PLASMA LIPOPROTEIN-ASSOCIATED ARYLESTERASE IS INDUCED BY BACTERIAL LIPOPOLYSACCHARIDE

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1. Introduction

The biological function of plasma arylesterase (ArE, EC 3.1.1.2) is not known, but it is associated to high density lipoprotein (HDL, α-lipoprotein) [1] and is implicated in normal lipid metabolism through fatty acid exchange of cholesterol esters [2]. In addition, the enzyme may participate in host defence against toxins (including endotoxins, lipopolysaccharides, LPS) [3,4, and Erdös as cited in 2], but there is little evidence for an actual degradation of LPS, as reviewed by Olitzki [5]. On the other hand, the amphiphilic nature of LPS is well established, including its affinity for cell membrane phospholipids [6,7]. Therefore we found it interesting to study the interaction of LPS with other amphiphilic structures, and we were able to show that LPS binds to the HDL-associated arylesterase in plasma in vitro, which led us to support the views of Skarnes [3] by suggesting that the initial degradation and detoxification of LPS in plasma is performed by the arylesterase [8].

This paper presents a new aspect of the host response to LPS challenge, namely the LPS-induced change of arylesterase activity in the blood of specific-pathogen-free mice, and our study suggests that the arylesterase may be classified as an acute phase plasma protein.

2. Experimental procedures

2.1. *Endotoxin*

Endotoxin or lipopolysaccharide (LPS) was obtained from cultures of *E. coli* type 0124 by phenol extraction and purification according to [9] and LPS from *Salmonella abortus equi* was a gift from Dr C. Galanos, Max Planck Institut für Immunbiologie, Freiburg, Germany. LPS was administered by intra-peritoneal injection of 0.5 ml LPS in water, and doses between 0.3 × LD-50 and 3 × LD-50 were tested. For BALB/c the LD-50 dose of *E. coli* and *Salmonella* LPS was about 175 μg and 50 μg, respectively.

† U. Back died on October 2, 1977

2.2. *Mouse strains*

Two SPF strains BALB/cABomFib and C57BL/6JBomFib maintained at the Fibiger Laboratory were used for our experiments. The strains were poly-associated in 1972 with a hexaflora composed of *Streptococcus faecalis, Lactobaccillus brevis, Aerobacter aerogenes, Staphylococcus epidermidis, Bacteroides* species and a yeast fungus. Conventional strains maintained at the State Serum Institute were: A67/Ssc, AKR/Ssc, C3H/FuAaSsc, C3H/HeJSsc, and C57B1/Ssc. The reported experiments are based upon experience with about 100 SPF mice and more than 1000 non-SPF mice. Dr F. Ørskov (The Coli Centre, State Serum Institute) determined that BALB/c SPF mice contained about 2300 colony-forming facultative anaerobic cells, about 100 000 to 10 000-fold less than BALB/c non-SPF and AKR/Ssc non-SPF mice.

2.3. *Analyses*

The arylesterase activity was monitored visually after crossed immunoelectrophoresis of 0.5 μl serum from individual mice at specified times after LPS-challenge. Agarose gel (Litex, Glostrup, Denmark) was prepared as 1% solution in Tris–veronal buffer pH 8.7 (24.5 mM veronal, 73 mM Tris, 0.36 mM calcium lactate). The first dimension was electrophoresed for 50 min at 10 V/cm, the second dimension into antibody-containing gel for 18 h at 2 V/cm [10]. The plates were pressed, washed, pressed, dried in cold air, and stained by a mixture of 1- and 2-naphthyl acetate and Fast Red TR-salt at pH 7.2 [1]. Mouse serum proteins were identified by their reaction with specific antihuman serum protein antibodies in crossed immunoelectrophoresis with intermediate gel as in [10].

2.4. *Antibodies*

These were rabbit immunoglobulins, either commercially available from DAKO-Immunoglobulins (Frederiksberg, Denmark) or prepared from 5 rabbits immunized with small doses of mouse serum according to [11] as immunoglobulin fraction concentrated 6 times relatively to the antiserum.

3. Results

When serum is obtained from mice after LPS treatment and analysed by crossed immunoelectrophoresis, we observe a change of the protein pattern which is characteristic for the acute phase response. Figure 1 shows the serum protein pattern of a specific-pathogen-free BALB/C mouse treated 24 h previously with a high dose of LPS (approximately LD-50). The major protein changes are indicated: Appearance of haptoglobin, increase of hemopexin, and complement C3, and increase and shift of high density lipoprotein

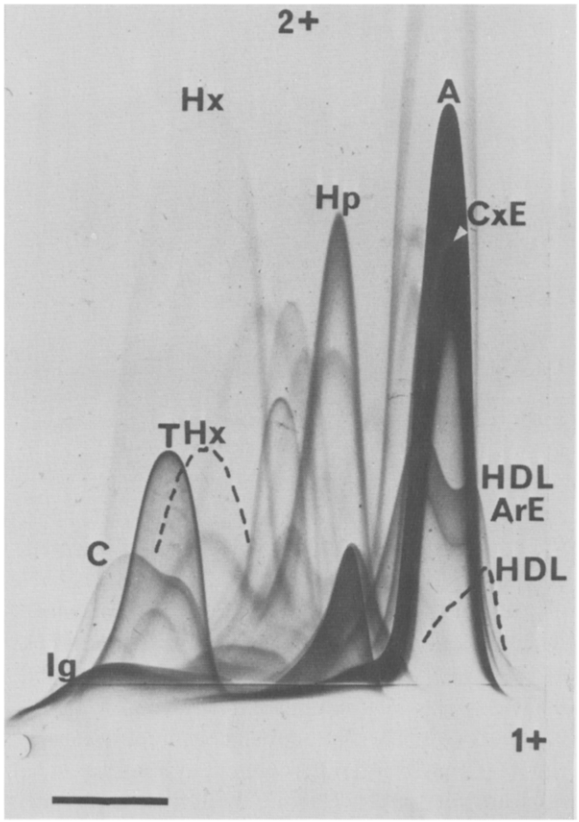

Fig.1. Crossed immunoelectrophoresis of 0.5 μl serum from an SPF BALB/c mouse. The mouse was treated with endotoxin (i.p. 50 μg *Salmonella abortus equi* LPS) 24 h previously. *Abbreviations:* A, albumin; ArE, high-density lipoprotein-associated arylesterase; CxE, carboxylic esterase; HDL, high-density lipoprotein; Hp, haptoglobin (absent in untreated SPF-mice), Hx, hemopexin; C, complement C3; Ig, immunoglobulins; T, transferrin. Broken lines indicate position of proteins in untreated SPF BALB/c mice. The bar represents 1 cm. (1+) indicates the first dimension electrophoresis, (2+) the second dimension in gel containing immunoglobulin fraction against mouse serum proteins (1 μl per cm^2).

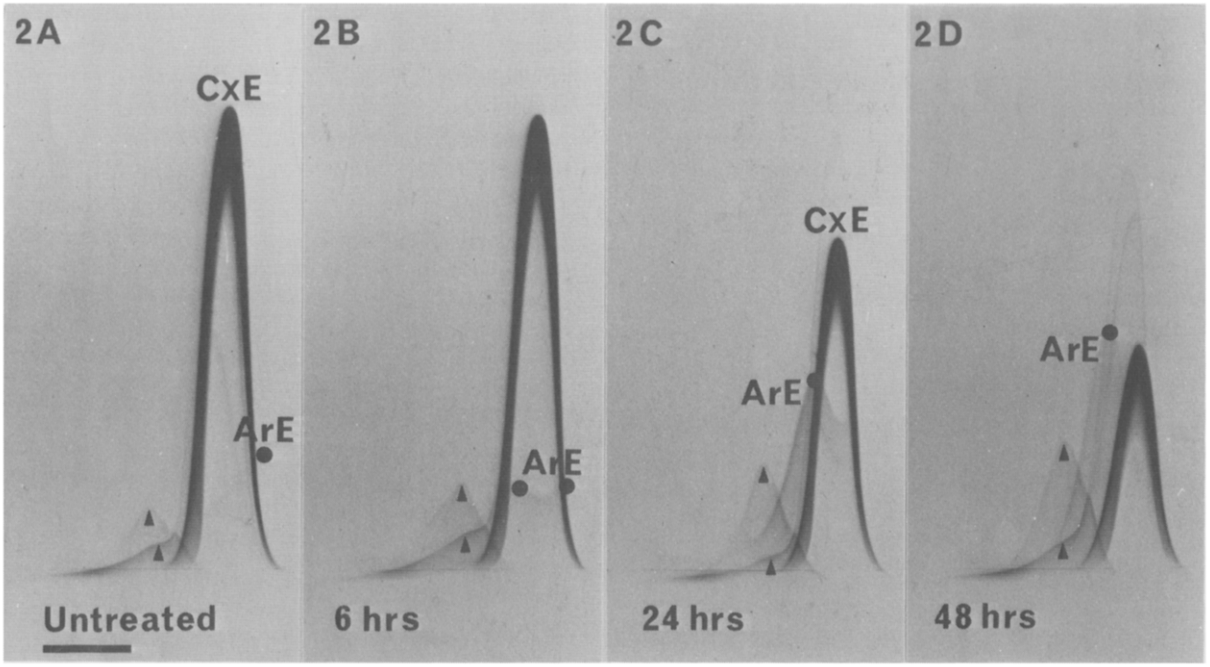

Fig.2. Crossed immunoelectrophoresis of serum from SPF BALB/c mice treated with endotoxin (enzyme stained plates, only anodal part is shown. The time after LPS challenge is indicated for each serum. The arylesterase activity outlines the HDL precipitate and is indicated by dots. Arrowheads indicate α-protein and cathodic foot of albumin which takes up the dye. Figure 2C is fig.1 prior to protein staining. Abbreviations as for fig.1.

(HDL, α-lipoprotein). Analyses of similarly treated animals of the same inbred strain show a remarkable identity of the changes. However, significant inter-strain variations, as well as the individual variations of individual mice of an out-bred strain were found.

The esterases of mouse serum are seen by histochemical stain after immunoelectrophoretic separation (fig.2A–D): HDL-associated arylesterase and non-specific carboxylesterase (earlier termed A and B esterase [12]). Cholinesterase (C esterase) is very weak and not seen in fig.2 (only the anodal part is shown). A specific quantitative analysis for arylesterase in mouse serum is desirable, but not easily feasible because of the overlapping activities and properties of the A and B esterases.

In contrast to human serum arylesterase [1], we find for mouse serum analysed with our antibodies that there is total coprecipitation of arylesterase and HDL.

In SPF-animals the arylesterase activity is very weak, but after LPS-treatment the activity increases. The plates of fig.2 are analysed in parallel and visual comparison reveals a considerable increase of arylesterase staining from 6 to 48 h after LPS-treatment. The area of the precipitate of the enzyme-HDL complex is increased and fig.3 shows the relative variation measured by planimetry which reflects the change in protein amount, provided no antigenic changes occur. However, since the precipitate is shifted towards the cathode we must conclude that the enzyme-HDL complex is modified as a result of LPS treatment, at least with respect to electrophoretic properties, and therefore the changes in fig.3 do not necessarily reflect changes in amount of HDL. The change in enzyme-HDL complex is related to the fall in carboxylic esterase (CxE) and the increase of haptoglobin (Hp) is taken as a characteristic parameter of the acute phase response (fig.3).

When the SPF-animals are transferred to conventional conditions to obtain the natural flora of

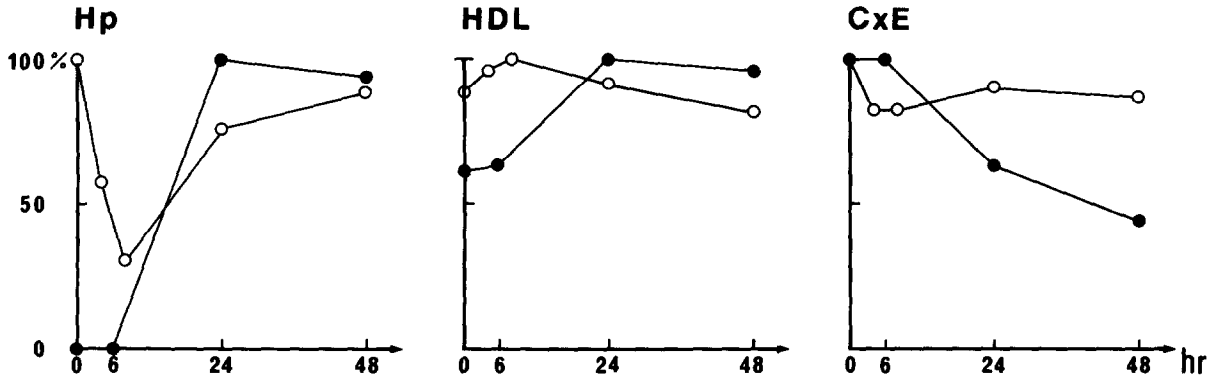

Fig.3. Time-course of specific proteins in SPF and non-SPF BALB/c mice. The area (proportional to amount of protein) of the haptoglobin (Hp), the α-lipoprotein (HDL), and the carboxylicesterase (CxE) precipitates in crossed immunoelectrophoresis before and after LPS-challenge, cf. figs.1 and 2. (•), SPF mice; (○), non-SPF mice.

endogenous gram-negative bacteria, which constantly release LPS, the arylesterase has a higher level than in the untreated SPF-animals. The same is also true for haptoglobin, and it is not possible to detect any increase in arylesterase activity by visual monitoring of the staining intensity after LPS-treatment. The situation in the untreated normal mice can be mimicked in SPF-mice when they are given small LPS doses repeatedly, and analogous results are obtained with the two 'high responder' strains BALB/C and C57BL under SPF and conventional conditions.

4. Discussion

Our results show that an increased level of plasma arylesterase is one of the adaptations of the mouse to the ever-present endotoxin. The experiments here with specific-pathogen-free and conventional mice show that LPS-challenge brings about changes of plasma proteins termed 'acute phase response', that there is a big difference between the response of SPF mice and conventional mice, and that the HDL-associated arylesterase is an inducible protein in SPF mice. The inducibility of the enzyme suggests that it may belong to the reactants, and since arylesterase may be regarded as a regular apo-lipoprotein of HDL (because of its association of HDL and its amphiphilic nature [13]) this enzyme is also the first example of induction of an apoprotein. The increase found here may be either specifically induced de novo synthesis or release of already formed enzyme, but our experiments cannot distinguish between these possibilities. After LPS-treatment the cellular decay is accellerated, and another possible explanation of the present result is that the HDL-associated arylesterase appears in HDL in plasma as a result of release from cellular location. The significance of the relative decrease of the other esterase, the carboxylic esterase, is not clear. There is a possibility that the decrease is an expression of consumption of enzyme, which may be important in regard to the extremely good LPS-resistance of mice compared to other species which do not have this esterase. The possible role of specific enzymatic degradation of endotoxin could involve cleavage of the ester-bound or amide-bound fatty acids (or both) of lipid A, the toxic part of LPS (for a recent review of LPS chemistry and biology, cf. [14]).

Other mammalian proteins have been shown to have an effect on endotoxins: Spleen esterase [15], serum lipoproteins and α_1-globulin [16], and α-globulin LPS inhibitor [17]. All of these proteins result in inactivation of endotoxin, but whether the possible degradation by HDL-associated arylesterase results in detoxified products is not known. Other unrelated results indirectly implicate arylesterase in detoxification, as patients with liver insufficiency have a significantly decreased arylesterase activity [18] in addition to persistently circulating endotoxin

[19]. Preliminary results on arylesterase in such patients seem to reveal a small group of patients with an increased level of arylesterase (in preparation).

Abd-El-Fattah et al. have observed the plasma protein changes in rats after challenge with the purified toxic component of endotoxin, lipid A, and have listed increases of acute phase proteins as we find in the present experiments, but they did not analyze for enzyme activities, nor did they describe any electrophoretic shift of lipoprotein with lipid A [20]. The increase in α-lipoprotein (HDL, fig.3) may be parallel to the 'acute phase response' of haptoglobin, arylesterase, etc. or it could be due to a change in lipoprotein antigenicity induced by the LPS in circulation. Skarnes has found that lipoprotein and enzyme coprecipitates with endotoxin when serum from treated mice, guinea pigs and rabbits is precipitated by specific antiserum against endotoxin [21]. The shifts in electrophoretic mobility and in precipitate morphology (fig.2) strengthen the notion of changes in the lipoprotein structure as a result of stimulation with bacterial lipopolysaccharide.

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