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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ADA128488

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: AFRRI SR82-19	2. GOVT ACCESSION NO.: AD-A128688
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): HEMATOPOIETIC RESPONSES TO LIPOPOLYSACCHARIDE IN C57BL/10Sn AND C57BL/10ScN STRAIN MICE	5. TYPE OF REPORT & PERIOD COVERED
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): T. J. MacVittie, M. L. Patchen, and R. I. Walker* (cont. on reverse)	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Armed Forces Radiobiology Research Institute (AFRRI), Defense Nuclear Agency, Bethesda, Maryland 20814	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NWED QAXM, MJ 00029
11. CONTROLLING OFFICE NAME AND ADDRESS: Director, Defense Nuclear Agency (DNA), Washington, DC 20305	12. REPORT DATE: December 1982
	13. NUMBER OF PAGES: 15
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Published in Stem Cells 2:24-33, 1982.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Lipopolysaccharide - Hemopoiesis - CFU-s - C57BL/10ScN

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Inbred mouse strains C57BL/10Sn.(Sn) and C57BL/10ScN (ScN) differ in response of their hematopoietic system to injection of lipopolysaccharide-W (LPS-W) in a manner similar to that observed for the LPS unresponsive C3H/HeJ and the paired responsive C3H/HeN strain mice. Responses of endogenous (E-CFU) stem cells as well as bone marrow and spleen-derived exogenous (CFU-s) stem cells, granulocyte-macrophage (GM-CFC) and macrophage (M-CFC) colony-forming cells were determined for Sn and ScN strain mice

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20. ABSTRACT (continued)

following an intraperitoneal injection of 10μg LPS-W. Sn strain mice responded characteristically in terms of every parameter measured. Marrow-derived parameters reflected release of nucleated cells and early decrease of CFU-s, GM-CFC, and M-CFC followed by return toward control values. Peak splenic responses were observed within 4-5 days, whereas E-CFU increased significantly within 24 hours after LPS-W. These responses were in marked contrast to those observed for the ScN strain mice, which were relatively unresponsive in terms of each parameter measured after LPS-W injection. These results show that the phenotypic expression of the defective LPS locus recently described in the ScN strain mice extends to those cells that control the response of the hematopoietic system to LPS, and is similar in every respect to those responses observed for the mutant LPS defective, C3H/HeJ strain mice.

Stem Cells

Editor-in-Chief: M.J. Murphy, Jr., Dayton, Ohio
Publishers: S. Karger, Basel
Reprint (Printed in Switzerland)

Stem Cells *2*: 24–33 (1982)

Hematopoietic Responses to Lipopolysaccharide in C57BL/10Sn and C57BL/10ScN Strain Mice

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Key Words. Lipopolysaccharide · Hemopoiesis · CFU-s · C57BL/10ScN

Abstract. Inbred mouse strains C57BL/10Sn (Sn) and C57BL/10ScN (ScN) differ in response of their hematopoietic system to injection of lipopolysaccharide-W (LPS-W) in a manner similar to that observed for the LPS unresponsive C3H/HeJ and the paired responsive C3H/HeN strain mice. Responses of endogenous (E-CFU) stem cells as well as bone marrow and spleen-derived exogenous (CFU-s) stem cells, granulocyte-macrophage (GM-CFC) and macrophage (M-CFC) colony-forming cells were determined for Sn and ScN strain mice following an intraperitoneal injection of 10μg LPS-W. Sn strain mice responded characteristically in terms of every parameter measured. Marrow-derived parameters reflected release of nucleated cells and early decrease of CFU-s, GM-CFC, and M-CFC followed by return toward control values. Peak splenic responses were observed within 4–5 days, whereas E-CFU increased significantly within 24 h after LPS-W. These responses were in marked contrast to those observed for the ScN strain mice, which were relatively unresponsive in terms of each parameter measured after LPS-W injection. These results show that the phenotypic expression of the defective LPS locus recently described in the ScN strain mice extends to those cells that control the response of the hematopoietic system to LPS, and is similar in every respect to those responses observed for the mutant LPS defective, C3H/HeJ strain mice.

Injection of lipopolysaccharide (LPS) elicits a variety of endotoxic, inflammatory, immunogenic, and hematopoietic responses within the animal. The variety of cell types involved in eliciting these reactions have made it difficult to understand the regulatory mechanisms involved. Recent experiments with the mutant C3H/HeJ and C57BL/10ScN strain mice have indicated that many of these responses to the LPS molecule are initiated by a common mechanism present in a variety of responsive cell

types. A number of studies have shown that, relative to response of normal C3H substrains, the hematopoietic system of the C3H/HeJ strain is markedly unresponsive to LPS in terms of plasma colony stimulating activity (CSA) [1–3] and humoral factors [4, 5], splenic and marrow stem cells, granulocyte-macrophage and macrophage colony-forming cells [1, 3, 6–8] and delayed mobilization of stem cells [9]. Few studies, however, have examined the extent of the phenotypic expression of a similar mutation in the C57BL/10ScN and C57BL/10ScCR strain mice [10–13]. To date, the C57BL/10ScN and derived C57BL/10ScCr strains have been shown to be unresponsive to the LPS molecule in terms of lethality, B-cell mitogenicity [10–13], glucose utilization, in vitro production of PGE_2 and LAF by macrophages, and in vivo production of acute-phase serum reactive protein [12, 13]. Recently *Benner* et al. [9], investigating hematopoietic responses, showed that the delayed mobilization and splenic accumulation of stem cells require the LPS receptor in both the C57BL/10ScN and C3H/HeJ strain mice. The studies reported here concerned the effect of the LPS mutation on the hemopoietic system of the C57BL/10ScN in comparison to the normal C57BL/10Sn strain mice, as measured by endogenous (E-CFU) and exogenous (CFU-s) stem cells and committed granulocyte-macrophage (GM-CFC) and macrophage (M-CFC) progenitor cells in response to a single injection of LPS-W.

Materials and Methods

Animals, Cell Suspensions, and Culture Technique

Femoral bone marrow (BM) and spleen (SPL) cells were obtained from male or female mice, 8–12 weeks old, of the strains C3H/HeN (Charles River Labs., Wilmington, Mass.), C3H/HeJ, C57BL/10Sn (Jackson Laboratories, Bar Harbor, Me.), and C57BL/10ScN (National Institutes of Health, Bethesda, Md.). Cell suspensions were prepared as previously described [14]. The double-layer agar culture technique used for detection of GM-CFC and M-CFC has been outlined in detail [14]. Pregnant mouse uterine extract (PMUE) and mouse L-cell-conditioned medium (LCM) were used as sources of CSA. Exogenous stem cells (CFU-s) were determined by the method of *Till and McCulloch* [15]. Endogenous stem cells (E-CFU) were determined by counting macroscopic spleen colonies from control and experimental mice at 9 days after 650 rad total-body irradiation.

LPS

Experimental animals were each injected intraperitoneally with 10 μg of lipopolysaccharide-W (LPS-W) derived from *Escherichia coli* 055:B5 (List Biological Lab., Inc., Campbell, Calif.) in 0.5 ml pyrogen-free saline. Control mice were injected intraperitoneally with 0.5 ml of pyrogen-free saline. Lipid A prepared by acid hydrolysis of *Salmonellla*

minnesota Re 595 lipopolysaccharide (List Biological Labs) was rendered soluble by addition of 0.5% triethylamine (TEA), which was subsequently diluted with pyrogen-free saline. A 0.5% TEA solution in saline was used as control solution.

Endotoxin Lethality

Groups of 5 age-matched C57BL/10Sn and C57BL/10ScN strain mice were injected intraperitoneally with selected doses of LPS-W. Three replicate experiments were performed. Deaths were recorded over a 4-day period.

Statistics

Data presented represent the mean values ± SEM of five replicate experiments. Cell suspensions from femurs and spleens of 2 mice were pooled for determination of each datum point for cellularity and exogenous assays, while 6 mice per variable were used for the endogenous spleen colony assay within each experimental replicate. Student's two-tailed t test was used for determining statistical significance of mean values.

Results

Effect of LPS-W on Femoral and Splenic Cellularity

Femoral cellularity in C57BL/10Sn (Sn) strain mice decreased significantly ($p < 0.005$) from control values within 24 h and remained so through 72 h after injection of LPS-W (table I). Similarly treated C57BL/10ScN (ScN) strain mice did not experience a significant decrease in femoral cellularity.

Splenic cellularity in the responsive Sn strain rose steadily after injection of LPS-W to reach peak values by the 4th day (table I). The ScN strain was unresponsive in terms of splenic cellularity.

Effect of LPS-W and Lipid A on Endogenous CFU (E-CFU)

E-CFU in the responsive Sn strain increased approximately 25- and 9-fold over respective controls within 24 h after injection of LPS-W and lipid A, respectively, while spleen weights were approximately doubled (table II). The ScN strain mice were relatively unresponsive in terms of E-CFU and spleen weight to either LPS-W or lipid A.

Effect of LPS-W on Femoral and Splenic Content of CFU-s, GM-CFC, and M-CFC

The femoral and spleen-derived CFU-s, GM-CFC, and M-CFC of Sn strain mice responded characteristically to the single injection of LPS-W (fig. 1–3). Femoral content of all three cell types decreased significantly from control values within 24 h, remained at these levels at least through

Table I. Alterations in bone marrow and spleen cellularity[a] after injection of 10 µg of LPS-W into C57BL/10Sn (Sn) and C57BL/10ScN (ScN) strain mice

Time h	Bone marrow		Spleen	
	Sn	ScN	Sn	ScN
0	2.11 ± 0.23	2.17 ± 0.19	13.57 ± 1.31	18.04 ± 1.41
24	1.03 ± 0.18[b, c]	2.04 ± 0.12	14.18 ± 1.43	19.44 ± 1.57
48	1.00 ± 0.10[b, c]	1.78 ± 0.13	17.40 ± 2.00	18.00 ± 2.04
72	1.35 ± 0.14[b, c]	2.25 ± 0.16	20.93 ± 1.90[c]	17.55 ± 1.97
96	1.90 ± 0.18	2.07 ± 0.16	25.19 ± 1.50[b, c]	14.55 ± 1.20
120	2.04 ± 0.20	2.09 ± 0.21	25.69 ± 1.77[b, c]	16.17 ± 1.34
168	1.89 ± 0.17	1.92 ± 0.23	23.36 ± 1.45[b, c]	14.48 ± 1.24

[a] Mean values × 10^7 (± SEM) of five replicate experiments; bone marrow is equivalent to one femur; spleen is total organ content. 3 mice per time point per experiment were used.
[b] Mean values differ significantly from their counterpart value (Sn versus ScN), $p < 0.001$.
[c] Mean values differ significantly from their respective control values, $p < 0.001$.

Table II. Endogenous stem cell response[1] and spleen weight[1] in C57BL/10Sn (B10/Sn) and C57BL/10ScN (B10/ScN) strain mice 24 h following a single injection of LPS-W[2] or lipid A[2]

	LPS-W		Lipid A	
	4	10 µg	0	10 µg
B10/Sn				
E-CFU	1.4 ± 0.3	37.0 ± 4.1	2.3 ± 0.6	23.0 ± 2.5
Spleen weight, mg	34.0 ± 3.0	68.0 ± 7.0	33.0 ± 3.0	61.0 ± 5.0
B10/ScN				
E-CFU	1.6 ± 0.4	3.6 ± 0.8	1.5 ± 0.4	2.1 ± 0.4
Spleen weight, mg	30.0 ± 4.0	36.0 ± 4.0	35.0 ± 5.0	32.0 ± 3.0

[1] Mean values (± SEM) of five replicate experiments; six spleens per replicate; mice received 650 rad TBI.
[2] LPS-W from *E. coli* 055:B5; lipid A from Re 595 *S. minnesota*, solubilized in 0.5% TEA.

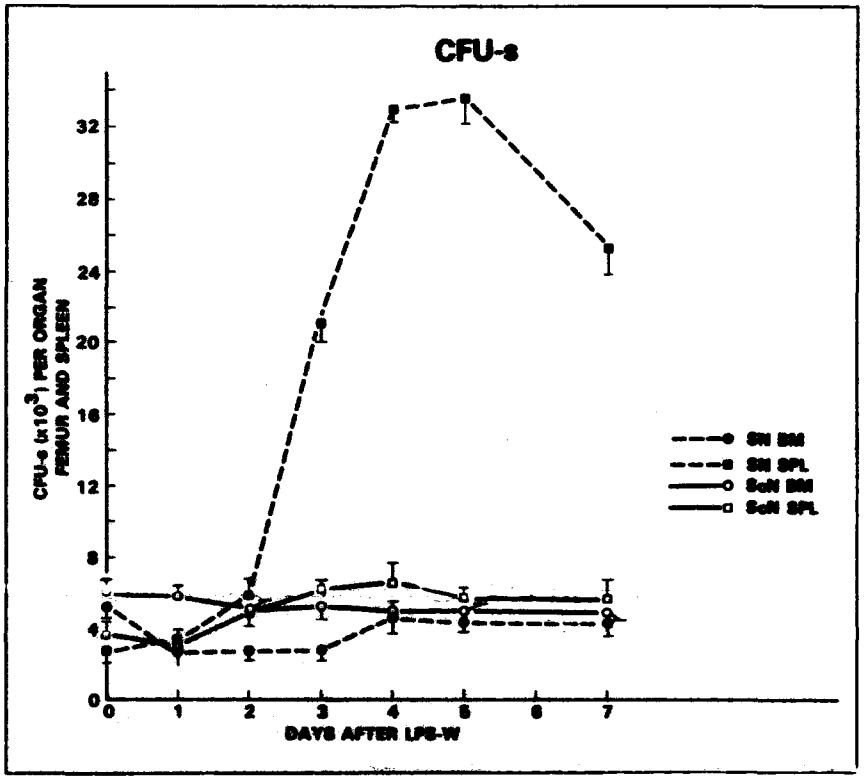

Fig. 1. Number of CFU-s per femur and spleen in C57BL/10Sn and C57BL/10ScN mice at various times after intraperitoneal injection of 10 μg of *E. coli* 055:B5 LPS-W. Mean values (± SEM) of five replicate experiments.

72 h, and returned toward control levels. Femoral content of CFU-s and GM-CFC in the ScN strain did not differ from control levels, whereas the M-CFC decreased over the 7-day period to 80% of control.

Splenic content of CFU-s, GM-CFC, and M-CFC in the responsive Sn strain mice rose to respective peak values of 13-, 120-, and 14-fold control within 4–5 days after LPS-W injection (fig. 1–3). In marked contrast, the CFU-s and GM-CFC of the ScN spleen were relatively unresponsive. The M-CFC, however, repeated the quantitative and qualitative response recently observed in the C3H/HeJ strain mice [3]. The M-CFC of ScN spleens rose within 24 h to values approximately 230% of control, remained elevated through 72 h, and then decreased to within control levels (fig. 3).

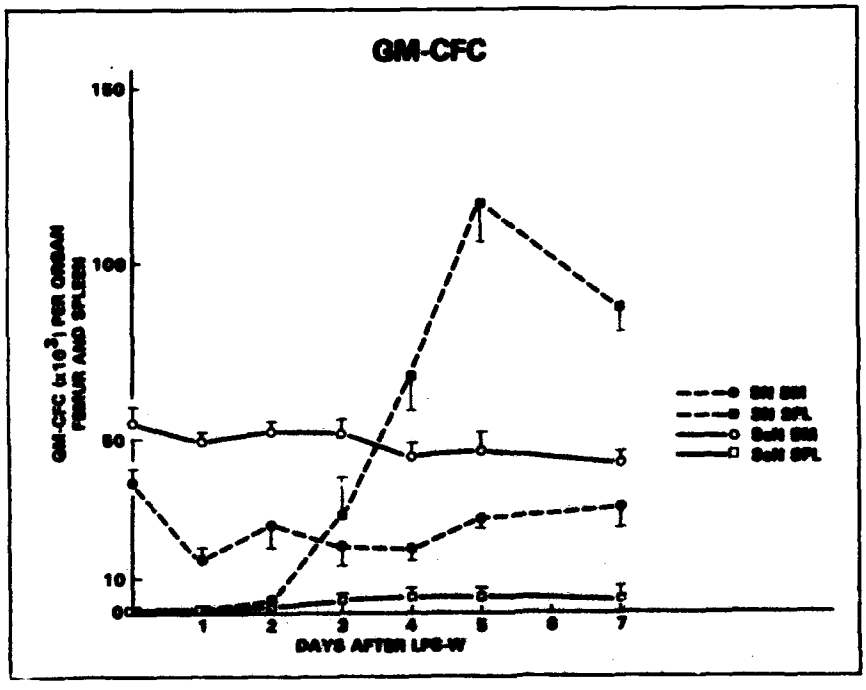

Fig. 2. Number of GM-CFC per femur and spleen in C57BL/10Sn and C57BL/10ScN mice at various times after intraperitoneal injection of 10 μg of *E. coli* 055:B5 LPS-W. Mean values (± SEM) of five replicate experiments.

Endotoxin-Induced Lethality

A dose of 220 μg LPS-W was lethal for 50% of the Sn strain mice, whereas 2,500 μg was required for 50% lethality in the ScN strain. Respective doses resulting in 100% lethality were 250 and 3,500 μg for the Sn and ScN strains.

Discussion

The mutation in the ScN strain mouse renders it hematopoietically unresponsive to low doses of LPS-W in a manner qualitatively identical to that observed in the hematopoietic system of the mutant C3H/HeJ strain mouse [1, 3, 6–9]. *Benner* et al. [9] recently showed that the normal delayed accumulation of CFU-s in the peripheral blood and spleen does not occur in the C57BL/10ScCR and C3H/HeJ strain mice. Our data confirm the

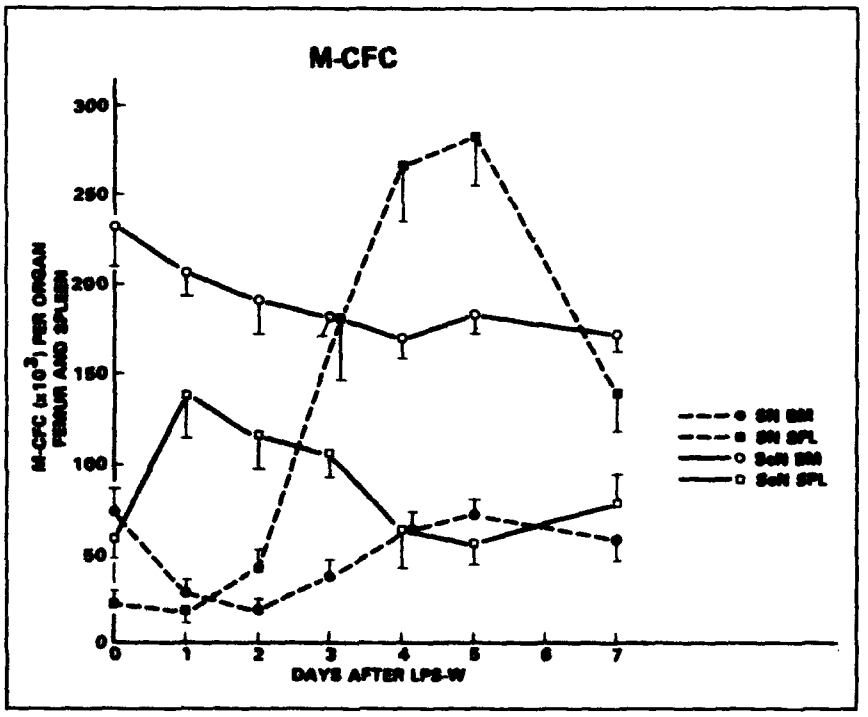

Fig. 3. Number of M-CFC per femur and spleen in C57BL/10Sn and C57BL/10ScN mice at various times after intraperitoneal injection of 10 μg of *E. coli* 055:B5 LPS-W. Mean values (± SEM) of five replicate experiments.

absence of accumulation of exogenous CFU-s in the spleen of the mutant ScN strain and also extend the phenotypic expression of the mutation to include defective responses within the splenic GM-CFC, M-CFC, and endogenous stem cell populations. The differential response pattern observed for the M-CFC population – that is, the early increase in the spleen and decreased number in the femur – mimics the similar response observed for this cell in the C3H/HeJ strain mice [3]. The response of the M-CFC in the mutant ScN and HeJ strains is perplexing. At present we can only speculate that a similar mechanism is operable within both strains [3]. Increases in M-CFC responses in the mutant HeJ to LPS have been observed in the spleen, circulation, and peritoneal exudate [3, 16]. The splenic increase may be the result of trapping M-CFC released from the marrow into the circulation with subsequent differentiation and loss of numbers within the splenic tissue [3]. Circulating numbers of M-CFC are increased in the HeJ

to the same relative degree as those in the responsive C3HeB/FeJ after a dose of endotoxin [3]. The peritoneal M-CFC response of the HeJ strain was characterized by a more rapid influx of M-CFC into the cavity than in the paired C3HeB/FeJ, although resultant peak values were similar in both strains [16]. A similar response has been observed in the ScN strain relative to its paired Sn strain [unpubl. observations]. In addition to this responsive pattern of the M-CFC in the mutant HeJ and ScN strains, a consistent, significantly greater concentration and total content of M-CFC exist in the organs of the control, mutant HeJ [16] and ScN mice relative to their paired C3HeB/FeJ, C3H/HeN, and Sn strains. Basal levels of CFU-s and GM-CFC are equivalent between the paired strains. These are interesting observations that require further study.

It is also apparent from these data that nucleated cells are not released from the marrow compartment to any significant degree and that the stem and progenitor cell populations in the marrow of the ScN mice are unaffected at the 10-μg dose level. These mutational effects are qualitatively, if not quantitatively, similar in many aspects to those responses recently described for the hematopoietic system of the C3H/HeJ strain mice [1–3, 6–9]. Yet to be determined is whether (a) the defective locus is expressed through a lack of surface receptors necessary for LPS reactivity [17–19], or (b) equivalent binding of the LPS molecule to reactive target cells takes place, but the defect lies in a consequent triggering event [10]. Whichever mechanism is invoked, the result is most probably the lack of synthesis and release of specific factors such as CSA and/or other regulatory molecules (lymphokines and/or monokines) capable of initiating proliferation and amplification of stem cells and progenitor cells.

As indicated in a recent report [3], the previously mentioned results and the data reported herein imply not that the mutant animal cannot respond to the LPS complex, but only that the mutant animal does not initiate the normal events in response to LPS in the low dose range used. The method of preparation or extraction of the LPS molecule as a bacterial cell wall component is of prime concern when considering its interaction with the LPS receptor and the consequent effects. Several laboratories have confirmed the results of *Skidmore* et al. [21] and *Sultzer and Goodman* [22], who demonstrated that (a) the unresponsive state of the C3H/HeJ strain mice, relative to LPS-induced B-cell mitogenesis, depended on the purity of the LPS molecule, and hence the extraction method used relative to its associated protein components, and (b) the associated protein was a potent mitogen and lymphocyte activator. These aspects have recently been em-

phasized relative to the hemopoietic effect of lipid A and LPS complexes by *Staber* et al. [4, 23], *Staber and Metcalf* [5], and *Benner* et al. [9]. Using a purified preparation of LPS, *Benner* et al. [9] showed that a functional LPS receptor is necessary for LPS-induced CFU-s accumulation in blood and spleen. However, this does not imply that the defective locus may not be bypassed or overridden by the presence of associated protein or other bacterial cell wall components held within the impure LPS molecule (phenol or butanol preparations) or as it is seen within killed gram-negative bacteria or during the infectious process.

Acknowledgements

Supported by Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Research Work Unit MJ 00029. The views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. The authors gratefully acknowledge the excellent technical assistance of Mr. *James Atkinson*, Mr. *Daniel Dodgen*, and Mr. *Richard Brandenburg*, as well as the professional and editorial assistance of Dr. *S.J. Baum* and Ms. *Junith Van Deusen* in the preparation of this manuscript. We also acknowledge the excellent typing of Ms. *Marianne Owens*.

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Received: October 13, 1981; accepted: April 1, 1982

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