Anaesthesia affects outcome of sepsis in mice

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Purpose: To determine whether the survival curves and rates differ between a mutant strain of mice (C3H/HeJ: endotoxin-resistant) and an endotoxin-susceptible strain (C3H/HeN) when severe sepsis (caecal ligation and puncture: CLP) is performed with different anaesthetics (ketamine or varied exposure to halothane).

Methods: The CLP was performed under a randomly chosen anaesthetic method out of inhalation of halothane in oxygen (15 min, 2 hr, or 6 hr) (100, 120, and 103 mice respectively) or a single injection of ketamine *im* (127 mice) in paired fashion in eight-week-old male mice of each strain. The daily survival rates were compared between the two strains until the 10th day after CLP for each anaesthetic and among the four anaesthetic methods for each strain using multiple comparisons.

Results: The C3H/HeJ had delayed death relative to the C3H/HeN with every anaesthetic method except a two hours halothane ($P < 0.01$ at day 1 and day 2–5 in halothane 15 min and 6 hr, $P < 0.05$ at day 4–6 in ketamine), however, the final survival rates for each method of anaesthesia were the same in the two strains. Regardless of the genetic susceptibility to endotoxin, short exposure to halothane resulted in the most rapid death compared with the other anaesthetic methods ($P < 0.05$), and two hours halothane showed the best survival.

Conclusion: Endotoxin susceptibility affected the septic course for each anaesthetic method, and the anaesthetic methods influenced the survival from sepsis in both strains.

Objectif : Déterminer si les taux et courbes de survie diffèrent entre deux lignées de souris, une mutante résistante à l'endotoxine (C3H/HeJ) et une souche susceptible à l'endotoxine (C3H/HeN) lorsqu'elles sont soumises à une septicémie sévère, provoquée par la ligature du caecum et sa perforation (CLP), sous différents agents anesthésiques, kétamine ou halothane d'une durée d'exposition variable.

Méthodes : La CLP a été pratiquée chez des groupes pairés de souris mâles âgées de 8 semaines provenant de chaque lignée et soumis à une technique anesthésique choisie aléatoirement: anesthésie d'inhalation à l'halothane dans l'oxygène (15 min, 2h, 6h) (100, 120 et 103 souris respectivement) ou injection *im* unique de kétamine (127 souris). Les taux de survie quotidiens ont été comparés entre les deux lignées de souris jusqu'au 10^e jour après CLP pour chacun des anesthésiques et entre les quatre protocoles anesthésiques pour chaque lignée en utilisant des comparaisons multiples.

Résultats : La lignée résistante à l'endotoxine est décédée de façon plus tardive que la lignée susceptible pour tout protocole anesthésique sauf pour l'anesthésie à l'halothane d'une durée de 2 heures ($P < 0,01$ au jour 1 et jours 2–5 dans les groupes halothane 15 min et 6 heures, et $P < 0,05$ aux jours 4–6 pour la kétamine); cependant, le taux final de survie a été le même pour chaque technique anesthésique et pour chaque lignée de souris. Indépendamment de la susceptibilité génétique à l'endotoxine, une exposition courte à l'halothane a entraîné les décès les plus rapides par rapport aux autres méthodes ($P < 0,05$), l'anesthésie de deux heures à l'halothane étant associée à la meilleure survie.

Conclusion : La susceptibilité à l'endotoxine a affecté l'évolution de la septicémie pour chaque technique anesthésique, et les techniques anesthésiques ont influencé la survie à la septicémie dans les deux lignées.

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THE elucidation of the pathophysiology of sepsis is essential to Critical Care Medicine because sepsis is still a major cause of morbidity and mortality in the intensive care unit.¹ During sepsis, macrophages perform a vital function in responding to initial events and regulating mediators,² such as tumour necrosis factor (TNF).

The mutant mouse strain C3H/HeJ is considered to be "endotoxin-resistant" as well as "susceptible to bacterial infection" because its macrophages do not produce tumour necrosis factor in response to endotoxin.³⁻⁵ Thus, the question arises as to how they would react to septic stimuli. Caecal ligation and puncture (CLP) is a well-established model of sepsis that closely resembles human sepsis.⁶ Endotoxin-resistant mice have been shown to have good survival rates after CLP compared with endotoxin-susceptible (syngeneic C3H/HeN) mice when CLP is performed under anaesthesia using a combination of ketamine and xylazine.⁷ However, other investigators⁸ have reported that there is no difference in mortality rates for these strains when CLP is performed during a brief exposure to methoxyflurane.

We considered whether the differences observed might result from the use of different anaesthetics, because the non-specific immune response to viral and/or bacterial stimulants involving macrophages and neutrophils differs whether ketamine or halothane anaesthesia is used.⁹ Further, we doubted whether varying the halothane exposure time would affect the early response to sepsis because mice exposed to halothane for two hours showed suppression of the non-specific immune-response.⁹

We hypothesized that different anaesthetics and different exposure times to anaesthetics would affect the survival rate of sepsis and the cause of the controversial results observed in endotoxin-susceptible and -resistant mice would be elucidated. To test the hypothesis, the effect of two different anaesthetics (halothane and ketamine) and different halothane exposure times on survival rates after CLP in endotoxin-resistant and -susceptible mice were examined.

Methods

Animals

The experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee of Gunma University. In total, 580 specific pathogen-free eight-week-old male (25–30 g) endotoxin-resistant and -susceptible mice were obtained from Japan SLC Incorporated (Hamamatsu, Japan). They were housed in a room maintained at a constant temperature (25°C) with free access to pellet food and water at all times.

Surgical procedures

The CLP procedure described by Baker *et al.*⁷ and Wichterman *et al.*⁶ was adopted. In brief, ethanol 70% was sprayed on the abdomen after anaesthesia as described in Experimental Procedures, and a 10-mm midline incision was made. The caecum was exposed and ligated just below the ileocaecal valve in such a way that the ligation permitted uninterrupted bowel continuity. Two through-and-through punctures of the caecum were made with a 20-gauge needle, and the caecum was gently squeezed to express a tiny amount of faeces. The abdominal incision was closed, and 1 ml physiological saline sc was administered for fluid resuscitation.

Experimental procedures

Caecal ligation and puncture was performed at the same time in 10 endotoxin-resistant and in 10 -susceptible mice under 200 mg·kg⁻¹ ketamine *im* anaesthesia (Park-Davis-Sankyo, Tokyo, Japan) or during halothane anaesthesia (1.5–2.0% in oxygen). In the halothane anaesthesia groups, three different exposure times (15 min, two hours, or six hours) were adopted. Twenty mice as a group were randomly assigned to one anaesthesia method out of four. The paired experiment (10 endotoxin-resistant and 10 -susceptible mice) was repeated until more than 50 mice of each strain were attained in each anaesthesia group (Table I). Each mouse in the halothane group was anaesthetised separately in a small box. After a surgical level of anaesthesia had been reached, the mouse was removed from the box and surgery was performed while the mouse inspired a halothane/oxygen mixture through an anaesthesia mask. In the groups exposed to halothane for two or six hours, the heads of mice were inserted into small holes placed 3 cm apart in an anaesthesia box after CLP was performed. Oxygen-halothane (5 L·min⁻¹) was insufflated into the anaesthesia box to maintain a stable level of anaesthesia for two or six hours. In the ketamine anaesthesia group, the mice received one intramuscular injection, and surgery was carried out. In each sham-operated group, paired 10 mice were anaesthetised with ketamine or halothane, and the surgical procedure was carried out in the same way as for the experimental group, with the exception of caecal ligation and puncture. After completion of surgery, the animals were given free access to food and water.

The distribution of experimental groups

A total of 560 mice were used in this study (experimental group = 480, sham operated group = 80, 30 mice were lost due to possible anaesthetic overdosing or surgical errors) see Table I.

TABLE I The distribution of mice

Group	anaesthesia	strains of mice	total	number of mice failure*	evaluated
Caecal ligation and puncture groups	halothane	endotoxin-resistant	50	0	50
	15 min	endotoxin-susceptible	50	0	50
	halothane	endotoxin-resistant	60	0	60
	2 hours	endotoxin-susceptible	60	0	60
	halothane	endotoxin-resistant	60	7	53
	6 hours	endotoxin-susceptible	60	10	50
	ketamine	endotoxin-resistant	70	6	64
		endotoxin-susceptible	70	7	63
	halothane	endotoxin-resistant	10	0	10
	15 min	endotoxin-susceptible	10	0	10
Sham operated groups	halothane	endotoxin-resistant	10	0	10
	2 hours	endotoxin-susceptible	10	0	10
	halothane	endotoxin-resistant	10	0	10
	6 hours	endotoxin-susceptible	10	0	10
	ketamine	endotoxin-resistant	10	0	10
		endotoxin-susceptible	10	0	10
total			560	30	530

*failure shows that the mice were lost due to possible anaesthetic overdosing or surgical errors.

Statistics

All animals were assessed daily to determine the survival rate until the 10th day after CLP (few animals died after 10 days). The differences in daily survival rates for endotoxin-resistant and -susceptible mice using each anaesthetic method were examined using Kaplan-Meier's method. The difference in the survival rate of each strain was compared for the four anaesthetic methods using multiple comparisons by Ryan's method.¹⁰ A $P < 0.05$ was considered significant.

Results

There were no deaths in the immediate post-surgical period in either the experimental or sham-operated groups, nor throughout 10 days in the sham-operated groups (Table II). Except for the group given halothane for 2 hours, the endotoxin-resistant mice showed a better survival rate than the endotoxin-susceptible mice immediately and at 24 hr after CLP in the group given halothane for 15 min, until five days in the group given halothane for six hours and for 4 to 6 days in the group given ketamine anaesthesia (Table II). However, the final survival rates for endotoxin-resistant and -susceptible mice in each of the four anaesthesia groups converged to a single value for each anaesthesia method (Table II). There was no difference in the final survival rate between two strains irrespective of anaesthesia methods.

When the survival curves of the two strains were compared for the four anaesthesia groups, they were influenced by anaesthesia methods by a similar trend in both strains (see Figure on page 364). Mice briefly exposed to halothane died most rapidly. In the groups

given halothane anaesthesia for a two or six hours, or those given ketamine anaesthesia, death was delayed. Mice given halothane anaesthesia for two hours showed the best survival throughout the observation period.

Discussion

The main finding of this investigation was that the endotoxin-resistant mice showed a better survival than the endotoxin-susceptible mice after CLP transiently irrespective of the anaesthesia method. Also, the anaesthetic method influenced the septic course regardless of the strain.

What is different from the previous studies?

Baker *et al.*⁷ and Kisala *et al.*⁸ separately reported survival from CLP in the endotoxin-resistant and -susceptible mice using different anaesthesia. In this study, the survival rate of the endotoxin-resistant mice was superior to that of the endotoxin-susceptible mice for four to six days after CLP with ketamine anaesthesia, similar to Baker's data, and mice briefly exposed to halothane showed a similar to those in Kisala's study (Table II). Thus, we concluded that the difference in Baker's⁷ and Kisala's results⁸ was caused by the different anaesthetics used in performing CLP. This is the first report showing that the controversial survival in severe sepsis between the endotoxin-resistant and -susceptible strains were caused by the anaesthetic methods used.

Why did anaesthesia affect septic course?

The anaesthetic method affected the septic course regardless of the mouse strain. This is the first report to

TABLE II Daily surviving numbers (survival rates) of mice for ten days from caecal ligation and puncture (CLP) in endotoxin-resistant (R) and -susceptible (S) mice which were anaesthetised with halothane (15 min, 2 or 6 hours) or ketamine.

<i>Anaesthesia</i>	<i>strains of mice</i>	<i>number of mice surviving from CLP (survival rates %)</i>				
		0 5 9	1 6 10	2 7 days after CLP	3 8	4
experimental groups						
	R	50	30 (60.0) 4 (8.0) 3 (8.0)	10 (20.0) 3 (8.0) 3 (8.0)	6 (12.0) 3 (8.0)	5 (10.0) 3 (8.0)
halothane 15 min	S	50	16 (32.0) 2 (4.0) 1 (2.0)	5 (10.0) 2 (4.0) 1 (2.0)	2 (4.0) 2 (4.0)	2 (4.0) 2 (4.0)
	R	60	51 (85.0) 25 (41.7) 17 (25.2)	38 (63.3) 22 (36.7) 17 (25.2)	33 (55.0) 21 (35.0)	30 (50.0) 19 (31.7)
halothane 2 hours	S	60	49 (81.7) 21 (35.0) 17 (25.2)	36 (60.0) 19 (31.7) 17 (25.2)	29 (48.3) 18 (30.0)	25 (41.7) 8 (30.0)
	R	53	49 (92.5) 14 (26.4) 3 (5.7)	32 (60.0) 8 (15.1) 3 (5.7)	24 (45.3) 5 (9.4)	21 (35.0) 4 (7.5)
halothane 6 hours	S	50	39 (78.0) 3 (6.0) 3 (6.0)	17 (34.0) 3 (6.0) 3 (6.0)	9 (18.0) 3 (6.0)	3 (6.0) 3 (6.0)
	R	64	51 (79.7) 20 (31.3) 9 (14.1)	27 (42.2) 19 (29.7) 8 (12.5)	24 (37.5) 12 (18.8)	22 (34.4) 10 (15.6)
ketamine	S	63	46 (73.0) 9 (14.3) 7 (11.1)	23 (36.5) 8 (12.7) 7 (11.1)	16 (25.4) 8 (12.7)	11 (17.5) 7 (11.1)
sham-operated groups						
halothane 15 min	R	10	no deaths until 10 days			
	S	10	no deaths until 10 days			
halothane 2 hours	R	10	no deaths until 10 days			
	S	10	no deaths until 10 days			
halothane 6 hours	R	10	no deaths until 10 days			
	S	10	no deaths until 10 days			
ketamine	R	10	no deaths until 10 days			
	S	10	no deaths until 10 days			

Strains of mice: R = endotoxin-resistant and S = endotoxin-susceptible strains

* $P < 0.05$, † $P < 0.001$, ‡ $P < 0.01$ compared with survival rates of endotoxin-susceptible mice in the same anaesthesia group by Kaplan-Meier's method.

show that different halothane exposure times affected the septic course, and we suggest that the immunosuppression by anaesthesia might be possible cause. A plethora of immunosuppressive reactions after general

anaesthesia has been reported. The main reactions include (a) decreased natural killer cell activity,¹¹ (b) alterations in lymphocyte populations and functions,¹² (c) decreased resistance to sepsis,¹² (d) decreased resistance to malignant tumours,¹³⁻¹⁵ and (e) depression of PMN function.¹⁶ The apparent discrepancy in survival curves between the short halothane exposure group and the other groups (Figure) suggested that prolonged anaesthesia would affect the outcome of CLP favourably compared with a brief halothane exposure which was maintained only during surgery. Short-term halothane exposure might affect macrophages and other immunocytes to a minimal degree, leading to full expression of the CLP inflammatory reaction which results in early death. The difference between halothane anaesthesia for two hours and the ketamine anaesthesia described by Tait *et al.*⁹ as well as this study might be caused by a difference in the suppressive potency of the anaesthetics on macrophages, because ketamine was also reported to suppress endotoxin-induced tumour necrosis factor alpha production in mice.¹⁷ A two-hour halothane exposure might be the optimum for macrophage and leukocyte suppression in mice, and a six-hour exposure may suppress immunocytes and other functions to such a degree that CLP survival becomes unequal to that in a two-hour halothane exposure. Echtenacher *et al.*¹⁸ reported that TNF played a crucial role in the initial hours after infection (within 8 hours in mice), because mice died when monoclonal antibody to TNF was administered within 8 hours of CLP; however, after 8 hours its administration did not affect the outcome.¹⁸ This might be one of reasons why a two-hour halothane exposure was superior to a six-hour halothane exposure.

Why did the endotoxin-susceptibility affect the septic course?

In this study, the endotoxin-resistant mice showed better (at least equal) survival than the endotoxin-susceptible mice transiently after CLP irrespective of anaesthesia methods. A single mutation (the *lps^d* mutation) on chromosome 4 accounts for the difference between endotoxin-resistant and -responsive mice.¹⁹ This region regulates the macrophage response to endotoxin, and mutation results in reduced production of TNF in the mutant strain in response to endotoxin stimulation. The difference in the survival rates for the two strains with each anaesthetic method (Table II) must be due to the difference in macrophage function, because anaesthesia affected both strains in the same way and all other functions except macrophage are considered to be unaltered in the mutant strain.^{3,19} TNF plays an important role in defending the body from

FIGURE Comparison of survival curves of endotoxin-resistant (upper half of the figure) and -susceptible mice (lower half of the figure) after caecal ligation and puncture (CLP) between exposure to a short period of halothane (squares), six hours halothane (circles), ketamine anesthesia (triangles), and two hours halothane (diamonds). Statistical difference compared with halothane short exposure group only by Ryan's method was shown; (* $P < 0.05$).

invading bacteria, as shown by the impaired clearance of inoculated bacteria in the urinary tract in endotoxin-resistant mice,²⁰ the high susceptibility to *Salmonella typhimurium* infection⁵ and live *E-coli* infection in endotoxin-resistant mice,²¹ and the increased survival of TNF-pretreated mice from infection.²¹ Accordingly, we considered that a mutant strain of mice deficient in macrophage TNF production in response to endotoxin could not survive a polymicrobial sepsis. Contrary to our assumption, death was delayed in endotoxin-resistant mice, and the reason for this will be explained as follows.

Ayala *et al.*²² reported that endotoxin-resistant mice showed an increase in blood TNF, IL-6, and IL-1 concentrations after CLP. However, their peritoneal macrophages obtained after CLP showed little response to further stimulation with LPS compared with those obtained from endotoxin-responsive mice. The CLP is a gradually-developing sepsis model which involves surgery and, therefore, it differs from a simple injection of live bacteria or endotoxin. Trauma (surgery) stress may lead to acute phase gene transcription by TNF- and IL-6-independent mechanism,²³ which suggests involvement of other factors such as interferon-gamma (IFN). In septic patients, elevated serum concentrations of IFN are observed,²⁴ and IFN primes the endotoxin-resistant macrophages to respond to septic stimuli.²⁵ This might be the reason why endotoxin-resistant mice showed better survival rates in CLP than endotoxin-susceptible mice, as endotoxin-resistant mice might secrete TNF in a beneficial degree for short-term survival.²²

Two-hour halothane exposure rendered endotoxin-susceptible mice capable of survival to the same degree as endotoxin-resistant mice, as suggested by Tait *et al.*⁹ Although deaths in endotoxin-resistant mice were delayed, the final survival rates in endotoxin-resistant and -susceptible mice were the same for each method of anaesthesia. This might be due to a difference in the endotoxin-resistant macrophage response to CLP compared with endotoxin-susceptible macrophage providing protection from early death, but no ultimate cure from infection.

To verify our findings, further study such as measurement of altered expression of TNF-mRNA by anaesthesia in immunocytes will be needed. However, the results obtained from this study suggest that suppression of immunofunction by anaesthetics might be beneficial in certain types and at certain stages of infection. In conclusion, the endotoxin susceptibility affected the severe septic course for each anaesthetic method, and anaesthesia methods influenced the septic course in both strains. When immunological

function is being evaluated, the effect of anaesthesia has to be considered.

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References

- 1 Parrillo JE, Parker MM, Natanson C, *et al.* Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. *Ann Intern Med* 1990; 113: 227–42.
- 2 Nathan CF. Secretory products of macrophages. *J Clin Invest* 1987; 79: 319–26.
- 3 Beutler B, Krochin N, Milsark IW, Leudke C, Cerami A. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* 1986; 232: 977–80.
- 4 Nakane A, Minagawa T, Kato K. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. *Infect Immun* 1988; 56: 2563–9.
- 5 O'Brien AD, Rosenstreich DL, Scher I, Campbell GH, MacDermott RP, Formal SB. Genetic control of susceptibility to *salmonella typhimurium* in mice: role of the LPS gene. *J Immunol* 1980; 124: 20–4.
- 6 Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock – a review of laboratory models and a proposal. *J Surg Res* 1980; 29: 189–201.
- 7 Baker CC, Niven-Fairchild T, Caragnano C, Kupper TS. Outcome following femur fracture and subsequent cecal ligation and puncture in endotoxin-sensitive (C3H/HeN) and endotoxin-resistant (C3H/HeJ) mice. *J Surg Res* 1991; 50: 170–4.
- 8 Kisala JM, Ayala A, Perrin MM, Stephan RN, Chaudry IH. Lethality and altered macrophage cytokine production following sepsis in endotoxin-tolerant mice. *Circ Shock (Abstract)* 1989; 27: 304.
- 9 Tait AR, Davidson BA, Johnson KJ, Remick DG, Knight PR. Halothane inhibits the intraalveolar recruitment of neutrophils, lymphocytes, and macrophages in response to influenza virus infection in mice. *Anesth Analg* 1993; 76: 1106–13.
- 10 Ryan TA. Significance tests for multiple comparison of proportions, variances, and other statistics. *Psychol Bull* 1960; 57: 318–28.
- 11 Markovic SN, Knight PR, Murasko DM. Inhibition of interferon stimulation of natural killer cell activity in mice anesthetized with halothane or isoflurane. *Anesthesiology* 1993; 78: 700–6.

- 12 *Hansbrough JF, Zapata-Sirvent RL, Bartle EJ, et al.* Alterations in splenic lymphocyte subpopulations and increased mortality from sepsis following anesthesia in mice. *Anesthesiology* 1985; 63: 267-73.
- 13 *Moudgil GC, Singal DP.* Halothane and isoflurane enhance melanoma tumour metastasis in mice. *Can J Anaesth* 1997; 44: 90-4.
- 14 *Waxler B, Zhang X, Wezeman FH.* Anesthetic agents modify tissue proteinase inhibitor content and tumor behavior. *J Lab Clin Med* 1994; 123: 53-8.
- 15 *Radošević-Stašić B, Udovič-Sirota M, Stojanov L, Ribari L, Rukavina D.* Growth of allogenic sarcoma in mice subjected to halothane anesthesia and/or surgical stress. *Anesth Analg* 1989; 69: 570-4.
- 16 *Nakagawara M, Takeshige K, Takamatsu J, Takahashi S, Yoshitake J, Minakami S.* Inhibition of superoxide production and Ca^{2+} mobilization in human neutrophils by halothane, enflurane, and isoflurane. *Anesthesiology* 1986; 64: 4-12.
- 17 *Takenaka I, Ogata M, Koga K, Matsumoto T, Shigematsu A.* Ketamine suppresses endotoxin-induced tumor necrosis factor alpha production in mice. *Anesthesiology* 1994; 80: 402-8.
- 18 *Echtenacher B, Falk W, Männel DN, Krammer PH.* Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J Immunol* 1990; 145: 3762-6.
- 19 *Rosenstreich DL, Vogel SN, Jacques AR, Wahl LM, Oppenheim JJ.* Macrophage sensitivity to endotoxin: genetic control by a single codominant gene. *J Immunol* 1978; 121: 1664-70.
- 20 *Hagberg L, Hull R, Hull S, McGhee JR, Michalek SM, Eden CS.* Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. *Infect Immun* 1984; 46: 839-44.
- 21 *Cross AS, Sadoff JC, Kelly N, Bernton E, Gemski P.* Pretreatment with recombinant murine tumor necrosis factor χ /cachectin and murine interleukin 1a protects mice from lethal bacterial infection. *J Exp Med* 1989; 169: 2021-7.
- 22 *Ayala A, Kisala JM, Felt JA, Perrin MM, Chaudry IH.* Does endotoxin tolerance prevent the release of inflammatory monokines (interleukin 1, interleukin 6, or tumor necrosis factor) during sepsis? *Arch Surg* 1992; 127: 191-7.
- 23 *Macada K, Schoeniger LO, Shimada M, Winchurch RA, Buchman TG, Robotham JL.* Regulation of acute phase gene expression following surgery and endotoxin administration in the anesthetized pig. *Anesthesiology* 1993; 79: 1324-37.
- 24 *Girardin E, Grau GE, Dayer J-M, Roux-Lombard P, The J5 Study Group, Lambert PH.* Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med* 1988; 319: 397-400.
- 25 *Beutler B, Tkacenko V, Milsark I, Krochin N, Cerami A.* Effect of γ -interferon on cachectin expression by mononuclear phagocytes. Reversal of the *lps*^d(endotoxin resistance) phenotype. *J Exp Med* 1986; 164: 1791-6.