New Phenotypic Marker for Lipopolysaccharide Responsiveness

SASTRY V. S. GOLLAPUDI, STEPHEN H. GREGORY, AND MILTON KERN*

Laboratory of Biochemistry and Metabolism, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205

Lipopolysaccharide-enhanced secretion of non-immunoglobulin proteins by bone marrow cells derived from responder, nonresponder, and low-responder mouse strains did not precisely correlate with the lipopolysaccharide responsiveness assessment based on the mitogenic reactivity of splenocytes. These findings suggest that enhancement of secretion of non-immunoglobulin protein may be useful for further characterization of lipopolysaccharide responsiveness.

Several strains of mice have now been identified which share the property that their B-cell populations do not exhibit the usual mitogenic response to lipopolysaccharide (LPS) (5, 14, 17). Genetic tests have established that mouse strains C3H/HeJ and C57BL/10ScCR are identical with regard to chromosomal location of the gene involved (6, 18) and that mouse strains C3H/HeJ and CBA/N are not identical with regard to the gene involved (1, 11). The genetic site for unresponsiveness to LPS in C57BL/10ScN mice remains unknown at this time.

It must be emphasized that nonresponder mice are simultaneously defective in other cellular responses to LPS besides that known for B-cell mitogenesis (8, 19). Furthermore, at least three kinds of cells have been shown to respond to LPS, i.e., B cells (2), macrophage cells (9), and fibroblasts (3). Therefore, the possibilities for expansion of phenotypes is great and should prove to be of considerable use in evaluating similarities and differences in nonresponder strains. In this report we present evidence establishing a new phenotype.

Recently, we observed that LPS enhances the secretion of non-immunoglobulin proteins by freshly isolated bone marrow cells during a 4-h incubation period (6a). It was established that the pertinent cells reside in bone marrow and are not detectable elsewhere, e.g., liver, spleen, thymus, lymph node, peritoneal exudate, reticulocytes, leukocytes, erythrocytes, appendix, and testis. The selectivity and nature of the stimulation have not been reported heretofore and therefore provide a novel way to measure the effect of LPS and, perhaps more importantly, establish a new phenotype.

The femur and tibia of mice (6 to 8 weeks of age) of the indicated strains were used as a source of bone marrow cells. The cells were collected in medium 199 containing 10% fetal

calf serum, penicillin (35 μ g/ml), and 0.02 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) at pH 7.5 (20, 22). Splenocytes were isolated by teasing the tissue apart with wire brushes (16). [3 H]thymidine incorporated into DNA was determined as previously described (21). LPS derived from *Salmonella abortus-equi* was purchased from Difco Laboratories, Detroit, Mich. Information supplied by the manufacturer indicated that it was prepared by hot phenol extraction. Secreted 3 H-proteins were rendered free of 3 H-immunoglobulin by adding carrier mouse immunoglobulin and antibody with specificity for mouse immunoglobulins. The non-immunoglobulin 3 H-protein was then isolated by removing the specific precipitate.

The secretion of proteins other than immunoglobulins by bone marrow cells of mouse strain C3H/HeN was markedly enhanced by incubation with LPS (Fig. 1A). In contrast, bone marrow cells from strain C3H/HeJ did not exhibit LPS-enhanced secretion (Fig. 1B). Although C3H/HeJ splenocytes are nonresponsive to the mitogenic activity of LPS, some LPS preparations contain a peptide contaminant which is mitogenic for C3H/HeJ (10, 15). Confidence that the LPS used in the present study was free of contaminating mitogenic agent was supported by the fact that C3H/HeJ spleen cells were nonresponsive toward the LPS employed (Fig. 2). Consequently, a survey of mouse strains for LPS enhancement of secretion of non-immunoglobulin proteins by bone marrow cells was performed (Table 1) and compared with the known pedigree of these strains as determined by splenocyte mitogenesis (5, 14, 17). Non-immunoglobulin protein secretion by all nonresponder strains was not enhanced by LPS, whereas the corresponding responder strains (C3H/HeN and C57BL/10J) exhibited enhance-

FIG. 1. Bio-Gel A-0.5m chromatography of secreted proteins. After washing with leucine-free medium 199 buffered with 0.02 M HEPES at pH 7.5, 10^7 cells were incubated for 4 h at 37°C in cell-washing medium supplemented with 10 μ Ci of [3 H]leucine and, where indicated, with 10 μ g of LPS in a total volume of 1.0 ml. At the end of the incubation period, cells were removed by centrifugation at 600 $\times$ g for 10 min, and the extracellular fluid was concentrated by vacuum dialysis against 0.15 M NaCl-0.01 M phosphate buffer at pH 7.4 (buffered saline). When dialysis was complete, 3 H-immunoglobulin was removed by coprecipitation; that is, carrier mouse immunoglobulin and antibody with specificity for mouse immunoglobulin were added, and the mixture was incubated overnight at 4°C. The specific precipitate then was removed by centrifugation at 600 $\times$ g for 10 min to yield 3 H-proteins free of 3 H-immunoglobulins. Batch preparations of such proteins prepared in the presence (filled symbols) and absence (open symbols) of LPS were applied to a Bio-Gel A-0.5m column (93 by 1.6 cm) previously equilibrated with buffered saline. After elution with buffered saline, samples of the eluate were assessed for radioactivity. In panel A bone marrow cells from the responder strain C3H/HeN were used, and in panel B the nonresponder strain C3H/HeJ was the source of cells.

FIG. 2. Effect of the concentration of LPS on thymidine incorporation. Filled symbols correspond to splenocytes derived from C3H/HeN, and open symbols correspond to splenocytes derived from C3H/HeJ.

TABLE 1. Comparison of mouse strains for LPS-mediated enhancement of secretion by bone marrow cells^a

Mouse strain	Pedigree ^b	Secretion enhancement ^c (cpm)	
		Without LPS	With LPS
C3H/HeJ	LPS nonresponder	17,300	18,300
C57BL/10ScCR	LPS nonresponder	40,400	30,100
C57BL/10ScN	LPS nonresponder	17,600	17,900
C3H/HeN	LPS responder	47,300	136,800
C57BL/10J	LPS responder	20,800	38,100
CBA/N	LPS low responder	48,000	143,000
CBA/J	LPS responder	41,900	40,400
CBA/CAHN	LPS responder	17,100	18,400

^a The procedure for determining the enhancement of secretion of non-immunoglobulin proteins by bone marrow cells is given in the legend to Fig. 1.

^b The pedigree is based on the LPS-mediated mitogenic response of splenocytes, and all pedigrees described in the literature have been confirmed by assessment using a preparation of *S. abortus-equi*. The LPS low-responder strain (CBA/N) is a B cell-deficient strain whose characteristics are defined in the text.

^c Results are given for the total radioactivity in the first major peak shown in Fig. 1.

ment. Unexpected results were obtained with mice of the CBA strain in that the mitogenic responder strains CBA/J and CBA/CAHN did not exhibit enhanced secretion. In contrast, enhancement of secretion by CBA/N bone marrow cells was readily detected despite the fact that CBA/N was a low-responder strain as determined from splenocyte mitogenesis. In this context, it is noteworthy that CBA/N splenocytes display somewhat unique characteristics in that in addition to exhibiting a low mitogenic responsiveness to LPS (11), the cells react poorly to other B cell-reactive agents like pneumococcus polysaccharide (1) and dinitrophenyl-Ficoll (13) and possess a more immature B cell population based on their surface immunoglobulins (12).

These results demonstrate a new phenotype for study of LPS responsiveness. Although the relationship of secretion enhancement to other measurements of LPS reactivities remains obscure, it is worth emphasizing that nonresponder mice, assessed on the basis of splenocyte mitogenesis, also are known to be nonresponsive to a variety of other LPS-related reactivities (19). Although this generalization holds for many biological tests, there are also some exceptions in which nonresponders, assessed via a splenocyte mitogenesis test, behaved as responders with regard to other biological tests (4, 7). In this sense the enhanced secretion shown by the low-responder strain CBA/N is not unusual. The finding herein that splenocyte mitogenic responders (CBA/J and CBA/CAHN) are nonre-

sponsive to LPS by another test has not been reported heretofore. At this juncture, we do not know whether the present finding is due to the existence of an unusual locus for responsiveness or nonresponsiveness toward LPS in these strains or is due to a low concentration of the pertinent cell within bone marrow populations such that the detection is not possible with the technique employed.

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