Sequential Patterns of Eicosanoid, Platelet, and Neutrophil Interactions in the Evolution of the Fulminant Post-traumatic Adult Respiratory Distress Syndrome

AVRAHAM I. RIVKIND, M.D.,* JOHN H. SIEGEL, M.D., PIETRO GUADALUPI, M.D.,† and MARGUERITE LITTLETON, D.N.Sc.

Thirty multiply injured blunt-trauma patients at high risk for development of ARDS (multisystem trauma including more than one organ or extremity, Injury Severity Score of 26 or more, hypotension and need for 1500 mL or more blood within the first hour after admission, and $Pa0_2 \leq 70$ torr) were studied sequentially with blood and physiologic evaluations beginning immediately after injury and every eight hours for eight days, or until death, to study the evolution of the ARDS process. Mixed venous blood samples were obtained for eicosanoids PGE_2, $PGF_2\alpha$, thromboxane B_2, PGI_2 (6-KetoPGF$_1\alpha$) and leukotriene B_4 (LTB_4). Platelet (PLAT), and neutrophil (WBC) counts were also done and plasma elastase was measured. At 7:00 AM each day patient neutrophils were obtained for a study of zymosan-activated superoxide production using a chemiluminescence assay. These data were correlated with physiologic measurements of the Respiratory Index (RI), per cent pulmonary shunt (QS/QT), and respiratory compliance measures. Seven patients developed a fulminant post-traumatic ARDS syndrome within 96 hours after injury. Twelve patients without ARDS developed sepsis (TS) four or more days after injury, and 11 had uncomplicated postinjury courses (TR). Compared to both TR and TS, ARDS had a significant ($p < 0.01$) rise in neutrophil superoxide production beginning on day 2 through day 4 after injury. This was preceded by rises in PGE_2 and LTB_4, which were significantly correlated with subsequent falls in PLAT and WBC and rises in TXB_2, PGF_1, and superoxide production and followed by increases in RI, QS/QT, and a fall in compliance. The significant difference in the pattern and sequence of events in ARDS compared to TR and TS patients suggests that in ARDS the earliest event may be related to peripheral release of PGE_2 and LTB_4 due to platelet activation and lung sequestration with release of $PGF_2\alpha$, and by aggregation and leukocyte adherence with release of elastase. However, fulminant ARDS mortality appears to be related to the subsequent amplification of the LTB_4 leukocyte activation with superoxide production that does not achieve significance before the second day after injury and rises to a maximum by day 4 after injury. These data suggest that post-trauma ARDS follows a different evolutionary pattern than that reported in animal models and is also different from that seen in human TS or TR patients. They also suggest that a therapeutic window may exist between platelet and white cell endothelial adherence-aggregation (sequestration) with proteolytic degranulation (elastase release) and the subsequent WBC activation producing toxic superoxides and lung gas exchange abnormalities.

From the Maryland Institute for Emergency Medical Services Systems (MIEMSS), and the Department of Surgery, University of Maryland at Baltimore, Baltimore, Maryland

THE ASSOCIATION OF ACUTE respiratory distress syndrome and trauma has been known since the late 19th century and continues to be a significant factor in morbidity and mortality. During World Wars I and II, the term traumatic wet lung was applied in combat victims with post-traumatic respiratory failure.[1,2] Recently the concept of PMN-related host autoinjury in ARDS has been established.[3-8] Much of the pathophysiology of the Adult Respiratory Distress Syndrome (ARDS) appears to be related to host autoinjury phenomena secondary to activated leukocytes. Neutrophil NADPH oxidoreductase plays a key role in this process[9] because it is the enzyme responsible for generating the precursor toxic oxygen species, mainly superoxide anion (O_2^-). Active oxygen compounds have at least in part been implicated in the endothelial injury associated with neutrophil-induced host autoinjury.

Neutrophils were implicated as important effector cells by studies that showed that dialysis membranes can activate the complement system, resulting in the release of inflammatory factors such as C_5a[10], which can initiate the cyclooxygenase and lipoxygenase pathways of eicosanoid production. It was also hypothesized that C_5a could cause vascular endothelial damage by aggregating and activating

*MIEMSS:Israeli Fellow of Hadassah University, Jerusalem, Israel.
†MIEMSS:Italian Fellow of University of Milan, Milan, Italy.

Presented at the 109th Annual Meeting of the American Surgical Association, Colorado Springs, Colorado, April 10–12, 1989.

Correspondence and reprint requests to: John H. Siegel, M.D., University of Maryland, MIEMSS, 22 S. Greene Street, Baltimore, MD 21201.

Accepted for publication: April 14, 1989.

neutrophils in the pulmonary capillaries.[9-11] Bronchoalveolar lavage fluids aspirated from lungs of patients with ARDS have been shown to contain more than 80% neutrophils, regardless of the cause of the lesion.[11] In some cases of ARDS, elastolytic activity is present in lung lavage fluid.[12] Furthermore, enzymes (probably neutrophil elastase) in the lung lavage fluids are capable of cleaving Hageman factor, prekallikrain, plasminogen, high molecular weight kininogen, C_3, C_4, C_5, and factor b of the complement system to fragments of the same molecular weight as the active components.[13] Alpha 1-antitrypsin in lung lavage fluids from patients with ARDS appears to be inactivated because the antiprotease inhibitor shield in the alveolar space is destroyed by neutrophil-generated oxidants and consumed by neutrophil proteases.[13,14] Therefore, while ARDS has been described in neutropenic patients, under most circumstances it appears that the introduction of neutrophils is critical to the perpetuation or evolution of the lung injury in ARDS.

It has been shown[15] that the lung also plays a major regulative role in prostaglandin metabolism. PGE_2 and $PGF_2\alpha$ (PGF_2), which are released by platelets and damaged tissue, are inactivated more than 90% during one single passage through the lungs. Extensive metabolism of PGF_2 has also been described in human lungs.[16] Prostacycline (PGI_2) is a potent vasodilator and antiaggregator metabolite of arachidonic acid. Plasma 6-keto prostaglandin $F_1\alpha$ (PGF_1), the stable hydrolysis product of PGI_2, was found elevated in experimental toxic and septic shock.[17-19] In human septic shock, elevated plasma levels of 6 keto-$PGF_1\alpha$ have been found and raise the possibility that the increased PGI_2 formation may play a role in human septic shock.[20] Plasma thromboxane B_2 (TXB_2), the stable hydrolysis product of thromboxane A_2, is also elevated in experimental endotoxic and septic shock,[18,21] as well as in patients dying of septic shock.[22] PGI_2 was significantly lower in ARDS and mean thromboxane B_2 was unchanged in these patients.[23] Leukotriene B_4 (LTB_4) is a potent leukocyte chemotaxin and accentuates the inflammatory process. There is evidence that LTB_4 has a major role in augmenting neutrophil endothelial adherence[24] and the leukotriene receptor antagonist, FPL-57231, has been reported to attenuate 0_2 radical induced lung injury.

The interaction between cyclooxygenase and lipoxygenase eicosanoid pathways in regulating the action and localization of neutrophils and in stimulating them to produce oxidant and proteolytic substances makes these mediators of prime consideration as effectors of the ARDS process.

The present study was undertaken to investigate prospectively the temporal relationships of the plasma eicosanoids (thromboxane, measured as TXB_2; and prostacycline, measured as PGF_1; PGE_2, PGF_2, leukotriene B_4) and the activation of neutrophils (which elaborate the proteolytic enzyme elastase and generate superoxides) to the development of fulminant nonseptic ARDS in posttrauma patients.

Materials and Methods

Patients

Thirty polytrauma patients at high risk for ARDS development were selected based on the criteria generated from a study of 1778 patients admitted during the years 1984 to 1985 to the Maryland Institute for Emergency Medical Services Systems (MIEMSS). From this group, which had a 7.4% incidence of ARDS, the factors common to those patients who developed ARDS were multisystem trauma including more than one organ or extremity, Injury Severity Score (ISS) greater than or equal to 26, hypotension and the necessity to replace at least 1500 cc of blood or colloid within the first hour after admission, and an admission $Pa0_2 \leq 70$ torr. Using these factors on admission, patients were entered into this prospective study and then were later divided into three groups based on their clinical outcomes: ARDS, trauma uncomplicated by ARDS or sepsis (TR), and septic trauma (TS). Sepsis was defined as a positive blood culture, autopsy or operative evidence of infection, or clinical, roentgentographic, and laboratory evidence of pneumonia. All patients were admitted to MIEMSS within one hour of the injury and all patients underwent surgery within two to four hours of admission. Blood samples were drawn for formed elements, eicosanoid isolation, and elastase measurement immediately on admission (A) and between one and two hours after admission and resuscitation (R). The admission sample was from the femoral vein, but the remaining samples were all mixed venous blood drawn from the pulmonary artery catheter. Subsequent blood samples were drawn three times daily at eight-hour intervals (7:00, 15:00, and 23:00 hours) for eight days after injury for analysis of prostaglandins, white cells (WBC), platelets, and free elastase. Beginning with the 7:00 AM sample on the first postinjury day, neutrophil superoxide production was determined by chemiluminscence assay and repeated at 7:00 hours each successive day. Informed consent was obtained from all patients or their families in accordance with institutional guidelines. Comprehensive respiratory and physiologic parameters were also measured on admission and then every eight hours at the times of the blood sample collections.

Chemiluminescence Assay

Chemiluminescence was performed by the method described by Allen and Loose,[25] as modified by us.[26] Briefly, leukocytes were isolated from whole blood by dextran sedimentation, followed by hypertonic lysis of contami-

nating RBCS. The leukocytes were then washed three times in Gey's balanced salt solution (GBSS, Gibco) and resuspended at a concentration of 2×10^6 per mL. The percentage of neutrophils was calculated by differential count and a leukocyte suspension containing 1×10^6 neutrophils was added to a chemiluminescence cuvette with 0.25 mL of 1.25 mmol luminol, and GBSS was then added to a total volume of 0.9 mL. Reaction was initiated by automatic addition of 0.2 mg washed preopsonized zymosan (Zymosan A, Sigma, St. Louis, MO) and the chemiluminescence response was monitored in a luminometer (LKB 1251) with measurements of output in millivolts performed each minute for 19 minutes. The peak response and 19-minute peak integral response rate and continuous response rate were recorded. Neutrophils were collected from normal volunteers with group O blood for testing of patient serum. Normal type AB serum was used to assay patient neutrophils.

TABLE 1. *Criteria for Diagnosis of Acute Post-traumatic ARDS*

Physiologic Criteria	ARDS Patient Means
$FIO_2 \geq 0.50$	0.65
PEEP $\geq$ 10 cm H_2O	16.0 ± 6
$PaO_2/FIO_2 \leq 250$	181.0 ± 91
QS/QT $\geq$ 25%	29.0 ± 8
Respiratory index $\geq$ 2.5	3.7 ± 2.7
Total static compliance $\leq$ 35 cm/L	22.0 ± 8

Clinical criteria: Evident respiratory distress with x-ray findings of unilateral or bilateral diffuse pulmonary infiltration or edema with a normal cardiac silhouette in the absence of primary infections pneumonia.

The overall mortality rate for the ARDS patients was 86%, but only 8% of the septic and 9% of the uncomplicated trauma patients died.

Opsonization of Zymosan

Zymosan was mixed with 0.9% normal saline and heated in a boiling water bath for one hour, centrifuged at 1600 g/10 minutes, washed three times, and then re-

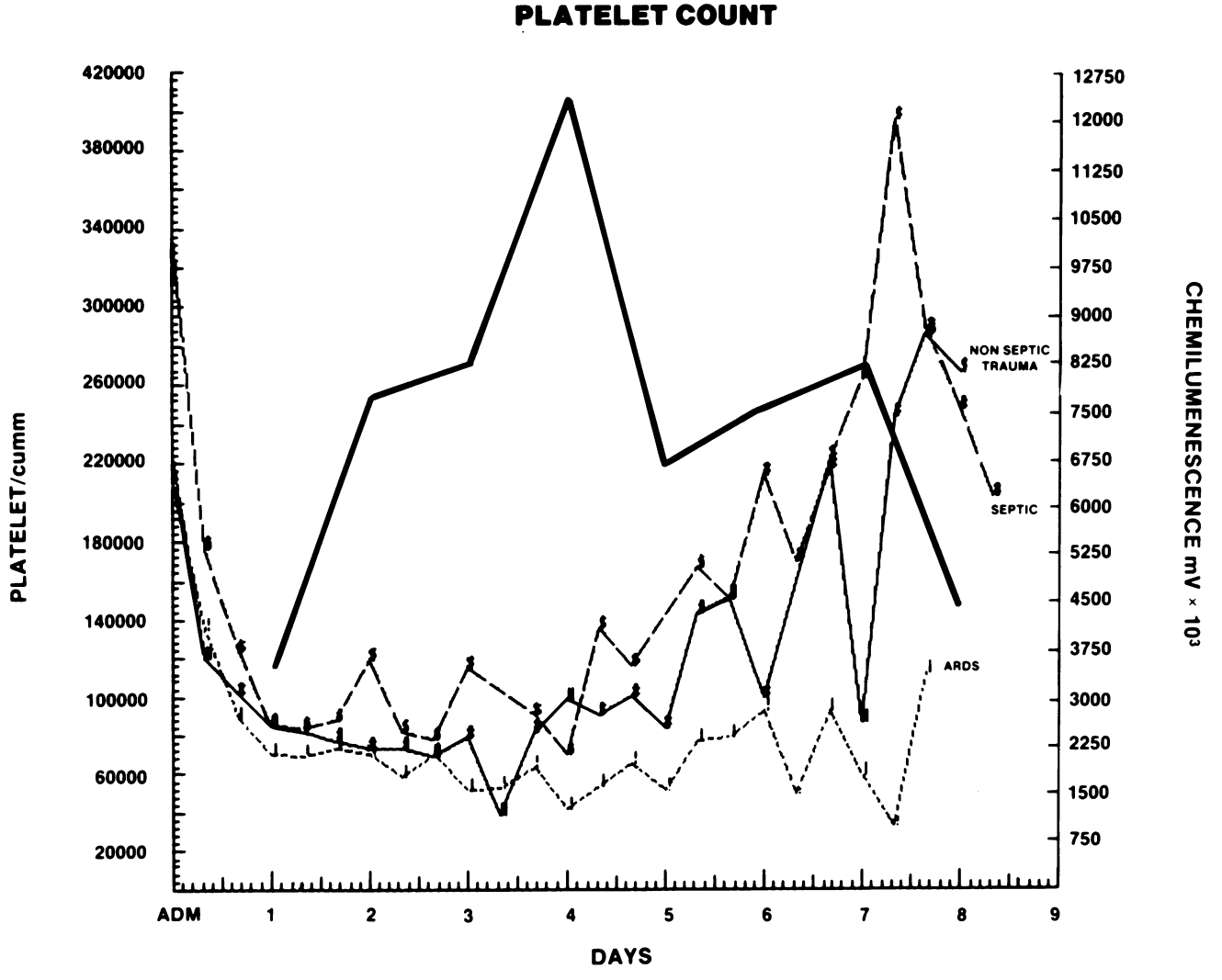
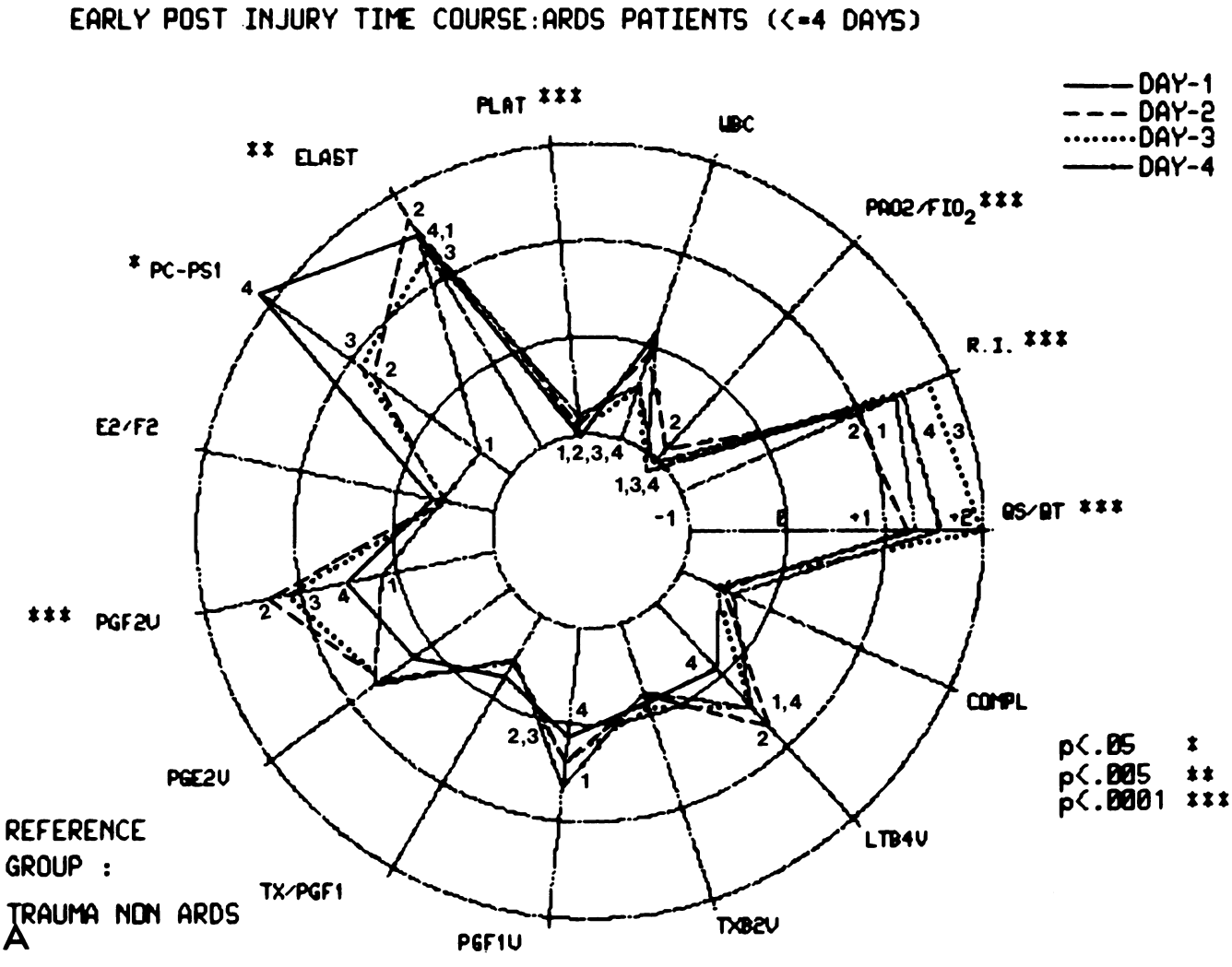


FIG. 1. Relationship of platelet responses in ARDS, nonseptic trauma, and septic trauma patients over an eight-day period after injury. Superoxide production by activated leukocytes (measured by chemiluminescence) in ARDS patients is shown (solid heavy line) for time comparison.



FIGS. 2A AND B. (A) Statistical circle diagram showing changes in ARDS patients normalized by mean and standard deviations (−1 to +2) of the nonseptic trauma patients (TR). Patterns represent 7:00 AM samples on days 1 to 4 after trauma. All differences noted as significant have been tested by simultaneous Bonferroni analysis of individual ANOVA determinations. All blood samples are from mixed venous pulmonary artery blood (V). See text for details. (B) Statistical circle diagram showing changes in trauma septic patients (TS) normalized by mean and standard deviation (−1 to +2) of the nonseptic trauma patients (TR). Legends as in Figure 2A.

suspended in 20% pooled normal serum or patient serum and incubated for 30 minutes at 37 C. The opsonized zymosan was then centrifuged at 1600 g/10 minutes, washed three times in Gey's media, and resuspended in Gey's media at a concentration of 2 mg/mL.

Eicosanoids Radioimmunoassay

Radioimmunoassay for prostaglandins was done by taking mixed venous blood samples in a polypropylene syringe containing 4.5 mmol EDTA and indomethacin 10 mcg/mL to prevent further prostaglandin synthesis. The samples were immediately centrifuged in a Beckman microcentrifuge (model 11, Beckman Instruments Inc., Irvine, CA) for three minutes at 20 C. After the plasma was pipetted into polypropylene tubes, it was stored at −70 C until extraction with ethylacetate using standard procedures. Briefly, three volumes of ethylacetate were added to the plasma; it was then shaken vigorously for ten minutes to insure thorough mixing and centrifuged at 3000 RPM, at 20 C for ten minutes to separate the organic and aqueous phases. The organic phase containing the eicosanoids was pipetted, dried, and reconstituted with a phosphate buffer. Determination of PGE_2, PGF_2, leukotriene B_4 (LTB_4), as well as the stable metabolites of thromboxane (TXB_2) and PGI_2 (PGF_1), were performed using commercially available radioimmunoassay kits (Advanced Magnetics Inc., Boston, MA).

Enzyme-Linked Immunosorbent Assay for Elastase

Briefly, the elastase α_1 protease inhibitor (E-α_1PI) concentration was determined on the concentrated fluids using the enzyme-linked immunosorbent assay described in detail by Neumann et al.[27-28] The samples were added to

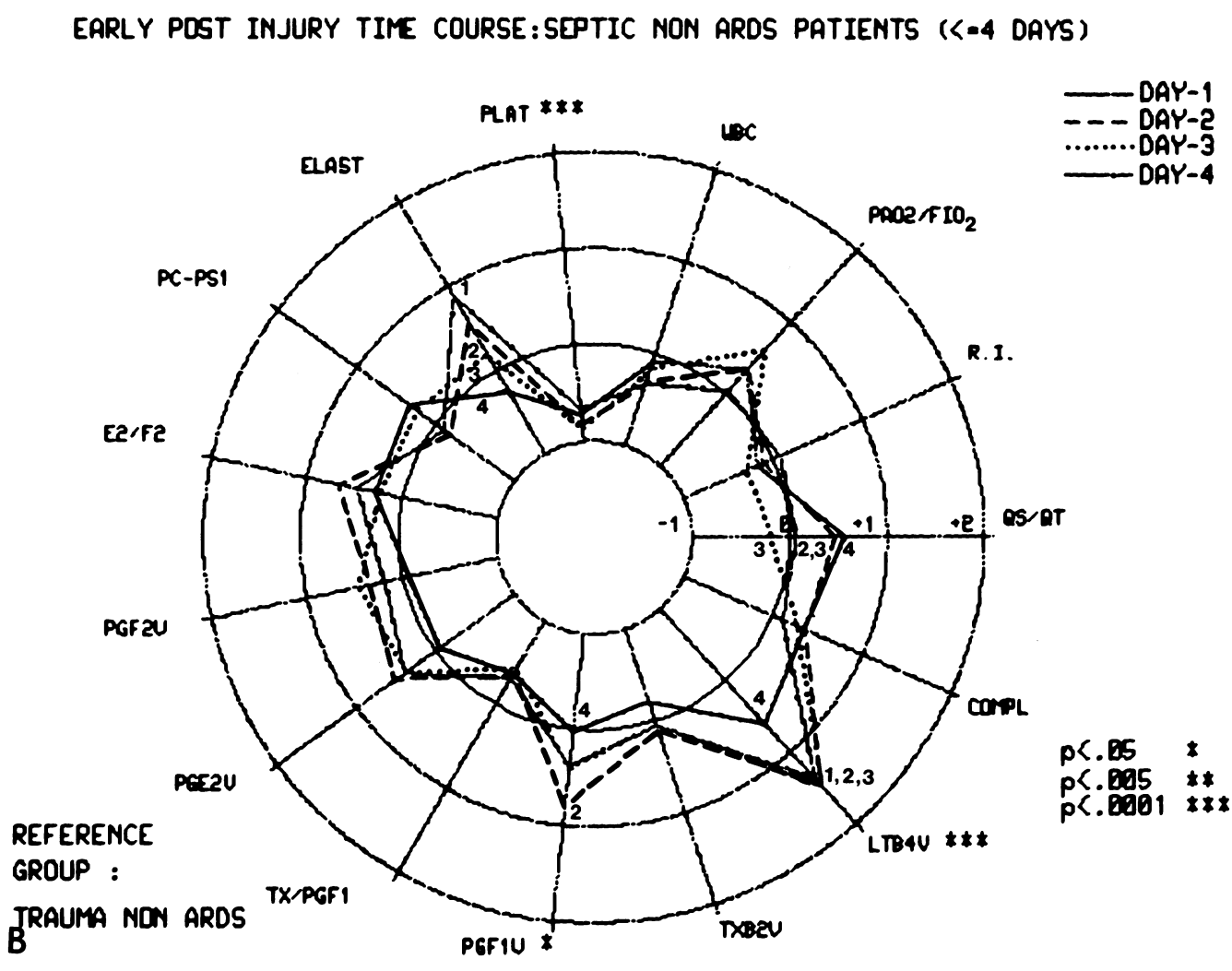


FIG. 2B.

microtitration plates coated with chep anti-elastase IGg. These antibodies do not cross react with cathepsin G and other neutrophil proteinases. After incubation and washing, the solid-phase bound E-α_1PI complexes were reacted with alkaline phosphatase-labeled rabbit anti-α_1PIIGg. After further washing p-nitrophenylphosphate was added to measure the amount of solid-phase bound E-α_1PI complex. The assay was calibrated using a standard solution of known E-α_1PI concentration. The preparation of this solution is described in detail in the report of Neumann et al.[27-28] Calibration curves identical to those reported by these investigators were always obtained. A commercial kit is available for measuring elastase (E. Merck, Darmstadt, West Germany).

Results

Thirty patients were entered into the protocol and followed clinically. Seven of these developed fulminant ARDS, 12 had post-trauma sepsis (TS), and 11 had uneventful trauma recoveries (TR). The criteria used for diagnosis of ARDS are shown in Table 1.

Comparison of the Daily Pattern of Initial Post-traumatic Responses in Trauma, Sepsis, and ARDS

In comparing the ARDS patients to the nonseptic trauma (TR) and septic trauma (TS) patients, observations of the platelet (PLAT), white cell count (WBC) and elastase (ELAST) responses in relation to the generation of leukocyte superoxides (measured by chemiluminescence) showed that all post-traumatic patients had a marked drop in platelets within the first day and that the thrombocytopenia persisted up to the fourth day. However, the ARDS patients maintained the thrombocytopenia throughout the entire eight days of study, whereas both the septic (TS) and nonseptic (TR) trauma patients tended to return to relatively normal platelet counts within the

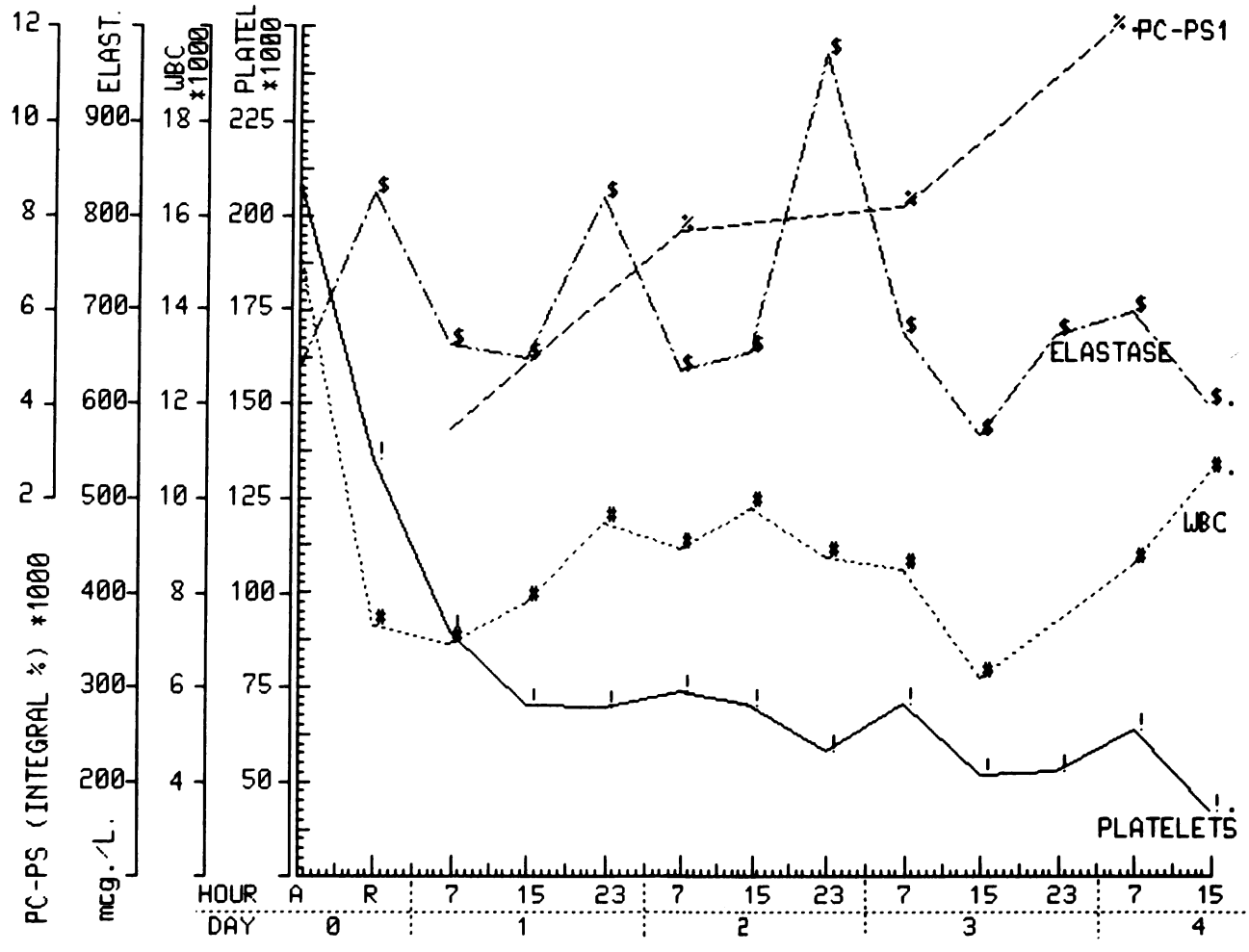


FIG. 3. Time sequence of data means from ARDS patients for first four days after injury. Shown are leukocyte chemiluminescence (PC-PS1), elastase, white blood cell count (WBC), and platelets. See text for details.

initial eight day post-injury period (Fig. 1). It can also be seen that the nadir of the platelet response in the ARDS patients corresponds quite closely to the maximum of superoxide production (heavy solid line). All post-trauma patients showed a fall in white count. However, in the ARDS and septic patients, there tended to be an increase in white count to super normal levels starting the fourth day after injury and reaching maximum by approximately the eighth day with the mean ranging between 24,000 and 26,000 cells/mm^3 for sepsis and ARDS, respectively. In contrast, the TR patients tended to return to roughly the levels found immediately after injury ($\leq$ 15,000 cells/mm^3). The mean plasma elastase was characteristically found to be elevated to levels exceeding 600 mcg/l in the ARDS patients and this was maintained for the first five days after injury when it fell to lower levels. In general, in both TS and TR patients the mean ELAST levels, although elevated, did not reach levels characteristic of the ARDS process.

The clinical pattern over the initial four days also appeared to be a nonseptic response. To compare the ARDS early response to that seen in either the TS or the TR patients, the patterns of seven physiologic variables ($Pa0_2$/$FI0_2$ ratio; respiratory index, RI; per cent pulmonary venoarterial admixture, QS/QT; static lung compliance, COMPL; platelet count, PLAT; white blood cell count, WBC; and the plasma elastase, ELAST) were compared to the superoxide production of activated patient cells studied in patient serum (PC-PS1). These were also compared to the pattern of pulmonary artery blood eicosanoids (leukotriene B_4, LTB_4; thromboxane, TXB_2; PGF_1, PGE_2, and PGF_2, as well as the ratio between thromboxane B_2 and PGF_1, TX/PGF_1, and the prostaglandin E_2/F_2 ratio) all done simultaneously at 7:00 AM on successive days. These data are shown in Figures 2A and B.

In Figure 2A, the early postinjury time course of the ARDS patients is shown for the first four days after injury and compared to the mean and standard deviation of those same variables in the reference group of TR patients. In Figure 2B, the same variables are compared between the TS and the TR patients. It was decided to look at this early segment of the response closely because after four

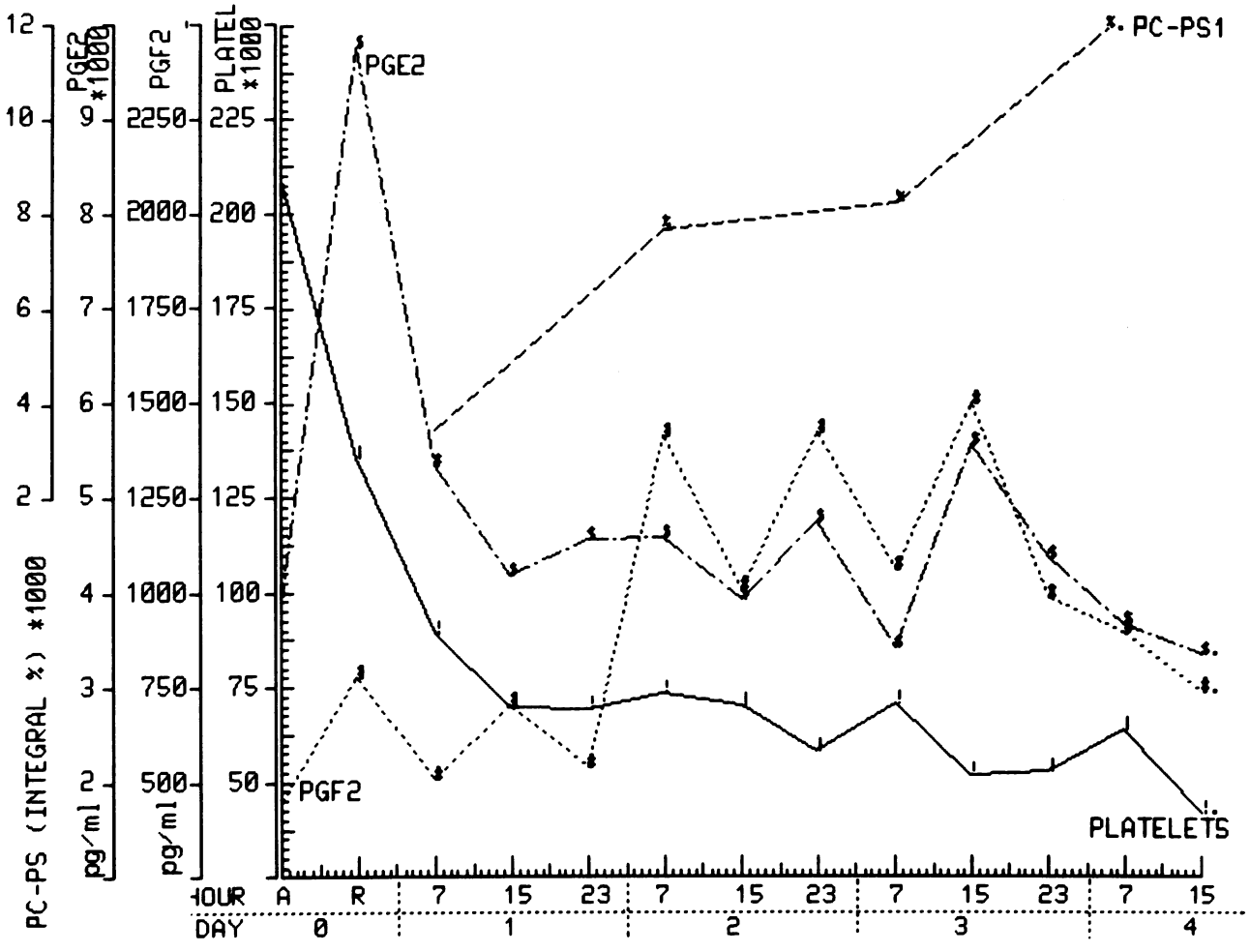


FIG. 4. Time sequence of data means from ARDS patients for first four days after injury. Shown are leukocyte chemiluminescence (PC-PS1), PGE_2, PGF_2, and platelets. See text for details.

days many of the ARDS patients became secondarily septic, so the response patterns were pathophysiologically confused. As can be seen in Figure 2A, the characteristic features of the fulminant ARDS patients were a marked fall in the $Pa0_2/FI0_2$ ratio ($Pa0_2/FI0_2$) to more than one standard deviation lower than the TR group with a concomitant rise in RI and QS/QT to between one and two standard deviations greater than TR, and these changes were simultaneously significant ($p < 0.0001$). In the ARDS patients, the compliance (COMPL) was reduced. With regard to the eicosanoids, the values for LTB_4 were increased but were not simultaneously significant. There were also increases in PGF_1 occurring in greatest magnitude at day 1. PGE_2 was also increased, but only PGF_2 was significantly increased beginning at day 2 until day 3, so that there was a fall in the E2/F2 ratio even though the absolute values for PGE_2 were always greater. There was a marked increase in superoxide formation with PC-PS1 being significantly increased ($p < 0.05$) from day 2 onward and an immediate early increase in elastase (ELAST) that was significant ($p < 0.005$) throughout all four days in the ARDS patients. The platelets were significantly ($p < 0.0001$) reduced through all four days. The white cell count that was slightly elevated on admission fell by the first day, but this was not significant when tested simultaneously with the other variables.

In contrast to ARDS (Fig. 2B), the patterns seen in the evolution of the septic syndrome in nonARDS patients during the first four days showed a very minimal increase in QS/QT and only slight rises in PAO_2/FIO_2, neither of which were significant, and compliance also was not significantly altered. Of the TS eicosanoids studied, only LTB_4 was consistently increased ($p < 0.0001$) thoughout the first three days after injury, thromboxane was minimally increased over TR, and PGF_1 was increased significantly on day 2. TS leukocyte superoxide production (PC-PS1) was not different from TR, although all trauma patients were increased over normal controls.[26] There were no other significant TS changes except in the platelet count, which was markedly reduced ($p < 0.0001$) in the

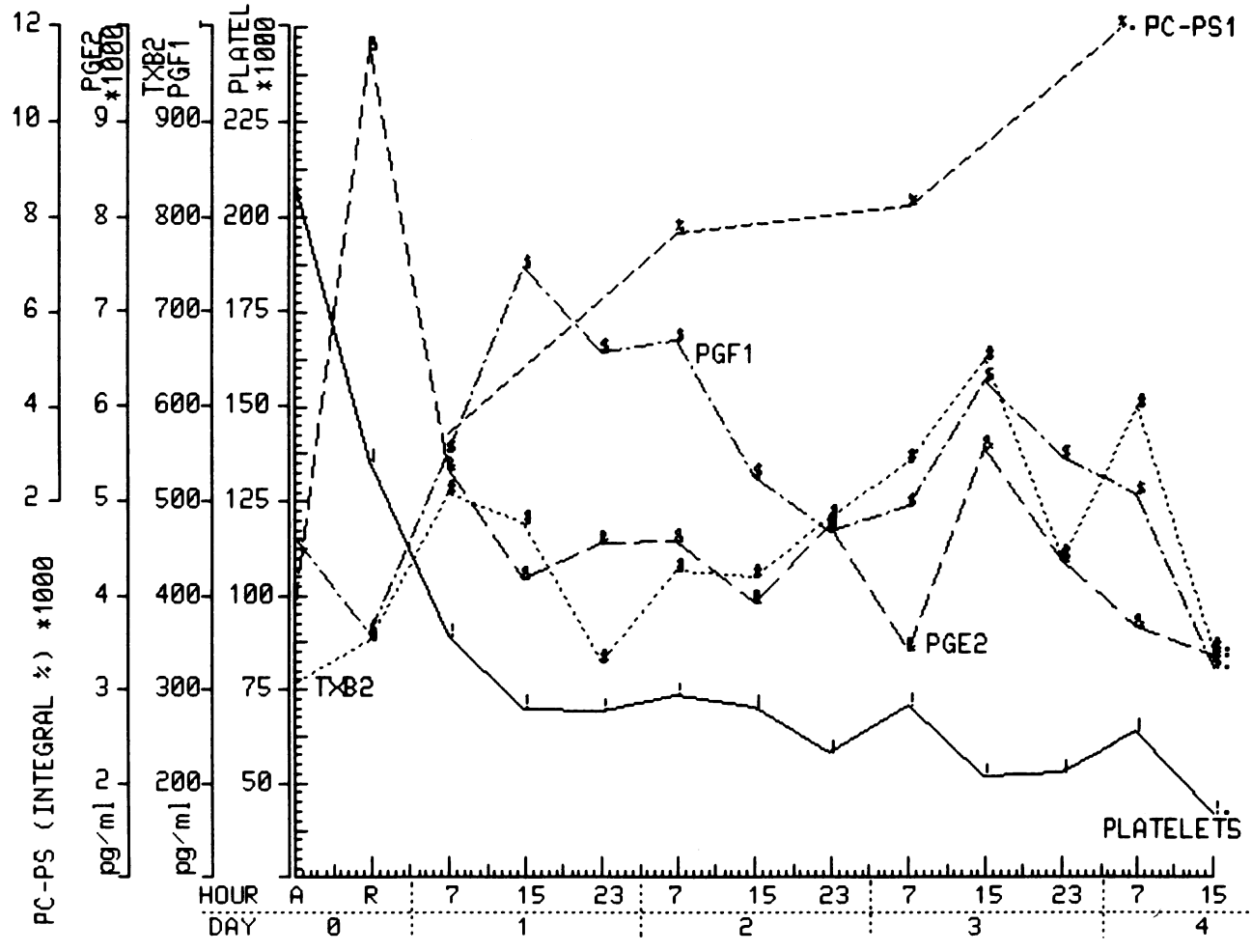


FIG. 5. Time sequence of data means from ARDS patients for first four days after injury. Shown are leukocyte chemiluminescence (PC-PS1), PGE_2, TXB_2, PGF_1, and platelets. See text for details.

first four days, but this thrombocytopenia was quickly reversed thereafter (Fig. 1). The TS white count, which was slightly decreased, began to rise by the fourth day and there was a leukocytosis in successive days. These differences in the initial four-day patterns between the fulminant ARDS and the septic non-ARDS patients, both compared to TR, suggest that a different process of activation of the eicosanoid and superoxide pathways might be occurring in ARDS. Therefore, it was believed to be advisable to carefully examine the ARDS patients with regard to the interrelationships between these physiologic mediators during the first four days after injury to understand the early evolution of the process characteristic of posttraumatic, nonseptic, fulminant ARDS.

Time Sequence Interrelationships in Fulminant Posttraumatic ARDS

Figure 3 shows the details of the temporal series sequence of changes in the levels of leucocyte superoxide production measured as the peak of the integral of the chemiluminescence response of activated patient cells in patient serum (PC-PS1), compared to the changes in the level of plasma elastase, the white cell count (WBC), and the platelet count during the first four days in the evolution of the ARDS patients. It can be seen that in the admission (A) sample there was already an increased level of elastase, even though neither the platelet count nor the white cell count have fallen from their immediate post-traumatic increased levels. After resuscitation (R) there was a further spike in elastase with an initial fall in both platelets and white cell count and these reached their nadir by 7:00 AM on the morning of the first day, at the time at which the first leukocyte superoxide determination was made. During the initial four-day period, the elastase continued to remain high, with fairly regular spikes at approximately 23:00 hours each day. The platelet count remained low and progressively declined and the white cell count, although fluctuating somewhat, remained low. There was a progressive increase in activated leukocyte superoxide production (PC-PS1), reaching its maximum at 7:00 AM on day 4.

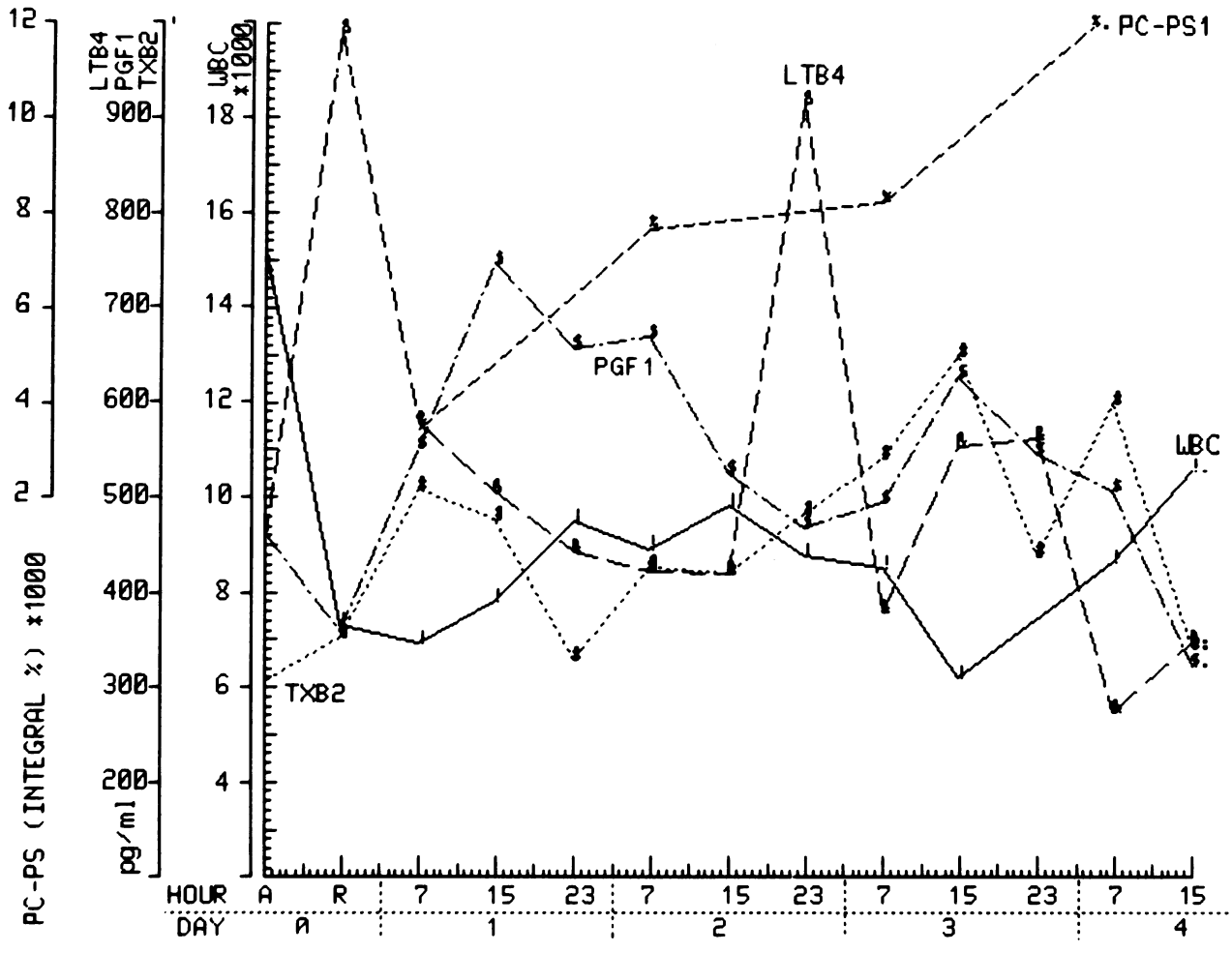


FIG. 6. Time sequence of data means from ARDS patients for first four days after injury. Shown are leukocyte chemiluminescence (PC-PS1), LTB_4, TXB_2, PGF_1, and white blood cell count (WBC). See text for details.

Figure 4 shows the relationship of the mixed venous prostaglandins PGE_2 and PGF_2 to the fall in platelets, as well as their relation to the increased superoxide production by the leukocytes (PC-PS1). As can be seen, the initial decline in the platelets was accompanied by a marked spike of PGE_2 during the resuscitation period. PGF_2 initially did not change, although all values were elevated above normal. The fall in PGE_2, once having been activated during injury and resuscitation, in general paralleled the decline in platelets with the mean rises preceding slightly in time the mean falls in platelet count during the remaining four days. PGF_2, however, showed a marked transition in levels, with a sharp increase occurring shortly after the initial decline of platelets and rising to approximately twice the initial levels beginning at 7:00 AM on day 2 and continuing for the next three days. This period of increased activity in PGF_2 was associated with a progressive rise in PC-PS1, but the two were not shown to be significantly correlated, although they were both simultaneously significant (Fig. 2A).

Figure 5 shows the relationship of the PGE_2 and platelet alterations to the production of thromboxane (measured by TXB_2) and the stable metabolite of PGIα, 6-keto-α-PGF_1 (PGF_1). The rise in thromboxane follows the spike in level of PGE_2 by approximately eight hours and remains increased over a 16-hour period; in a similar fashion the rise in PGF_1 can also be shown to follow the PGE_2 spike and is inversely related to successive falls in platelets over an 8- to 16-hour period. Both of these events preceded the rise in superoxide production from the initial level at 7:00 AM on day 1, to the rise on day 2 at 7:00 AM. The maximum increase in PC-PS1, which occurred at 7:00 AM on day 4, was again preceded by similar increases in thromboxane and PGF_1 and by a smaller secondary spike in PGE_2, which was accompanied by a small further decrease in platelets. These interrelationships will be discussed below.

The white cell to eicosanoid relations are shown in Figure 6, which demonstrates the interrelationships between LTB_4, white cell count (WBC), PC-PS1, thromboxane

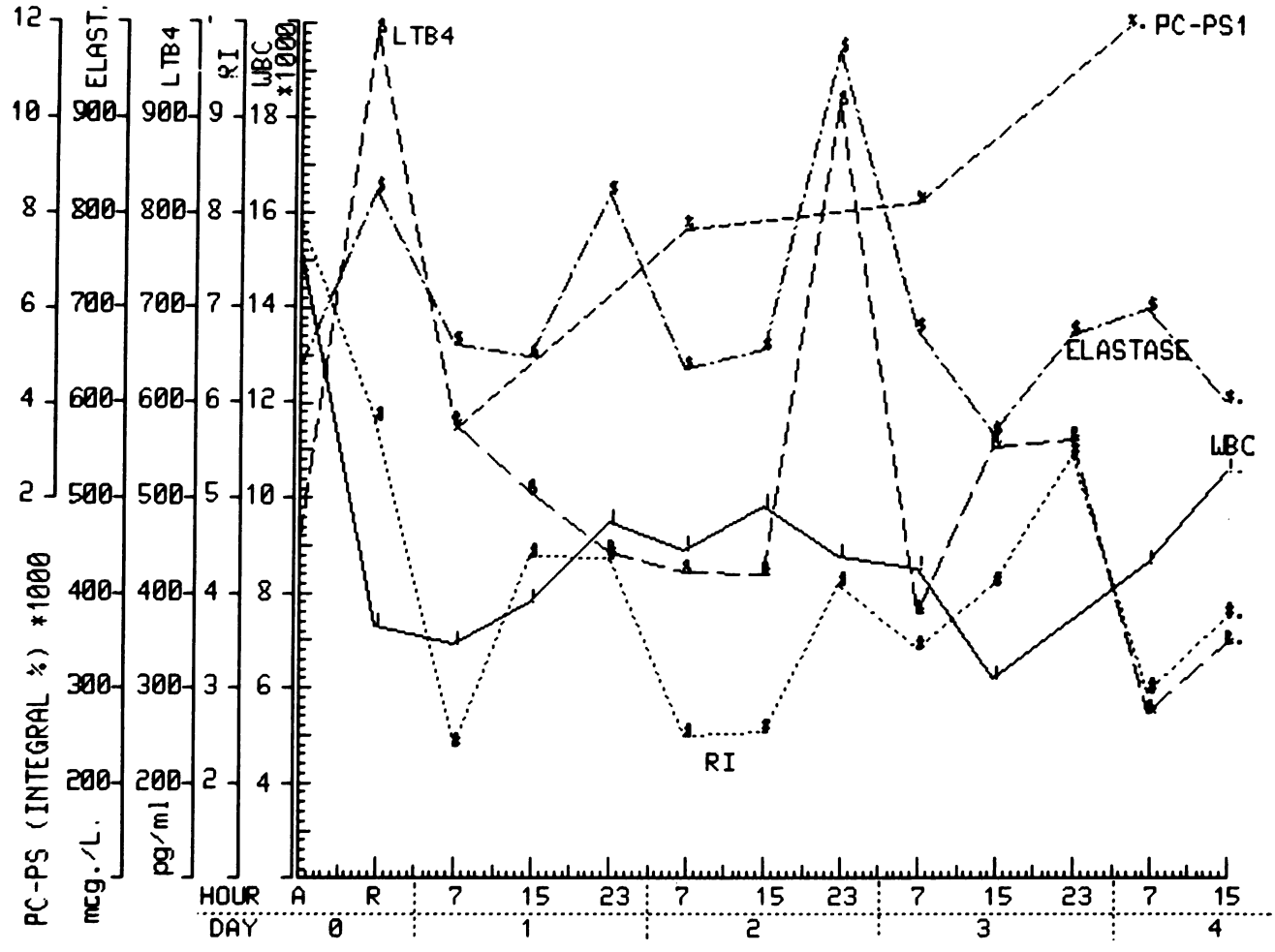


FIG. 7. Time sequence of data means for ARDS patients for first four days after injury. Shown are leukocyte chemiluminescence (PC-PS1), elastase, white blood cell count (WBC), and respiratory index (RI). See text for details.

(TXB_2), and PGF_1. Before the rise in PC-PS1, it can be seen that as with PGE_2, the LTB_4 spike preceded the rise in thromboxane and PGF_1 by 8 to 16 hours both in terms of the primary response, as well as in the secondary LTB_4 spike response, and also preceded by 8 to 16 hours the fall in white cell count. This suggests that in ARDS, leukotriene generation may play an important role in both sequestration and activation of the leukocytes to increased superoxide production.

Finally, Figure 7 shows the interrelationship between LTB_4, elastase, white cell count, PC-PS1, and the level of the respiratory index (RI) that has been shown to reflect the severity of the physiologic derangement in ARDS.[29] The previously delineated relationships between LTB_4 and the white cell count are shown here also to be related, at least in part, to spikes in the plasma elastase. With the exception of the initial admission (A) point on the RI function, which is elevated largely due to the high incidence of contusive atelectasis and pneumothorax on admission, the postinjury, postresuscitation rises in RI are seen to follow the declines in white cell count and to generally occur after the spikes in mixed venous LTB_4. They also appear to be roughly synchronous with the rises in plasma elastase. These findings suggest that the prostanoid elaboration and leukocyte activation interact with the leukocyte degranulation to produce the final alterations in membrane permeability, which is reflected in the increase in the alveolar-arterial gradient to PaO_2 ratio that forms the basis of the RI measurement.

Statistical Correlations as a Guide to Mechanisms of Prostanoid, Superoxide, and Formed Element Interactions in ARDS

Table 2 shows the relationships of significance between the various prostanoids and formed blood elements (WBC, PLAT), as well as the superoxide and respiratory index relationships characteristic of patients with fulminant post-traumatic ARDS. In Table 2 the dependent variable is shown under the Y term, with the independent

TABLE 2. *Eicosanoid, Platelet, WBC, and Superoxide Production Correlations in ARDS Patients*

GRP	Depend	Indepent					Scheffé		Select	Note
	Y =	X	N	r^2	F	p(Reg)	p(B1)	p(Bo)	Days	
A	PGE_2	PLAT	48	0.184	10.4	0.005	0.01	0.0001	$T \leq 4$	
B	PGE_2	LTB_4-24	54	0.257	18.0	0.0001	0.001	0.0001	$T \leq 4$	
	PGE_2	$\underline{LTB_4\text{-}16}$	62	0.291	24.6	0.0001	0.0001	0.0001	$T \leq 4$	
	PGE_2	$\sqrt{LTB_4\text{-}8}$	66	0.110	7.9	0.01	0.02	NS	$T \leq 4$	
	PGE_2	LTB_4	84	0.366	47.4	0.0001	0.0001	0.0001	$T \leq 4$	
C	LTB_4	PGE_2-24	53	0.289	20.7	0.0001	0.0005	0.01	$T \leq 4$	
	LTB_4	PGE_2-16	61	0.221	16.8	0.0002	0.001	0.002	$T \leq 4$	
D	WBC	$-LN(LTB_4\text{-}24)$	82	0.150	14.1	0.0005	0.001	0.0001	$T \leq 8$	
	WBC	$-LN(LTB_4\text{-}16)$	89	0.225	25.2	0.0001	0.0001	0.0001	$T \leq 8$	
	WBC	$-LN(LTB_4\text{-}8)$	89	0.194	21.0	0.0001	0.0001	0.0001	$T \leq 8$	
	WBC	$-LN(LTB_4)$	94	0.148	16.0	0.0002	0.005	0.0001	$T \leq 8$	
E	PC-PS1	$-LN(WBC\text{-}24)$	20	0.226	5.3	0.05	NS	NS	$T \leq 4$	
F	TXB_2	LTB_4-8	66	0.111	8.0	0.01	0.05	0.0001	$T \leq 4$	
G	$-LN(TXB_2)$	WBC	95	0.157	17.3	0.0001	0.001	0.0001	$T \leq 8$	
H	TXB_2	PLAT-16	92	0.166	18.0	0.0001	0.0001	NS	$T \leq 8$	
	TXB_2	PLAT-8	87	0.285	33.8	0.0001	0.001	0.05	$T \leq 8$	
	TXB_2	$PLAT^2$	87	0.121	11.7	0.002	0.005	0.0001	$T \leq 8$	
I	PLAT	TXB_2-24	73	0.138	11.4	0.002	0.01	0.0001	$T \leq 8$	
	PLAT	TXB_2-16	79	0.195	18.6	0.0001	0.001	0.0001	$T \leq 8$	
J	$-LN(PGF_1)$	PLAT	51	0.336	24.7	0.0001	0.0001	0.0001	$T \leq 4$	
K	PGF_1	$LN(TXB_2)$	83	0.099	8.9	0.005	0.02	NS	$T \leq 4$	
L	RI	$-(WBC\text{-}16)$	31	0.264	10.4	0.005	0.02	0.0001	$T \leq 4$	
	RI	$-(WBC\text{-}8)$	39	0.373	22.0	0.0001	0.001	0.0001	$T \leq 4$	
	RI	$-(WBC)$	51	0.216	13.5	0.001	0.005	0.0001	$T \leq 4$	

NS, not significant.

variable and its linear or log relationship, as well as the sign of that relationship under the X term. The number of observations, N, used to make the determination and the overall r^2 of the relationship are shown together with the F ratio. A p value for the entire regression is shown as well as the simultaneous comparison of all contrasts developed by the Scheffé methodology for the intercept (B_0) and the β-coefficient of the independent variable term (B_1). In addition, the selection period is shown indicating the number of days over which the observations for this particular regression were done. These were generally studied for the initial 4 days only, but in a few instances the relationship was strong enough to be carried out for the full eight-day period as a means of reinforcing it. The regressions are divided into groups A through L that show the correlations of individual independent–dependent variable pairs, primarily to examine the time relationships in which a given dependent variable is modified by a deterministic temporal relation to the independent variable, Eg: WBC = -LN(LTB_4-8). This means that the influence of the negative log (-LN) of the level of the independent variable (LTB_4) eight hours before (-8) the determination of the dependent variable, in this case the white count (WBC), is examined. If no minus value is shown after the independent variable name, it means that the relationship being examined is the one with no deterministic time delay (zero time) between the independent and dependent variables.

From the observations shown in group A it can be seen that there is a strong relationship between platelet count (PLAT) and the level of PGE_2, which is highly significant even though the variability explained (r^2) is not great. Similarly in group B, there is a strong relationship over time between PGE_2 and LTB_4 from minus 24 hours through 0 time. Interestingly, this particular relationship is even strong from minus 16 to minus 24 hours when the dependent and independent variables are reversed in group C. This suggests that there is a continuous interaction between the factors elaborating PGE_2 and the elaboration of LTB_4, perhaps through the medium of platelet activating factor (lyso PAF-PAF) released concomitantly with the platelet formation of PGE_2 and related to the white cell elaboration of LTB_4 (see Discussion). In group D one can see that there is an inverse relationship (negative log) between LTB_4 and the change in white count (WBC), showing that a rise in LTB_4 is associated with a fall in WBC over the subsequent full 24-hour period (from minus 24 hours to zero hours), indicating a broad interaction between these two variables with the LTB_4 being deterministic with regard to white cell aggregation. Similarly in E the fall in white cell log concentration level in the plasma tends to predict by 24 hours a subsequent rise in PC-PS1, suggesting a prolonged period of WBC activation in response to the previously described, long time-constant, LTB_4 to leukocyte relationship. In F the LTB_4 increase is seen to precede the rise in TXB_2 by eight hours,

whereas in G the white cell fall is seen to be directly related to the TBX_2 rise (shown by the negative log function), again reflecting through the medium of the white cell, the driving factor of the leukotriene stimulation.

In H, the platelet to thromboxane relations are explored and the change in platelets is shown to predict the rise in thromboxane over a prolonged period of time ranging from immediate (zero hour) to 16 hours before the event, which suggests that a significant aspect of the thromboxane component is also mediated by a decline in platelets with their sequestration and subsequent activation. There is also a similar relationship between thromboxane and platelets in group I when these are reversed for the minus 16- and minus 24-hour periods, suggesting that there is a interaction between the thromboxane already released and further reductions in the platelet count, so that the platelet aggregation effect is multiplied. In J and K, the relationships of platelets and thromboxane are examined with regard to their effect on PGF_1, the PLAT fall is related to the log of the PGF_1 rise, but the TXB_2 has a direct relationship to PGF_1 levels. These data are consistent with the fact that platelet aggregation and thromboxane release have been suggested to induce prostacycline release by endothelial cells, measured indirectly as PGF_1. Finally in L, the relationship of the white cell changes to the respiratory index are explored over a 16-hour period. As is shown, there is a broad time relationship over which the fall in WBC is significantly related to the subsequent rise in respiratory index, suggesting that the white cell aggregation, degranulation, and liberation of superoxides are indeed the process mediators that affect the permeability changes reflected in the rise in respiratory index.

As is shown in Table 2, all of these relationships are significant. In all but one (PC-PS1 = -LN(WBC-24), the Scheffé evaluation of the β coefficient and intercept were also significant, and in this correlation the relationship was limited by the number of observations because the superoxide chemiluminescence measures (PC-PS1) were only made once daily, whereas the other measurements were made on a three-times daily basis. These data strongly suggest that there is a sequence of interaction between the prostanoids and the formed elements of white cells and platelets that amplify and enhance each other's action to finally result in activation of leukocytes with increased superoxide production. This, in turn, may, in conjunction with the high levels of elastase released by the leukotriene-stimulated white cells, ultimately damage the capillary exchange surface so that impairment of respiratory gas exchange occurs. The RI increase is also seen to be significantly related to the increase in pulmonary shunt and a reduction in total static compliance as shown in Figure 2A. Thus it can be inferred that the sequence and interaction between these mediators and the time delay between the initial leukocyte activation and their amplified activation represent the key factors in the induction of the fulminant ARDS syndrome.

Discussion

The mechanisms of interaction that may be influential in the production of the fulminant post-trauma ARDS syndrome are suggested by the sequence of elaboration of the lipoxygenase and cyclooxygenase prostaglandins, their relationship to the presence of plasma elastase, to the decreasing platelet and white cell counts, and to the subsequent formation of activated neutrophils capable of producing superoxides (chemiluminescence) in response to opsonized zymosan stimulation. These are outlined in the time relationship diagrams shown in Figures 8 and 9, created from the significant regression relations shown in Table 2.

In Figure 8 and Table 2, the earliest response pattern following resuscitation from major injury producing shock, in association with tissue trauma, in those patients who developed the fulminant ARDS syndrome was characterized by a large spike in the plasma concentrations of prostaglandin PGE_2 and LTB_4, which occurred immediately after resuscitation (R) with volume expanders. The relationship between a given level of PGE_2 and of LTB_4 was found to be highly significant (Table 2) over a 24-hour period, regardless of which variable was made time dependent, which suggests a strong interaction in the peripheral tissue elaboration of these two eicosanoids coming from both cyclooxygenase and lipoxygenase pathways. As shown in the correlation diagram (Figs. 4 and 8), beginning with resuscitation (R) the fall in PGE_2 was significantly correlated ($p < 0.005$) with the PLAT falls. This (PLAT) was in turn correlated ($p < 0.0001$) with the subsequent rise in TXB_2 over an 8- to 16-hour period. The TXB_2 changes are directly ($p < 0.005$) related, and the PLAT falls are inversely ($p < 0.0001$) related to the rise in PGF_1, suggesting a cascade-type response with the platelet adherence and activation liberating TXB_2 and this activating PGF_1 release.

The initial elaboration of LTB_4 prostaglandin was associated with a similar spike in plasma elastase and with the initiation of the fall in both the platelet count and in the white cell count. However the increase in the plasma concentrations of LTB_4 preceded by eight hours the initial nadir of the WBC count (Fig. 9). Indeed, the statistical relationship between the fall (or rise) in WBC and the rise (or fall) in LTB_4 in the preceding 24-hour period was shown to be highly significant ($p < 0.0005$ to $p < 0.0001$). This suggests that the synthesis of LTB_4 may play a deterministic role in the white cell aggregation and sequestration in ARDS. As can also be seen from Figure 9 (and Fig. 6), the peak rises (and falls) in thromboxane TXB_2 in the pulmonary venous blood follow the leukotriene spikes (and troughs) by approximately eight hours, and

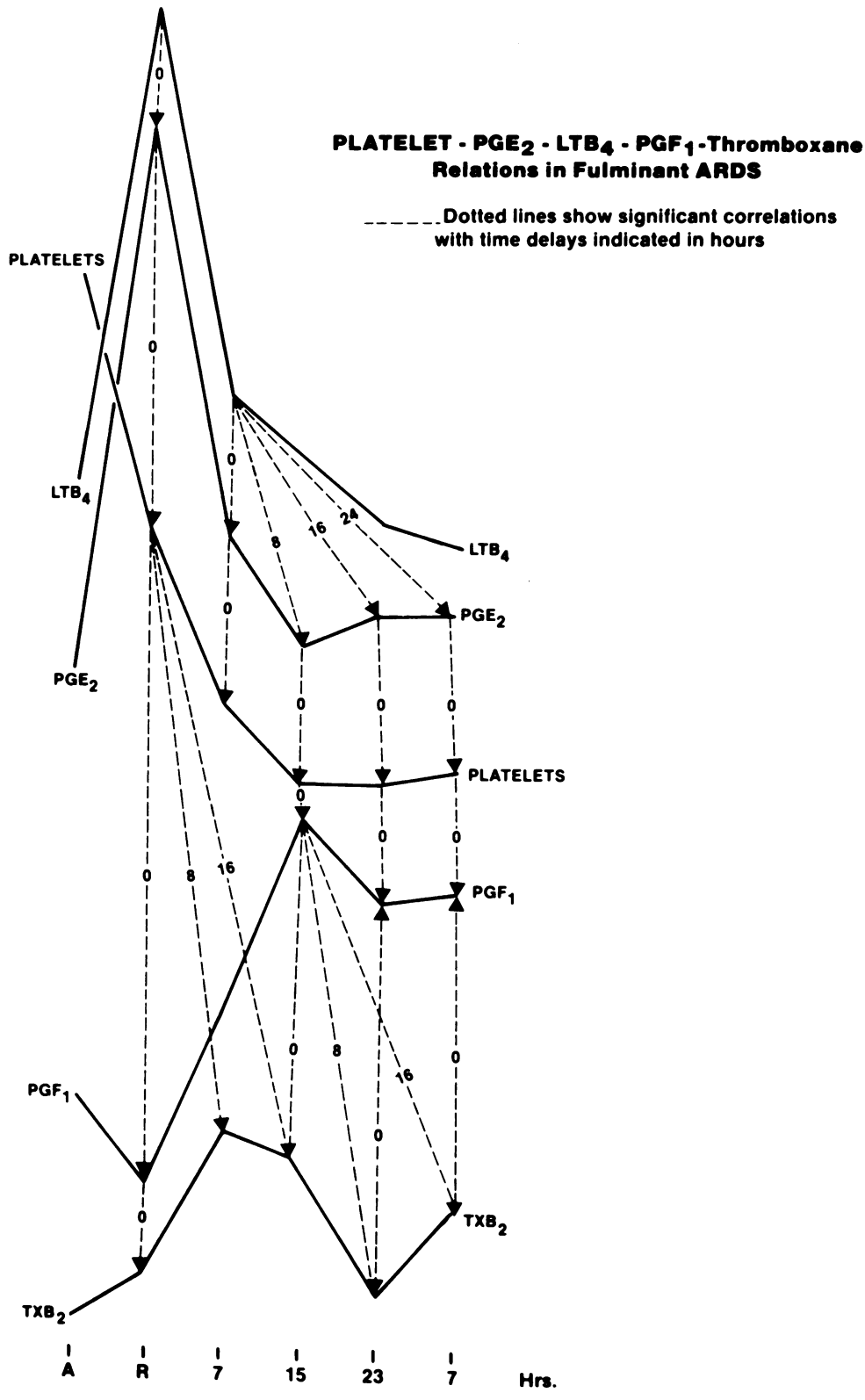


FIG. 8. Schematic of significant correlations between platelets, PGE_2, LTB_4, PGF_1, and TXB_2 in patients with fulminant ARDS. Taken from statistical probability data in Table 2. See text for details.

this cascade relationship was also shown to be significant ($p < 0.01$), as was the inverse time-zero relation between WBC and TXB_2 ($p < 0.0001$), so that as the LTB_4 reduced the WBC, the TXB_2 rose.

Moreover the LTB_4-mediated fall in WBC was correlated with a subsequent rise in superoxide production 24 hours later ($p < 0.05$) as the leukocytes become activated (evidenced by the increased chemiluminescence, PC-PS1).

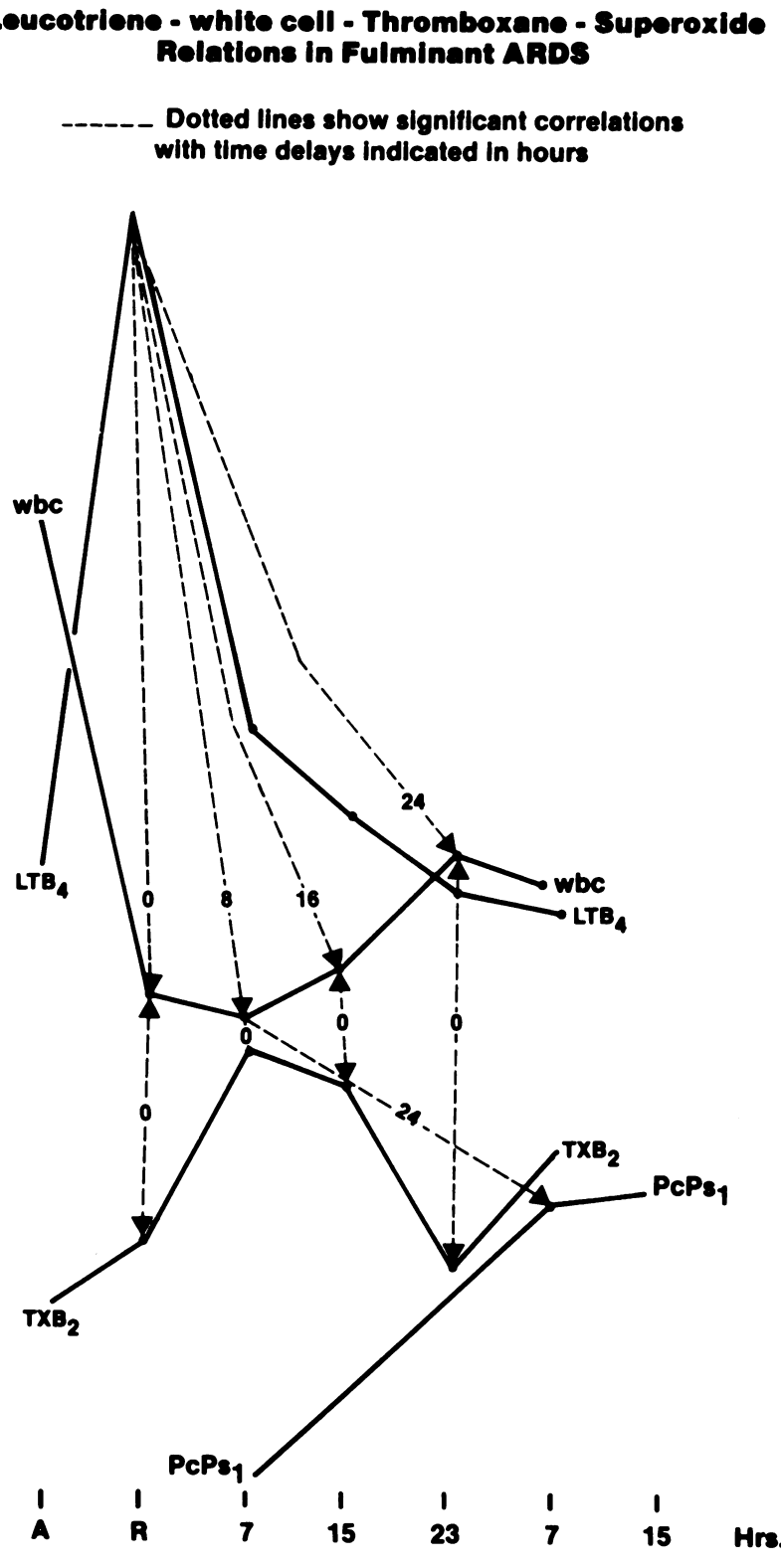


FIG. 9. Schematic of significant correlations between leukocytes, LTB_4, TXB_2, and leukocyte superoxide production (PC-PS1) measured by chemiluminescence. Taken from statistical probability data in Table 2. See text for details.

In the ARDS patients the immediate postresuscitation (R) spike in LTB_4 was followed by a somewhat smaller peak of LTB_4 occurring between the 15:00 hours and 23:00 hours sample on day 2. This LTB_4 rise also preceded a subsequent spike in TXB_2 and the markedly increased rise in superoxide production (PC-PS1) occurring at the 7:00 AM sample on day 4 also shows the same time pattern of activation. Because the sequence of activation and

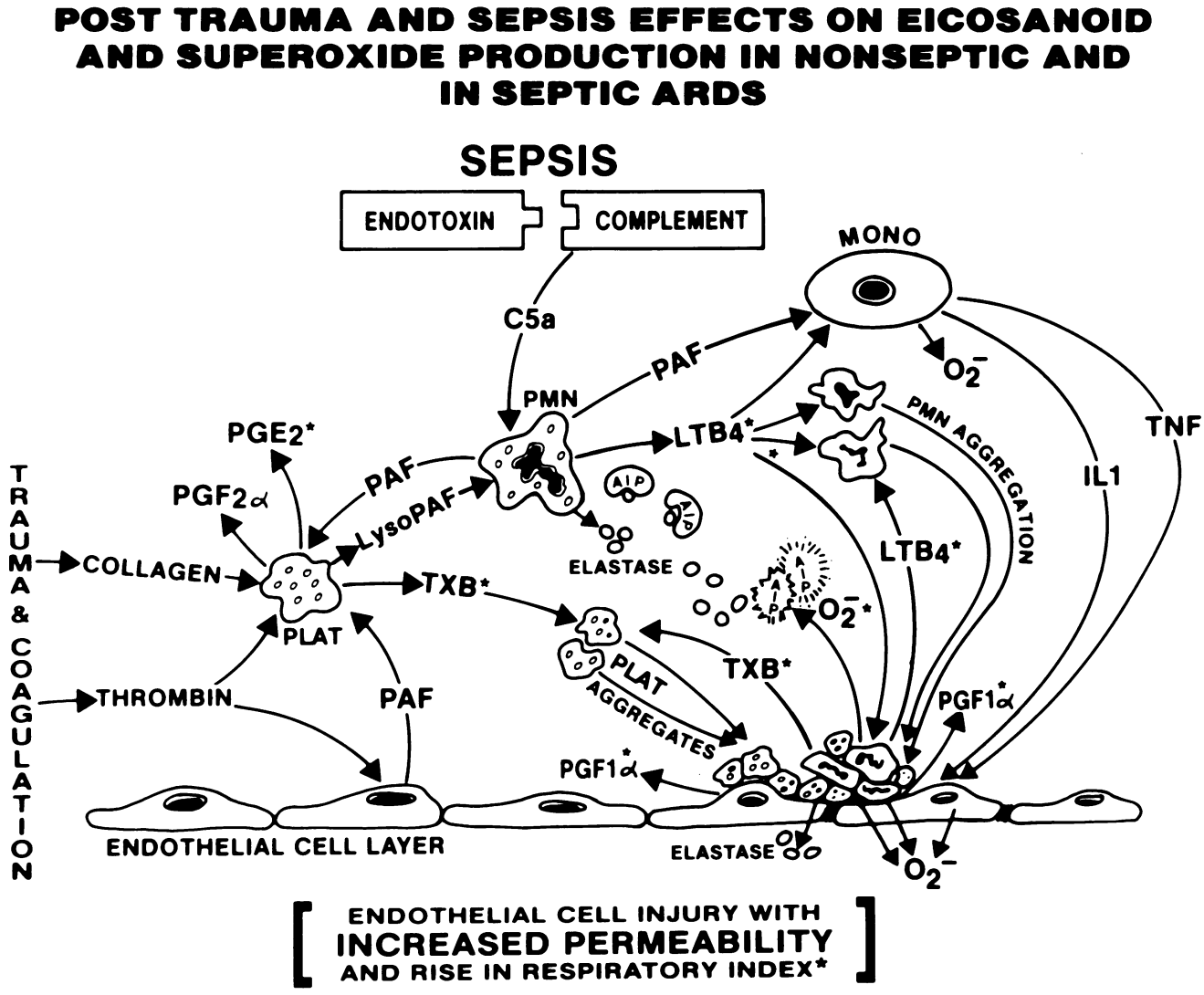


FIG. 10. Diagram of proposed post-traumatic effects on eicosanoid, elastase, and leukocyte superoxide production in ARDS. Comparison of nonseptic trauma activation with possible septic activation. Legend: PAF, platelet-activating factor; PMN, polymorphonuclear neutrophil; MONO, monocyte; AIP, α_1 antiproteinase; O_2-, superoxide; IL1, interleukin 1; TNF, tumor necrosis factor; and TXB, thromboxane. Remaining abbreviations are in text. Asterisks show significant relations seen in this study. See Discussion for details.

of sequestration of both platelets and WBCs are correlated with subsequent TXB_2 and PGF_1 rises, it is also important to note that, in general, the spikes in LTB_4 and in PGF_1 taken together correspond to the peaks in elastase seen in the plasma. It also would appear important that the marked increases in respiratory index, reflecting the level of pulmonary dysfunction,[29] tend to follow the LTB_4 spikes by 16 to 24 hours and are significantly, but inversely, correlated over a 16-hour period with the changes in WBC levels ($p < 0.005$ to $p < 0.0001$). This appears consistent with the progressive significant increase in elevation of leukocyte chemiluminescence seen in those patients on days 2, 3, and 4 (Fig. 2A). While the explained variability between the LTB_4 levels, the subsequent fall in WBC levels, and the rise in TXB_2 explain only a small portion of the total variability, as does the relationship of TXB_2 to the alterations in platelet levels; nevertheless, they are all highly significant. The interacting pattern of these responses appears consistent with the hypothesis elaborated in Figure 10 based on our data and careful examination of the literature.

Faist and colleagues[30] present considerable evidence that injured tissue releases large quantities of PGE_2. These PGE_2 levels were elevated not only in the immediate admission sample taken from the ARDS patients, but there was also a marked spike on resuscitation as tissue by-products presumably are washed from the areas of low flow ischemia and contusion related to the soft-tissue trauma. In addition, the studies of Smith, Ingram, Kocsis, and Silver[31] and others[32] have shown that the prostaglan-

dins PGE_2 and PGF_2 are also formed and released from activated platelets by the action of thrombin, especially in a collagen-enriched suspension (all compatible with tissue trauma). In the ARDS patients the spikes in both of these prostaglandins also occur during the immediate postresuscitation phase that corresponds with the initial fall in platelets, which is consistent with the platelet-aggregation response demonstrated to occur under these circumstances by Zucker and Borelli.[33]

The known action of thrombin-activated platelets on leukotriene production in damaged tissues, demonstrated by DelMaschio et al.[34] and by MacLouf[35] in response to platelet-derived 12-HPETE, is also consistent with the initial LTB_4 spike on resuscitation. This is also compatible with the initial resuscitation spike in plasma elastase because, as reported by DelMaschio,[34] the amplification of leukocyte degranulation by activated platelets can occur independently of arachidonate metabolism. This suggested to these workers that these two white cell activation processes can proceed through separate mechanisms and certainly may be separated in time. This observation is also consistent with the studies reported by Braquet[36] that suggest that platelet-leukocyte cooperation occurs due to the donation of lyso-PAF from activated platelets to leukocytes to produce PAF, which in turn initiates further platelet aggregation and induces leukocyte release of LTB_4 with further leukocyte recruitment, activation, and later formation of superoxides and other arachidonate metabolites. This secondary activation of the cyclooxygenase pathway would also be consistent with the release of thromboxane (TXA_2) from the leukocytes with further TXA_2 (measured by TXB_2)-mediated platelet aggregation and adherence to lung capillary endothelium.

Also, the initial circulating leukotriene response tends to induce chemotaxis and aggregation of white cells, and their adherence follows in time the platelet adherence to the capillary endothelium.[37] The leukotriene elaboration would be expected to induce secondary activation of the adhered leukocytes with superoxide production and further release of leukocyte granular elastase,[38,39] and this is consistent with the continued rise in superoxide production, even as the plasma elastase remains high or declines somewhat during the subsequent four days after injury. It is also consistent with the persistently maintained thrombocytopenia and leukopenia, which occurs in these patients during this period of time.

In addition other mechanisms not investigated here may also play an important role in the induction of various forms of ARDS. It has been independently demonstrated that endotoxin-mediated complement activation can occur through the release of C_{5a}-activated leukocytes with induction of cyclooxygenase and lipoxygenase pathways, so that LTB_4, as well as thromboxane and PGF_1 production, will be induced.[40,41] This mechanism may play a role in the postinjured patient, in whom circulating endotoxins are present, but is more likely to play the major role in the evolution of the septic process. This is suggested by Figure 2B, which shows that in septic patients there is a persistent LTB_4 increase that is highly significant by the first postinjury day and is maintained at elevated levels through the remaining four days of the preseptic period, declining as the secondary septic manifestations of leukocytosis occur. Because in septic patients the rise in superoxide production appears to occur later than that seen in the patients with a fulminant nonseptic ARDS, this may suggest that there may be different mechanisms of activation of leukotrienes in the two conditions and, moreover, the persistent thrombocytopenia found in the ARDS patients did not occur in the trauma patients who became septic without ARDS (Fig. 1). This may imply a greater role for platelet–leukocyte interactions in ARDS than in sepsis.

Finally, as is suggested in Figure 10, leukocyte activation of monocytes (possibly through the mediation of leukocytes stimulated by PAF[36] or by direct action of leukotrienes[40]) may result in the secondary induction of monocyte cytokines such as interleukin-I (IL1) or tumor necrosis factor (TNF). TNF has been implicated in the permeability changes associated with ARDS.[42] Because this is a secondary rather than primary response, it suggests that although not measured in our study, this summation response may be in part related to the progressive rise in white cell chemiluminescence and may be important in the maintenance of the progressive ARDS process in those patients with fulminant disease because, as demonstrated earlier, this group of patients had persistent ARDS resulting in a 86% mortality rate.

The relationship between the time sequence of these observations is consistent with the pulmonary leukosequestration noted by Anner et al.[43] and by Dunham, Shepro, and Hechtman[44] with regard to the sequence of leukotriene induction of TXB_2 from capillary endothelium and the relationship of the activation of these eicosanoids to the accumulation of neutrophils in the pulmonary capillary bed. Anner et al.[43] showed that inhibition of thromboxane synthesis limited the neutrophil accumulation of the lungs. However, LTB_4 has been shown by Hoover et al.[24] to have a direct action on the pulmonary endothelium and has been implicated by Dahlen et al.[45] and others[46] to promote plasma leakage from capillaries and postcapillary venules.

The progressive rise in superoxide production in the fulminant ARDS patients (which is preceded by rises in PGF_2, LTB_4, thromboxane, and PGF_1 occurring between days 2 and 3 after injury) also suggests an amplifying effect of the initial response because this is associated with a persistent thrombocytopenia, as well as by a marked rise in PGF_2 (Fig. 4), which may be related to continuing

platelet activation. These data suggest, although they do not prove, that the platelet activation, perhaps through release of lyso-PAF, may further stimulate the leukocyte production of leukotrienes and superoxides to continue the ARDS process. In addition, as suggested by Wiess[39] in his review of tissue injury by neutrophils, the critical determinant related to tissue destruction may be the magnitude of the zone of oxidation of the α-1 proteinase inhibitors, which allows large amounts of neutrophil degranulated elastase to attack injured basement membranes. A variety of studies by Wiess and his colleagues,[39] by Bruch and Bieth,[47] as well as by Kramps, Van Twisk, and Dijkman,[48] and by Campbell and Campbell[49] have suggested that in the presence of excessive neutrophil superoxide production, with inactivation of these antiproteinases (AIP), there is synergistic damage to the endothelial cells and basement membrane. Their studies demonstrate that the combined action of activated oxygen and elastase allows the activated neutrophil to exert its maximal destructive effect when adherent to the endothelium, as occurs in these ARDS patients with maximum pulmonary leukocyte sequestration. This is shown diagramatically in Figure 10.

Finally these studies suggest that there may be a considerable time delay between the initial platelet activation and leukocyte sequestration in response to the peripheral tissue-released eicosanoids and the full activation of the sequestered neutrophils to produce excessive amounts of superoxides with progressive pulmonary damage. This is evidenced in the progressive cyclic increases in respiratory index and the increase in the RI/(QS/QT) ratio, which has been shown by Laghi et al.[29] to be prognostic of death from ARDS. These data suggest that effective administration of a combination of inhibitors of the platelet-leukocyte amplification process (PAF), the lipoxygenase pathway of leukotriene formation, and possibly of thromboxane synthesis may be effective in preventing or ameliorating the clinical ARDS syndrome and its subsequent mortality in these nonseptic patients with early fulminant ARDS, if administered within the initial 24- to 48-hour period following injury.

This hypothesis also is consistent with the studies of the relationship of thromboxane to prostacycline ($PGF_1\alpha$) release in the adult respiratory distress syndrome by Deby-Dupont and colleagues.[50] They noted that not only was there a rise in TXB_2 in the patients with ARDS, but also that there was an imbalance of the TBX_2-to-$PGF_1\alpha$ ratio, even though both prostanoids were increased. A similar relationship was found in our data, but only after the third postinjury day at the time that the superoxide production rose to its highest levels. Before that, as shown in Figure 5, the PGF_1 levels exceeded the thromboxane levels, which gradually rose as the amplification process proceeded. This phenomenon has been attributed by Deby-Dupont to an imbalance between the thromboxane synthesized by the platelets entrapped with the leukocytes and the prostacyclins synthesized in endothelial cells as a protective mechanism to inhibit platelet aggregation and to induce vasodilatation to counteract the vasoconstrictor effect of thromboxane. The significance of this interrelationship, if correctly stated in our data, is also that a significant time period elapses before the TXB2/PGF1 ratio is reversed, which also provides additional support for the presence of a therapeutic window following injury, during which effective therapy could be instituted to prevent activation of pathologic levels of the eicosanoid pathways.

References

1. Brewer LA, Burbank B, Samson PC, et al. The "wet lung" in war casualties. Ann Surg 1946; 123:343–363.
2. Burford TH, Burbank B. Traumatic wet lung. J Thorac Surg 1945; 14:415–424.
3. Larsen GL, Parrish DA, Henson PM. Lung-defense: the paradox of inflammation. Chest 1983; 83(Suppl 5):15–55.
4. Babior BM. Oxidants from phagocytes: agents of defense and destruction. Blood 1984; 64:959–966.
5. Dowe JE, Short A, Sibbald WJ, Driedger AA. Pulmonary accumulation of polymorphonuclear leukocytes in the adult respiratory distress syndrome. Crit Care Med 1982; 10:712–718.
6. Zimmerman GA, Renzetti AD, Hirc HR. Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome. Am Rev Respir Dis 1983; 127:290–300.
7. Tate RM, Repine JE. Neutrophils and the adult respiratory distress syndrome. Am Rev Respir Dis 1983; 128:552–559.
8. Hallgren R, Borg T, Venge P, Modig J. Signs of neutrophil and eosinophil activation in adult respiratory distress syndrome. Crit Care Med 1984; 12:14–18.
9. Sacks T, Moldow CF, Craddock PR, et al. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. J Clin Invest 1978; 61:1161–1167.
10. Craddock PR, Fehr J, Brigham KL, et al. Complement and leukocyte mediated pulmonary dysfunction in hemodialysis. N Engl J Med 1977; 296:769–774.
11. Hammerschmidt DE, Harris PD, Wayland H, et al. Complement induced granulocyte aggregation in vivo. Am J Pathol 1981; 102: 146–150.
12. Lee CT, Fein A, Lippman M, et al. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory distress syndrome. N Engl J Med 1981; 304:192–196.
13. McGuire W, Spragg RG, Cohen AB, Cochrane CG. Studies on the pathogenesis of the adult respiratory distress syndrome. J Clin Invest 1982; 69:543–553.
14. Cochrane CG, Spragg R, Revak SD. Pathogenesis of adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 1983; 71:754–761.
15. Piper PJ, Vane JR, Wyllie JH. Inactivation of prostaglandins by the lung. Nature 1970; 225:600–604.
16. Jose P, Niederhauser U, Piper PJ, et al. Degradation of prostaglandin $F_2\alpha$ in the human pulmonary circulation. Thorax 1976; 31:713–719.
17. Bult H, Beetens J, Herman AG. Blood levels of 6-keto prostaglandin $F_1\alpha$ during endotoxin-induced hypotension in rabbits. Eur J Pharmacol 1980; 63:47–56.
18. Butler PR, Jr, Wise WC, Halushka PV, Cook JA. Thromboxane and prostacycline production in septic shock. Adv Shock Res 1982; 7:133–145.
19. Rampart M, Bult H, Herman AG. Effect of complement activation on the biosynthesis of prostacyclin PGI_2 by rabbit peritoneum in vitro. Arch Int Pharmacodyn Ther 1981; 253:327–329.

20. Halushka PV, Reines HD, Barrow SE, et al. Elevated plasma 6-keto-prostaglandin $F_1\alpha$ in patients in septic shock. Crit Care Med 1985; 13:451–453.
21. Wise WC, Cook JA, Eller T, Halushka PV. Ibuprofen improves survival from endotoxic shock in the rat. J Pharmacol Exp Ther 1980; 215:160–164.
22. Reines HD, Halushka PV, Cook JA, et al. Plasma thromboxane levels are elevated in patients dying with septic shock. Lancet 1982; ii:174–175.
23. Slotman GJ, Burchard KW, Gann DS. Thromboxane and prostacyclin in clinical acute respiratory failure. J Surg Res 1985; 39: 1–7.
24. Hoover RL, Karnovsky MJ, Austen KF, et al. Leukotriene B_4 action on endothelium mediates augmented neutrophil/endothelial adhesions. Proc Natl Acad Sci 1984; 81:2191–2193.
25. Allen RC, Loose LD. Phagocytic activation of a luminol dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem Biophys Res Commun 1976; 69:245–252.
26. Rivkind AI, Littleton M, Siegel JH, et al. Neutrophil superoxide formation and the pattern of physiologic relationships as early biologic markers of the evolution of the fulminant posttraumatic adult respiratory distress syndrome. Circ Shock 1989 (in press).
27. Neumann S, Hennrich N, Gunzer G, Lang H. Enzyme-linked immunoassay for elastase from leukocytes in human plasma. J Clin Biochem 1981; 19:232–241.
28. Neumann S, Hennrich N, Gunzer G, Lang H. Enzyme-linked immunoassay for human granulocytic elastase in complex with α_1 proteinase inhibitor. *In* WH Horl, Heidland A, eds. Proteases. New York: Plenum Publishing, 1984; 379–390.
29. Laghi F, Siegel JH, Rivkind AI, et al. The respiratory index/pulmonary shunt relationship:quantification of severity and prognosis in the posttraumatic adult respiratory distress syndrome. Critical Care Medicine 1989 (in press).
30. Faist E, Mewes A, Baker CC, et al. Prostaglandin E_2 (PGE_2)-dependent suppression of interleukin α (IL-2) production in patients with major trauma. J Trauma 1987; 27:837–847.
31. Smith JB, Ingerman C, Kocsis JJ, Silver MJ. Formation of prostaglandins during the aggregation of human blood platelets. J Clin Invest 1973; 52:965–969.
32. Hamberg M, Samuelsson B. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. Proc Nat Acad Sci 1974; 71:3400–3404.
33. Zucker MB, Borrelli J. Platelet clumping produced by connective tissue suspensions and by collagen. Proc Soc Exp Biol Med 1962; 109:779.
34. DelMaschio A, MacLouf J, Grovazier E, et al. Activated platelets stimulate human neutrophils functions. Nouv Rev Fr Hematol 1985; 27:275–278.
35. MacLouf J, Fruteau de Laclos B, Borgeat P. Stimulation of leukotriene biosynthesis in human blood platelets by platelet derived 12-hydroperoxy-icosatetraenoic acid. Proc Nat Acad Sci 1982; 79:6042–6046.
36. Braquet P, Touqui L, Shen TY, Vargaftig BB. Perspectives in platelet-activating factor research. Pharmacologic Reviews 1987; 39:97–145.
37. Schlag G, Redl H. Morphology of the posttraumatic human lung after traumatic injury. *In* Zapol WM, Falke E, eds. Pathophysiology and Therapy of Severe Acute Lung Disease. New York: Dekker, 1985. pp. 161–183.
38. Garcia JGN, Noonan TC, Jubiz W, Malek AB. Leukotrienes and the pulmonary microcirculation. Am Rev Resp Dis 1987; 136: 161–169.
39. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320:365–376.
40. Cybulsky MI, Chan MK, Movat HZ. Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, and tumor necrosis factor and their implication in gram-negative infection. Lab Invest 1988; 58:365–378.
41. Demling RH. The role of mediators in human ARDS. J Crit Care 1988; 3:56–72.
42. Stephens KE, Ishizaka A, Larrick JW, Raffin TA. Tumor necrosis factor causes increased pulmonary permeability and edema. Am Rev Respir Dis 1988; 137:1364–1370.
43. Anner H, Kaufman RP, Kobzik L, et al. Pulmonary leukosequestration induced by hind limb ischemia. Ann Surg 1988; 206: 162–167.
44. Dunham B, Shepro D, Hechtman HB. Leukotriene induction of TXB_2 in cultured bovine aortic endothelial cells. Inflammation 1984; 8:303–321.
45. Dahlen SE, Bjork J, Heaguist P, et al. Leukotrienes promote plasma leakage and leukocyte adhesion in post capillary venules: in vivo effects with relevance to the acute inflammatory response. Proc Nat Acad Sci 1981; 78:3887–3891.
46. Harlan JM. Leukocyte-endothelial interactions. Blood 1985; 65:513–525.
47. Bruch M, Bieth JG. Influence of elastin on the inhibition of leukocyte elastase by α_1-proteinase inhibitor and bronchial inhibitor: potent inhibition of elastin-bound elastase by bronchial inhibitor. Biochem J 1986; 238:269–273.
48. Kramps JA, VanTwisk C, Dijkman JH. Oxidative inactivation of bronchial antileukoprotease by triggered polymorphonuclear leukocytes. Am Rev Resp Dis 1987; 135:290 (abstract).
49. Campbell EJ, Campbell MA. Pericellular proteolysis by neutrophils in the presence of proteinase inhibitors: effects of substrate opsonization. J Cell Biol 1988; 106:667–676.
50. Deby-Dupont G, Braun M, Lamy M, et al. Thromboxane and prostacyclin release in adult respiratory distress syndrome. Intensive Care Med 1987; 13:167–174.

Discussion

DR. HERBERT B. HECHTMAN (Boston, Massachusetts): I am pleased to discuss this paper because it represents the results of a very large effort to shed light on a most perplexing subject.

Dr. Siegel's group has again shown us the advantages of careful clinical and investigative work in bringing to light many of the mediators that might well play a role in the adult respiratory distress syndrome. The overall message that we derive from these data is that severe trauma unleashes a host of inflammatory agents that target the lungs.

My first question is: are the lungs uniquely involved the first several days after trauma? Is there malfunction of other organs, and if so, do you believe that these events are also mediated by the same inflammatory agents? That there are multiple possible pathways of injury with amplification and feedback loops is anticipated and represents the marvel of the body's homeostasis.

The basic ingredients for lung injury appear to be severe tissue trauma, hypotension, and volume infusion. Do the authors concur that hemorrhagic and traumatic injury with fluid replacement are similar to ischemia and reperfusion? It is likely that in both settings oxygenation products, that is the eicosanoids and oxygen-free radicals, play key roles. Platelets may be of unique importance after vascular injury and activation of the clotting sequence while neutrophils are vital to most settings of adult respiratory distress.

Finally the recent advent of information about neutrophil endothelial adhesion proteins and the availability of monoclonal antibodies to block this adhesion may have already provoked your potential or current use of such agents. I wonder if Dr. Siegel might comment about this as a potential therapeutic strategy as well as the use of agents such as free radical and thromboxane inhibitors, for example, mannitol, an agent that might be available to us now.

I am sure the information contained in this paper will fuel a number of future studies. It has been an honor to have been asked to comment upon it.

DR. BASIL A. PRUITT, JR. (Fort Sam Houston, Texas): I, too, would like to compliment Dr. Siegel on a carefully performed clinical evaluation