Leukocyte Level as a Potential Factor Determining Hepatic Phagocytosis Following Surgery[1] (36643)

THOMAS M. SABA AND WILLIAM A. SCOVILL[2]

Departments of Physiology and Surgery, University of Illinois, College of Medicine, Chicago, Illinois 60612

Macrophage or reticuloendothelial (RE) participation in host defense has been clearly demonstrated (1). This physiological function of the reticuloendothelial system (RES) has been reported in bacterial infection (2, 3), neoplastic disease (4–6), and a variety of immunologic events (7, 8). These observations not only focus attention on RE cells as a mechanism of resistance, but clearly demonstrate the necessity to understand the biologic factors which regulate its activity in both normal and pathological states.

Several studies have documented a state of RE depression following surgery. In this regard, surgical manipulation of humans (9) and animals (10) will induce an alteration of RE phagocytic activity as demonstrated by delayed clearance from the vascular space and decreased hepatic uptake of foreign particulate matter. RE depression following surgery (9, 10) appears to be mediated by a decrease in circulating plasma opsonic activity (11) which serves as a physiologic regulator for intravascular phagocytosis. This relationship between opsonic activity and RE function has been emphasized as a potential factor mediating the postoperative state of host resistance (11). Support for this potential functional relationship between opsonins and resistance is the observation that colloid induced "RE blockade," which is due to a depletion of opsonin (12), will decrease host resistance (1, 13). Furthermore, a depletion of serum opsonic activity exists in humans with neoplastic disease (14, 15), an abnormal state in which the defense mechanism is definitely altered.

While correlations between opsonin and RE changes after surgery have been made, the basis for the postsurgery depression in the opsonic system has not been delineated. Recent studies have demonstrated that the vascular entrance of foreign colloids will induce a significant but transient leukopenia which is temporally related to a transient depletion in the serum opsonin level (16). This response is not particle specific in that leukopenia has also been noted following intravenous injection of various foreign colloids (16, 17) and bacterial preparations (18).

Since opsonin depletion has been demonstrated during leukopenic states, and since postsurgery opsonin changes can be related to the level of hepatic phagocytosis, the following study was undertaken to delineate the relationship between the peripheral leukocyte level and the plasma opsonic and hepatic phagocytic alterations following surgery. Furthermore, since hepatic blood flow is a major factor limiting the degree of hepatic particle clearance (22), the potential involvement of hepatic blood flow changes in the hepatic clearance alterations following surgery was also evaluated.

Materials and Methods. Male Holtzman rats (250–350 g) maintained on Rockland Lab-Tek Chow and tap water *ad libitum* were used in all studies. The surgical stress employed was a midline laparotomy approximately 2.5 cm in length followed by 15–30 sec of gentle intestinal manipulation as previously described (11), and the incision was

[1] This investigation was supported by the U.S. Public Health Service Grant AM 14382-02.

[2] Resident in the Department of Surgery and a candidate for MS in Surgery at the University of Illinois College of Medicine, Chicago, IL.

[3] Please send reprints request to Dr. T. M. Saba, Department of Physiology, University of Illinois College of Medicine, Chicago, IL.

closed with 9 mm stainless steel clips. All surgical procedures were performed after shaving and scrubbing of the abdomen under light ether anesthesia, and controls were anesthetized in a similar manner.

Hepatic phagocytosis was evaluated by an isotopic colloid uptake technique in which the degree of hepatic phagocytosis following intravenous colloid injection was determined. This method is based on the ability of the RES to phagocytize circulating foreign colloids and has been used extensively to evaluate RE function (3, 20, 22). The test colloid employed was a radioiodinated lipid emulsion referred to as the gelatinized "RE test lipid emulsion" (19). This emulsion is exclusively removed from the vascular compartment by the cells of the RES, especially the hepatic Kupffer cells (12, 19) as confirmed by electron microscopy (21). The test emulsion was prepared as an anhydrous base with glycerol, ^{131}I labeled triolein (Mallinckrodt Nuclear, St. Louis) and soya lecithin in a ratio of 10:10:1 by weight, respectively. Prior to injection, 0.3% gelatin supplemented sterile 5% dextrose and water solution was added to the anhydrous lipid base to provide 10% emulsion (10–12). All emulsion preparations were incubated at 37° for 20 min prior to intravenous injection to effect gelatinization.

Hepatic Kupffer cell phagocytosis of the intravenously injected colloid was determined by taking randomly spaced liver samples 15 min following colloid injection at a dose of 50 mg/100 g body weight (11, 12). The liver uptake was calculated as the percentage of the injected dose (%ID) removed on a total organ uptake basis in both normal rats and in rats over a 4 day postsurgery interval.

Hepatic blood flow was determined by an isotopic clearance technique utilizing the "fractional clearance rate" (k) as an index of hepatic sinusoidal blood flow (22). In this technique, the test colloid having a specific activity of 0.05 μCi/mg was injected intravenously at a tracer dose of 5 mg/100 g body weight. At this tracer dose the rate limiting factor for the clearance of the colloid is liver blood flow (22). The "fractional clearance rate" (k) can be calculated from the expression; $k = 0.693/t_{1/2}$, where k represents the fraction of the blood volume which is cleared of the test colloid per minute and $t_{1/2}$ represents the half-time for the biologic disappearance of the test colloid from the vascular space. The fractional clearance rate (k) multiplied by the blood volume is a measure of hepatic sinusoidal blood flow (22). Blood volume was determined by extrapolation of the disappearance curve to zero time and applying the dilution principle. Hepatic blood flow was expressed as ml/min, ml/min/100 g body weight, and ml/min/g liver weight.

The serum opsonic activity was determined by an *in vitro* bioassay technique which has been previously described (12). Liver slices were prepared from normal rats and incubated in a medium containing 1 ml of the experimental serum, 2 ml of Krebs–Ringer phosphate buffered to pH 7.4, 100 USP units of heparin and 2000 μg of the gelatinized ^{131}I "RE test lipid emulsion" (1% emulsion; 0.1% gelatin). Incubation was carried out under a gas phase of 95% O_2 and 5% CO_2 in a Dubnoff metabolic shaker at 37° for 30 min. Following incubation, the liver tissue slices were removed, washed in cold isotonic saline, weighed and analyzed for ^{131}I colloid uptake with a Nuclear-Chicago Autogamma crystal scintillation system. Serum opsonic activity was evaluated in terms of its ability to stimulate Kupffer cell phagocytosis as previously described (12) and control levels represent levels in nonsurgically stressed anesthetized rats.

The peripheral leukocyte levels were determined using standard hematologic techniques. In this regard, timed peripheral blood aliquots obtained from the tail were diluted with 0.1 N HCl prior to cell count determinations in an AO Bright-Line hemacytometer. Leukocyte (WBC) levels were expressed as total WBC counts/mm^3 and as percentage of control (presurgery level). All data were statistically analyzed with Student's t test employing a confidence limit of 5% and correlation coefficient (r) was also determined for the relationship of the variables evaluated.

Results. As presented in Fig. 1, the hepatic uptake of the intravenously injected RE test

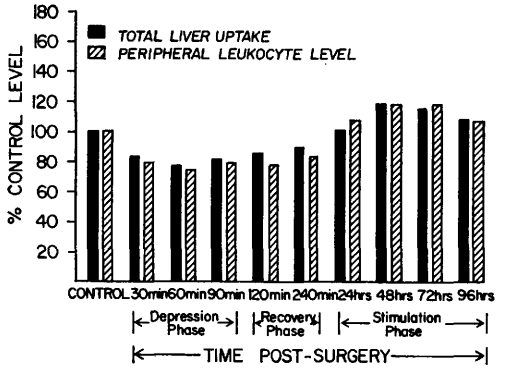

FIG. 1. Hepatic phagocytic activity and peripheral leukocyte level following surgery. Data are all expressed as the percentage (%) of presurgery control level. Total liver uptake is the percentage of the injected dose (% ID) cleared by the liver by 15 min postinjection using a standard 50 mg/100 g intravenous test dose of colloid. In absolute terms, the control liver uptake was 63.71 ± 3.68% ID as determined in 16 rats. Control leukocyte level was 17,963 ± 417 cells/mm³ as determined in 28 rats. Each postsurgery group had a total of 6 rats for liver uptake and leukocyte determinations. For all points the correlation coefficient $r = 0.9801$ ($p < .001$).

lipid emulsion was significantly depressed ($p < .05$) during the initial 3–4 hr interval following surgery with the point of maximum 22% depression at 60 min. Recovery of hepatic phagocytic activity and an apparent enhanced liver phagocytic activity existed by 24 and 48 hr. The close temporal relationship between the liver phagocytic activity and the peripheral leukocyte level over the 4 day postsurgery interval ($p < .001$) is depicted. In this regard, hepatic uptake depression (22%) was related to a leukopenic state (25% decrease) while recovery of the leukocyte level was reflected in a restoration of hepatic uptake.

Presented in Fig. 2 is the effect of surgery on the peripheral leukocyte level in relationship to the plasma opsonic activity. As compared to the control white blood cell count of 17,963 cells/mm³, the leukocyte levels at 30, 60, 90, 120, and 240 min were significantly ($p < .05$) depressed to approximately 75–80% of control. The plasma opsonic activity was also significantly ($p < .05$) depressed at the same time intervals following surgery with a 40% reduction at 60 min. The potential association between plasma opsonic activity and peripheral leukocyte level is further reflected in their close temporal relationship ($p < .01$) over the 4 day interval (Fig. 2). However, the apparent drop in opsonic activity at 72 hr was significant ($p < .05$) and consistently observed even in the presence of no leukopenia.

Evidence to support the concept that the depression in hepatic clearance capacity was not merely related to an impaired vascular perfusion of the liver is presented in Table I. In this regard, while maximum RE changes were observed at 60 min and 48 hr postsurgery, it can be seen that no significant changes were apparent in hepatic sinusoidal blood flow at 60, 180 min, and 48 hr following surgery compared to liver blood flow in the nonsurgically stressed controls (Table I) on either a total flow (ml/min) or per gram (ml/min/g) basis.

Discussion. In the present study, changes in the peripheral leukocyte level were evaluated in relationship to the serum opsonin level and hepatic clearance capacity following surgery. The findings demonstrate an association between these three parameters follow-

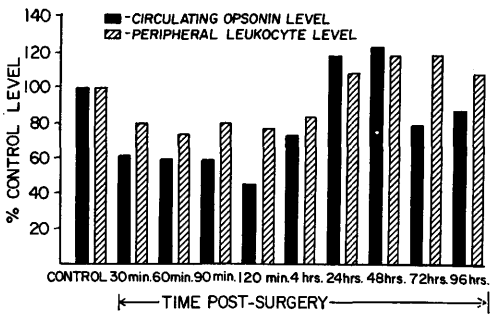

FIG. 2. Circulating opsonin and leukocyte levels following surgery. Data are expressed as the percentage (%) of presurgery control level. Control opsonin level in terms of phagocytosis was 11.44 ± 0.82 % ID/100 mg tissue as determined in 22 separate determinations. Control leukocyte level was 17,963 ± 417 cells/mm³ as determined in 28 rats. Relative to opsonin levels each postsurgery point represents 5–12 determinations. For all points the correlation coefficient $r = 0.8028$ ($p < .01$).

TABLE I. Liver Sinusoidal Blood Flow[a] During Periods of RE Depression, and RE Hyperactivity Following Laparotomy and Intestinal Manipulation.

Experimental[b] parameter	Nonsurgically stressed group (controls)	Postsurgery experimental group (hr): 1	3	48
ml/min	13.13 ±1.54	12.65 ±0.53	13.30 ±1.00	14.80 ±3.80
ml/min/g	1.14 ±0.15	1.19 ±0.07	1.23 ±0.13	1.64 ±0.49
ml/min/100 g	4.48 ±0.51	4.60 ±0.22	4.82 ±0.37	5.92 ±1.52

[a] All data are expressed as mean ± SE of the mean with 4 rats assessed at each time interval.

[b] Liver sinusoidal blood flow is presented as ml/min of total flow, ml/min/g of liver tissue, and ml/min/100 g of body weight.

ing surgery. Whether the concomitant fall in peripheral leukocyte level is temporally or causally related to the diminution in opsonic activity and RE phagocytic function can only be speculated at this time. However, recent findings clearly demonstrated the intimate role played by serum or plasma factors called opsonins or "recognition factors" in the regulation of RE cell phagocytosis (1, 12, 14, 15). Decreased circulating opsonic activity during a period of RE depression following surgery as indicated by this finding supports the concept of an opsonin deficit as a major factor mediating the postsurgery phagocytic depression (11, 23). The lack of a change in liver blood flow during the period of postsurgery hepatic clearance depression is in contrast to the findings of Donovan (9) which suggest a critical role for hepatic vascular impairment in the postsurgery RE depression observed in humans. This observation does, however, confirm previous data by Saba and DiLuzio (10) on the stability of liver blood flow following celiotomy in the rat.

Whether mobile phagocytes, *i.e.*, leukocytes, have a functional regulating influence on phagocytosis by fixed macrophages of the RES remains to be determined. Grogan (24) has noted that the administration of antilymphocyte serum resulted not only in lymphopenia but also blockade of the RES. The blockade was reversed by the administration of normal serum, but not by heated serum (24), which suggests a depletion of a thermolabile factor (1) possibly opsonin. Williams (25) noted that chronic drainage of the thoracic duct lymph resulted in leukopenia and also in depletion of serum opsonic factors as reflected in the impaired follicular localization of antigen. Blockade of the RES following colloid injection (12) which causes a profound decrease in the peripheral leukocyte level (16) also results in a concomitant reduction in plasma opsonic activity (12). During or following various bacterial infections in animals and man (3, 21, 26), in which polymorphonuclear leukocyte levels would alter, there is a marked increase in RE phagocytic activity. However, Wagner, Ilio and Hornick (26) could not relate the increased phagocytosis after infection in man to the leukocyte level.

The mechanism for the leukocyte changes following surgery have not been delineated. The release of adrenal cortical steroids into the plasma in response to tissue trauma as well as the leukopenic effect of steroids are well known. Schildt and Löw (27) measured corticosterone levels in mice subjected to trauma. In this model, corticosterone or ACTH given in amounts to stimulate posttrauma levels were found to have a significant depressing effect on the clearance capacity of the RES. These findings correlate well with the study by Wiener *et al.* (28) relative to steroid-induced RE blockade. These latter findings concluded that the effect of steroids was at the surface attachment phase of phagocytosis which would suggest a possible impairment in opsonin or oth-

er humoral factors involved in this phase of the clearance process. While a previous report (10) speculated on the potential binding of opsonic protein by adrenal steroids, experimental observations supporting this mechanism are not available.

Thus, while the mechanism mediating depression of both the peripheral leukocyte level and circulating opsonin level following surgery have not been elucidated, a significant correlation between hepatic phagocytosis, serum opsonic activity and leukocyte level has been demonstrated. In this regard, it is tempting to make an association between both leukopenia and depletion of plasma opsonic activity in the etiology of the depressed RE phagocytic activity following surgery. The significance of these findings to host resistance alterations following surgical intervention or tissue trauma remains to be clarified.

Summary. The peripheral leukocyte level and serum opsonic activity were evaluated in relationship to the hepatic Kupffer cell clearance capacity following surgery. Hepatic phagocytic clearance was significantly depressed during the early postsurgery period, with recovery and hyperfunction being apparent by 24 and 48 hr postsurgery. The level of RE function after surgery was closely related to alterations in both the circulating level of opsonins and the peripheral leukocyte level. However, a significant role for hepatic vascular alterations in the host surgery RE depression was not demonstrated. While previous studies demonstrate the importance of opsonins as a regulator of intravascular phagocytosis, the present data further suggest a potential relationship of fixed RE cell functional activity to the blood leukocyte level following surgery. The significance of these findings to altered host defense mechanisms against bacterial infection and tumor growth following surgical intervention remains to be clearly established. The evidence appears to suggest that circulating leukocytes may be an important factor in the regulation of fixed RE cell phagocytic activity mediated via the regulation of plasma opsonic activity.

1. Saba, T. M., Arch. Intern. Med. **126**, 1031 (1970).

2. Snell, J. F., Ann. N.Y. Acad. Sci. **88**, 56 (1970).

3. Biozzi, G., and Stiffel, C., *in* "Progress in Liver Diseases" (H. Popper and F. Schaffner, eds.), Vol. 3, p. 166. Grune & Stratton, New York (1970).

4. Old, L. J., Clarke, D. A., Benacerraf, B., and Goldsmith, J., Ann. N.Y. Acad. Sci. **88**, 264 (1960).

5. Stern, K., *in* "Reticuloendothelial Structure and Function" (J. H. Heller, ed.), p. 233. Ronald Press, New York (1960).

6. Old, L. J., Benacerraf, B., Clarke, D. A., and Carswell, B. A., Cancer Res. **21**, 1281 (1961).

7. Rowley, D., Experientia **22**, 1 (1966).

8. Halpern, B. N., J. Pharm. Pharmacol. **11**, 321 (1959).

9. Donovan, A. J., Arch. Surg. **94**, 247 (1967).

10. Saba, T. M., and DiLuzio, N. R., Surgery **65**, 802 (1969).

11. Saba, T. M., Proc. Soc. Exp. Biol. Med. **133**, 1132 (1970).

12. Saba, T. M., and DiLuzio, N. R., Amer. J. Physiol. **216**, 197 (1969).

13. Fisher, B., and Fisher, B. R., Surg. Clin. N. Amer. **42**, 335 (1962).

14. Pisano, J. C., DiLuzio, N. R., and Salky, N. K., J. Reticuloendothel. Soc. **7**, 386 (1970).

15. Pisano, J. C., DiLuzio, N. R., and Salky, N. K., Nature (London) **226**, 1049 (1970).

16. Goldman, J., masters thesis (Spon. Saba, T. M.), Univ. of Illinois at the Medical Center, 1970.

17. Banaszak, B. H., Kampine, J., and Smith, J. J., Amer. J. Physiol. **209**, 532 (1966).

18. Rogers, D. E., Bacteriol. Rev. **24**, 50 (1960).

19. DiLuzio, N. K., and Riggi, S. T., J. Reticuloendothel. Soc. **1**, 136 (1964).

20. Benacerraf, B., Biozzi, G., Halpern, B. N., and Stiffel, C., *in* "Physiopathology of the Reticuloendothelial System" (B. N. Halpern, ed.), p. 57. Thomas, Springfield, IL (1957).

21. Salky, N. K., DiLuzio, N. R., P'Pool, D. B., and Sutherland, A. J., J. Amer. Med. Ass. **187**, 744 (1964).

22. Saba, T. M., J. Reticuloendothel. Soc. **7**, 406 (1970).

23. Saba, T. M., Nature (London) **228**, 781 (1970).

24. Grogan, J. B., J. Reticuloendothel. Soc. **6**, 411 (1969).

25. Williams, G. M., Immunology **11**, 467 (1966).

26. Wagner, H. N., Iio, M., and Hornick, R. B., J. Clin. Invest. **42**, 427 (1963).

27. Schildt, B. B., and Löw, H., Acta Endocrinol. **67**, 141 (1971).

28. Weiner, J., Cottrell, T. S., Margaretten, W., and Spiro, D., Amer. J. Pathol. **50**, 187 (1967).

Received Jan. 28, 1972. P.S.E.B.M., 1972, Vol. 140.