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α_2 macroglobulin of the rat, an acute phase protein, mitigates the early course of endotoxin shock

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Summary. Normal rats and rats with high levels of α_2 macrofetoprotein (α_M FP), an acute phase globulin induced by pretreatment with $BaSO_4$ i.p., were injected with sublethal doses of endotoxin. One hour survival was better in the group with high levels of α_M FP (36%) than in controls (9%). All rats receiving purified α_M FP i.p. survived. Recovery of mean arterial blood pressure, expressed as the surface area under the curve, was significantly better in the groups with high α_M FP levels. Leakage of i.v. administered human albumin was the same in control and $BaSO_4$ pretreated rats. $BaSO_4$ induces peritonitis which could explain the albumin leakage. Experiments were repeated therefore in rats pretreated with adrenalin which also initiates the production of α_M FP. In this group, 1 h survival after endotoxin administration was 100% and albumin leakage was significantly less than in rats receiving either endotoxin only or $BaSO_4$-pretreatment. In early endotoxin shock prostaglandins, including PGE_2 a potent vasodilatator, are released into the circulation. From previous data it is known that α_M FP prevents the vasodilatation and increased vascular permeability caused by PGE_2. Rats with high levels of α_M FP had a smaller fall in diastolic blood pressure after PGE_2 administration than did controls with normal α_M FP levels. The effects of α_M FP on the haemodynamic events in early endotoxin shock could well be due to inhibition of PGE_2 activity.

Keywords: endotoxin shock, α_2 macrofetoprotein (α_M FP), prostaglandin E_2

Many authors explain some of the phenomena associated with hepatic injury such as coagulation disorders, thrombopenia and shock by attributing them to bacterial endotoxins, originating in the intestines and spilling over to the diseased liver (Liehr *et al.* 1978; Camara *et al.* 1983; for a review see Nolan & Camara 1982).

In hepatitis induced by galactosamine-HCl (Gal-N), systemic endotoxemia is observed regularly together with coagulation defects and shock (Grün *et al.* 1977; Liehr *et al.* 1978). However, it has been shown that an acute-phase reactant in the rat, α_2 macrofetoprotein (α_M FP, α_2 macroglobulin of the rat) protects the liver against many of the toxic effects of galactosamine (Gal-N) (Van Gool *et al.* 1978). Depending on the plasma level of α_M FP, liver enzyme leakage was significantly lower and less necrosis and inflammatory infiltration was observed histologically. Complete resistance to galactosamine was observed at plasma levels of 2000 μg/ml (normal value 60–80 μg/ml). However, α_M

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FP had no effect on the primary biochemical lesion (uridine diphosphate glucose depletion) even where there was complete resistance. This primary biochemical lesion is temporary and the intermediates formed are excreted by the kidney. These and other findings (Liehr *et al.* 1978; Camara *et al.* 1983) suggest that other factors also are of importance in Gal-N hepatitis. One possibility is that the protection given by α_M FP is due to an anti-endotoxin effect, thus mitigating the sequences of endotoxaemia. We showed previously that α_M FP has a strong preventive effect on inflammatory oedema caused by mediators such as bradykinine, 5-hydroxytryptamine and prostaglandin E_2 (Van Gool *et al.* 1977; Ufkes *et al.* 1983). This is interesting because prostaglandins and plasmakinins are activated during endotoxin shock. The aim of this study is to investigate whether: (a) α_M FP protects against endotoxin shock; (b) α_M FP inhibits the circulatory effects of PG E_2.

Materials and methods

Male Wistar rats (TNO-Zeist), weighing 250–300 g, were used throughout the experiments. α_M FP production was stimulated by injection of 2 ml 40% w/v $BaSO_4$ suspension in sterile 0.9% NaCl intraperitoneally (i.p.) 24 h previously. This produces a localized chemical peritonitis, which provokes a strong acute phase reaction resulting in high levels of α_M FP and other acute phase proteins. Controls received 2 ml 0.9% NaCl i.p. In later experiments α_M FP production was stimulated by two injections of 0.2 mg adrenalin subcutaneously 24 h before the experiment (Van Gool *et al.* 1984). Rats were anesthesized with 0.08 ml per 100 g bodyweight pentobarbital (Nembutal® 60 mg/ml). Blood pressure was recorded continuously, electromanometrically from a carotid artery. After a control period to stabilize the blood pressure, rats received a sublethal dose of 2 mg lipopolysaccharide (LPS) per 100 g bodyweight (E. coli serotype 0126: B 8 TCA extract, Sigma) intravenously. Thereafter blood pressure was recorded for 60 min or less if the rat died. Body temperature was maintained at 37 ± 0.5°C with a heating lamp. Five rats received 12.5 mg purified α_M FP i.p. 2 h before challenge with LPS.

In other experiments, rats received 1 ml purified human albumin (50 mg/ml) (Nordic Diagnostics, batch 772) intravenously. Blood samples were taken at 5, 15, 30, 60 and 90 min after injection. In the experimental groups LPS was given after taking the first sample. Plasma levels of human albumin were measured by radial immunodiffusion using a commercially available method (Partigen® plates Albumin from Behring-werken AG Marburg, West Germany). No cross reaction with rat albumin exists. Finally rats with low and high levels of α_M FP received 1 μg of PG E_2 by retrograde injection into the aortic arch, each dose being washed in with 0.2 ml 0.9% NaCl. PG E_2 was stored as a stock solution of 500 mg/2 ml in ethanol at -20°C. Solutions prepared from stock by dilution with 0.9% NaCl were used immediately. All values are given as mean $\pm$ SEM; statistical analysis by Student's *t*-test for unpaired data.

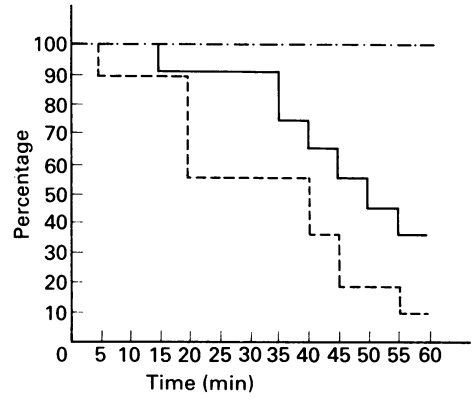

Fig. 1. Survival of rats after endotoxin administration (2 mg LPS/100 g body weight i.v.). -----, Control rats ($n=11$) α_M FP 68 ± 30 μg/ml; ——, rats pretreated with $BaSO_4$ ($n=11$) α_M FP 1622 ± 270 μg/ml; -·-·-·-, Rats after i.p. administration of 12.5 mg purified α_M FP 2 h before endotoxin administration α_M FP 400 ± 88 μg/ml. Values are given as mean $\pm$ SEM.

Results

Arterial pressure after endotoxin administration

Fig. 1 summarizes the survival of 11 rats with normal levels of α_M FP (68 ± 30 μg/ml; Control group), 11 rats with high levels of α_M FP induced by injection of $BaSO_4$ (1622 ± 270 μg/ml) (acute phase group) and five rats who received 12.5 mg purified α_M FP i.p. 2 h before challenge with LPS, inducing α_M FP plasma levels of 400 ± 88 μg/ml.

After 20 min, five out of 11 rats in the control group had died whereas in the group with $BaSO_4$-induced acute phase reaction, only one animal died. After 1 h 10 out of 11 rats in the control group were dead, a survival of 9%. In the acute phase group seven rats died, a survival of 36%. All rats with purified α_M FP i.p. survived at least 1 h.

Fig. 2 shows the mean arterial pressure during the experiment. In all groups we observed a fast initial lowering of arterial

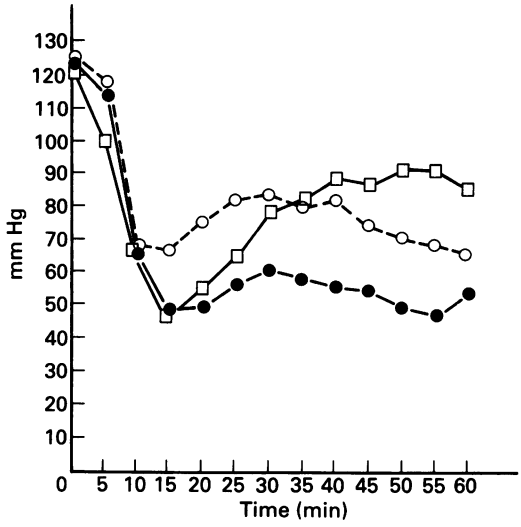

Fig. 2. Mean arterial pressure after endotoxin administration (2 mg LPS/100 g bodyweight). ●, Control rats ($n=11$) endotoxin only; ○, Endotoxin 24 h after $BaSO_4$ i.p. ($n=11$); □, Endotoxin 2 h after i.p. administration of 12.5 mg purified α_M FP ($n=5$).

Table 1. Absolute values (mean ± SEM) of the mean arterial pressures after endotoxin administration (2 mg LPS/100 g body weight)

Time after endotoxin administration (min)	Endotoxin only		Endotoxin 24 h after $BaSO_4$ i.p.		Endotoxin 2 h after 12.5 mg purified α_M FP i.p.	
	No of rats	mm Hg	No of rats	mm Hg	No of rats	mm Hg
0	11	124±7	11	125±5	5	120±6
5	10	114±11	11	118±7	5	100±33
10	10	65±5	11	67±8	5	67±15
15	10	48±6	11	66±12	5	51±8
20	6	49±5	10	75±12	5	55±8
25	6	56±5	10	82±12	5	66±10
30	6	60±5	10	83±13	5	79±12
35	6	57±5	10	80±13	5	83±12
40	6	55±6	8	82±14	5	89±11
45	4	54±6	7	74±12	5	88±9
50	2	59, 39*	6	70±16	5	92±8
55	2	55, 36*	5	68±18	5	91±10
60	1	54*	4	66±24	5	85±10

* Values for individual rats.

pressure during the first 15 min after endotoxin administration, followed by a period of stabilization at a low level in the controls and a gradual rise of the blood pressure in the acute phase group and the group with purified α_M FP. It is clear that rats with an acute phase reaction due to $BaSO_4$, attain higher blood pressure levels than do controls. Rats receiving purified α_M FP stabilized at a higher level than the group with $BaSO_4$-induced acute phase reaction which is in accordance with the data on mortality. Due to the high mortality in the control group these curves are not fully representative because they show only the means of animals alive at one point in time. A better representation is achieved by calculating for each rat the area under the blood pressure curve as a measure of the severity of the shock. For rats receiving endotoxin only, the mean area was 2225 ± 365 mm^2 ($n=11$). After $BaSO_4$-induced acute phase reaction the mean area rose to 4179 ± 670 mm^2 ($n=11$, $P<0.02$ compared with endotoxin controls). Rats receiving α_M FP i.p. 2 h before endotoxin administration had a mean surface area of 4818 ± 357 mm^2 ($n=5$, $P<0{\cdot}001$ compared with endotoxin controls). Absolute values for the mean arterial pressures are given in Table 1.

Effect of α_M FP on leakage of plasma proteins after endotoxin administration

Endotoxin (LPS) induced a rapid albumin loss from the intravascular space in rats with low α_M FP levels (89 ± 22 μg/ml) (Fig. 3). However, the group with $BaSO_4$-induced acute phase reaction with high levels of α_M FP (4398 ± 858 μg/ml) showed the same rapid disappearance of albumin. Thus the acute phase reaction has no protective effect on protein leakage in this situation. However, when the acute phase proteins were induced by adrenalin administered one day previously, a good protection against albumin loss after endotoxin administration was obtained.

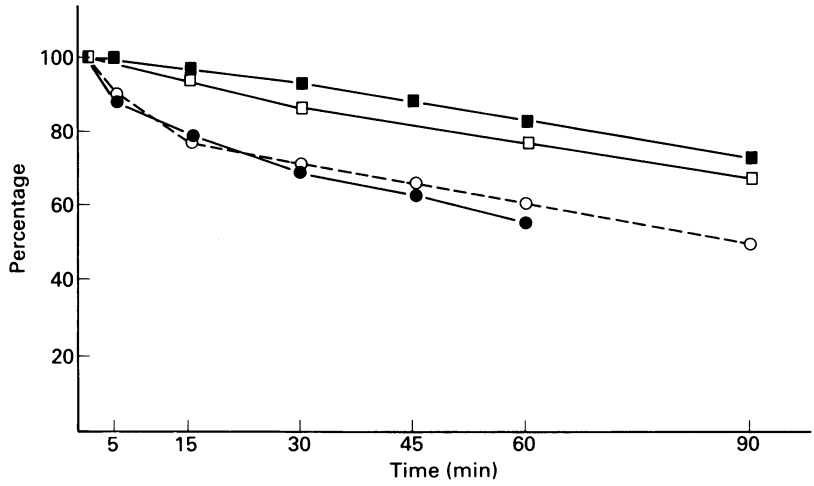

Fig. 3. Albumin disappearance in normal rats and rats after endotoxin administration (2 mg LPS/100 g body weight). All values are given as mean ± SEM. a, (■) Albumin disappearance in normal rats ($n=4$) α_M FP 79 ± 12 μg/ml. Endotoxin was given to the following experimental groups; b, (□) Endotoxin 24 h after adrenalin s.c. ($n=5$) α_M FP 2070 ± 168 μg/ml; c, (○) Endotoxin 24 h after $BaSO_4$ i.p. ($n=7$) α_M FP 4398 ± 858 μg/ml; d, (●) Endotoxin only, ($n=7$) α_M FP 89 ± 22 μg/ml. $t=5$: a *vs* c, $P<0.005$; a *vs* d, $P<0.001$. $t=15$: a *vs* c and d, $P<0.005$; b *vs* c and d $P<0.01$. $t=30$: a *vs* b, $P<0.025$. $t=60$: a *vs* b, $P<0.01$. A $t=30$ and $t=60$, a *vs* c and d, $P<0.001$; b *vs* c, $P<0.005$; b *vs* d, $P<0.001$.

Table 2. Maximal decrease in mean diastolic blood pressure (mean ± SEM) after intraarterial administration of 1 μg PGE_2

Experimental Groups	*n*	Maximal decrease in diastolic blood-pressure mm Hg	*P*
Normal rats			
α_M FP 68 ± 8 μg/ml	8	50 ± 6	—
Rats 24 h after $BaSO_4$;			
α_M FP 3190 ± 718 μg/ml	8	38 ± 3	n.s.
Rats 24 h after adrenalin			
α_M FP 3383 ± 647 μg/ml	8	27 ± 3	< 0.005 *vs* normal controls

In the adrenalin treated rats, albumin loss after endotoxin administration was significantly less at 15 min ($P < 0.01$) than in the other two experimental groups. This difference persisted throughout the 60 min period. Compared with normal controls not receiving endotoxin, albumin loss from the extravascular space was significantly higher at 15, 30 and 60 min in all three groups (Fig. 3).

None of the rats which received endotoxin only were alive at 90 min but all animals both in the control group and in the adrenalin-treated group still survived at this time. Three out of seven rats treated with $BaSO_4$ were alive at 90 min.

Effect of α_M FP on PG E_2-induced hypotension

The mean maximal fall in diastolic blood pressure after intra-arterial administration of 1 μg PG E_2 was compared in normal rats and in rats treated with $BaSO_4$ or adrenalin 24 h previously. This dose of PG E_2 produces a mean maximal fall in diastolic blood pressure of 50 mm Hg in normotensive rats (Armstrong *et al.* 1976). Mean maximal fall in normal control rats was 50 ± 6 mm Hg (Table 1). In rats pretreated with $BaSO_4$ or adrenalin, the vasodepressor effect of PG E_2 was respectively 38 ± 3 and 27 ± 3 mm Hg, i.e. lower than in controls (Table 2).

Discussion

Our results indicate that previous induction of an acute phase reaction, monitored by α_M FP as a typical acute phase protein, markedly improves survival after endotoxin shock (Fig. 1). To induce a strong acute phase reaction, i.p. administration of $BaSO_4$ (40% w/v) is a useful and simple technique; α_M FP rises with 24 h from 50–100 μg/ml to about 4000 μg/ml. Other acute phase reactants also are stimulated (haptoglobin, α_1 antitrypsin, α_1 acidglycoprotein) (Van Gool *et al.* 1984). The effect of purified α_M FP in our endotoxin model was marked. There was no mortality among α_M FP-treated animals in the first hour whereas in the $BaSO_4$-treated group, survival was only 36%. Thus α_M FP alone, even at lower plasma levels, had a stronger protecting effect than did the $BaSO_4$-induced acute phase reaction, even though the $BaSO_4$ induces higher α_M FP levels (Fig. 1). α_M FP did not protect against the fall in blood pressure during the first 15 min after endotoxin administration in any of the three experimental groups. However, after 15 min the mean arterial pressure (MAP) in controls remained at a low level or dropped further, while in the α_M FP-treated rats it began to rise and then stabilized.

The fact that all rats receiving purified α_M FP intraperitoneally survived and had a

better recovery of blood pressure levels than did the $BaSO_4$-treated group is understandable, because intraperitoneal administration of $BaSO_4$ causes sterile peritonitis. $BaSO_4$-treated rats were thus at a disadvantage compared to those receiving α_M FP intraperitoneally, for they already had a leaking peritoneal surface. Adrenalin can be used to initiate the production of several acute phase proteins without the complication of peritonitis (Van Gool *et al.* 1984). This is illustrated by Fig. 3 which shows that albumin leakage after endotoxin administration is significantly less in rats pretreated with adrenalin. The level of α_M FP at the moment of endotoxin injection was 2070 ± 168 μg/ml. This confirms that the difference between the $BaSO_4$-treated rats and those receiving α_M FP intraperitoneally is attributable to peritonitis.

During endotoxin shock an increased synthesis of prostaglandins from arachidonic acid is found in many species, including rats (Wise *et al.* 1980; Cook *et al.* 1980). Many studies have recorded a markedly improved survival of animals treated with inhibitors of prostaglandin synthesis such as acetylsalicylic acid or indomethacin (Anderson *et al.* 1975; Cook *et al.* 1980). When endogenous arachidonic acid stores are depleted by feeding rats a diet devoid of essential fatty acids, the rats become highly resistant to endotoxin toxicity (Cook *et al.* 1979; 1980). In rats, endotoxin interacts with serum HDL *in vivo* to form a stable complex (Freudenberg *et al.* 1980). These complexes are cleared mainly in the liver, initially by the Kupffer cells (Freudenberg *et al.* 1982). When Kupffer cells are stimulated by endotoxin, prostaglandins are released predominantly E_2 but also $F_{2\alpha}$, prostacyclin and thomboxane (Bhatnagar *et al.* 1982; Birmelin & Decker, 1984). Prostaglandins E and F are significantly increased in early endotoxin shock (Anderson *et al.* 1975; Fletcher *et al.* 1976). Fletcher & Ramwell (1977) found elevated prostaglandin E and F levels within 1–2 min after injection of endotoxin, concommitant with the fall in blood pressure.

PGE_2 is a potent vasodilatator and increases vascular permeability to protein. In the rat, *in vivo* vascular resistence is reduced in the mesenteric vascular bed after PGE_2 administration (Gerber & Nies 1979). It is relevant to the effect of α_M FP on early haemodynamic events in endotoxin shock, that α_M FP is a strong inhibitor of the inflammatory response mediated by PGE_2. Both oedema formation, extravascular accumulation of ^{121}I human serum albumin and vasodilatation are considerably decreased after $BaSO_4$-pretreatment (Van Gool *et al.* 1979; Ufkes *et al.* 1983). So too are other inflammatory mediators released in endotoxin shock, such as bradykinin and serotonin (Aasen *et al.* 1978; Emau *et al.* 1984).

High levels of α_M FP in rats reduce the PGE_2-induced fall in systemic blood pressure (Table 2). In conclusion α_M FP, a typical acute phase reactant, has a beneficial effect on the early course of haemodynamic events during endotoxin shock that can be explained by inhibition of prostaglandin E_2 activity.

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