtained from the non-survivors. Even the lowest MPFL (256.0 ± 30.7 μg/ml) noted at 24 hours for the survival group (Figure 1) was still significantly ($p<0.01$) higher than the agonal MPFL. Furthermore, MPFLs in the survival group began to increase after the first 24 hours, such that at 48 and 72 hour post ERHS the values were 313.36 ± 11.52 μg/ml and 301.67 ± 17.74 μg/ml, respectively. These alterations in plasma fibronectin level noted between the groups of survivors and non-survivors were not due to differences in the level of

heat stress for there were no significant differences ($p<0.05$) in the mean maximum temperatures or total thermal areas between these two groups (Table 1).

Grouping rats according to their pre-heat PF level (Figure 2) indicated that rats with levels exceeding 300 µg/ml had reduced mortality rates as compared to rats with levels below this value. A comparison of mortality rates for rats above and below the 300 µg/ml value (Figure 3), demonstrated a significant ($p<0.05$) reduction in mortality rate for rats with PF values exceeding 300 µg/ml prior to ERHS. Further examination of the association between pre-heat PF level and mortality rate following heat stress demonstrated that mortality rate did not vary significantly ($p<0.05$) when PF level was below 300 µg/ml (Figure 2). These changes in mortality rates were not due to differences in the heat exposure experienced by the various groups for no significant differences ($p<0.05$) in the heat stress parameters were noted (Table 1).

Discussion:

As observed in other forms of stress, elevated PF level appears to correlate with a favorable prognosis following ERHS. The increased MPFLs immediately following heat stress likely reflects breakdown of the fibronectin protein by proteolytic enzymes released as a result of tissue damage. Since the immunoturbidometric assay cannot discriminate between the complete protein and split products, PF levels appear high. When this has been noted in studies of other forms of stress, plasma

obtained immediately following the stress did not support in vitro phagocytosis (18). Thus, even though the immunoturbidometric values up to 12 hours following heat exposure were elevated (Figure 1), fibronectin's physiological function is likely suppressed.

Several findings indicate that PF level may be an important parameter impacting on the ERHS mortality rate. First, MPFLs prior to heat stress are higher in survivors than in non-survivors. Second, survivors maintain significantly higher MPFLs following ERHS. Furthermore, mortality rate was significant lower in rats whose pre-heat PF level exceeded 300 µg/ml (Figu 3). However, since mortality rate did not significantly chang in groups of rats whose MPFLs were below 300 µg/ml (Figure 2), an exclusive role for PF in heat stress resistance is in question. In this study, mortality rate continued to drop as PF level prior to heat stress decreased below 250 µg/ml (Figure 2). If PF level played an exclusive role in reduced heat stress mortality, an increase in the ERHS mortality rate would be expected in groups of rats with reduced PF levels. Since this was not noted, it may be that when PF levels are in the elevated condition (i.e. > 300 µg/ml) there are other factors present in addition to enhanced RES clearance which are important in the noted resistance to ERHS which are not active in animals with lower PF levels. This may relate in some manner to plasma co-factors recently described which are known to amplify the opsonic activity of fibronectin (3). Thus, thermotolerance may represent

a combination of factors associated with elevated levels of PF and enhanced RES clearance. Since PF is synthetized by vascular endothelial cells (20), maintenance of PF at elevated levels may be indicative of an alteration in the general metabolism of the endothelium which in some way is associated with thermotolerance.

To summarize, elevated PF levels prior to ERHS appear to correlate with reduced ERHS mortality rate. Therefore, knowledge of its level may have utility as an indicator for the presence of thermotolerance.

Figure 1. Comparison of MPFLs between survivors and non-survivors following ERHS. *significantly different ($p < 0.01$) than survivor value.

FIGURE 1

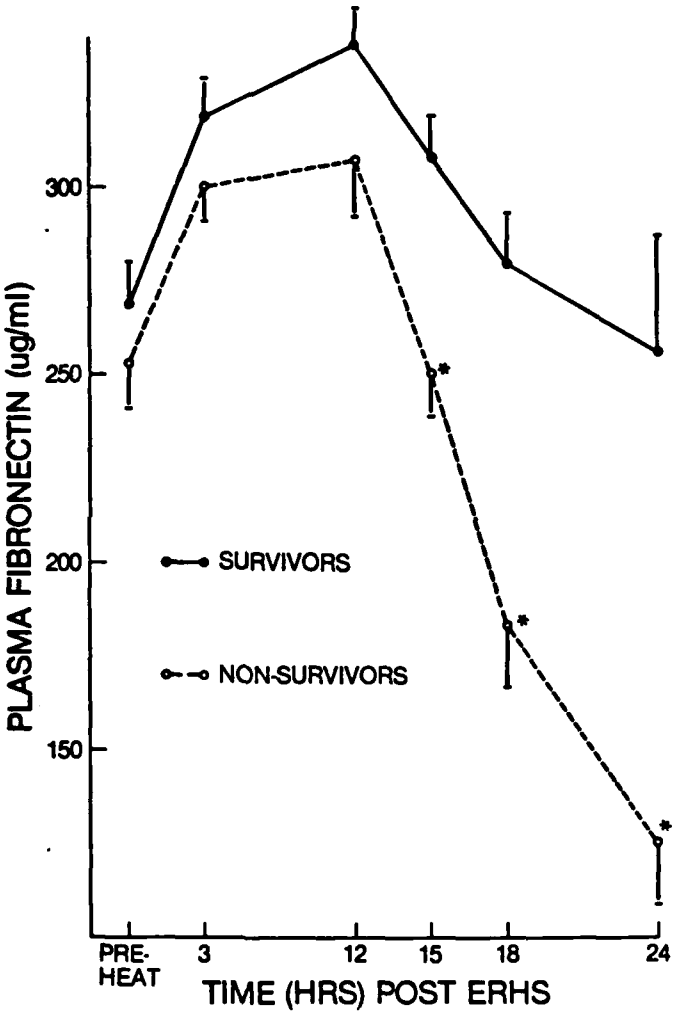

PLASMA FIBRONECTIN (ug/ml)
300
250
200
150
SURVIVORS
NON-SURVIVORS
*
*
*
PRE-HEAT
3
12
15
18
24
TIME (HRS) POST ERHS

Figure 2. Correlation between pre-heat plasma fibronectin level and ERHS mortality rate. Values within parenthesis represent the number of rats in each group. There were no deaths in the group of rats whose plasma fibronectin values prior to ERHS ranged between 350-400 μg/ml.

FIGURE 2

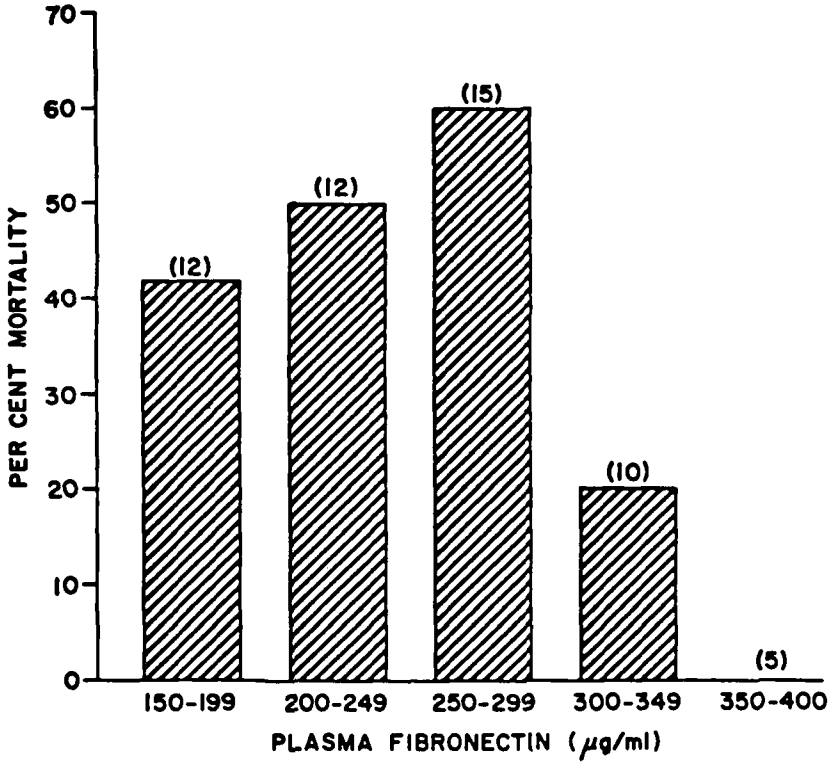

70
60
50
40
30
20
10
0
PER CENT MORTALITY
(12)
(12)
(15)
(10)
(5)
150-199
200-249
250-299
300-349
350-400
PLASMA FIBRONECTIN (μg/ml)

Figure 3. Comparison of the ERHS mortality rate between groups of rats with pre-heat PF values above and below 300 μg/ml. *significantly different ($p>0.05$).

FIGURE 3

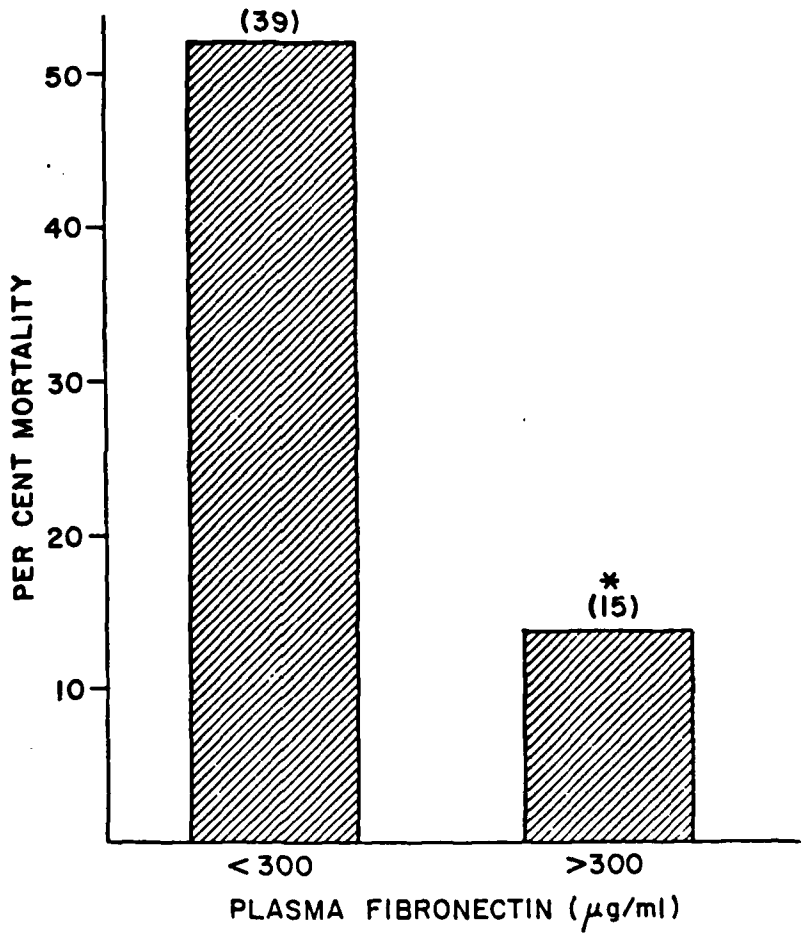

(39)
50
40
30
20
10
PER CENT MORTALITY
*
(15)
< 300
>300
PLASMA FIBRONECTIN (μg/ml)

Table 1. Comparisons of the Heat Stress Parameters* Among the Various Rat Groupings

			Pre-Heat Stress Plasma Fibronectin (μg /ml) Level				
	Survivors	Non-Survivors	150-199	200-249	250-299	300-349	350-400
Mean max. rec. temp.	42.46 ± 0.06	42.57 ± 0.06	42.38 ± 0.11	42.60 ± 0.06	42.47 ± 0.09	42.51 ± 0.08	42.55 ± 0.13
Mean thermal area	44.07 ± 0.45	45.34 ± 0.65	43.86 ± 0.86	44.03 ± 0.71	44.95 ± 0.88	45.38 ± 0.73	45.63 ± 0.88

*values are means ± standard error; no significant differences ($p<0.05$) noted

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ANIMAL RESEARCH

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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