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SYNOVIAL INFLAMMATORY RESPONSE TO BACTERIAL ENDOTOXIN**

The ability of bacterial endotoxins to produce local inflammation has been recognized as one of the many biologic activities of these intriguing products of the gram-negative bacterial cell.[1,2] This property of endotoxin, however, has not been studied extensively with the highly purified materials now available. Preliminary observations are reported here on the inflammatory response elicited by intra-synovial injections of endotoxin into rabbit suprapatellar bursae. These studies were designed to determine the sensitivity of this technique for detecting endotoxin contamination as a possible cause of the inflammatory reaction produced by intra-synovial injection of foreign proteins.[3] Because the synovial cavity proved extremely reactive to endotoxin-induced inflammation, the initial observations were expanded and compared with other known biologic properties of endotoxin. Repeated intravenous injections of endotoxin produce a relative resistance to many of its biologic effects, particularly the ability to produce fever and to cause death. This relative resistance has been termed 'tolerance' to endotoxin.[4] The inflammatory reaction in 'tolerant' rabbits was of particular interest.

MATERIALS AND METHODS

Most of the experiments utilized two highly purified endotoxins, one derived from *Proteus vulgaris* ("E" pyrogen, Organon Laboratories, Morden, Surrey, England, supplied by Dr. W. J. Tindall), and the other a preparation of *Salmonella abortus equi* (Lipexal, supplied by Dr. Paul Calabresi). In some experiments, crude typhoid vaccine was used to produce endotoxin tolerance and bacterial immunity.[5]

One-half milliliter of the materials to be studied was injected into the suprapatellar bursae of 3-4 kg. albino rabbits anesthetized with pentobarbital. At an appropriate time after injection the animals were re-anesthetized and the synovial exudate was harvested by entering the bursae with a #19 gauge needle attached to a 2 ml. plastic syringe filled with sterile saline. The joint was massaged as the saline was repeatedly injected and withdrawn from the bursae. The final volume of exudate-saline was

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** This work was supported in part by a grant for arthritis research from the John A. Hartford Foundation, and by grants AM 07348 and AI 01564 from the U. S. Public Health Service.

Received for publication 25 May 1965.

measured, white cells counted with a Model A Coulter electronic particle counter, and differential counts prepared from the centrifuged exudate by resuspending the cells in a small amount of rabbit serum and brushing the cell suspension onto #00 cover slips with a camel hair brush. The films were stained with Wright's stain. From these data, total quantitative and qualitative joint exudate could be determined. In previous experience with this method of studying inflammation, the normal rabbit suprapatellar bursal wash yielded less than 100,000 cells, mostly lymphocytes; at six hours following intra-bursal injection of pyrogen-free saline the mean exudate, mostly polymorphonuclear leukocytes, was 1.95 million cells, with 95 per cent confidence limits extending to 4.7 million. Similar values were obtained with sterile homologous rabbit serum.[3]

Endotoxin tolerance, as indicated by striking resistance to the fever-producing action of these materials, was produced by two methods used extensively in previous studies: (1) Daily intravenous injection of 1.5 ml. of a 1:10 dilution of a stock typhoid vaccine for seven days,[5] and (2) a single intravenous injection of one microgram of the Organon Laboratory proteus endotoxin.[6] In other experiments, 0.1 microgram of the proteus endotoxin was given intravenously for 7 or 14 days before intra-synovial testing with endotoxin.

In experiments designed to detect the persistence of endotoxin in biologic materials or the emergence of some type of tissue inflammatory agent in response to endotoxin, serum or suprapatellar bursae washings were obtained at intervals after endotoxin injection. These supernates were assayed for their inflammatory activity by injection of 0.5 ml. of the material into the bursae of normal rabbits. Details of these methods are included in the text.

RESULTS

Comparison of the synovial inflammatory reaction of proteus endotoxin and pyrogen-free crystallized ovalbumin

Our initial concern with the synovial inflammation produced by endotoxin was to assess the sensitivity of this response, since contaminating endotoxins have been frequently implicated as a cause of the inflammatory reaction produced by foreign soluble proteins and polysaccharides. A twice recrystallized ovalbumin (Worthington Biochemical Company) was determined to be nonpyrogenic after intravenous injection of 100 mg. into rabbits. The synovial inflammatory effect of this ovalbumin (5 mg.) was contrasted with that of .0005 μg. of the proteus endotoxin. The amount of the two materials used was selected on the basis of preliminary experiments that indicated an approximately equivalent 6-hour inflammatory response, but the data in Table 1 indicate that the endotoxin was somewhat more potent in this experiment. It was hoped that the time sequence of evolution and resolution of the synovial inflammatory reaction of the two substances would demonstrate some differences by which the two inflammatory stimuli could be distinguished. The characteristics of the two types of inflammation proved, however, to be nearly identical as in-

dicated in Table 1, although the endotoxin produced a larger response. The apparent delay in endotoxin inflammation at two hours was inconstant in other experiments. At 48 hours, the endotoxin-injected joints had a residual total cell count of one million, almost 100 per cent large monocytes, but

TABLE 1. EVOLUTION OF SYNOVIAL EXUDATIVE REACTION AFTER INTRASYNOVIAL INJECTION OF PROTEUS ENDOTOXIN (.0005 μg.) OR OVALBUMIN (5 mg.)

	Endotoxin		*Ovalbumin*	
Time after injection	*Total WBCx10⁶*	*% Granulocytes*	*Total WBCx10⁶*	*% Granulocytes*
2 hr.	2.0	95	7.0	97
6 hr.	42.0	95	15.8	94
24 hr.	11.6	54	2.2	27
48 hr.	0.9	12	2.3	9

the difference from the ovalbumin group was not distinctive. The type and intensity of inflammation with ovalbumin and endotoxin were very similar to that observed after heterologous whole serum.[8]

Detection of circulating endotoxin by synovial inflammation

Beeson and others have demonstrated that a febrile response could be obtained from the serum of animals shortly after injection of endotoxin. This bioassay has proven to be a sensitive technique for determining the amount of endotoxin present in the circulation and results obtained by this method on the plasma clearance of endotoxin have been confirmed by studies with radioactively tagged material.[7] Serum from rabbits injected with endotoxin was similarly assayed for its inflammatory propreties. In one experiment, serum was obtained from a donor rabbit before intravenous injection of 1.0 ml. of undiluted stock typhoid vaccine, and again five minutes and two hours later. This amount of vaccine was selected because it has been used extensively in observations on the clearance of endotoxin, and it is known to be largely removed from the plasma within one hour.[8] After collection of the sera from the donor, 0.5 ml. amounts of each sample were injected into the eight bursae of four normal recipient animals, and the 6-hour inflammatory response examined (Table 2). The mean value of the 6-hour total exudate for the control serum was 2.2 million. The serum obtained five minutes after vaccine produced marked inflammation with a mean exudate of 7.9 million cells ($p = .001$). At two hours, when this endotoxin preparation has been shown to be almost totally

cleared from the circulation, the inflammatory response (mean of 2.9 million) was almost at control levels.

Similar experiments with the purified proteus endotoxin are shown in the scatter diagram (Fig. 1). Large rabbits (4-5 kg.) were injected intravenously with one microgram of endotoxin, and serum from 10 ml. of blood was obtained before and at various times after the injections. The

TABLE 2. SYNOVIAL INFLAMMATION PRODUCED BY SERUM FROM A DONOR RABBIT BEFORE, AND FIVE MINUTES AND TWO HOURS AFTER, AN INTRAVENOUS INJECTION OF 1.0 ML. TYPHOID VACCINE

Total synovial exudate (millions) in normal recipients six hours after intra-bursal injection of the donor serum		
Before	*5 minutes after vaccine*	*2 hrs. after vaccine*
1.5	10.7	0.8
0.9	8.6	0.9
2.5	8.4	4.6
2.4	6.3	3.4
2.0	14.1	5.3
3.6	7.8	3.2
2.3	4.1	2.5
2.2	3.0	2.3
2.17 (Mean)	7.87 (Mean)	2.87 (Mean)

serum samples were injected into the bursae of normal rabbits and the inflammatory response observed six hours later. The response drops to almost control levels within two hours.

Comparison of inflammatory response to endotoxin in saline or in normal rabbit serum

Serum of normal rabbits contains a factor that enhances the pyrogenicity of small amounts of endotoxin. When endotoxin is mixed with rabbit serum at 4°C. or room temperature, its potency in producing fever is increased about tenfold as compared with the same dose in saline.[9] The nature of this fever-enhancing factor is obscure, and its role in the local inflammatory response has not been studied. Concentrations of proteus endotoxin (.0005 μg and .00005 μg/0.5 ml) were prepared in saline or sterile normal rabbit sera, and both were left at room temperature for 30 minutes. As indicated previously, both saline and normal rabbit serum produce similar and negligible degrees of inflammation.[8] The endotoxin-saline was injected in the left knee and the endotoxin-serum in the right to compare their ability to produce synovial inflammation. Three

rabbits were studied at each dose level. The six-hour inflammation produced was practically indentical. With the larger amount of endotoxin, mean values for endotoxin in saline was 14.5 million exudate cells, compared to 12.1 million for endotoxin in serum. With the smaller concentrations, means were 8 and 10 million, respectively. Although the number

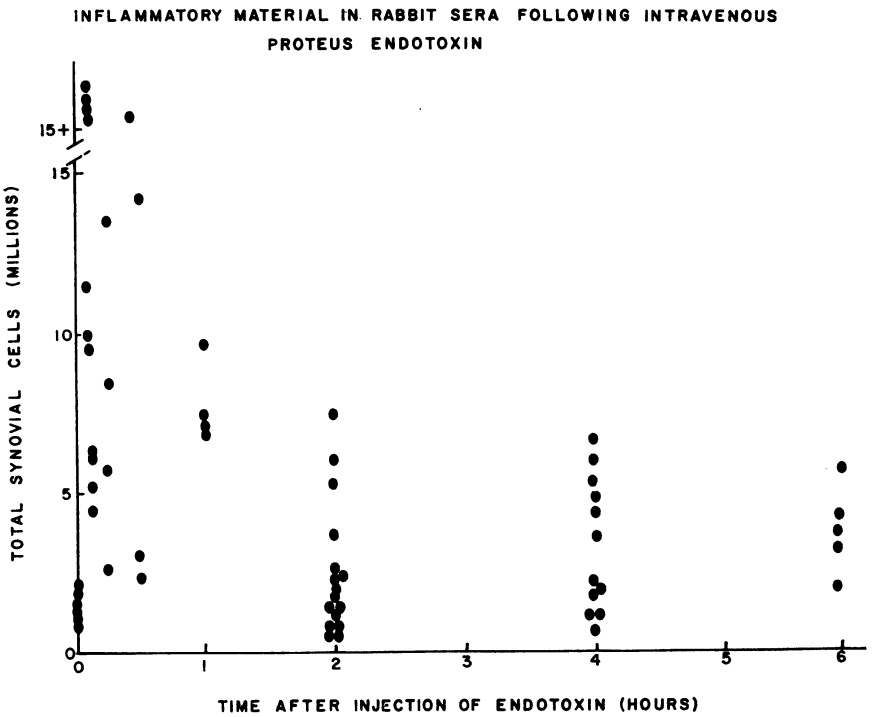

FIG. 1. Total six-hour synovial exudate produced in recipient knee joints by 0.5 ml. of serum (obtained from donor rabbits 5 minutes to 6 hours after intravenous injection of 1 μg. of Proteus endotoxin).

of experiments was too small to allow valid statistical analysis, there was nothing to suggest that serum factors enhance the local inflammatory response to endotoxin.

Sensitivity of the synovial inflammatory mechanism to endotoxin

Tenfold dilutions in saline of both the purified proteus and the salmonella preparations were prepared and assayed for their ability to produce significant synovial inflammatory reactions in six hours. It is of interest that the reported minimal pyrogenic dose in rabbits of both these endotoxin

preparations (about .001 μg/kg.) happens to be similar.[6,10] The results are plotted in Figure 2 and contrasted with the range of values obtained with saline alone in 32 rabbits. Clear-cut inflammatory responses with both preparations were obtained with 0.5 ml. of a 1:10,000 dilution of an

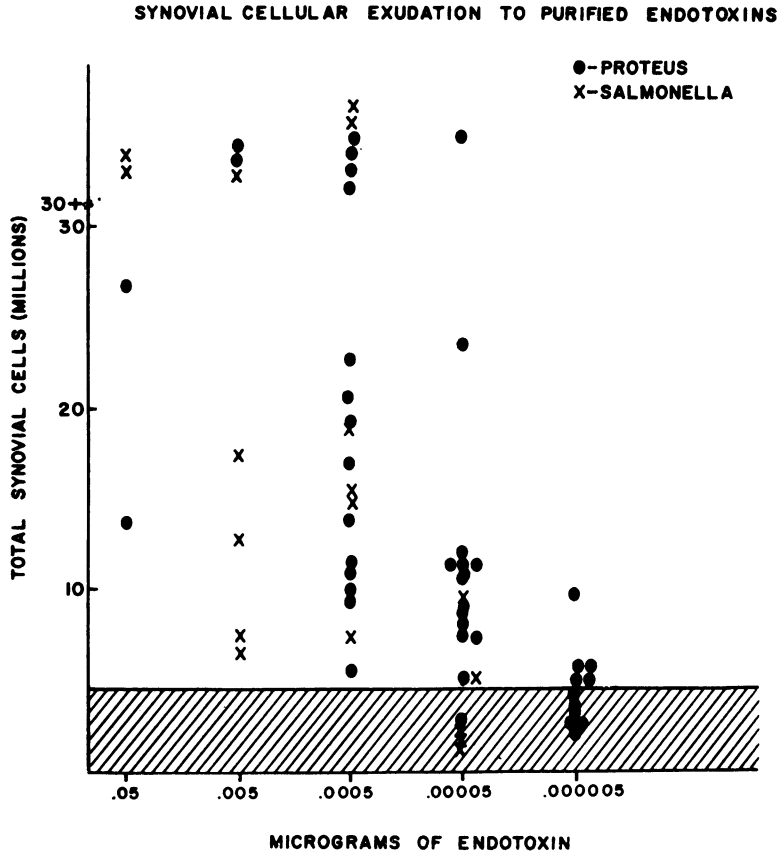

FIG. 2. Total synovial exudate produced in rabbit knee joints six hours after injection of graded doses of two purified endotoxin preparations. The lined area represents the inflammatory response to injection of saline alone.

initial preparation containing 1 μg. per ml. (.00005 μg. of purified endotoxin), Even the lowest dilution may exhibit some activity.

Because of the report by Larson and his colleagues[11] that endotoxin produces a marked inflammatory response when injected into the lateral skin of the rabbit, intradermal injection of 0.2 ml. of the dosages used in Figure 2 were made. No gross inflammatory changes were evident.

Studies in endotoxin-tolerant rabbits

The synovial inflammatory response to purified endotoxin was observed in several groups of animals treated intravenously by purified or crude endotoxin with regimens that are known to produce partial tolerance to the febrile response. Fourteen rabbits (in two groups) were injected intravenously daily for a week with 1.5 ml. of 1:10 stock typhoid vaccine, a dosage known to produce a significant reduction in fever.[5] When the joints were challenged one day later with .0005 μg. of the purified proteus endotoxin, the mean six-hour exudates were 14.7 and 14.9 million. The mean exudate induced by endotoxin in the joints of two groups of control animals, given a similar series of saline injections intravenously, was 16.6 and 7.0. In the second experiment, the response was greater ($p = .05$) in the animals receiving the vaccine. Both animals receiving vaccine and controls from the second experiment were re-injected with endotoxin in the bursae two and four weeks later, when agglutinating antibodies to the typhoid bacillus were high in the vaccine-injected group (titers 1:80 to 1:1280). The mean total exudate in both groups ranged from 14 to 21 million cells/joint, with no difference between the immune and control rabbits.

A more pronounced refractoriness to the pyrogenic effect of endotoxin may be produced rapidly by a single large inoculation of endotoxin. Eighteen to 24 hours after an intravenous injection of 1 μg. of the proteus endotoxin, rabbits have been found to be completely unresponsive to a dose of endotoxin (from the same or heterologous microorganism) capable of producing a monophasic fever of 0.7-1.0°C. in normal rabbits.[6] The mechanism of this refractoriness is unknown, but it does not appear to depend solely upon accelerated clearance since the febrile responsiveness to endotoxin is not restored after blockade of the RES by Thorotrast.[®6] In two experiments, rabbits were injected into the bursae with .0005 μg. of endotoxin 24 hours after 1 μg. of the material intravenously. The individual and mean data are shown in Table 3; the endotoxin-treated group was more responsive to intrabursal inoculation of endotoxin than were the controls which had received saline intravenously the day before ($p = .05$). In another experiment, a group of rabbits received 0.1 μg. of proteus endotoxin daily for 13 days. No 'tolerance,' as indicated by a change in their febrile responses to this amount of endotoxin, was observed. Joint inflammation was again greater in the endotoxin-treated group.

In most of these experiments the blood white cell and differential counts were measured at the time of the intra-synovial challenge with endotoxin. The total granulocyte count was higher in the endotoxin-treated animals (and this difference was often statistically significant), but the differences

were not great. It is possible, however, to correlate grossly the unchanged or enhanced synovial response to endotoxin in tolerant rabbits with a modest blood granulocytosis. Whether this is a fortuitous association is uncertain, but there seemed to be no direct relationship in individual animals between blood granulocyte level and the degree of synovial exudative reaction.

Attempts to demonstrate auto-inflammatory factors in serum or synovial washings after endotoxin injection

Because such minute quantities of endotoxin were capable of initiating a brisk and sustained polymorphonuclear inflammatory reaction (Fig. 2),

TABLE 3. COMPARISON OF SYNOVIAL INFLAMMATORY RESPONSE TO .0005 μg. PROTEUS ENDOTOXIN IN ANIMALS ACUTELY "TOLERANT" TO THE ENDOTOXIN*

Total synovial exudate (millions) six hours after intra-bursal endotoxin	
"Tolerant"	*Controls*
25.2	40.3
19.9	13.9
52.7	10.1
70.0	20.5
21.6	19.2
22.2	23.1
25.5	11.5
21.8	9.8
29.0	15.3
30.0	30.0
43.5	27.8
26.5	16.7
27.8	43.5
16.7	26.5
46.6	47.5
34.6	8.7
14.5	5.9
18.2	4.8
24.5	6.5
35.2	2.4
6.9	25.5
9.6	10.7
28.3 (Mean)	19.1 (Mean)

* The "tolerant" group received 1 mg. of endotoxin intravenously 24 hours before the intra-synovial challenge. Controls received saline intravenously.

it seemed possible that endotoxin triggers the liberation of an auto-inflammatory agent from normal host tissues. Many of the effects of endotoxin are now known to be mediated by the release of biologically active products from various cells. Atkins and Wood have shown that after intravenous injection, endotoxin liberates an endogenous fever-producing material[8] probably largely derived from polymorphonuclear leukocytes.[15, 18] Under the conditions of their experiments, maximum concentrations of circulating endogenous pyrogen (EP) were present in the serum 1-3 hours after the intravenous injection of endotoxin (typhoid vaccine). Later work, with a variety of purified endotoxins from other Gram-negative organisms, has confirmed this observation.[14] Accordingly, an attempt was next made to determine whether sera containing EP might similarly produce an inflammatory reaction.

The donor rabbits were each given a single intravenous inoculation of endotoxin and bled at intervals (5 minutes to 6 hours) thereafter. As shown in Figure 1, serial samples of their sera contained progressively less inflammation-inducing factor (presumably endotoxin). It should be noted that there was no secondary rise of an endogenous inflammatory substance in the 2 and 4-hour samples, though sera drawn at these times contain maximal amounts of endogenous pyrogen.[8] The points in the 2 and 4-hour columns are derived from three separate experiments.* Particular care was exercised to prevent loss of a heat-labile substance in these experiments; the donor serum was clotted and centrifuged at 40°C. and injected into the test animals after overnight refrigeration.

In view of the events that follow the intravenous inoclulation of endotoxin, analogous experiments were designed to determine if the disappearance of injected endotoxin from the synovial cavity was similarly followed by the appearance of newly generated tissue inflammatory products. In a preliminary experiment, a small dose of proteus endotoxin (.0005 μg.) was injected into both knees of four donor rabbits. At intervals thereafter (1 minute, 30 minutes, 2 hours, and 6 hours), a donor was sacrificed and both bursae washed with 2.0 ml. of saline. The exudate cells were then pooled, counted, and centrifuged. The supernatant material (in 0.5 ml. aliquots)was immediately injected into the two suprapatellar bursae of three normal recipients. The recipients' exudate was then examined six hours later (Fig. 3). It should be noted that the amount of endotoxin used was at least 10 times the minimal inflammatory dose (see

* These experiments were repeated because in a preliminary experiment (not plotted) two rabbits responded to 4-hour serum with a definite inflammatory response. Presumably this single observation represented a contaminant, since it was not seen in subsequent work, presented in Figure 1.

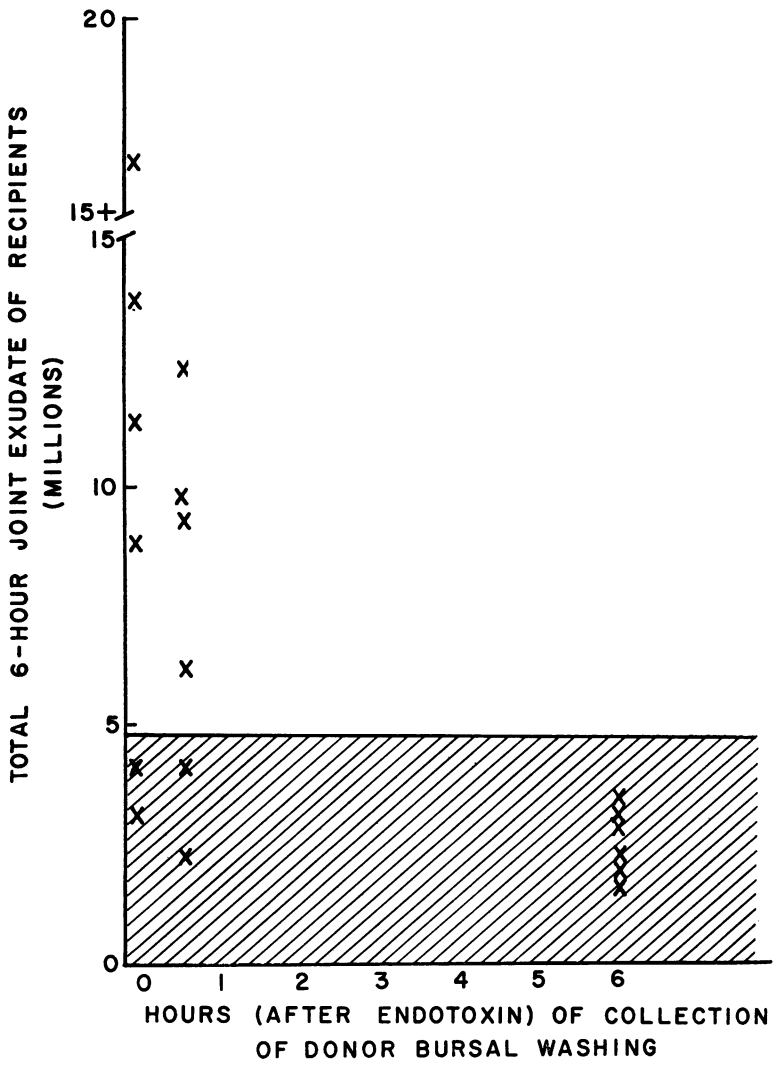

FIG. 3. Total synovial exudate produced in recipient knee joints six hours after injection of bursal washings from endotoxin-injected donor rabbits. Washings were collected from donors at intervals after intrabursal inoculation of .0005 μg. of Proteus endotoxin.

Fig. 2). In this experiment, bursal washings of the donor rabbits collected one minute and 30 minutes after endotoxin produced an inflammatory reaction in the recipient bursae (due presumably to residual endotoxin). Washings obtained six hours after endotoxin, on the other hand, evoked only a minimal reaction that did not exceed control levels induced by

saline (less than four million exudate cells in each of the six joints). It is of interest that the cellular composition of the donor washings was inversely related to their capacity to induce inflammation. The six-hour donor exudate contained 12 million granulocytes/bursae, whereas the one minute and 30 minute samples contained no significant numbers of cells.*

TABLE 4. RESULTS OF TWO SEPARATE EXPERIMENTS DESIGNED TO DETECT A SECONDARY INFLAMMATORY MATERIAL IN BURSAL WASHINGS OF RABBITS AFTER INTRASYNOVIAL ENDOTOXIN.*

Total synovial cells, millions Experiment 1		*Total synovial cells, millions Experiment 2*	
15 minutes	*2 hours*	*15 minutes*	*2 hours*
2.5	3.9	6.3	4.4
5.4	7.1	5.5	1.8
3.2	2.4	17.6	4.1
7.3	2.1	13.2	1.6
6.5	2.6	30.0	3.9
5.8	2.8	15.5	1.9
2.8	3.1	7.3	1.7
4.3	2.8	8.3	2.8
5.0	4.4	6.1	1.9
3.3	4.4	5.4	0.3
4.7	3.0	12.4	2.8
5.8	2.0	17.4	0.6
			3.7
			1.9
4.7 (Mean)	3.3 (Mean)	12.1 (Mean)	2.4 (Mean)

* Donor rabbits received .0005 μg. proteus endotoxin intra-synovially, and their bursae were washed 15 minutes or two hours later. Values in the table represent the inflammation produced by the bursal washings 6 hours after injection into the knee joints of normal rabbits.

The experiments were next performed in which the inflammatory reaction produced by bursal washings 15 minutes and two hours after endotoxin were compared (corresponding to the period when uncleared endotoxin and EP, respectively, are present in approximately equally pyrogenic amounts in the blood of rabbits given an intravenous dose of endotoxin).[8] In both these experiments (Table 4), the two-hour donor bursal washings

* In the same experiment, the two-hour donor washing produced marked inflammatory reactions from 7 to 25 millions in the six knees of the recipients, responses somewhat greater than those produced by washings of the donor bursae at one and 30 minutes. Other experiments, described below, failed to confirm that observation.

produced mild inflammation comparable to that evoked by saline alone in 25 recipient bursae; one recipient reacted with slightly elevated response of 7.1 million cells. In the two experiments, the 15-minute donor bursal washings contained inflammatory material, presumably endotoxin. Statistical analysis of both experiments indicated that the 15-minute washings evoked a greater degree of inflammation than did the two-hour washings ($p = .05$ and .01, respectively).

From these data, endotoxin seems to be fixed to cells, degraded, or removed from the synovial cavity within two hours and, as the inflammatory reaction evolved in the bursae, there was no conclusive evidence that a secondary autogenous inflammatory substance was released.

DISCUSSION

The study of the many interactions between host and Gram-negative bacteria or their endotoxins continues to intrigue biologists and physicians, and this interest seems properly placed. Because of the ubiquity of these organisms in air, water, and in the intestinal tract and the frequency with which some members of the group cause disease in man and animals, it seems reasonable to believe that unusual and effective host reactions have evolved to contain and destroy these organisms. An evaluation of these phenomena should contribute both to our understanding of disease and to our therapeutic efforts in Gram-negative infections.

The local inflammatory response so rapidly elicited by Gram-negative microorganisms has been studied episodically. Fruhman in recent studies has emphasized that peritoneal polymorphonuclear exudation occurs with amounts of endotoxin that are much smaller than with any other substance tested.[3] When large dosages of endotoxin are given intraperitoneally, Cohn has demonstrated that this exudation is paradoxically impeded, and the virulence of intraperitoneal staphylococci consequently enhanced.[15] The decrease in granulocyte migration with large doses of endotoxin has been confirmed by Fruhman.[3]

Our studies were performed in the knee joints of rabbits because of our interest in synovial inflammation. Also, since the rabbit has been so extensively used in studies of other reactions to endotoxin, comparisons could be made between these systemic responses and local inflammation produced by endotoxin. The synovial joint may be a particularly sensitive site for these studies, since the lining cells are loosely arranged and the capillaries of the synovium have been shown by electron microscopy to be peculiarly fenestrated, perhaps more readily permitting cellular migration.[16]

Several pertinent points emerge from our studies. We were unable to demonstrate a decreased local exudative response to intra-synovial endotoxin in animals that are hyporeactive or 'tolerant' to other reactions to endotoxin; indeed, these animals exhibited somewhat greater reactivity. Preservation of the local inflammatory response in tolerant animals makes good biologic sense. The phenomenon of tolerance has been demonstrated in most of the systemic endotoxin reactions: fever, leukopenia, hypotension, and the generalized Shwartzman reaction.[17] In these situations, tolerance is clearly of benefit to the host.

The disparity between local and generalized reaction to endotoxin in tolerance is perhaps more apparent than real. Tolerance is a state induced by repeated intravenous inoculations and involves mechanisms whereby endotoxin is more rapidly cleared from the bloodstream by cells of the RES.[18] Humoral factors are known to participate in this process, presumably acting as opsonins, though their nature and function is still unclear.[19] Since it is likely that antibodies play a role in producing tolerance to endotoxins, it is not surprising that tolerance does not interfere with the extravascular inflammatory response to these agents and, indeed, might enhance it. The local mobilization of leukocytes is well-known to be augmented where antibodies (either cellular or circulating) are present, as in the tuberculin and Arthus reactions, respectively. On the other hand, the reduction in systemic signs of inflammation (fever, leukocytosis, etc.) associated with tolerance to intravenously injected endotoxin, reflects a corresponding reduction in the effective dosage of this agent, brought about by its more rapid disposal.

If the local inflammatory response were important in localizing and destroying Gram-negative bacterial invaders, there would be little benefit to the host becoming hyporeactive to this effect of endotoxin. Consistent with this view, highly immunized animals retained their synovial reactivity to endotoxin.

Another pertinent and somewhat surprising observation is the minute quantity of purified endotoxin that could elicit a synovial inflammatory reaction. Six hours after injection of .00005 μg. of two different endotoxin preparations, rabbit joints often yielded 10 million polymorphonuclear leukocytes. This is approximately 100 million molecules of endotoxin, presuming a molecular weight of one million, or a 10:1 ratio of molecules of endotoxin to granulocytes. Even this ratio is probably conservative, since the polymorphonuclear inflammation persists for more than a day. Although it is not known how long a given exudative granulocyte can persist in a rabbit knee, human granulocytes in rheumatoid arthritic knees have a half life of three or four hours.[20] By analogy, it seems possible that

one molecule of endotoxin may call forth more than one polymorphonuclear leukocyte.

These quantitative considerations suggest that the endotoxin molecule sets off a series of local cellular inflammatory events, rather than directly mobilizing granulocytes into the synovial cavity. Other studies indicate clearly that endotoxin releases a large number of enzymes and other substances with biologic activity from various tissues—in part, perhaps, by its ability to rupture cell lysosomes. Recently, Moses and co-workers have shown that granulocytes themselves release materials that cause inflammation in the rabbit ear chamber.[21] Since synovial cells may be modified macrophages rich in cytoplasmic lysosomes, it seems quite plausible that these cells as well as granulocytes would release auto-inflammatory substances in response to endotoxin. Attractive as such speculations are, we could not demonstrate any auto-inflammatory materials in serum containing endogenous pyrogen or in synovial washings; endotoxin was rapidly removed or inactivated and no secondary agent capable of inducing inflammation could be detected by this rather sensitive system for assaying such a response. Recent observations in our laboratory indicate, however, that endogenous pyrogen prepared from rabbit peritoneal leukocytes incubated in saline can induce a significant inflammatory exudate in the knee joint. If these preliminary results are confirmed, a more determined search for *in vivo* activation of an auto-inflammatory cycle must be undertaken.

The extraordinary reactivity of the joint cavity to endotoxin has led to some speculations on the role of this or similar mechanisms in human arthritis. Most of the clinical enigmas of gonococcal arthritis and chronic meningococcemia could be explained by the potent endotoxins possessed by these organisms. The acute migratory arthritis often seen in these patients, with effusions that are almost invariably sterile, may represent an intense inflammatory reaction overwhelming the bacterial invader from the bloodstream. On the other hand, there is a protracted form of gonococcal arthritis that responds rather slowly to penicillin therapy although cultures become negative almost immediately, suggesting that endotoxin released from the dead organisms may sustain the inflammation. Braude has made a similar suggestion.[22] The arthritis associated with ulcerative colitis and regional enteritis, diseases in which the intestinal barrier to Gram-negative organisms is impaired, may be similarly related to endotoxin.

In a much broader area of speculation, Gram-negative bacterial endotoxin is not the only complex polysaccharide to which the body is exposed. Perhaps as an evolutionary necessity, the body appears to have developed a complex immunological and biochemical apparatus for degrading this

molecule.[7, 14] Other macromolecules that might gain access to the body may not be similarly susceptible to its enzymes, and could thus conceivably contribute to the pathogenesis of the more common inflammatory arthritic diseases. Though such speculations are far removed from the observations on endotoxin reported in this paper, they can perhaps be justified by the very paucity of speculations on the pathogenesis of such major clinical diseases as rheumatoid and psoriatic arthritis.

SUMMARY

The inflammatory reaction produced by endotoxin has been investigated in the suprapatellar bursae of rabbits. In extremely small amounts (.00005 μg.) purified endotoxin preparations were capable of eliciting a polymorphonuclear exudate in the bursae.

Because such small quantities of endotoxin produce a sustained inflammation (less than 10 molecules/PMN), it seemed likely that release of inflammatory products from tissues might play a role in this response. Yet serum and synovial washings from animals given endotoxin, obtained at a time when leukocyte pyrogen is known to be present, did not evoke inflammation.

Animals made tolerant or refractory to the pyrogenic action of endotoxin by repeated intravenous injections or by a single large injection, respectively, had undiminished inflammatory responses when endotoxin was injected into their bursae, as did animals with a specific humoral immunity. The preservation of the local tissue reaction to endotoxin in both tolerant and immune rabbits seems to be in keeping with the general biological importance of this reaction as a defense mechanism of the host against bacterial invasion.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Elizabeth Maloney and Miss Dorothy Maloney for their technical assistance.

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