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Complement split product C5a mediates the lipopolysaccharide-induced mobilization of CFU-s and haemopoietic progenitor cells, but not the mobilization induced by proteolytic enzymes

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Abstract. Intravenous (i.v.) injection of mice with lipopolysaccharide (LPS), and the proteolytic enzymes trypsin and proteinase, mobilizes pluripotent haemopoietic stem cells (CFU-s) as well as granulocyte-macrophage progenitor cells (GM-CFU) and the early progenitors of the erythroid lineage (E-BFU) from the haemopoietic tissues into the peripheral blood. We investigated the involvement of the complement (C) system in this process. It appeared that the early mobilization induced by LPS and other activators of the alternative complement pathway, such as *Listeria monocytogenes* (*Lm*) and zymosan, but not that induced by the proteolytic enzymes, was absent in C5-deficient mice. The mobilization by C activators in these mice could be restored by injection of C5-sufficient serum, suggesting a critical role for C5.

The manner in which C5 was involved in the C activation-mediated stem cell mobilization was studied using a serum transfer system. C5-sufficient serum, activated *in vitro* by incubation with *Lm* and subsequently liberated from the bacteria, caused mobilization in both C5-sufficient and C5-deficient mice. C5-deficient serum was not able to do so. The resistance of the mobilizing principle to heat treatment (56°C, 30 min) strongly suggests that it is identical with the C5 split product C5a, or an *in vivo* derivative of C5a. This conclusion was reinforced by the observation that a single injection of purified rat C5a into C5-deficient mice also induced mobilization of CFU-s.

Haemopoietic stem cells (CFU's), granulocyte-macrophage progenitor cells (GM-CFU) and the early progenitor cells of erythrocytes (E-BFU) occur in the peripheral blood in extremely low numbers (Goodman & Hodgson, 1962; Hara & Ogawa, 1977; Rickard *et al.*, 1971). Their frequency in the blood can be increased by intravenous (i.v.) injection of certain substances which stimulate their mobilization out of the haemopoietic tissues into the blood

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stream. These substances include lipopolysaccharides (LPS), zymosan, proteolytic enzymes, polyanions and lectins (Vos, Buurman & Ploemacher, 1972; Quesenberry *et al.*, 1973; Ross *et al.*, 1976; van der Ham, Benner & Vos, 1977; Cline & Golde, 1977; Vos & Wilschut, 1979).

In mice, depending on the agent injected, peak CFU-s numbers are found 10 min to 6 hr after injection. LPS and zymosan, in addition, cause a 'delayed' accumulation of CFU-s and haemopoietic progenitor cells in the peripheral blood and spleen, reaching peak numbers about 5 days after injection of the agent (Vos *et al.*,1972; Staber & Johnson, 1980; Benner *et al.*, 1981; Molendijk, Ploemacher & Erkens-Versluis, 1982). Early CFU-s mobilization by LPS, zymosan and proteolytic enzymes, but not by polyanions, can be prevented by prior decomplementation with the complement (C) activating factor of cobra venom (CoF) (Wilschut *et al.*, 1979). LPS and zymosan are also incapable of early mobilization of CFU-s in complement C5-deficient mice (Benner *et al.*, 1981). The delayed CFU-s accumulation in blood and spleen by these agents, on the other hand, was not affected in C5-deficient mice.

The experiments described in this paper were designed to explore the role of the C system in the early mobilization of CFU-s and haemopoietic progenitor cells by LPS and proteolytic enzymes in more detail. The results suggest that C5a is the active principle in the LPS-induced mobilization of CFU-s and haemopoietic progenitor cells. C5a did not appear to be required for mobilization of the various types of colony-forming cells by proteolytic enzymes.

MATERIALS AND METHODS

Mice and rats

Male and female mice of the following strains were used: AKR (C5-deficient), DBA/2 (C5-deficient), (C3H/Law × DBA/2)F1 (C5-sufficient), B10.D2/oSn (C5-deficient), B10.D2/nSn (C5-sufficient) and (C57BL/Rij × CBA/Rij)F1 (C5-sufficient). The AKR, DBA/2 and (C3H/Law × DBA/2)F1 mice were purchased from the Radiobiological Institute TNO, Rijswijk, The Netherlands, B10.D2/oSn mice from Jackson Laboratories, Bar Harbor, Maine, U.S.A., B10.D2/nSn mice from Bantin and Kingman, Aldbrough, U.K., and (C57BL/Rij × CBA/Rij)F1 mice from the Laboratory Animals Center of the Erasmus University, Rotterdam, The Netherlands. For the spleen colony assay male mice were used. The age of all donor and recipient mice ranged between 12 and 24 weeks. Lewis rats, 8–12 weeks old, were purchased from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands.

Mobilizing agents

LPS from *Salmonella abortus equi* was kindly provided by Dr C. Galanos, Max-Planck Institut für Immunobiologie, Freiburg, F.R.G. The properties and source of the proteinase and trypsin used were described previously (Vos *et al.*, 1972; van der Ham *et al.*, 1977; Vos & Wilschut, 1979; Wilschut *et al.*, 1979). Mobilizing agents were dissolved in pyrogen-free phosphate buffered saline (PBS). They were injected i.v. in a volume of 0·5 ml PBS.

Cell suspensions

After killing the mice by exposure to carbon dioxide, blood samples were collected by cardiac puncture and immediately heparinized in plastic tubes containing preservative-free lithium heparin (Sigma, Detroit, Michigan, U.S.A.).

For cell culture, one volume of heparinized blood was mixed with one volume of 0·2% methylcellulose in α-median (α-modification of Dulbecco minimal essential medium). Red blood cells were allowed to sediment for 30 min at room temperature. The leucocyte-rich

plasma was pipetted off and the cells were washed twice in α-medium containing 2% fetal calf serum (FCS).

Cell culture

Aliquots of 5×10^5 or 10^6 nucleated blood cells were cultured in 35 × 10 mm plastic tissue culture dishes at 37°C in a fully humidified incubator in an atmosphere of 5% CO_2 in air. The culture medium consisted of α-medium supplemented with 0·8% methylcellulose, 1% de-ionized bovine serum albumin (No A-9647; Sigma, St. Louis, Missouri, U.S.A.), 10% FCS, 10^{-4} M 2-mercaptoethanol (Merck-Schuchardt, Hohenbrunn, F.R.G.), 20% mouse spleen Con A conditioned medium and 2·0 U of sheep plasma erythropoietin (Connaught Laboratories Ltd., Willowdale, Ontario, Canada). Granulocyte/macrophage colonies, containing at least 50 cells, were counted on day 7 of culture, erythroid bursts on day 10. All cultures were done in triplicate.

Irradiation

Whole body irradiation was performed with a ^{137}Cs (Gammacell 40, Atomic Energy of Canada Ltd., Ottawa, Canada) at a dose rate of 1·29 Gy/min. (C57BL/Rij × CBA/Rij)F1 mice received 9 Gy, AKR and (C57BL/6J × DBA/2)F1 mice 8·5 Gy.

Spleen colony assay

The spleen colony assay of Till & McCulloch (1961) was performed as described previously (Molendijk *et al.*, 1982). The spleens were taken out 8 days after irradiation and i.v. injection of the blood samples.

Complement activation *in vitro*

For the *in vitro* activation of C, mouse or rat serum was used from non-heparinized blood that was allowed to clot for 90 min at room temperature. One ml of serum was mixed with 0·5 ml of saline containing $3·2 \times 10^9$ heat-inactivated (56°; 60 min) *L. monocytogenes* bacteria. This mixture was incubated for 30 min at 37°C. After incubation the bacteria were pelleted by centrifugation at 5000 r.p.m. for 10 min at 4°C. The remaining bacteria were removed from the supernatant by filtration through a 0·22 μm filter (Millipore, Bedford, Massachusetts, U.S.A.). Control serum from the same bleeding was mixed with saline only, but otherwise similarly treated.

Heat inactivation of complement

Mouse serum was heat-decomplemented by incubation in a waterbath for 30 min at 56°C.

Assay for alternative complement pathway activity

The alternative complement pathway activity of the serum samples activated by *L. monocytogenes* bacteria was determined as described previously (van Dijk, Rademaker & Willers, 1980; van Kessel *et al.*, 1981).

Purification of rat C5a

One hundred and seventy five ml of fresh rat plasma collected in 2 mM-EDTA, 1 unit/ml of trasylol, 2 mM benzamidine and 0·5 mM fluryl-methyl-sulphonyl fluoride (PMSF) was precipitated with a final concentration of 6% polyethyleneglycol-6000 during 60 min at 0°C. After centrifugation (15 min, 5000 ***g***) the precipitate was dissolved in 35 ml Tris-HCl buffer (0·01 M Trizma base, containing the above mentioned inhibitors and adjusted to a PH of 7·8 with HCl) dialyzed overnight at 4°C against the same buffer, and applied on a 1·5 × 45 cm DEAE Sephacel column which was equilibrated in dialysis buffer. After collection of

50 fractions of 5 ml each, bound C5 was eluted using a linear salt gradient. The C5 haemolytic activity (Daha *et al.*, 1982), which was eluted from the column with a conductivity between 6 and 8 mS, was pooled, dialyzed against 0·01M acetate buffer, pH 6·3 and subjected to cation-exchange chromatography of a 1·5 × 20 cm Sulphopropyl-C50 column. C5 haemolytic activity, eluted from the column with a linear salt gradient and found between 3 and 5 mS, was concentrated to a volume of 5 ml and subjected to gelfiltration on a 2·5 × 90 cm Sephacryl S300 column. C5 haemolytic activity which filtered with an apparent molecular weight of 185,000 daltons was pooled and applied on a column of Sepharose 4B to which anti-rat-C3 and anti-rat-H were coupled (Daha *et al.*, 1979; Daha & van Es, 1982) to remove minor contaminants of rat-C3 and rat-H from the C5 preparation. The final C5 preparation was homogeneous on SDS-PAGE analysis.

To obtain C5a, 3 mg purified C5 an isotonic Veronal buffered saline was reacted during 60 min at 37°C with 1×10^9 intermediates bearing the classical pathway C5 convertase EAC1423 (Daha, Hazevoet & Van Es, 1983). The intermediates were then removed by centrifugation and the small molecular weight C5a was obtained by gelfiltration on Sephadex-G75. The final material was dialysed against distilled H_2O, freeze-dried and finally resuspended in 2 ml pyrogen-free 0·15 M NaCl.

RESULTS

Mobilization of CFU-s, GM-CFU and E-BFU in C5-deficient mice by LPS

Complement C5-deficient mice, e.g. DBA/2, B10.D2/oSn and AKR (Cinader, Dubiski & Wardlaw, 1964; Rosen, 1975), are incapable of CFU-s mobilization upon i.v. injection of LPS (Benner *et al.*, 1981). We investigated whether the mobilization of GM-CFU and E-BFU is similarly defective in C5-deficient mice. Therefore, C5-deficient DBA/2, B10.D2/oSn and AKR mice and genetically related, C5-sufficient (C3H × DBA/2)F1 mice and B10.D2/nSn mice were injected with LPS or PBS. In the control mice, injection of LPS led to the mobilization of CFU-s as well as GM-CFU and E-BFU (Table 1). The increase in GM-CFU and E-BFU numbers was proportionally smaller than that of CFU-s. In the C5-deficient mice, mobilization of all three cell types was weak or absent (Table 1). The data from the C5-sufficient and C5-deficient mouse strains differ significantly ($P < 0.0001$).

Mobilization of CFU-s in C5-deficient mice by proteolytic enzymes

Injection of C5-deficient mice with the proteolytic enzymes trypsin and proteinase did induce mobilization of CFU-s into the peripheral blood. Both enzymes were found to cause a dose dependent increase of blood CFU-s numbers in normal as well as C5-deficient mice (Table 2).

Mobilization of CFU-s in C5-deficient mice injected with C5-sufficient serum

Injection of C5-deficient DBA/2 mice with 1 ml serum from C5-sufficient (C57BL × CBA)F1 mice enabled these DBA/2 mice to respond to injection of LPS with CFU-s mobilization. DBA/2 mice, injected with 300 μg LPS i.v. 1 hr after infusion of the C5-sufficient serum, had 306 ± 100 CFU-s/ml blood at 30 min after LPS injection, whereas control DBA/2 mice injected with the same amount of LPS had 64 ± 19 CFU-s/ml blood.

CFU-s mobilization by serum-containing activated complement

The fact that LPS is an activator of the alternative C pathway, and the observation that LPS-induced CFU-s mobilization is absent in decomplemented and C5-deficient mice, suggest that activation of C5 via the alternative C pathway may be important for the LPS-induced

Table 1. *Mobilization of CFU-s, GM-CFU and E-BFU by LPS in the C5-deficient mouse strains DBA/2, B10.D2/oSn and AKR*

Mouse strain	C5†	CFU-s/ml blood*		GM-CFU/ml blood		E-BFU/ml blood	
		PBS	LPS	PBS	LPS	PBS	LPS
DBA/2	–	42 ± 4‡	72 ± 4	43 ± 5	80 ± 6	20 ± 2	19 ± 4
(C3H × DBA/2)F1	+	16 ± 3	730 ± 61	86 ± 9	664 ± 39	104 ± 13	284 ± 15
		21 ± 6	1000 ± 88	50 ± 3	532 ± 28	13 ± 2	93 ± 7
B10.D2/oSn	–	44 ± 5	72 ± 10	78 ± 9	42 ± 13	14 ± 6	14 ± 3
		40 ± 7	92 ± 5	23 ± 2	18 ± 2		
B10.D2/nSn	+	48 ± 5	760 ± 92	75 ± 9	259 ± 7	24 ± 5	61 ± 6
		51 ± 5	594 ± 45	110 ± 19	779 ± 29		
AKR	–	52 ± 7	21 ± 7	14 ± 5	17 ± 3	9 ± 4	8 ± 2
		29 ± 4	80 ± 4	18 ± 8	12 ± 5	16 ± 6	20 ± 4

* Blood was taken 30 min after i.v. injection of 300 μg LPS or 0·5 ml PBS.
† C5-sufficient mouse strains are indicated by +, C5-deficient mouse strains by –.
‡ Arithmetic mean ± SEM.

Table 2. CFU-s mobilization by trypsin and proteinase in the C5-deficient mouse strains DBA/2, B10.D2/oSn and AKR

Mouse strain	C5†	CFU-s/ml blood*				
		PBS	0·4 mg trypsin	2·0 mg trypsin	0·1 mg proteinase	0·5 mg proteinase
DBA/2	–	46 ± 8	119 ± 9	367 ± 97	150 ± 44	640 ± 65
		52 ± 11	71 ± 9	100 ± 7	319 ± 20	594 ± 43
(C3H × DBA/2)F1	+	45 ± 7	89 ± 48	240 ± 112	209 ± 38	570 ± 65
		16 ± 3	45 ± 12	160 ± 32	404 ± 15	547 ± 58
B10.D2/oSn	–	37 ± 9	30 ± 8	143 ± 52	81 ± 8	381 ± 37
		40 ± 7	91 ± 11	160 ± 21	216 ± 18	405 ± 22
B10.D2/nSn	+	53 ± 6	81 ± 11	90 ± 29	186 ± 9	440 ± 40
		48 ± 5	97 ± 6	180 ± 16	187 ± 13	400 ± 32
AKR	–	52 ± 7	61 ± 6	106 ± 14	150 ± 22	586 ± 74
		29 ± 4	142 ± 8	180 ± 16	300 ± 18	686 ± 78

* Blood was taken 30 min after i.v. injection of trypsin, proteinase or PBS.
† C5-sufficient mouse strains are indicated by +, C5-deficient mouse strains by –.
‡ Arithmetic mean ± SEM.

CFU-s mobilization. To investigate this putative mediatory role of C, normal mouse serum (NMS) was incubated *in vitro* with *Listeria monocytogenes* (*Lm*) which has also been shown to activate C via the alternative pathway (Van Kessel *et al.*, 1981). C activation was measured by determining the remaining alternative pathway C activity (van Dijk *et al.*, 1980). Figure 1 shows that *in vitro* incubation of (C57BL × CBA)F1 serum with *Lm* led to a complete C consumption as determined in the haemolytic assay. Neither before nor after incubation with *Lm* did the serum of C5-deficient mice show haemolytic activity.

Injection of mice with *Lm*-treated serum from C5-sufficient mice was found to cause a 4- to 7-fold increase in the blood CFU-s numbers within 30 min (Table 3). Serum which had been heat-inactivated (30 min 56°C) to destroy the C enzymes and subsequently incubated

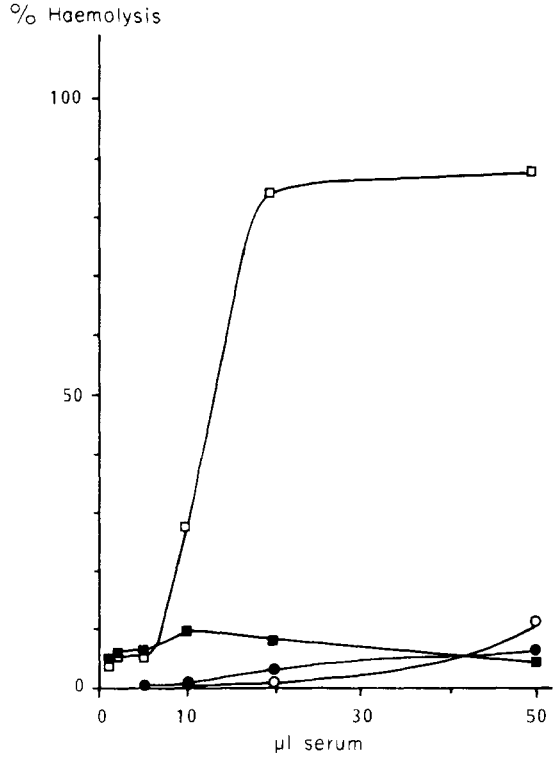

Fig. 1. Haemolytic complement activity of normal (C57BL × CBA)F1 (□) and DBA/2 (○) serum, and Lm-activated (C57BL × CBA)F1 (■) and DBA/2 (●) serum.

Table 3. CFU-s mobilization by *Listeria*-treated normal mouse serum

Serum donor	C5†	Recipient	C5†	CFU-s/ml blood*		
				NMS‡	*Lm*-NMS	INACT-*Lm*-NMS
(C57BL × CBA)F1	+	(C57BL × CBA)F1	+	87 ± 5§	326 ± 13	85 ± 9
				26 ± 7	304 ± 22	46 ± 6
DBA/2	−	(C57BL × CBA)F1	+	73 ± 8	92 ± 7	92 ± 4
				59 ± 4	61 ± 6	
AKR	−	(C57BL × CBA)F1	+	55 ± 7	56 ± 6	79 ± 6
				78 ± 6	90 ± 5	160 ± 6
(C57BL × CBA)F1	+	DBA/2	−	149 ± 10	669 ± 22	128 ± 4
				105 ± 11	695 ± 48	118 ± 9

* Blood was taken 30 min after i.v. injection of 0·75 ml serum.

† C5-sufficient mouse strains are indicated by +. C5-deficient mouse strains by −.

‡ NMS means normal mouse serum; *Lm*-NMS means NMS incubated with *Listeria* bacteria; INACT-*Lm*-NMS means NMS from which the complement enzymes were inactivated by treatment for 30 min at 56°C and was subsequently incubated with *Listeria* bacteria.

§ Arithmetic mean ± SEM.

with *Lm* had no significant activity. *Lm*-treated serum from C5-deficient DBA/2 and AKR mice could not cause an increase in the blood CFU-s numbers.

Since the observed CFU-s mobilization in C5-sufficient mice injected with *Lm*-treated NMS might be due to activation of the recipients C system, we also injected C5-deficient DBA-2 mice with *Lm*-treated C5-sufficient (C57BL × CBA)F1 serum. Also under these conditions, *Lm*-treated serum caused CFU-s mobilization (Table 3). The somewhat increased numbers of CFU-s in the peripheral blood of the NMS-infused control mice are at the upper limit of the normal range found in untreated control mice. As to the normal variation in background circulating CFU-s numbers, in each experiment a control group was included from the same supplier and delivery.

Not only *Lm*-activated NMS mobilized CFU-s, but also *Lm*-activated normal rat serum was able to do so. This is shown in Table 4 for Lewis rat serum infused in C5-deficient DBA/2 mice.

C5a, in contrast to most other C components, including native C5, is resistent to heat-inactivation for 30 min at 56°C (Hugli & Müller-Eberhard, 1978). We made use of this property to determine whether C5a may be the intermediate in the LPS-induced mobilization of CFU-s. Indeed, NMS from C5-sufficient mice that had been incubated with *Lm*, and subsequently heat-inactivated, was still able to mobilize CFU-s. This was found in C5-sufficient (C57BL × CBA)F1 recipients as well as in C5-deficient DBA/2 recipients (Table 5).

Table 4. CFU-s mobilization by *Listeria*-treated rat serum

				CFU-s/ml blood*		
Serum donor	C5†	Recipient mice	C5†	PBS	NRaS‡	*Lm*-NRaS
Lewis rat	+	DBA/2	–	27 ± 6§	25 ± 6	270 ± 48

* Blood samples were taken 30 min after i.v. injection of 0·75 ml serum.

† Lewis rats are C5-sufficient, DBA/2 mice are C5-deficient.

‡ NRaS means normal rat serum; *Lm*-NRaS means NRaS that has been incubated with *Listeria* bacteria.

§ Arithmetic mean ± SEM.

Table 5. CFU-s mobilization by heat inactivated *Listeria*-treated normal mouse serum

				CFU-s/Ml blood*		
Serum donor	C5†	Recipient	C5†	NMS‡	*Lm*-NMS	*Lm*-NMS-INACT
(C57BL × CBA)F1	+	(C57BL × CBA)F1	+	86 ± 7§	495 ± 21	333 ± 17
				124 ± 8	686 ± 28	630 ± 20
(C57BL × CBA)F1	+	DBA/2	–	24 ± 7	146 ± 18	172 ± 35
				23 ± 5	197 ± 38	172 ± 38
				21 ± 14	368 ± 38	225 ± 74

* Blood samples were taken 30 min after i.v. injection of 0·75 ml serum.

† C5-sufficient mouse strains are indicated by +, C5-deficient mouse strains by –.

‡ NMS means normal mouse serum; *Lm*-NMS means NMS incubated with *Listeria* bacteria; *Lm*-NMS-INACT means NMS that was incubated with *Listeria* bacteria and was subsequently treated for 30 min at 56°C to inactivate the complement enzymes, except C5a.

§ Arithmetic mean ± SEM.

CFU-s mobilization by purified rat C5a

The possible mediatory role of C5a in murine CFU-s mobilization was further analysed by infusing C5-deficient DBA/2 mice with purified rat C5a. Rat C5a was used because no isolation procedures are available for mouse C5a, as yet. It was found that purified rat C5a caused a dose-dependent accumulation of CFU-s in the peripheral blood (data not shown). After i.v. injection of 25 μg purified rat C5a, maximum numbers of circulating CFU-s were found after 15 to 30 min (Table 6). The peak numbers of CFU-s induced by purified rat C5a were of the same order of magnitude as those caused by injection of C5-sufficient mice with LPS or by injection of *Lm*-activated NMS or *Lm*-activated normal rat serum (c.f. Tables 1, 3 and 4).

Table 6. CFU-s mobilization in C5-deficient DBA/2 mice injected with purified rat C5a

	Min after injection of purified rat C5a*				
PBS	5	15	30	60	120
61 ± 11†	160 ± 32	323 ± 60	240 ± 56	218 ± 53	95 ± 15
26 ± 4	68 ± 17	222 ± 39	399 ± 113	230 ± 38	52 ± 8
P value‡	$P = 0{\cdot}030$	$P = 0{\cdot}0024$	$P = 0{\cdot}014$	$P = 0{\cdot}011$	$P = 0{\cdot}035$

* Blood samples were taken at the indicated intervals after i.v. injection of 25 μg purified rat C5a.

† Figures represent the arithmetic mean ±1 SEM of the number of CFU-s per ml blood.

‡ *P*-values (calculated by the two-sided Student *t*-test) refer to comparison of the figures found at the various intervals after injection of rat C5a and the figures obtained after injection of PBS.

DISCUSSION

A variety of studies show that i.v. injection of mice with bacterial LPS induces the mobilization of CFU-s from the haemopoietic tissues into the peripheral blood within minutes of injection (Vos *et al.*, 1972; Vos & Wilschut, 1979). This early mobilization is not restricted to CFU-s since more mature cell types are also mobilized, although not all to the same extent (Ploemacher *et al.*, 1980). The present studies extend these observations by showing that GM-CFU and E-BFU are also rapidly mobilized by LPS. It can thus be concluded that the early LPS-induced mobilization of haemopoietic cells neither has cell-type specificity nor is restricted to particular differentiation stages.

In a previous paper we have concluded that the C system is involved in the early LPS-induced mobilization of CFU-s, since LPS-induced CFU-s mobilization is deficient in mice that have been pre-treated with CoF (Wilschut *et al.*, 1979) and also in C5-deficient mice (Benner *et al.*, 1981). In the present paper it appears that this role of the C system is not restricted to the mobilization of CFU-s. The mobilization of GM-CFU, E-BFU (Table 1) and mature cell types (data not shown) is also highly defective in C5-deficient mice. This suggests that C is involved in the LPS-induced mobilization of all types of haemopoietic cells.

In the LPS-non-responder mouse strains C3H/HeJ and C57BL/10.ScCr, which lack a serologically identifiable structure ('LPS-receptor') that is present in all LPS-responder strains (Forni & Coutinho, 1978), the late but not the early mobilization of CFU-s upon LPS injection is absent (Benner *et al.*, 1981). Together with the above data concerning the role of C, this observation suggests that not the LPS-receptor, but one or more C components may

be the active principle(s) which account for the rapid mobilization of haemopoietic cells after LPS injection.

In the present paper we show that *in vitro* activation of C5-sufficient NMS and normal rat serum by *Lm* leads to the formation of product(s) that can induce CFU-s mobilization in C5-deficient mice (Table 3 and 4). Such *Lm*-activated serum is still active after heat-inactivation for 30 min at 56°C (Table 3). As C5a and its nonspecific helper factor, in contrast to most other C components, are resistant to heat inactivation (Hugli & Müller-Eberhard, 1978; Perez & Goldstein, 1981), this observation suggests that C5a or an *in vivo* derivative of C5a is the active principle in the LPS-induced early mobilization of CFU-s and haemopoietic progenitor cells. This might be related to the chemotactic activity of C5a (Snyderman *et al.*, 1969; Fernandez *et al.*, 1978), supposing that CFU-s and haemopoietic progenitor cells have a binding site for this fragment.

Recent data suggest that spleen colonies measured at 7–8 days after i.v. injection of CFU-s and colonies measured on days 12–13 are derived from (partly) different subpopulations of CFU-s (Bertoncello, Hodgson & Bradley, 1985). As we determined the spleen colonies on day 8 only, the data presented refer to this particular subpopulation only. It would be of interest to investigate whether CFU-s that give rise to days 12–13 colonies behave similarly.

The mechanism of translation of such a chemotactic stimulus is not entirely known. When the active substance reaches the surface of the cell, an esterase is activated and the hexosemonophosphate shunt is stimulated. Subsequently, calcium enters the cytoplasm and the cytoplasmic contractile proteins assemble (Loor, 1980). Thus, both the energy and the means required for the movement of the cell are provided.

Alternatively, the haemopoietic cells might be sent passively into the circulation, being brought about by other cells which are known to be susceptible to the chemotactic activity of C5a, such as neutrophils and macrophages (Snyderman *et al.*, 1969; Snyderman, Shin & Hauseman, 1971). Furthermore, it cannot be excluded that the mobilization by C5a is mediated by its property to induce contraction of smooth muscle, or the fact that it is an anaphylatoxin inducing the release of histamine from mast cells and basophils (Hugli & Müller-Eberhard, 1978), which may lead to increased vascular permeability.

It is remarkable that the mobilization of CFU-s and haemopoietic progenitor cells by trypsin and proteinase is not decreased in C5-deficient mice, since previous studies have shown that decomplementation of mice by high doses of CoF does inhibit CFU-s mobilization by these enzymes (Wilschut *et al.*, 1979). Proteolytic enzymes, however, have been shown to activate C3 directly (Bokisch, Müller-Eberhard & Cocchrane, 1969; Molenaar *et al.*, 1974), so that the effects of these enzymes on CFU-s mobilization in C5-deficient mice are most likely to be explained by C3-activation and, consequently, release of C3a, which is also an anaphylatoxin, but which has only limited chemotactic activity (Snyderman *et al.*, 1969; Hugli & Müller-Eberhard, 1978). The inability of CoF-treated mice to respond to trypsin and proteinase is probably due to exhaustion of C3 (as well as later components of the C cascade), so that CoF-treated mice cannot generate C3a and C5a, and thus cannot mobilize CFU-s.

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