The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/25762

Please be advised that this information was generated on 2017-06-30 and may be subject to change.
Elimination of Various Subpopulations of Macrophages and the Development of Multiple-Organ Dysfunction Syndrome in Mice

Grard A. P. Nieuwenhuijzen, MD; Maarten F. C. M. Knapen, MD; Thijs Hendriks, PhD; Nico van Rooijen, PhD; R. Jan A. Goris, MD, PhD

Objective: To evaluate the role of specific macrophage subpopulations in the development of zymosan-induced multiple-organ dysfunction syndrome by selective elimination of liver, splenic, alveolar, and peritoneal macrophages.

Design: Randomized animal trial.

Setting: Central animal laboratory at the University Hospital Nijmegen, Nijmegen, the Netherlands.

Animals: Male C57Bl/6 mice.

Interventions: Elimination of macrophages was accomplished by administration of multilamellar liposomes that contained dichloromethylene bisphosphonate (C12MBP). Intravenous, intratracheal, and intraperitoneal administrations induced an elimination of liver and splenic, alveolar, and peritoneal and omental macrophages, respectively. Zymosan (1 mg/g) was injected intraperitoneally at day 0. The liposomes that contained C12MBP were administered before and after zymosan challenge. At day 12, all surviving mice were killed.

Main Outcome Measures: The body weights, temperatures, and mortality rates of the mice were monitored daily.

Results: The liposomes that contained C12MBP, administered intravenously before or after zymosan challenge, did not induce significant changes in the body weight, temperature, or mortality rate. The ROW of the liver was significantly decreased in both treatment groups. Elimination of liver and splenic macrophages after zymosan challenge induced an increased ROW of the lung and a decreased ROW of the liver. The liposomes that contained C12MBP, administered intratracheally before zymosan challenge, completely prevented deaths. The body weights, temperatures, and ROWs of the mice were not changed. The liposomes that contained C12MBP, administered intraperitoneally, did not change the body weight, temperature, or ROW. The liposomes that contained C12MBP, administered intraperitoneally before zymosan challenge, increased the mortality from 50% to 90%.

Conclusions: These data show that the elimination of specific macrophage subpopulations and the elimination on specific time points in this model had differential effects, indicating a differential role of specific macrophage subpopulations, either protective or detrimental, in the development of multiple-organ dysfunction syndrome.

Arch Surg. 1997;132:533-539

N NORMAL host-defense mechanisms, an inflammatory response is teleologically beneficial and is meant to protect the host against injurious events. On the contrary, in pathological conditions (eg, the systemic inflammatory response syndrome [SIRS], the adult respiratory distress syndrome [ARDS], and the multiple-organ dysfunction syndrome [MODS]), an excessive generalized inflammatory response may have detrimental effects on the host.1,2 Macrophages play a pivotal role in normal host-defense mechanisms because of their antimicrobial phagocytic activity, antigen-presenting capacity, and regulation of the inflammatory response by their secretion of various immunoregulatory substances.3 However, evidence has been accumulating with regard to an excessive activation of macrophages that could induce a generalized dysregulated excessive inflammatory response, leading to the development of SIRS, ARDS, or MODS.4-6 This hypothesis is supported by various observations. First, administration of macrophage-derived cytokines to healthy volunteers produces a syndrome that closely resembles SIRS.7-9 Second, experimental and clinical data indicate a concurrent activation of macrophages during the development of SIRS, ARDS, and MODS, as demonstrated by increased levels of macrophage-derived products (eg, tumor necrosis factor [TNF], interleukin-1, and neopterin) with a positive correlation of elevated levels with the sub-

See Invited Commentary at end of article
MATERIALS AND METHODS

ANIMALS

Inbred, specific pathogen-free, male C57Bl/6 mice (Charles Rivers, Wiga, Germany) (age range, 6-8 weeks) were adapted to handling in their cages for 7 days prior to the start of the actual experiment. Throughout the experiment, all mice had free access to water that was acidified with hydrochloric acid (to pH 3) and to standard mice laboratory chow (RMH-GS pellets [irradiated at 10 kGy], Hope Farms, Woerden, the Netherlands). The room temperature was kept constant at 21°C, and a 12-hour lighting cycle was maintained. The experiments were approved by the Animal Ethics Committee of the Medical Faculty of the University of Nijmegen, Nijmegen, the Netherlands.

ZYMOSAN

Zymosan A (Sigma Chemical Co, St Louis, Mo), sterilized by use of γ-irradiation (5 kGy), was suspended (25 mg/mL) by high-frequency vibration in liquid paraffin (60-80 mPa, European Pharmacopoeia PA.5.68.81 CP846021). The suspension was sterilized by incubation in a water bath at 100°C for 80 minutes. One day before use, the suspension was heated to 40°C and dispersed by high-frequency vibration for 15 minutes. Sterility was confirmed by culture on a blood agar medium. The zymosan suspension was administered IP in a dose of 1 mg/g body weight.

PREPARATION OF Cl2MBP-CONTAINING LIPOSOMES

Multilamellar liposomes were composed of phosphatidylcholine and cholesterol (molar ratio, 6:1) and contained Cl2MBP dissolved in phosphate-buffered saline (PBS [0.25 mg/mL]). They were prepared as described earlier.22 In brief, dichloromethylene bisphosphonate (Cl2MBP or clodronate) is selectively delivered intracellularly to macrophages by a liposome carrier; this results in a destruction of the cell. Other cells are not structurally or functionally affected by this technique.23 By varying the route of administration, liposomes can be targeted to eliminate macrophages in specific tissues.22

We have applied this technique in the zymosan-induced generalized inflammation model. In rodents, intraperitoneal (IP) challenge with zymosan, a particulate cell wall product of the yeast Saccharomyces cerevisiae, induces a typical triphasic illness with an early hyperdynamic response with concurrent bacterial translocation from the gut and, after a recovery phase, a late bacteria-independent hypodynamic MODS-like phase with a generalized inflammatory response and organ dysfunction.24,25

Recently, we have shown that elimination of liver and splenic macrophages can abrogate the early hyperdynamic response in this model, despite an increased bacterial translocation.26 The purpose of the present study is to determine the role of specific macrophage subpopulations (ie, liver, spleen, alveolar, or peritoneal macrophages) in the development of MODS by studying the consequences of selective elimination of those macrophage subpopulations, either prior to or after the challenge with zymosan, for the clinical syndrome and organ damage in the late phase of this model.

RESULTS

Elimination of liver and splenic macrophages was confirmed by the absence of acid phosphatase activity of liver and splenic tissue (Figure 1) in the mice that were treated...

ARCH SURG/VOL 132, MAY 1997

334
For each route of liposome administration, verification of macrophage elimination was performed on 3 animals, and the results were compared with those for untreated mice. Elimination of macrophages in the liver and spleen was verified by analyzing acid phosphatase activity in 8-μm cryostat tissue sections of liver and spleen 2 days after IV injection of Cl₃MBP-containing liposomes. Acid phosphatase activity was demonstrated by incubation with 1% naphthol-AS-BI—phosphate and 4% hexazotized para-rosanilin for 30 minutes at 37°C. Elimination of alveolar macrophages was verified by quantification of macrophages in the alveolar lavage fluid at 2 days after IT administration of Cl₃MBP-containing liposomes. After the lungs were dissected free, alveolar lavage was performed 3 times with 1 mL of PBS that contained 0.38% citrate. After staining with trypan blue, cells were counted using a hemacytometer chamber (Bürker counter, Tamson Co, Zoetermeer, the Netherlands). Cytopsin preparations confirmed that more than 95% of the alveolar cells were macrophages. Elimination of the peritoneal macrophages was verified by quantification of macrophages in the peritoneal lavage fluid. The liposomes that contained Cl₃MBP were administrated IP at days –4 and –1. At day 0, peritoneal lavage was performed with 4 mL of PBS that contained 0.38% citrate. Quantification of cells was performed as described previously.

**EXPERIMENTAL DESIGN**

Three series of experiments were performed to assess the effects of macrophage elimination in the various tissues. Within each series, 3 groups of mice were used: a control group that received only zymosan and 2 experimental groups that received the Cl₃MBP-containing liposome suspension either before or after administration of zymosan.

Thus, 3 groups of 15 animals were used to study the effects of IV liposome administration. The experimental groups received liposomes either 2 days before or 4 days after zymosan challenge. The effects of IT liposome administration were studied in 3 groups of 20 mice each. The experimental groups received liposomes either 3 days before or 4 days after zymosan challenge. Three groups of similar size were used to examine the effects of IP liposome administration, for which the experimental groups received liposomes either both 4 days and 1 day before or 1 day and 4 days after zymosan challenge.

In all mice, body weight and rectal temperature were measured daily. The clinical condition with possible symptoms such as loss of hemorrhagic fluid from the nose, conjunctiva, and mouth, lethargy, anorexia, hyperventilation, tachypnea, and loss of liquid stools, and mortality were monitored. On day 12, all surviving mice were bled and killed by cervical dislocation; the lungs with the trachea, kidneys, liver, and spleen were dissected free and weighed. Relative organ weights (ROWs) were calculated by the following formula: \( \text{ROW} = \frac{(\text{organ weight/body weight})}{100} \).

**STATISTICAL ANALYSIS**

Comparisons were only made between treatment groups and their specific control groups. Statistical analysis of biological parameters (body temperature and body weight) was performed using the distribution-free curve analysis according to Koziol et al. Since the zymosan-induced illness is characterized by distinct phases, comparisons were made separately for the course of the biological parameters in the acute phase (days 0–4) and late phase (days 8–12) of the model. The nonparametric Kruskal-Wallis and Wilcoxon 2-sample tests were used for statistical analysis of the ROWs. Noncontinuous data (mortality rates) were analyzed by use of the Fisher exact or \( \chi^2 \) tests, when appropriate. Differences between groups were considered to be statistically significant at \( P<.05 \).

IV with Cl₃MBP-containing liposomes. Reduction of alveolar and peritoneal macrophages was confirmed by quantification of the macrophage cell concentration in the alveolar and peritoneal lavage fluid, respectively. Mice that were treated IT with Cl₃MBP-containing liposomes demonstrated a mean macrophage concentration of \( 6.4\times10^5 \) cells per milliliter, while control mice displayed a concentration of \( 27.0\times10^5 \) cells per milliliter. Intraperitoneal administration of Cl₃MBP-containing liposomes achieved a mean macrophage concentration of \( 2.4\times10^3 \) cells per milliliter, while a concentration of \( 40.7\times10^2 \) cells per milliliter was found in the peritoneal lavage fluid of the control mice.

Intraperitoneal administration of zymosan induced a typical triphasic illness in all control animals as described earlier. In the early hyperdynamic phase (days 0–4), the animals became hypothermic, lethargic, and anorectic; they hyperventilated, lost weight and hemorrhagic fluid from the nostrils and conjunctivae, and had diarrhea. Thereafter, the condition of the surviving animals improved temporarily. However, after 8 days, the clinical condition worsened progressively. In this late hypodynamic phase (days 8–12), the animals became more lethargic and hypothermic, hyperventilated, and started to lose weight and hemorrhagic fluid from the nostrils and conjunctivae again. The mortality of the control animals varied among the 3 experiments, with overall survival rates of 47%, 75%, and 50% in the control mice with IV, IT, and IP treatment, respectively (Figure 2). Inspection of the lungs of the mice that died in the late phase or that were killed at day 12 demonstrated extremely hyperemic lungs with hemorrhagic spots and occasionally massive hemorrhagic infarction. The abdomen showed signs of an extensive fibrinous peritonitis with massive adhesions. The ROWs of the lungs, liver, and spleen were dramatically increased compared with the control values (Figure 3).

Elimination of liver and splenic macrophages by IV administration of Cl₃MBP-containing liposomes before or after challenge with zymosan did not induce significant changes in the subsequent course of body weight and temperature (data not shown). No significant changes were observed in the mortality between the control and Cl₃MBP-containing liposome–treated groups: overall survival rates were 47% in the control mice and 60% and 53% when mice were treated with liposomes before or after zymosan challenge, respectively (Figure 2). Intraperitoneal administration of liposomes either 2 days before or 4 days after zymosan challenge. The effects of IT liposome administration were studied in 3 groups of 20 mice each. The experimental groups received liposomes either 3 days before or 4 days after zymosan challenge. Three groups of similar size were used to examine the effects of IP liposome administration, for which the experimental groups received liposomes either both 4 days and 1 day before or 1 day and 4 days after zymosan challenge.

In all mice, body weight and rectal temperature were measured daily. The clinical condition with possible symptoms such as loss of hemorrhagic fluid from the nose, conjunctiva, and mouth, lethargy, anorexia, hyperventilation, tachypnea, and loss of liquid stools, and mortality rate were monitored. On day 12, all surviving mice were bled and killed by cervical dislocation; the lungs with the trachea, kidneys, liver, and spleen were dissected free and weighed. Relative organ weights (ROWs) were calculated by the following formula: \( \text{ROW} = \frac{(\text{organ weight/body weight})}{100} \).

**STATISTICAL ANALYSIS**

Comparisons were only made between treatment groups and their specific control groups. Statistical analysis of biological parameters (body temperature and body weight) was performed using the distribution-free curve analysis according to Koziol et al. Since the zymosan-induced illness is characterized by distinct phases, comparisons were made separately for the course of the biological parameters in the acute phase (days 0–4) and late phase (days 8–12) of the model. The nonparametric Kruskal-Wallis and Wilcoxon 2-sample tests were used for statistical analysis of the ROWs. Noncontinuous data (mortality rates) were analyzed by use of the Fisher exact or \( \chi^2 \) tests, when appropriate. Differences between groups were considered to be statistically significant at \( P<.05 \).

IV with Cl₃MBP-containing liposomes. Reduction of alveolar and peritoneal macrophages was confirmed by quantification of the macrophage cell concentration in the alveolar and peritoneal lavage fluid, respectively. Mice that were treated IT with Cl₃MBP-containing liposomes demonstrated a mean macrophage concentration of \( 6.4\times10^5 \) cells per milliliter, while control mice displayed a concentration of \( 27.0\times10^5 \) cells per milliliter. Intraperitoneal administration of Cl₃MBP-containing liposomes achieved a mean macrophage concentration of \( 2.4\times10^3 \) cells per milliliter, while a concentration of \( 40.7\times10^2 \) cells per milliliter was found in the peritoneal lavage fluid of the control mice.

Intraperitoneal administration of zymosan induced a typical triphasic illness in all control animals as described earlier. In the early hyperdynamic phase (days 0–4), the animals became hypothermic, lethargic, and anorectic; they hyperventilated, lost weight and hemorrhagic fluid from the nostrils and conjunctivae, and had diarrhea. Thereafter, the condition of the surviving animals improved temporarily. However, after 8 days, the clinical condition worsened progressively. In this late hypodynamic phase (days 8–12), the animals became more lethargic and hypothermic, hyperventilated, and started to lose weight and hemorrhagic fluid from the nostrils and conjunctivae again. The mortality of the control animals varied among the 3 experiments, with overall survival rates of 47%, 75%, and 50% in the control mice with IV, IT, and IP treatment, respectively (Figure 2). Inspection of the lungs of the mice that died in the late phase or that were killed at day 12 demonstrated extremely hyperemic lungs with hemorrhagic spots and occasionally massive hemorrhagic infarction. The abdomen showed signs of an extensive fibrinous peritonitis with massive adhesions. The ROWs of the lungs, liver, and spleen were dramatically increased compared with the control values (Figure 3).

Elimination of liver and splenic macrophages by IV administration of Cl₃MBP-containing liposomes before or after challenge with zymosan did not induce significant changes in the subsequent course of body weight and temperature (data not shown). No significant changes were observed in the mortality between the control and Cl₃MBP-containing liposome–treated groups: overall survival rates were 47% in the control mice and 60% and 53% when mice were treated with liposomes before or after zymosan challenge, respectively (Figure 2). Intraperitoneal administration of liposomes either 2 days before or 4 days after zymosan challenge. The effects of IT liposome administration were studied in 3 groups of 20 mice each. The experimental groups received liposomes either 3 days before or 4 days after zymosan challenge. Three groups of similar size were used to examine the effects of IP liposome administration, for which the experimental groups received liposomes either both 4 days and 1 day before or 1 day and 4 days after zymosan challenge.
The latter mortality was mainly due to the fact that the animals died in the early phase of this model. With only 2 animals surviving in this group, no reliable conclusions could be made with respect to organ damage. In the animals that were treated with liposomes after zymosan challenge, no statistically significant changes in the ROWs were observed (Figure 3).

**COMMENT**

This study shows that elimination of Kupffer cells and splenic macrophages (portal macrophages), either before or after zymosan challenge, is associated with lower, although not significantly, late deaths and significantly lower relative liver weights, indicating less liver damage. This is consistent with studies that have documented that excessive Kupffer cell activation is associated with alterations in liver function. Concomitantly, relative lung weights were higher when portal macrophages were eliminated after zymosan challenge. The latter observation may be explained in view of other studies that have hypothesized that ARDS and MODS could result from dysfunctioning Kupffer cells, which spill over endotoxin to the lung and induce an excessive activation of alveolar macrophages; this phenomenon has been coined the “liver-lung axis.” Indeed, alveolar macrophages are releasing much more TNF in response to lipopolysaccharide than Kupffer cells. Thus, by this mechanism, elimination of portal macrophages could lead to an activation of alveolar macrophages that results in increased lung damage.

Our data did not show adverse effects on the mortality rate by eliminating portal macrophages. This is in contrast with a study in the cecal ligation and puncture model, in which Kupffer cell blockade was achieved with gadolinium chloride. While increasing systemic immunity, this method of macrophage blockade increased the mortality rate. These discordant results could be explained because gadolinium chloride predominantly blocks phagocytic activity of Kupffer cells, leaving macrophage secretory activity relatively intact. The method, which we applied, physically eliminates almost all Kupffer cells, leaving no function intact. Furthermore, while the zymosan-induced generalized inflammation (ZIGI) model is largely independent of bacteria, the cecal ligation and puncture model is a slowly progressive bacteremic model, in which an intact host defense against bacteria is probably important. Apparently, the effects of manipulating macrophage function on the host defense can be detrimental in models or clinical situations that depend on the intact phagocytic activity of the host defense.

In this respect, our data of elimination of peritoneal macrophages are illustrative. Elimination of peritoneal macrophages before zymosan challenge induced a dramatic increase in the mortality rate, suggesting that in the early phase of this model, the function of peritoneal macrophages is essential for the host defense against intruding microorganisms or injurious agents like zymosan. However, elimination of peritoneal macrophages after zymosan challenge did not induce such effects since the mortality rate and ROWs were not significantly different. So, there appears to be a differential involvement of peritoneal macrophages in the development of MODS in this model: a protective role in the early phase and an indif-

---

*Figure 1.* Acid phosphatase activity of macrophages in mouse splenic tissue sections 48 hours after intravenous administration of liposomes that contained dichloromethylene bisphosphonate. Top, Section of normal mouse spleen (original magnification ×80). Bottom, Section of mouse spleen treated with liposomes that contained dichloromethylene bisphosphonate. Most macrophages are eliminated; only some macrophages in the white pulp remained (original magnification ×100).
Figure 2. Effects of intravenous (IV), intratracheal (IT), and intraperitoneal (IP) dichloromethylene diphosphonate-containing liposomes on survival rates. Solid circles indicate control mice; open circles, mice treated with liposomes that contained dichloromethylene diphosphonate before zymosan challenge; and open triangles, mice treated with liposomes that contained dichloromethylene diphosphonate after zymosan challenge.

Figure 3. Relative organ weights (ROWs) at 12 days after injection of zymosan. White bars indicate healthy animals; gray bars, zymosan-treated control mice; light blue bars, mice treated with liposomes that contained dichloromethylene diphosphonate before zymosan challenge; dark blue bars, mice treated with liposomes that contained dichloromethylene diphosphonate after zymosan challenge (data are expressed as mean±SEM, Wilcoxon 2-sample test; P<.01 [asterisks]); IV, intravenous; IT, intratracheal; and IP, intraperitoneal.

Figures 2 and 3 illustrate the effects of different routes of DICMB administration on survival rates and organ weights, respectively, following zymosan challenge.

A different role in the late phase. In the ZIGI model, an early depression of peritoneal macrophage production capacity for TNF, interleukin-1, and interleukin-6 was observed during the first days after zymosan challenge, while they regained their capacity to produce cytokines in the late phase.21 Cecal ligation and puncture has also been shown to induce an early depression of peritoneal macrophage TNF production capacity and antigen presentation.22 Furthermore, these observations are interesting when comparing them with the observation of an increased susceptibility to a septic challenge after hemorrhage, since hemorrhage has been shown to decrease antigen-presenting and phagocytic activity while leaving the ex vivo secretory capacity of peritoneal macrophages relatively intact.23,24 Although it remains speculative, it could be that in the early phase, elimination of peritoneal macrophages before zymosan challenge enhances the effects of this early overall depression of peritoneal macrophage function, resulting in an increased mortality rate, comparable with the observed increased susceptibility for a septic challenge after hemorrhage. Furthermore, IP administration of DICMB-containing liposomes does not only eliminate IP and omental macrophages but also, to a lesser extent, liver and splenic macrophages.25 However, it appears unlikely that the observed effects that are seen after IP administration of DICMB-containing liposomes before zymosan challenge could be attributed to partial elimination of these macrophages, since elimination of Kupffer cells and splenic macrophages alone did not induce adverse effects. Hence, it seems that the observed high early mortality rate should be specifically attributed to the elimination of peritoneal macrophages.

Although not preventing organ damage or altering the course of the syndrome, it is striking that the elmi-
nation of alveolar macrophages before zymosan chal-

lenges completely prevented mortality. This observation was consistently found in several pilot experiments (data not shown). This is in line with clinical data that have shown elevated levels of macrophage-derived cytokines (eg, TNF and interleukin-1) in bronchoalveolar lavage fluids in critically ill patients in whom ARDS develops.23 However, late elimination of alveolar macrophages did not alter the clinical course or mortality rate, nor did it influence organ damage. Thus, it appears that early alveolar activation is asso-
ciated with mortality, while MODS appears to be indepen-
dent of late alveolar macrophage activation. Other investiga-
tors, however, have shown experimentally that lung injury in the cecal ligation and puncture model was associated with a gradual increase in alveolar macro-
phage activation.24 Evidence for a less important role of the activated alveolar macrophage in the evolution of ARDS is supported by the clinical observation that early ARDS was associated with an increased number of macro-
phages, while a sustained elevated number of macro-
phages correlated with a decreased risk of death.25 These data suggest that early alveolar macrophage activation is associated with the onset of ARDS, while late alveolar mac-
rophyage activation is associated with a resolution of ARDS.

A major finding of this study was that selective elimi-
nation of any macrophage subpopulation, either before or after zymosan challenge, could not prevent both organ dam-
age and death in this model. This could be explained by several hypotheses. First, during the development of MODS in this model, repopulation of some macrophages could al-
ready have been achieved in the late phase. Repopulation from the bone marrow requires 7 days for Kupffer cells and, de-
pending on the type of macrophage, 7 days (red pulp mac-
rophages) to 60 days (marginal zone macrophages) for splenic macrophages.26 Alveolar macrophage repopu-
lation starts after 5 days and is complete after 18 days.27 Re-
population of peritoneal macrophages takes 7 days, and re-
population of macrophages of the omentum takes 7 to 28 days.28 When those macrophages were eliminated before zymosan challenge, their reappearance would be almost complete in the late phase, while it would be only partial when those macrophages were eliminated after the zyms-

Hence, the reappearance of those macro-

phages in this phase could trigger the development of MODS if the agents or events that keep the macrophages acti-

vated are still present. Second, there are also indications in other models that the nondepleted macrophages dem-

strate an increased activity.22 Thus, the elimination of 1 subpopulation of macrophages could result in a compensa-
tory activation of other macrophages or cell popula-
tions with an enhancement of the inflammatory response. In this respect, the observed increased lung organ damage after elimination of portal macrophages is an interesting phenomenon. Furthermore, elimination of peritoneal and alveolar macrophage populations was not complete; thus, the remaining macrophages could have hypotheti-
cally induced MODS in this model. On the other hand, the dra-
ic effects of intra-alveolar and IP liposomes before zy-
mosan challenge suggest a significant contribution of the depletion of those macrophages to these phenomena.

The results could also be interpreted to support the notion that macrophage activation is not an important feature in the development of MODS. Indeed, the mac-
rophyage hypothesis has been questioned because of re-
cent clinical data, indicating that preventive enhance-
ment of macrophage activity by polymyxin B2 glucopyranose glucan is beneficial for patients who are undergoing major surgery or multiple trauma.39 On the other hand, our data indicate that elimination of Kupffer cells and splenic macrophages attenuates liver damage. Furthermore, elimination of alveolar macrophages before zymosan challenge could fully prevent zymosan-
induced death, indicating an important role for these macro-
phages in the development of MODS.

Our data further show that the elimination of certain macrophage subpopulations and the elimination on spe-
cific time points in this model has differential effects, in-
dicating a differential role of specific macrophage subpopu-
lations, either protective or detrimental, in the development of MODS. Peritoneal macrophages appear to be im-
portant in the host-defense mechanisms in the early phase, while contributing to the development of MODS in the late phase of this model. Kupffer cells and splenic macro-
phages appear to play an important pathophysiological role in all phases, while alveolar macrophages appear to be det-

tamental in the early phase. Thus, it remains to be deter-

mined which specific macrophage functions or popula-
tions are essential and need to be stimulated, and which are detrimental and need to be inhibited.

The Cl2MBP used in this study was provided by Boeh-
ringer Mannheim GmbH, Mannheim, Germany.

Corresponding author: Grard A. P. Nieuwenhuijzen, MD, Department of Surgery, St Elisabeth Hospital, Hil-
varenbeekseweg 60, PO Box 90151, 5000 Tilburg, the Neth-
erlands.

REFERENCES

1. Goris RJA, te Boekhorst TPA, Nugterink JKS, Gimbrelre JSF. Multiple organ fail-
127:1451-1462.
4. Beai AL, Cerra FB. Multiple organ failure in the 1990s: systemic inflammatory
5. Deltch EA. Multiple organ failure: pathophysiology and potential future therapy.
6. Border JR. Seeps, multiple systems organ failure, and the macrophage. Arch
7. Butler LD, Layman NK, Cain RL, et al. Interleukin-1-induced pathophysiology:
induction of cytokines, development of histopathological changes, and immu-
8. Tracey KJ, Beutler B, Lowry SF. Shock and tissue injury induced by recombi-
kine levels in human septic shock: relation to multiple-system organ failure and
in patients after major vascular surgery, hemorrhagic shock, and severe trauma.
tumor necrosis factor protects from the lethal effects of endotoxin. Science.
1990;229:969-971.
348:550-552.
13. Staines HF, Pearce MK, Torisu M. Characteristics of alveolar macrophages


20. McMasters KM, Cheadle WG. Regulation of macrophage TNF-α, IL-1α and IL-6 mRNA expression during peritonitis is site dependent. *J Surg Res.* 1993;54:426-430.


---

**Invited Commentary**

The MODS that progresses to multiple-organ failure is a serious problem for patients who undergo surgical procedures. Once multiple-organ failure develops, the mortality rate is high. Thus, information about the factors that are involved in producing this problem will eventually be beneficial for our patients. In this issue of the *Archives*, Nieuwenhuijzen and coworkers provide important information about macrophage activity in various organs (e.g., the liver and spleen, the lungs [alveolar macrophages], and peritoneal cavity). Macrophages in these organs or locations were decreased in number by liposome-mediated depletion, and elimination of Kupffer cells (from the liver) and splenic macrophages decreased liver damage, but increased lung damage. Thus, with zymosan-induced peritonitis, elimination of peritoneal macrophages increased the zymosan-associated mortality rate. The elimination of Kupffer cells (from the liver) and splenic macrophages decreased liver damage, but increased lung damage. Thus, with zymosan-induced peritoneal inflammation, peritoneal macrophages are protective, whereas alveolar macrophages are deleterious. There were also time differences of early and late effects.

Thus, what is the message for all of us who take care of patients who undergo surgical procedures? The most important message is that the host-defense systems are complex, and they can be protective in some circumstances while deleterious in others. There will not be simple solutions to such problems. Clinically it is unlikely that there will be a magic bullet. So-called magic bullets that have been subjected to randomized, prospective clinical trials have failed to reduce the mortality rate in patients. These agents include interleukin-1 receptor antagonist antiendothoxins, anti-TNF antibodies, soluble TNF receptors, anti-platelet activating factor, and other agents. Thus, we must strive to prevent MODS and multiple-organ failure by excellent patient care—operations without complications, minimal surgical procedures when possible, and maximum organ support. The more we learn from studies such as the one by Nieuwenhuijzen and coworkers and those of Goris et al. have described previously on this topic, the more likely it is that we will be able to do something about overwhelming inflammation.

Inflammation is necessary to heal wounds, control infection, and promote survival. Also, as Goris et al. have described previously, overwhelming inflammation in patients is deleterious. The final question is: Can we fool Mother Nature and modulate a necessary but excessive response, namely, inflammation?

**Arthur E. Baue, MD**

**St Louis, Mo**