Neutropenic Responses to Intradermal Injections of Escherichia coli

Effects on the Kinetics of Polymorphonuclear Leukocyte Emigration

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Killed *Escherichia coli* organisms injected intradermally into rabbits induced significant neutropenia and provoked a rapid rise in body temperature. Both the magnitude and the duration of the neutropenia were dose-dependent. After recovery from neutropenia, the rabbits became refractory to its redevelopment when subsequently given an equivalent dose of *E coli*. The influence of neutropenia and the subsequent refractory period on the rate of polymorphonuclear leukocyte (PMN) emigration into inflammatory sites was examined. Killed *E coli* organisms (6×10^8 per site) were injected into two groups of 20 intradermal sites in each rabbit. The first group (Group F) preceded the second (Group S) by 6 hours. The kinetics of PMN emigration, quantitated with ^{51}Cr-labeled cells, differed in the two groups. In Group S sites an intense PMN influx was measured at 0–4 hours, and subsequently the extent of PMN emigration rapidly declined. In Group F sites a minute PMN influx was detected during the first 4 hours, coinciding with a marked neutropenia. The maximal PMN influx into Group F sites was measured between 6 and 10 hours. Microscopic sections at 4 hours showed a scanty PMN infiltrate and numerous bacteria in the dermis of Group F sites, while extensive phagocytosis of bacteria by PMNs was apparent in Group S sites. By comparing the extent of bacterial phagocytosis in 4-hour-old sites with the magnitudes of PMN emigration between 6 and 10 hours in both groups, we concluded that the phagocytic elimination of killed *E coli* was not a major mechanism regulating the cessation of local PMN emigration. Instead, we propose that tachyphylaxis or desensitization of sites to inflammatory factors released from *E coli* is the responsible mechanism. (Am J Pathol 1986, 124:1–9)

THE INTRADERMAL injection of *Escherichia coli* organisms is a model frequently employed in studying the pathophysiologic responses of the host to microorganisms.[1] Various inflammatory parameters, including vascular permeability,[2] hyperemia,[3] leukocyte emigration,[4–6] hemorrhage,[7] platelet aggregation,[8] and microthrombosis[9] can be rapidly and in some instances simultaneously quantitated with the use of radioisotope techniques. The use of multiple sites in an animal provides an internal control; however, systemic influences produced by a large total dose of *E coli* as a result of multiple lesions must be minimized by a protocol of serial injections in kinetic studies, or by employing relatively small individual doses. Under these conditions, animals maintain normal or slightly elevated levels of circulating polymorphonuclear leukocytes (PMNs), and the rate of PMN accumulation into intradermal sites of killed[1] or live (M. K. W. Chan and H. Z. Movat, unpublished observations) *E coli* injection reaches an early maximum between 2 and 3 hours, with only a low rate of influx measured after 6 hours. These kinetics of PMN accumulation are similar to those observed in sites of injection of various chemotaxins and chemotaxinigens[10] and likewise are independent of the dose.

In sites of infection infiltrating PMNs limit the spread of microorganisms by phagocytosis[11] and subsequent bacterial killing.[12,13] In the process microvascular injury may occur secondary to the release of PMN

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lysosomal enzymes and oxygen radicals[14] or bacterial products.[15,16] The resulting hemorrhage and enhanced vascular permeability are directly related to the magnitude of the PMN influx into lesions induced by bacteria.[17,18]

Neutropenia is a frequent feature of clinically significant Gram-negative infections.[19,20] PMNs are shunted from the circulating and bone marrow pools into the marginal pool,[21] which makes them unavailable for the defense of the host. Neutropenia occurs early in the course of clinical infections, at a time coinciding with the maximal rate of PMN influx observed in experimental models, with both killed[1] and live *E coli* (M. K. W. Chan and H. Z. Movat, unpublished observations). Because of the importance of PMNs in the early defense against microorganisms, we have employed a model where neutropenia with a greater than 80% decrease in the number of circulating PMNs is induced in rabbits 1-3 hours after 20 intradermal injections of killed *E coli* and have studied its effects on the kinetics of PMN accumulation into local lesions.

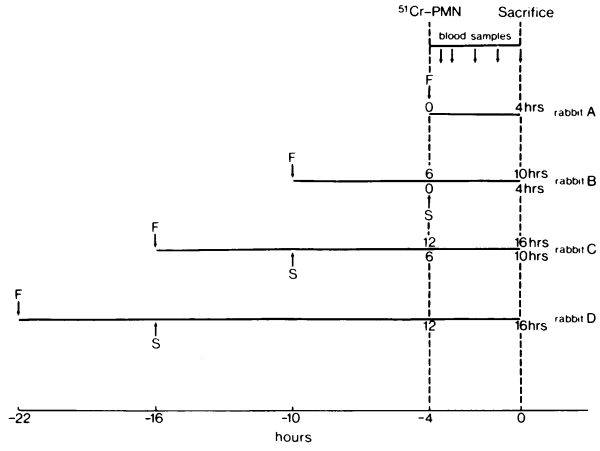


Figure 1—Protocol of intradermal injections of killed *E coli*. Groups of 20 intradermal injection sites were used in 4 rabbits in each experiment (Rabbits A–D). Group F injections (F) preceded Group S injections (S) by 6 hours. The ages of the intradermal sites during which PMN influx was measured for each rabbit are indicated above the lines for Group F and below the line for Group S injections. A single preparation of ^{51}Cr-labeled PMNs from a donor rabbit was aliquoted and transfused into the experimental rabbits 4 hours before sacrifice. Blood samples were obtained as indicated.

Materials and Methods

Bacteria

E coli 055:B5 were grown in RPMI 1640 for 18 hours at 37 C, washed twice with pyrogen-free saline (Baxter Travenol Laboratory, Malton, Ontario, Canada) and quantitated spectrophotometrically at 540 nm, using a standard curve that was verified by pour plate colony counts. The bacteria were killed with 0.5% formaldehyde (18–36 hours at 4 C) and washed twice with saline. They were injected intradermally (6×10^8 *E coli* in 0.2 ml saline per site) into female New Zealand albino rabbits weighing 2.5–3.0 kg.

Systemic Influences of Intradermal *E coli* Lesions

Changes in the numbers of circulating leukocytes and in the rectal temperature were followed in four groups of 4 rabbits after the intradermal injections of formalin-killed *E coli*, 6×10^8 bacteria per site: 1) 3 sites at 0 hours, 2) 3 sites at 0, 2, 4, 5, 6, and 7 hours, 3) 20 sites at 0 hours, and 4) 20 sites at 0 and 6 hours. Heparinized venous blood samples were obtained from the marginal ear veins, and the total leukocyte counts were determined with a hemacytometer. Blood films were stained with Wright's stain, and 200-cell differential counts were performed. The results are expressed as percent of control leukocytes remaining in the circulation. Control values were determined from the mean of two measurements prior to each experiment. Rectal temperatures were obtained by insertion of a lubricated mercury thermometer 6–8 cm into the rectums of trained rabbits, maintained for 1–2 minutes until no further rise in temperature was apparent. The mean normal body temperature was 39.4 ± 0.1 (SEM) C, and rabbits with control temperatures greater than 40 C were not used.

Quantitation of PMN Emigration

The total number of PMNs accumulating over 4-hour intervals in two groups of intradermal sites was estimated. In each group 20 intradermal sites were injected with formalin-killed *E coli* (6×10^8 per injection), the first group (Group F) preceded the second group (Group S) by 6 hours. The injection protocol is illustrated in Figure 1. Four sets of 4 rabbits were used. PMNs that were labeled with $Na_2{}^{51}CrO_4$ were obtained from blood collected from the central ear artery of a donor rabbit into acid citrate dextrose. They were isolated by sedimentation with hydroxyethyl cellulose and density gravity centrifugation on Percoll as described by Issekutz and Movat.[4] By this method the PMNs constituted greater than 90% of the leukocytes. They were resuspended at a concentration of 1×10^8 leukocytes/ml in Ca^{2+}-, Mg^{2+}-free Tyrode's buffer containing 10% platelet-poor rabbit plasma and labeled for 20 minutes at 37 C with 100 μCi $Na_2{}^{51}CrO_4$ (New England Nuclear, Lachine, Quebec, Canada, specific activity 200–500 mCi/mg). After labeling the cells were centrifuged (250*g* for 10 minutes) and resuspended in leukocyte-poor plasma containing unlabeled erythro-

cytes and 1% hydroxyethyl cellulose (saved from the initial sedimentation). The cells were resedimented for removal of the ^{51}Cr-labeled erythrocyte contaminant, because hypotonic lysis of erythrocytes may damage the PMNs and precludes optimal circulation and accumulation in inflammatory lesions.[5] More than 80% of the ^{51}Cr-labeled PMNs were recovered after resedimentation, and the ^{51}Cr-labeled erythrocyte contaminant constituted less than 1% of the total radioactivity, as determined by 0.84% NH_4Cl lysis of erythrocytes. Therefore, essentially all of the ^{51}Cr radioactivity was associated with the PMNs.

The ^{51}Cr-labeled PMN preparation was aliquoted, transfused intravenously into 4 experimental rabbits, and allowed to circulate for 4 hours. Blood samples were obtained at 3.5, 3, 2, 1, and 0 hours before sacrifice, and the blood PMN specific activity was calculated by dividing the ^{51}Cr radioactivity in one milliliter of blood by the number of circulating PMNs per milliliter.

$$\text{Blood PMN specific activity} = \frac{^{51}\text{Cr-cpm per milliliter blood}}{\text{PMNs per milliliter blood}}$$

Four hours after the injection of ^{51}Cr-PMNs the rabbits were sacrificed, their skins were removed,[17,18] and the ^{51}Cr radioactivity in intradermal sites was measured in a γ-spectrophotometer at a setting of 70–400 kev. The total number of PMNs accumulating over the 4-hour period was estimated by dividing the ^{51}Cr radioactivity in a site by the blood PMN specific activity at 2 hours, the midpoint of the accumulation.

$$\text{PMNs per site} = \frac{^{51}\text{Cr cpm per site}}{\text{blood PMN specific activity (2 hours)}}$$

We have previously shown that ^{51}Cr-labeled PMNs marginate identically to the host's unlabeled PMNs in response to an intravenous injection of endotoxin.[22] In order to examine the efficiency of ^{51}Cr-PMN influx into inflammatory sites, we induced pleural exudates in 4 rabbits, and the PMN specific activity in blood and that in 2-hour exudates were compared. The results summarized in Table 1 show comparable specific activities in the circulation and in the exudates, with the mean and SEM of the 4 ratios being 0.94 ± 0.15. This indicates that the transfused ^{51}Cr-labeled PMNs accumulate in sites of inflammation with the same efficiency as the host's PMNs.

Microscopy

Skin biopsies were fixed in universal fixative (2% gluteraldehyde, 10% formaldehyde, pH 7.4). Semithin (1–2-μ) sections of tissues embedded in hydroxyethyl methacrylate were cut and stained with azure-eosin.

Results

Systemic Responses to Intradermal Injections of *E coli*

A neutropenia was observed within 1 hour after the intradermal injection of *E coli*. In rabbits in which three sites were treated, each with 6×10^8 organisms (Figure 2), the neutropenia was mild and short-lived, compared with animals with 20 injection sites (Figure 3A). In these animals a decrease in the numbers of circulating PMNs was observed as soon as 30 minutes, with the nadir (86% decrease) at 2 hours. At 3 hours a trend toward recovery was apparent; and by 4–6 hours neutrophilia developed and persisted for at least 20 hours. The number of circulating mononuclear leukocytes (Figure 3A and B) declined gradually to less than 30% of control levels and recovered by 24–28 hours.

After recovery from neutropenia the rabbits did not redevelop neutropenia when subsequently given the same dose of *E coli*. In rabbits with triplicate intradermal injections at 2, 4, 5, 6, and 7 hours the number of circulating PMNs did not decrease after a transient neutropenia of 2 hours' duration (Figure 2), and neutrophilia occurred after 3 hours despite further injections. Injections of a larger dose of *E coli* at 4 hours elicited neutropenia (results not shown). The profile of PMNs circulating in rabbits with 20 intradermal sites inoculated with *E coli* at 0 and 6 hours is shown in Fig-

Table 1—^{51}Cr-PMN Specific Activity in the Circulation and in Inflammatory Pleural Exudates

Rabbit	Stimulus	Specific activity (^{51}Cr cpm/PMN)		
		Blood	Exudate	Ratio of exudate to blood
1	50% ZAP,* 5 ml	2.93×10^{-4}	2.69×10^{-4}	0.92
2	50% ZAP, 5 ml	6.40×10^{-4}	5.91×10^{-4}	0.92
3	10^{-6}M FMLP,† 5 ml	6.40×10^{-4}	3.92×10^{-4}	0.61
4	10^{-6}M FMLP, 5 ml	2.03×10^{-4}	2.67×10^{-4}	1.32

* ZAP, zymosan-activated plasma diluted with pyrogen-free saline.
† FMLP, formyl-methionyl-leucyl-phenylalanine in pyrogen-free saline.

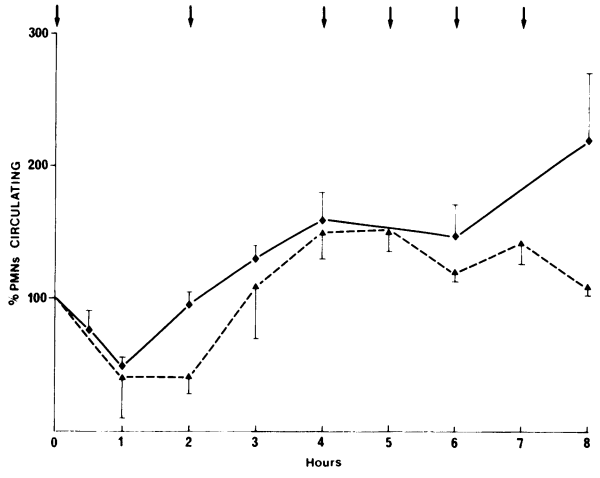


Figure 2—The numbers of PMNs circulating expressed as a percentage of control values in rabbits with triplicate intradermal injections of formalin killed *E coli* (6 × 10^8 per site) at 0 hours (*solid line*) and at 0, 2, 4, 5, 6, and 7 hours (*hatched line*). The means and standard error of 4 rabbits are given. The injection times are indicated by *arrows*.

ure 3B. After recovery from an initial neutropenia a gradual decrease in the number of circulating PMNs was observed (Figure 3B) from 280% control at 6 hours to 71% at 10 hours. This gradual decrease seems to be the result of a massive PMN emigration into *E coli* sites between 6 and 10 hours after injection.

An increase in the rectal temperature (Figure 3C) was measured within 30 minutes after the injection of 20 sites, with the peak occurring at 4 hours (Figure 3C). A second peak was observed after the injection of a second group of 20 sites at 6 hours.

PMN Emigration Into Intradermal *E coli* Sites

The PMN emigration into Group F sites during the first 4 hours was very small (0.5 × 10^6 PMNs per site) (Figure 4). This time period coincided with a severe neutropenia (Figure 3). Microscopic sections at 4 hours showed a sparse PMN infiltrate and numerous bacteria in the dermis (Figure 5A and B). The maximal PMN accumulation (1.8 × 10^7 PMNs per site) occurred when Group F sites were between 6 and 10 hours of age (Figure 4). PMNs were observed microscopically emigrating through the walls of venules and forming an early microabscess at 10 hours (Figure 6). Between 12 and 16 hours the PMN influx declined (7.9 × 10^6 PMNs per site).

Group S injections followed Group F by 6 hours. By this time rabbits had recovered from neutropenia, and neutrophilia was present (Figure 3B). A refractoriness to neutropenia had also developed. In contrast to Group F sites, the maximal PMN emigration into Group S sites

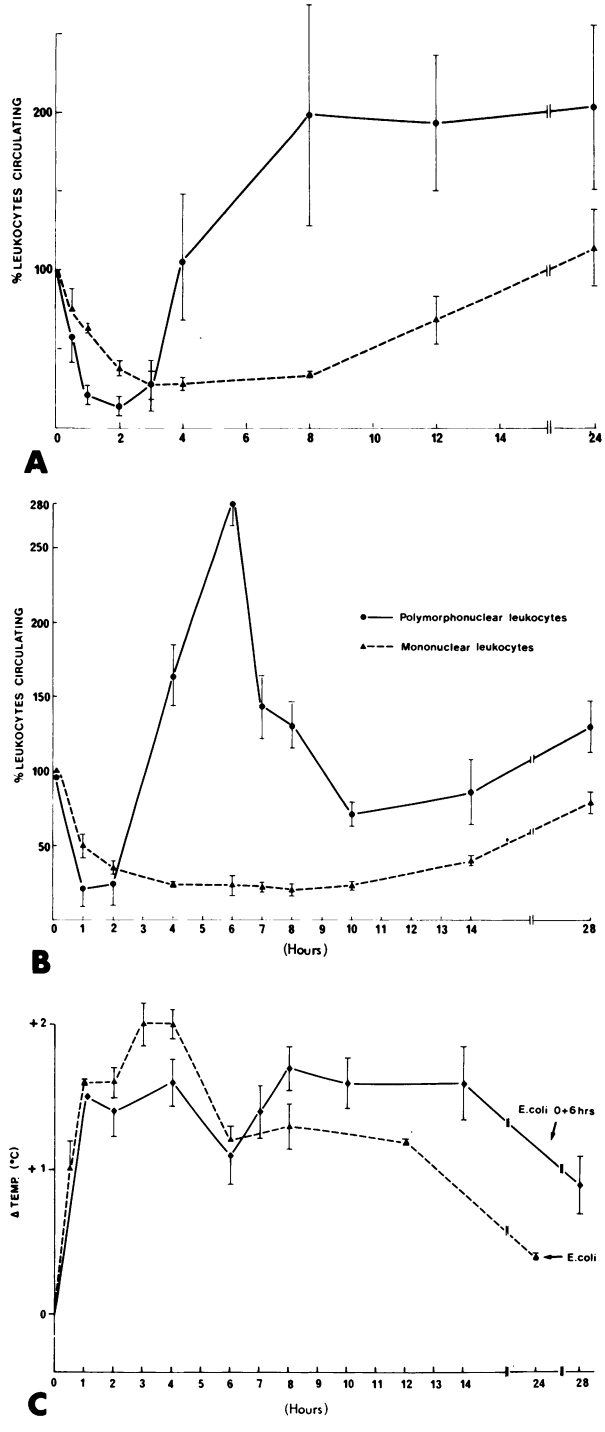


Figure 3—The numbers of leukocytes circulating (PMNs and mononuclear leukocytes) expressed as a percentage of control values after the injection in 20 intradermal sites of formalin-killed *E coli* (6 × 10^8 CFU per site). **A.**—Injections at 0 hours. **B.**—Consecutive injections at 0 and at 6 hours. The error bars represent the standard errors of the means for 4 rabbits. After the intradermal injection of 20 sites with saline, the numbers of PMNs and mononuclear leukocytes circulating remained within 30% of control for a 24-hour period. **C.**—Febrile responses (mean ± standard error) in 4 rabbits to 20 intradermal injections of *E coli* at 0 hours (▲) and consecutive injections at 0 and at 6 hours (◆). The rectal temperatures of rabbits given saline remained within 0.4 C of control.

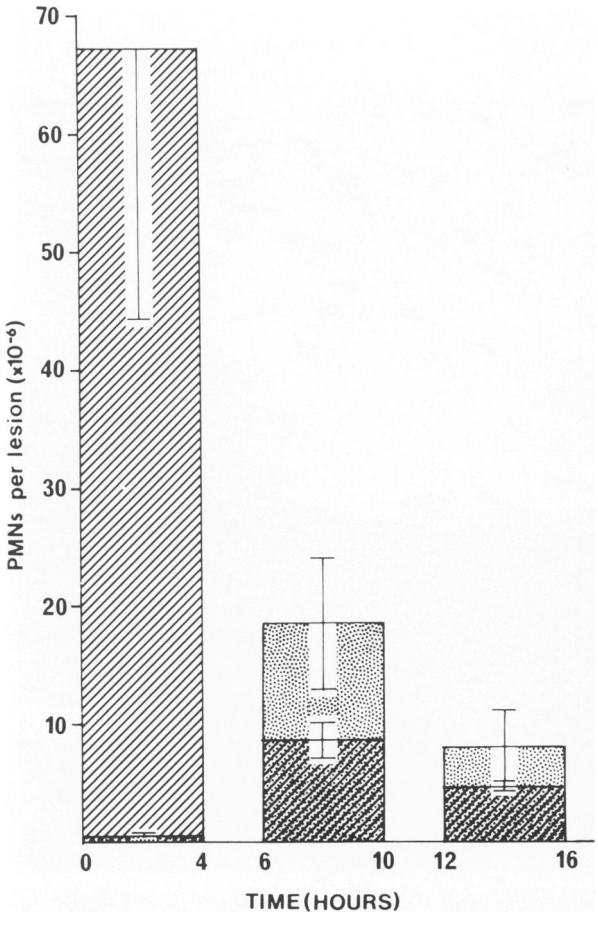


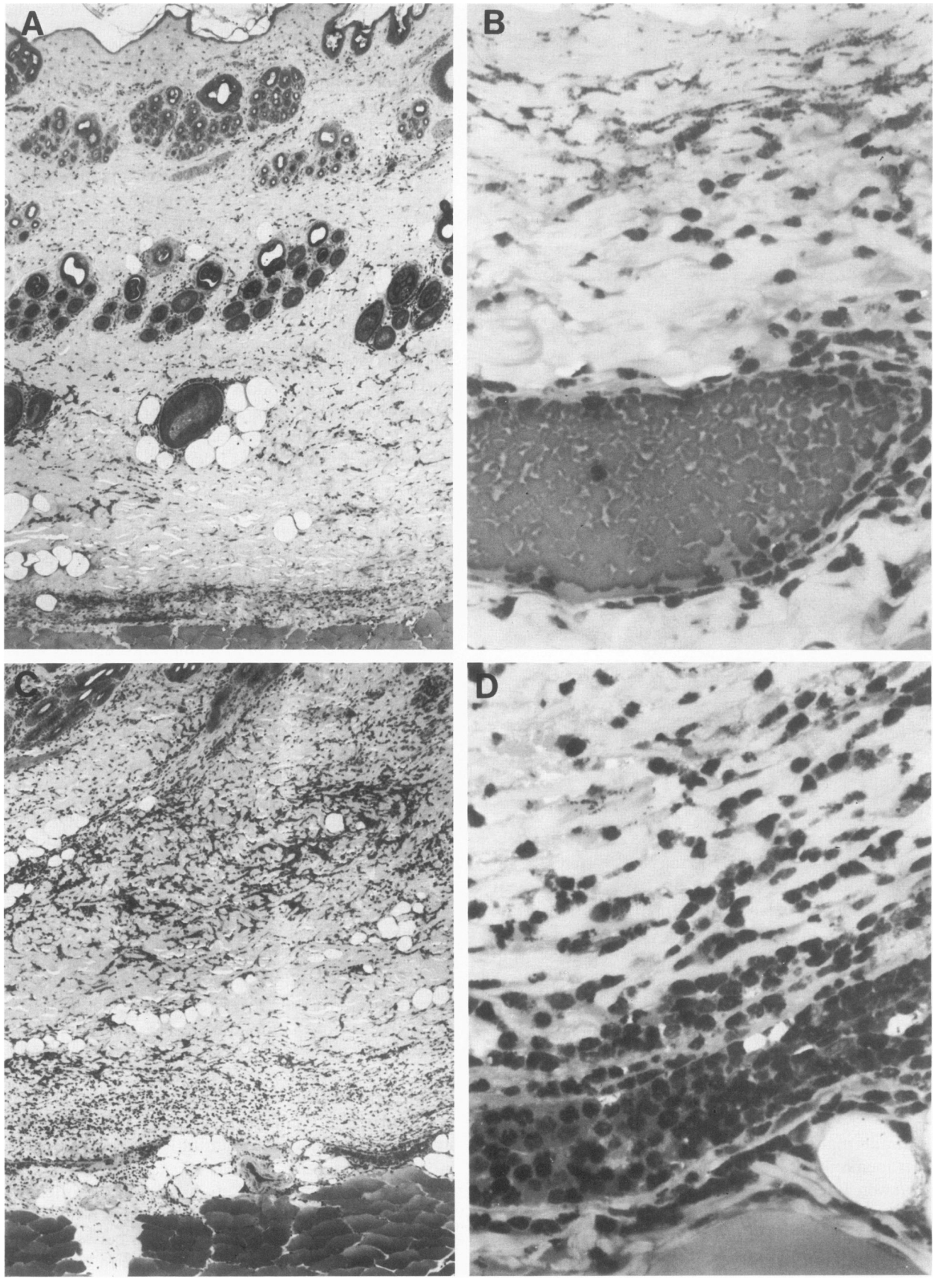
Figure 4—The kinetics of PMN emigration into intradermal sites injected with *E coli*, as indicated in Figure 1. The means and standard errors (n = 4) of Group F (*dotted bars*) and Group S (*hatched bars*) sites are plotted for each 4-hour interval. Four rabbits were used for each time period.

was measured during the first 4 hours after their injection (Figure 4). The influx was massive, with 6.7×10^7 PMNs accumulating per site. Microscopic sections of 4-hour Group S sites showed an intense PMN infiltrate in the dermis and extensive phagocytosis of bacteria (Figure 5C and D). The PMN accumulation into Group S sites between 6 and 10 hours (Figure 6) decreased approximately eightfold (8.5×10^6 PMNs per site), and a further decline was observed in 12–16-hour sites (4.5×10^6 PMNs per site) (Figure 4).

Discussion

Intradermal injections of *E coli* elicited in rabbits a neutropenia and fever. Even when small numbers of *E coli* were injected intradermally (6×10^8 bacteria per site, in triplicate), a mild and very transient neutropenia was detected. Profound systemic effects, including a rapid development of fever and large shifts in the numbers of circulating leukocytes, were observed when the number of injections was increased from 3 to 20. Patients with Gram-negative infections, and particularly those that develop septic shock, which is associated with a high morbidity[23] and mortality,[25,26] frequently exhibit similar clinical features early in the course of their disease.[19,20] Neutropenias encountered in Gram-negative infections are thought to be mediated predominantly by endotoxins. Our knowledge in this area is based almost exclusively on experiments in which purified lipopolysaccharides extracted from the outer membranes of Gram-negative bacteria were injected intravenously. Neutropenias induced by endotoxins are dose-dependent, with the bulk of the circulating PMNs sequestered in the lung microvasculature.[22] Concomitantly, PMNs from the marrow storage pool are shunted to the lung marginal pool[21] and do not recirculate following recovery. The characteristics of neutropenias induced by intradermal injections of *E coli* can be reproduced by intravenous injections of endotoxin. A single intravenous injection of *E coli* 055:B5 endotoxin (100–300 μg/kg) induces in rabbits a neutropenia of a magnitude and duration similar to that following 20 intradermal injections of *E coli*, except that its onset is more rapid (T. Bedrossian and M. Cybulsky, unpublished observations). Absorption of endotoxin released from *E coli* into the circulation can occur *via* lymphatics or diffusion directly through venular and capillary walls. In addition to endotoxin,[18,26] *E coli* release formylated chemotactic peptides[27]; however, these produce transient neutropenias after intravenous injections, with complete recovery usually by 10–15 minutes.[28]

After recovery from neutropenia and during the subsequent neutrophilia, rabbits were refractory to the redevelopment of neutropenia when given another injection of the same dose of *E coli*. After a mild neutropenia of 2 hours' duration, further triplicate injections of *E coli* did not decrease the number of circulating PMNs (Figure 2). In fact, the circulating PMN profile in rabbits with serial triplicate injections at 0, 2, 4, 5, 6, and 7 hours is quite similar to that seen in rabbits with a single triplicate injection (Figure 2). A gradual decrease in the numbers of circulating PMNs from 280% control at 6 hours to 71% at 10 hours was observed in rabbits after a second 20 intradermal sites were injected with *E coli* (Figure 3B). We believe that this decrease is primarily the result of a massive PMN emigration into *E coli* sites, rather than sequestration in the lung vasculature. Quantitative studies with ^{51}Cr-labeled PMNs indicated that 1.7×10^9 PMNs (1.8×10^7 per site $\times$ 20 Group F, 6–10 hours after injection + 6.7×10^7 per site $\times$ 20 Group S, 0–4 hours after injections) accumulated in *E coli* sites between 6 and 10 hours and accounts for the decrease in circulating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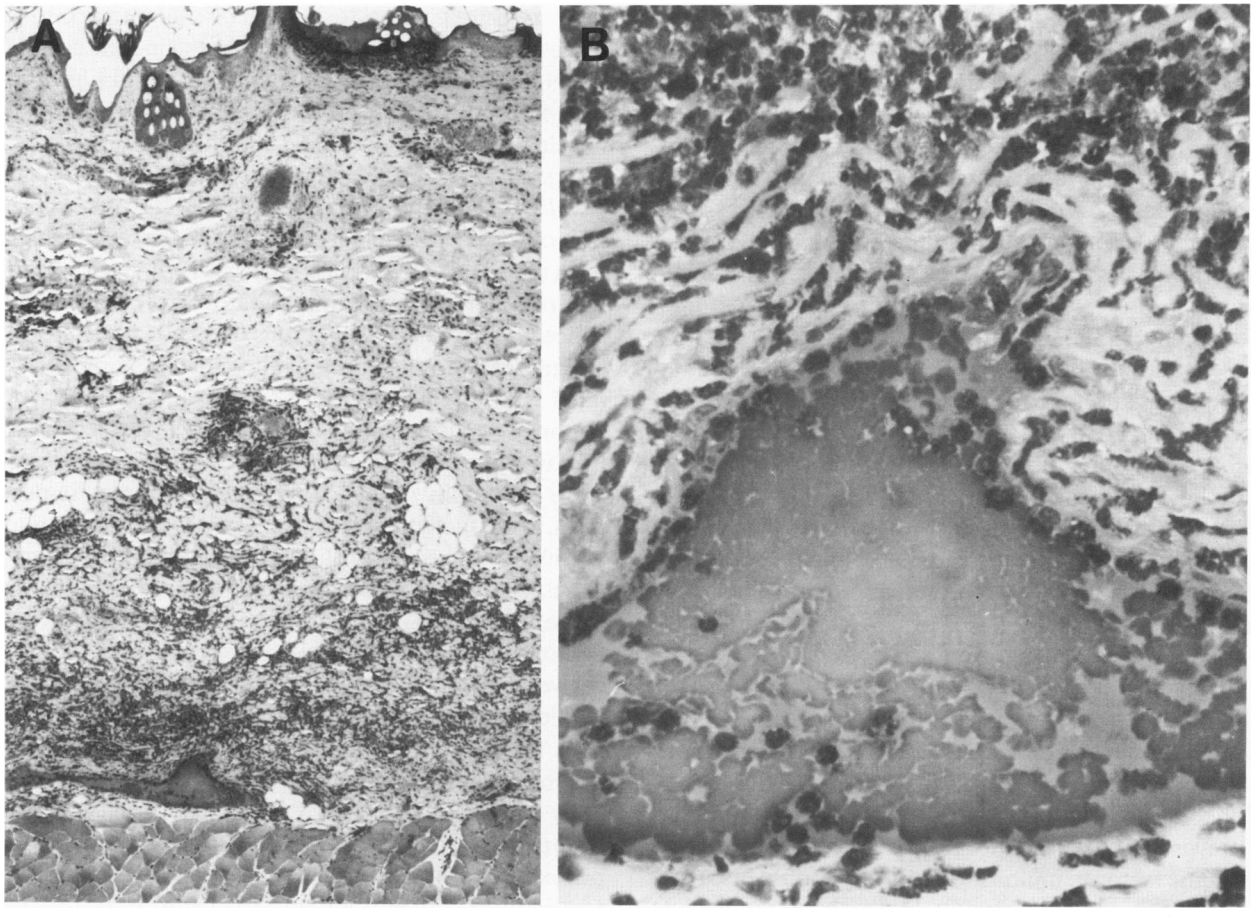

Figure 6—Low- (**A**) and high- (**B**) power photomicrographs of a 10-hour Group F site. A moderate to severe PMN infiltrate is seen, predominantly above the panniculus carnosus muscle. (**A**, ×48) PMNs are adhered to and migrating through the wall of a venule. Bacteria have been phagocytosed and are not apparent in the dermis. (**B**, ×480)

PMNs. The neutropenia encountered following Group F injections cannot be attributed to a migration of circulating PMNs into inflammatory sites, because a low influx (1×10^7 PMNs, 0.5×10^6 per site × 20 Group F sites) was measured with ^{51}Cr-labeled PMNs during the first 4 hours. As mentioned earlier, endotoxemia induces the sequestration of circulating PMNs in the pulmonary microvasculature[22] and shunting of PMNs from the marrow storage pool to the lung marginal pool.[21] Because sequestered PMNs do not recirculate,[22] the recovery from neutropenia and subsequent neutrophilia which usually occur within 6 hours after an intravenous injection of endotoxin are the result of recruitment of new PMNs from the bone marrow. These cells may have adapted to endotoxemia and not sequester in the pulmonary microvasculature on reexposure.

Previous studies have determined that the bulk of PMN emigration into intradermal sites injected with killed *E coli* occurs within the first 4 hours and the maximal rate of PMN emigration is at 2–3 hours.[1,4] However, we have observed a very low PMN influx into Group F sites during this time. (Initially this was an incidental finding when one of the authors [I. J. C.] attempted to inhibit ^{51}Cr-PMN accumulation in multiple *E coli* injection sites by administering locally nonsteroidal inflammatory drugs. The clue on what was happening came from a total and differential leukocyte count of the blood.) Local factors, which include the stimulus for PMN emigration and changes in blood flow within inflammatory sites, cannot explain the low influx. In the experiments described, the dose of *E coli* injected into each site was identical, thus ensuring an equivalent local stimulus. Modulations of the blood flow to

Figure 5—Representative photomicrographs of intradermal *E coli* sites 4 hours after injection. **A.**—In Group F sites a sparse PMN infiltrate is apparent predominantly above the panniculus carnosus muscle. (×48) **B.**—Group F sites show numerous bacteria in the dermis and several PMNs in the process of migrating through the walls of a venule. (×480) **C.**—Group S sites show an intense PMN infiltrate in the dermis and subcutaneous tissues. (×48) **D.**—Large numbers of PMNs are adherent in the lumen of veins and have migrated through their walls into the interstitial tissues of Group S sites. In contrast to Group F sites, few *E coli* organisms are apparent in the dermis and have been phagocytosed by infiltrating PMNs. (×480)

inflammatory sites by exogenous prostaglandins are known to directly influence the magnitude of PMN emigration.[29,30] However, the kinetics of hyperemia in sites of killed *E coli* injection appear to be independent of the local leukocyte accumulation, as was demonstrated in rabbits made neutropenic with nitrogen mustard.[17] On the basis of these studies, the hyperemic responses elicited by Group F or Group S injections are not likely to be significantly different. In addition to local factors, the extent of PMN emigration into inflamed tissues is dependent on systemic factors. These may influence the delivery of PMNs to sites of inflammation or the ability of PMNs to migrate into extravascular tissues. During the first 4 hours after Group F injections a marked neutropenia was observed, and the number of PMNs remaining in the circulation at 2 hours was less than 25% of control. The severe reduction in the numbers of circulating PMNs would result in decreased PMN delivery to tissues. The delivery of PMNs may also have been affected by a redistribution of the cardiac output, as is often observed in patients with septic shock.[20] The cardiac output is usually increased; however, the blood flow to the skin is reduced as a result of a peripheral vasoconstriction. Finally, functional changes may have affected PMN adhesion to the endothelial cells and migration into inflamed extravascular tissues. The relative contribution of each systemic factor to inhibiting PMN emigration into 0–4-hour Group F sites remains to be determined. We believe that neutropenia played a major role because of its magnitude and transient nature and because after recovery from neutropenia intense PMN emigration was measured simultaneously in Group F and Group S sites (6–10-hour Group F sites and 0–4-hour Group S sites, Figure 1, rabbit B). Systemic factors other than neutropenia may have also contributed to the extensive inhibition of PMN emigration observed; however, their influence was transient.

The maximal PMN emigration into Group S sites was in the first 4 hours (Figure 4). Injections were made in these sites after rabbits had become refractory to neutropenia. By 6 hours the bulk of the PMN emigration was over, and the influx measured between 6 and 10 hours was eightfold lower than between 0 and 4 hours. In contrast, the maximal PMN emigration into Group F sites was between 6 and 10 hours of age (Figure 4). Its magnitude was relatively low, approximately fourfold lower than the maximal influx into Group S sites and only twice that of Group S sites of the same age. Thus, it appears that a compensatory massive PMN influx did not occur into Group F sites after 6 hours. This is interesting in view of the differences in histologic appearance of the sites at 4 hours. A scanty PMN infiltrate and numerous bacteria were apparent in the dermis of Group F sites, and an intense PMN infiltrate with extensive phagocytosis of *E coli* was seen in Group S sites. In spite of the differences in the magnitudes of PMN emigration and the extent of bacterial phagocytosis in 4-hour sites, the PMN influx into 6–10-hour-old sites was of comparable magnitude in both groups. This suggests that the phagocytic elimination of killed *E coli* is not a major mechanism regulating the cessation of local PMN emigration. An alternative mechanism may be the desensitization or tachyphylaxis of sites to a particular dose of inflammatory agents.[31–33] This concept arose from observations of an attenuated PMN emigration into intradermal sites that were prestimulated with an identical dose of the same chemotactic factor. When different factors were used, PMN emigration was not affected. Because *E coli* are known to release endotoxin[18,26] and formylated chemotactic peptides,[29] the tachyphylaxis of sites to these agents may account for the diminished PMN emigration after 6 hours. Activation of complement by *E coli* appears not to be a major mechanism for PMN recruitment, as demonstrated in rabbits depleted of complement by cobra venom factor.[17] Tachyphylaxis of sites to endotoxin would also explain why endotoxin from degraded bacteria that is egested from PMNs[16] after 4 hours does not maintain a continuing PMN emigration. Tachyphylaxis may occur at the level of accessory cells such as tissue macrophages, which upon stimulation with endotoxin may synthesize and secrete interleukin-1, a potent inducer of PMN emigration,[34] and/or at the level of endothelial cells which have been shown to regulate PMN adherence *in vitro*.[35–38] Tachyphylaxis can be overcome by reinjecting the same factor at a higher dose. (M. I. Cybulsky and I. G. Colditz, unpublished observations). This situation may be relevant with live *E coli*, where bacterial multiplication and a gradual increased release of endotoxin and chemotactic peptides may occur during the neutropenic period, overcome local tachyphylaxis, and produce a massive PMN influx after recovery from neutropenia.

The importance of an early PMN influx into lesions induced with live microorganisms remains to be investigated. A rapid PMN influx may be critical in controlling the growth and spread of bacteria and may reduce eventual tissue injury. In studying these phenomena, the systemic reponses to local processes should not be disregarded.

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