LIPOPROTEIN CHANGES INDUCED BY BACTERIAL LIPOPOLYSACCHARIDES IN 'NON-RESPONDER' C3H/HeJ MICE

T. C. BØG-HANSEN*, Birgit HERTEL-WULFF** and U. BACK+,†

*The Protein Laboratory, University of Copenhagen, Denmark, **The Immunobiology Laboratory, Blood Grouping Department, The State Serum Institute, Copenhagen, Denmark and +The Collaboratory Centre for Reference and Research on Escherichia coli (WHO), The State Serum Institute, Copenhagen, Denmark

Received 12 March 1979
Revised version received 26 June 1979

1. Introduction

Bacterial lipopolysaccharides (LPS, endotoxins) cause many physiologic changes in mammals [1], except in one outstanding mouse strain, C3H/HeJ, that has been shown to be resistant towards LPS. Its unresponsiveness is genetically determined by a single autosomal dominant gene [2], and includes the following normal aspects of endotoxicity: polyclonal B-cell activation [3], adjuvant action [4], mitogenicity [2], endotoxic shock [5], enhancement of non-specific resistance to infections [6], polymorphonuclear leukocyte changes [7], protection against X-irraditation [8], inhibition of tolerance induction [9], and induction of glucocorticoid antagonizing factor [10].

We have been interested in the immediate LPS detoxifying potential of weakened mammals in relation to the pathogenicity of LPS, and we showed that the plasma HDL (high density lipoprotein)-associated arylesterase [11] binds to LPS in vitro [12] and is induced in vivo in SPF (specific pathogen-free) mice in concert with characteristic lipoprotein changes [13]. These findings may strengthen the views of Skarnes on the immediate enzymatic degradation of LPS by lipoprotein esterase [14], but on the other hand there is little – if any – direct evidence of degradation of LPS, as reviewed in [15].

We chose the C3H/HeJ non-responder strain for tests of the immediate reactions towards LPS, and this paper shows that LPS induces characteristic changes in this strain in plasma lipoprotein (HDL) in addition to a considerable increase of HDL-associated arylesterase activity. We conclude that lipoprotein is part of the general response against LPS and that C3H/HeJ is not a non-responder as concerns this response.

2. Experimental procedures

2.1. *Endotoxins*

E. coli type 026 LPS (trichloroacetic acid-extracted) and *E. coli* type 055:B5 LPS (phenol-extracted) were obtained from Difco Laboratories, Detroit, MI, USA. *E. coli* type 0124 LPS was prepared by phenol extraction as described in [13]. LPS was administered by intraperitoneal and intravenous injection of specified amounts of LPS in 0.2 μl saline.

2.2. *Mouse strains*

Strains were kept at conventional conditions at the State Serum Institute: C3H/HeJ/Ssc, C3H/FuAa/Ssc, and C57/B16/Ssc. The reported experiments are based upon experience with about 100 LPS non-responder C3H/HeJ mice. The non-responsiveness of the C3H/HeJ was determined according to the lacking of mitogenicity of phenol-extracted LPS (055 and 0124). Figure 1 shows the in vitro proliferative response towards LPS (026, 055, and 0124) as well as towards PHA (see 2.4) of spleen cells from C3H/HeJ and C57/B16.

†U. Back died on October 2, 1977

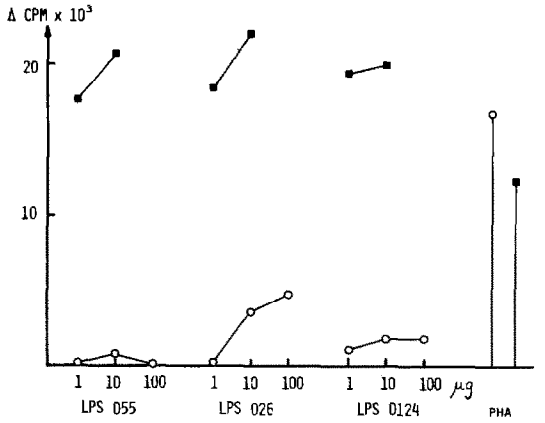

Fig.1. Mitogen-induced DNA synthetic response of C3H/HeJ (○) and C57/B16 (■) spleen cells. Δ cpm values given after substraction of cpm obtained in cultures without addition of any stimulator. The cpm values of non-stimulated C3H/HeJ or C57/B16 cultures were 2256 ± 142 cpm or 1999 ± 698 cpm, respectively. The maximum response on day 3 of culture with LPS extracts, and the maximum response on day 2 with PHA is given. The stimulation shown here with LPS in C57/B1 and with PHA (both strains) is within the range normally observed in our experiments [18].

2.3. *Preparation of lymphocyte suspensions*

Single cell suspensions were made from spleens as described previously [16,17].

2.4. *In vitro functional assay*

The in vitro lymphoproliferative response was performed as described previously [18]. Shortly, 2×10^5 lymphocytes were cultured in 0.2 ml of RPMI 1640 medium with or without LPS or PHA (phytohemagglutinin) for from 1 to 6 days at 37°C and 5% CO_2, the last 18 h in the presence of [^{3}H]-thymidine. Incorporation of labelled thymidine was determined by liquid scintillation counting.

2.5. *Analyses*

Crossed immunoelectrophoresis and visual estimation of HDL-associated arylesterase [11] was performed as described earlier [13]. Rabbit immunoglobulins for immunoelectrophoresis of mouse serum proteins were prepared from 5 rabbits immunized with small doses of mouse serum according to [19] and were concentrated 6 times relative to the antiserum.

Results

LPS was given to C3H/HeJ mice by intravenous and intraperitoneal injections and we analyzed the immediate reactions in plasma by crossed immunoelectrophoresis. We noted a characteristic shift in the lipoprotein pattern. Figure 2 shows details of the shifts of HDL and HDL-associated arylesterase of mice treated with LPS 026. Figure 2A is the normal pattern found in untreated mice. HDL is visualized not by lipid staining, but by specific staining for the HDL-associated arylesterase (A). Only the anodal part of the gel is shown and unspecific carboxylic esterase ('B-esterase' [20], B) and α_2-globulins (stained by unspecific dye-uptake, G) are taken as fix-points. Cholinesterase ('C-esterase' [20]) is very weak and not seen here in this anodal part of the gel.

Individual mice were killed and the blood was taken for analysis. Figure 2B–D shows the pattern 4, 8, and 32 h after intraperitoneal injection of LPS. After 4 h there appears an extra peak of HDL (fig.2B, arrow). This peak also carries arylesterase activity. After 8 h there is a complete shift of the HDL towards the cathode, and the shift becomes more and more pronounced until the HDL peak appears between the B-esterase and the α_2-globulins (fig.2D). There is still an extra peak on the cathodic side of HDL after 32 h. After about 2 days the HDL reappears in the normal position on the anodic side of B-esterase (not shown).

When we judged the staining intensity of arylesterase visually (fig.1) we found an increase in the activity after LPS treatment with a maximum about 24 h after challenge. A quantitative analysis for arylesterase in mouse plasma is desirable, but not easily feasible because the overlapping activities of A and B-esterases.

To ascertain that these changes were not effects of contaminating mitogenic protein in LPS 026 we tested a similar commercial phenol-water extracted LPS (*E. coli* 055) and our own LPS phenol-extracted from *E. coli* type 0124. We found similar changes induced with these LPS in the electrophoretic properties of HDL. Also the arylesterase activity increased considerably as estimated visually to give a maximum one day after LPS treatment.

Several doses were tested. Figure 2 is characteristic of the pattern observed with intraperitoneal injections

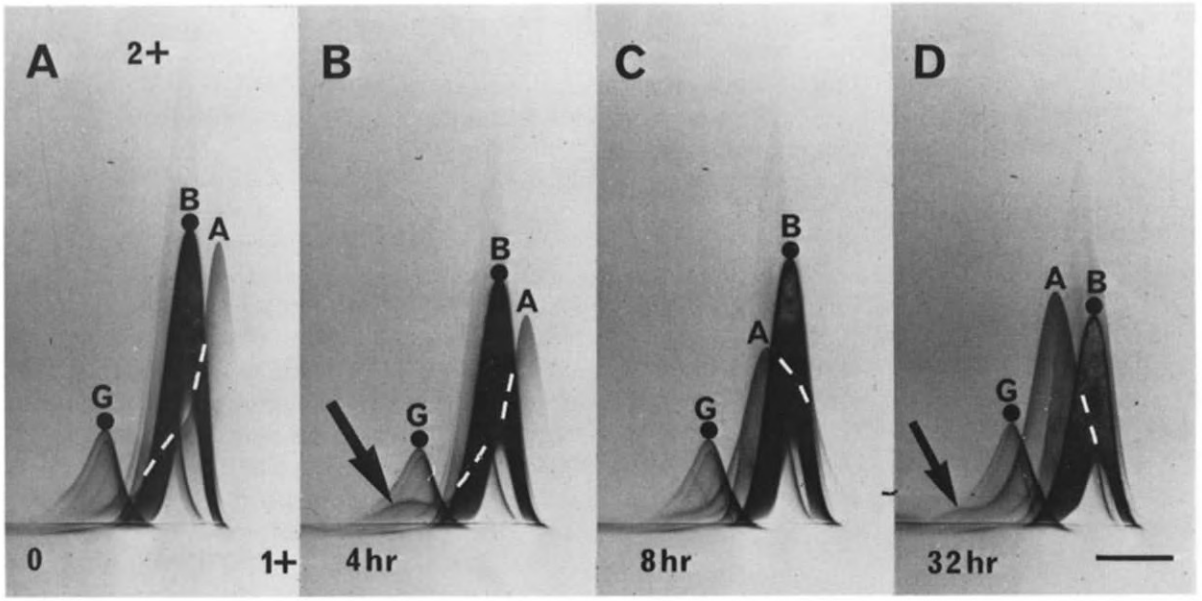

Fig.2. Crossed immunoelectrophoresis of serum from C3H/HeJ mice treated with endotoxin (enzyme-stained plates, only anodal part is shown). The time after LPS challenge (250 μl LPS type 026 given intraperitoneally) is indicated for each serum. The arylesterase activity outlines the HDL precipitate (marked with A, dashed lines and arrows). B and G (•) signify carboxylic esterase (B-esterase) and α_2-globulins respectively. The amount of serum analyzed was 0.5 μl and 1 μl antibody preparation was used per cm^2. The first and the second dimension electrophoresis are indicated by 1+ and 2+. The bar indicates 1 cm.

of LPS in doses that give a lethal response in other strains of mice (e.g., 1–3 times the LD_{50} in C3H/Fu responder mice and C57/B16). However, for each LPS there was a characteristic time-dependent electrophoretic shift of HDL. When the LPS was given by intravenous injection, the time course of the lipoprotein response was much faster but the same general features of the electrophoretic shifts and the increase of arylesterase activity were maintained.

Crossed immunoelectrophoretic analysis allows

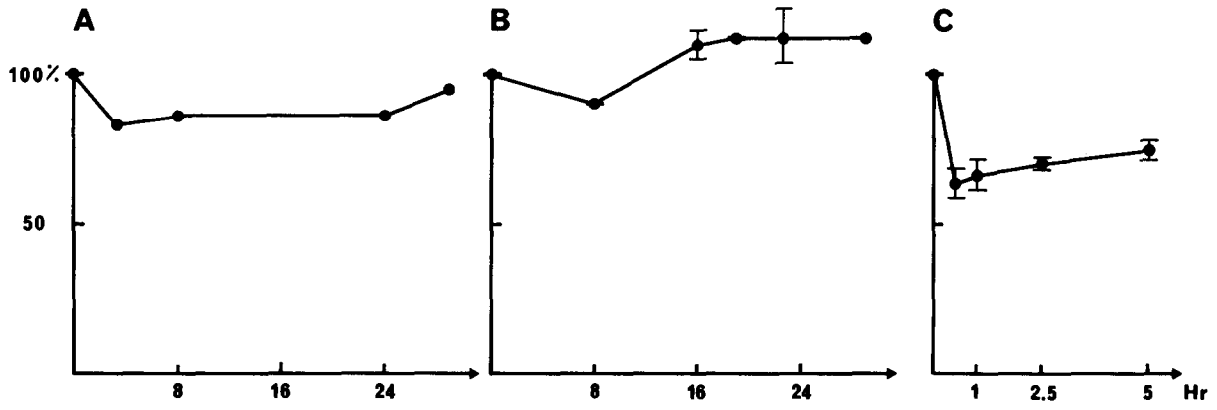

Fig.3. Time course of the relative HDL changes in C3H/HeJ mice after challenge with LPS type 026 (A, 250 μg given intraperitoneally), LPS type 0124 (B, 250 μg given intraperitoneally), and LPS type 0124 (C, 100 μg given intravenously). The area of the HDL precipitate (roughly proportional to the HDL concentration) was measured by planimetry. Each point represents one or two animals and the range of measurements is indicated. The HDL in untreated animals figures as 100%.

quantitation of proteins and we quantitated lipoprotein by measurement of the area enclosed by the HDL precipitate. Figure 3 shows the relative amounts of HDL observed in animals treated at different times before bleeding. Figure 3A shows that LPS 026 given intraperitoneally induces a small but remaining drop in HDL. LPS 0124 given in the same way induces only a temporary drop in HDL followed by an increase to more than the normal amount after about 16 h (fig.3B). With intravenous challenge the drop in HDL is considerably more pronounced and occurs within the first 30 min after challenge. The HDL level slowly rises to the normal level after one day (not shown).

4. Discussion

When we used crossed immunoelectrophoresis to study the immediate response towards LPS we found that C3H/HeJ, the non-responder mouse strain, exhibited changes in lipoprotein and arylesterase activity as seen before in normal responder mice [13]. These changes were observed with Boivin-extracted (026) as well as with Westphal-extracted (055 and 0124) LPS which excludes the mitogenic protein [4,21] as cause of our results, and thus we could demonstrate that C3H/HeJ is capable of a non-immune response towards LPS.

Therefore, the induction of arylesterase and the change of HDL may be parts of a general response which is independent of the immune response and independent of the expression of the Lps gene [22].

Recently Haas et al. showed that the C3H/HeJ is also capable of an immunological response towards LPS. The suppression of plaque-forming cells (PFC) formation was readily induced with LPS and found to be transferrable with spleen cells of LPS-treated C3H/HeJ mice [23]. Consequently we find that there are now good reasons to discontinue the use of the general terms 'non-responder' and 'unresponsive' without specific clarification.

Earlier we noted a remarkable difference between SPF (specific pathogen-free) and non-SPF animals with respect to the lipoprotein-arylesterase response. When normal strains (BALB/c and C57B1) were kept at SPF conditions the responses were similar to those described here, but when transferred to conventional conditions these strains showed a high level of arylesterase and the electrophoretic shifts of lipoprotein were less pronounced [13]. Another aspect, which may also be noted in fig.2 here, is the relative decrease of the other esterase, the carboxylic esterase (fig.2B–D). There is a possibility that the decrease is an expression of consumption of enzyme but there is no direct evidence for any enzymatic degradation of LPS [15]. On the other hand, our results bring actuality to the early experiments of Skarnes who found that lipoprotein and enzyme coprecipitated with endotoxin when serum from LPS-treated animals was precipitated by specific antiserum against endotoxin [24]. In relation to this we note that the literature is highly suggestive of reactions between endotoxins and plasma proteins, including detoxifying proteins and lipoproteins [14,24–26], and in spite of the known blood cell receptors for LPS [27,28] the circulating LPS is found primarily in the cell-free plasma fraction ([29] and Marina Freudenberg, personal communication).

The electrophoretic shift and change in morphology seen here for HDL and HDL-associated arylesterase after LPS treatment in vivo is analogous to the electrophoretic shift and change in morphology of lipoproteins or other amphiphilic proteins: After partial or total delipidation of lipoproteins [30,31], after staining of lipoproteins with lipophilic stains [32,33], and after reaction of membrane proteins with non-ionic or charged detergents [34,35] where in all cases the lipid constituents of the proteins are changed. The latter reaction has even been developed into a very sensitive analytical assay for intrinsic membrane proteins named 'charge shift electrophoresis' [36,37]. We interprete the changes of plasma lipoprotein after LPS treatment as charge shift changes in the lipid components of HDL due to LPS. Based upon the amphiphilic nature of HDL we find that HDL may serve as a general 'amphiphilic sink' for components of lipid nature like LPS.

Acknowledgements

Drs C. Galanos, K. L. Fennestad, Marina Freudenberg, Barbara and K. Jann, Ida and F. Ørskov are thanked for their interest and for providing biological material. Ms Pia Jensen, Birgit B. Pedersen, Grethe Rønholt,

Birgit Rumler and Mr W. Pedersen are thanked for their technical and secretarial assistance. The study was supported by the Thorwald Madsen Foundation (UB), the Warwara Larsen Foundation (UB), and the Danish Medical Research Council Grant no. 512-8729 (BHW).

References

[1] Milner, K. C., Rudbach, J. A. and Ribi, E. (1971) in: General Characteristics in Microbial Toxins. (Weinbaum, G., Kadis, S. and Ajl, S. J. eds) vol. 5, p. 1, Academic Press, New York.
[2] Watson, J. and Riblet, R. (1974) J. Exp. Med. 140, 1147–1161.
[3] Coutinho, A., Gronowicz, E. and Scultzer, B. M. (1975) Scand. J. Immunol. 4, 139–143.
[4] Goodman, M. G., Parks, D. E. and Weigle, W. O. (1978) J. Exp. Med. 147, 800–813.
[5] Sultzer, B. M. (1972) Infect. Immun. 5, 107–113.
[6] Chedid, L., Parant, M., Damais, C., Parant, F., Juy, D. and Galleli, A. (1976) Infect. Immun. 13, 722–727.
[7] Sultzer, B. M. (1969) J. Immunol. 103, 32–38.
[8] Urbaschek, R., Mergenhagen, S. E. and Urbaschek, B. (1977) Infect. Immun. 18, 860.
[9] Skidmore, B. J., Chiller, J. M., Weigle, W. O., Riblet, R. and Watson, J. (1976) J. Exp. Med. 143, 143–150.
[10] Moore, R. N., Goodrum, K. J., Couch, R. E. and Berry, L. J. (1978) Infect. Immun. 19, 79.
[11] Brogren, C. H. and Bøg-Hansen (1975) Scand. J. Immunol. 4, Suppl. 2, 37–51.
[12] Back, U., Møller, B. B. and Bøg-Hansen, T. C. (1976) Lancet II, 188–191.
[13] Bøg-Hansen, T. C., Krog, H. H. and Back, U. (1978) FEBS Lett. 93, 86–90.
[14] Skarnes, R. C. (1970) J. Exp. Med. 132, 300–316.
[15] Olitzki, A. (1972) Enteric Fevers. Causing Organisms and Host's Reactions. S. Karger, Basel.
[16] Hertel-Wulff, B. and Rubin, B. (1977) Scand. J. Immunol. 6, 933–944.
[17] Hertel-Wulff, B. and Rubin, B. (1978) Scand. J. Immunol. 7, 11–18.
[18] Hertel-Wulff, B. and Rubin, B. (1976) Eur. J. Immunol. 6, 418–424.
[19] Harboe, N. and Ingild, A. (1973) Scand. J. Immunol. 2, Suppl. 1, 161–164.
[20] Augustinsson, K. B. (1958) Nature 181, 1768–1780.
[21] Skidmore, B. J., Morrison, D. C., Chiller, J. M. and Weigle, W. O. (1978) J. Exp. Med. 142, 1488–1508.
[22] Watson, J., Kelly, K., Largen, M. and Taylor, B. A. (1978) J. Immunol. 120, 422–424.
[23] Haas, G. P., Johnson, A. G. and Nowotny, A. (1978) J. Exp. Med. 148, 1081–1086.
[24] Skarnes, R. C. (1968) J. Bact. 95, 2031–2034.
[25] Moreau, S. C. and Skarnes, R. C. (1973) J. Infect. Dis. 128, 122–133.
[26] Johnsson, K. J., Ward, P. A., Goralnick, S. and Osborn, M. J. (1977) Am. J. Pathol. 88, 559–574.
[27] Adye, J. C., Springer, G. F. and Murthy, J. R. (1973) Z. Immun. Forsch. 144, 491–496.
[28] Springer, G. F. and Adye, J. C. (1975) Infect. Immun. 12, 978–986.
[29] Herring, W. B., Herion, J. C., Walker, R. I. and Palmer, J. G. (1963) J. Clin. Invest. 42, 79–87.
[30] Glangeaud, M. C., Pastier, D., Ayrault-Jarrier, M. and Polonovski, J. (1974) Biochimie 56, 245–253.
[31] Cham, B. E. and Knowles, B. R. (1976) Clin. Chem. 22, 305–309.
[32] Edwards, J. A. and Muckle, T. J. (1976) Clin. Chim. Acta 67, 35–42.
[33] Muckle, T. J. (1976) Clin. Chim. Acta 73, 57–61.
[34] Bjerrum, O. J. and Lundahl, P. (1974) Biochim. Biophys. Acta 342, 69–80.
[35] Bjerrum, O. J., Bhakdi, S., Bøg-Hansen, T. C., Knufermann, H. and Wallach, D. F. H. (1975) Biochim. Biophys. Acta 406, 489–504.
[36] Helenius, A. and Simons, K. (1977) Proc. Nat. Acad. Sci. USA 74, 529–532.
[37] Bhakdi, S., Bhakdi-Lehnen, B. and Bjerrum, O. J. (1977) Biochim. Biophys. Acta 470, 35–44.