Involvement of the Acute Phase Protein α1-Acid Glycoprotein in Nonspecific Resistance to a Lethal Gram-negative Infection*

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Resistance to Gram-negative infection can be induced by pretreating animals with several agents such as turpentine and interleukin (IL-1). Because these agents are powerful inducers of acute phase proteins, we wondered whether these proteins, more particularly α1-acid glycoprotein (α1-AGP), are involved in nonspecific resistance to infection. Turpentine and IL-1 protect completely when given 48 and 12–48 h before the challenge, respectively. α1-AGP induction in the serum reached peak values 48 h after turpentine and 12–48 h after IL-1 injection. Administration of α1-AGP, 2 h before a challenge of Klebsiella pneumoniae, significantly increased the survival. Numbers of bacteria cultured from blood and organs were significantly lower in mice pretreated with a protective dose of turpentine, IL-1, or α1-AGP. These data suggest that α1-AGP is a possible mediator in turpentine- or IL-1-induced protection because time points of maximal induction of α1-AGP by turpentine or IL-1 and of optimal protection by α1-AGP coincide. Transgenic overexpression of rat α1-AGP protected mice from a K. pneumoniae infection. Bacterial counts in blood and organs were significantly lower in transgenic mice, and only in control mice were large necrotic areas, apoptosis, and blood clots observed in the spleen. Our data suggest that α1-AGP prevents Gram-negative infections and may be an essential component in nonspecific resistance to infection.

Septic shock, or systemic inflammatory response syndrome, is characterized by inadequate tissue perfusion. It is caused by an overwhelming infection (1), and several bacterial organisms have been identified to induce that syndrome (2). The disease is accompanied by a high death rate, exceeding 50%, depending on the type of organism involved. Treatment with antibiotics has proven to be effective, but because bacteria are becoming increasingly resistant to antibiotics, alternative solutions have to be found. One possibility is to identify endogenous molecules that are involved in increasing nonspecific resistance to infection and to evaluate their therapeutic use. The natural resistance of the host to infection can be increased by injection of various substances, most of which are of bacterial origin, such as lipopolysaccharide and muramyl peptides (3–5). However, because of their toxicity, these immunomodulatory substances cannot be used therapeutically in humans. Substances that increase nonspecific resistance are able to stimulate mononuclear phagocytes to secrete interleukin (IL)-1β and tumor necrosis factor (TNF) (6). It was demonstrated that IL-1, and to a lesser extent TNF, are able to induce natural resistance to infection (7–12). However, resistance can only be induced if certain time intervals are respected. For example, bacillus Calmette-Guérin has to be administered 2 weeks, lipopolysaccharide between 6 and 48 h, and IL-1 24 h prior to a lethal challenge of Klebsiella pneumoniae (4). To study septic shock, a lethal infection of K. pneumoniae is a relevant model because these Gram-negative bacteria were recognized, besides Escherichia coli, Proteus, Pseudomonas and Serratia, as some of the most frequent culture isolates (13).

α1-Acid glycoprotein (α1-AGP) is a highly glycosylated protein of 43 kDa with a pl of 2.7 (14). It is an acute phase protein normally found at a concentration of 0.2–0.4 mg/ml (20). During an acute phase condition, the concentration rises 2–5 times, making it one of the predominant proteins in the serum (14). Like most acute phase proteins, α1-AGP is induced both by cytokines and corticosteroids (21). IL-6 and IL-1 have proven to be very powerful inducers of α1-AGP both in vitro and in vivo (22). Although α1-AGP is an abundant protein, its real physiological significance is not fully understood. Inhibition of platelet aggregation (23) and of neutrophil function (24, 25) have been reported. Also, α1-AGP was found to inhibit selectively the transport of molecules through the endothelial layer (26, 27). In vivo protective effects of α1-AGP have been described (28–30).

We were interested in finding whether acute phase proteins are involved in nonspecific resistance to infection against K. pneumoniae. Therefore we studied the possible protection by turpentine oil and IL-1, two well known and very strong inducers of the acute phase response (31–34), and by α1-AGP itself, and we compared the kinetics of induction of α1-AGP in the serum and of optimal protection against K. pneumoniae.

We describe that α1-AGP significantly protects against a lethal infection with K. pneumoniae. This activity of the acute

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phase protein was observed in normal mice using purified α₁-AGP as well as in transgenic mice that overexpress rat α₁-AGP.

MATERIALS AND METHODS

Animals—Female C57BL/6 mice (Iffa-Credo, Saint Germain-sur-l’Arbresle, France) were used at the age of 8–12 weeks. Rat α₁-AGP transgenic mice were generated and described previously (35). They were generated by injecting genomic DNA into (C57BL/6 × DBA/2)F1 zygotes, and the resulting transgenic mice were back-crossed eight generations into a C57BL/6 background. Heterozygous transgenic mice from the line 9.5–5 constitutively produce about 2 mg/ml α₁-AGP. This is 10-fold more than wild-type (wt) animals. The colony was propagated by breeding heterozygous transgenic mice with C57BL/6 female mice; the offspring, containing heterozygous transgenics and wt littermates, was genotyped at weaning age by enzyme-linked immunosorbent assay. 100 μl of blood was collected from retro-orbital bleeding, after which serum was prepared. α₁-AGP was purified by phenol extraction (36) and coated on the bottom of an enzyme-linked immunosorbent assay plate. After washing, rat α₁-AGP was detected using an anti-rat α₁-AGP polyclonal antibody (generated by H. Baumann in rabbits) (1/1,000) and subsequent alkaline phosphatase-conjugated anti-rabbit antibody (1/1,000) and serum was prepared. 1-AGP was measured by nephelometry using a goat anti-human α₁-AGP polyclonal antibody.

Clearance of Bacteria—36 h after injection of K. pneumoniae, mice were anesthetized by intraperitoneal injection of tribromoethanol. Blood was taken by heart puncture. For preparation of plasma, 450 μl of blood was added to 50 μl of sodium citrate (0.1 M). Immediately thereafter, mice were killed by cervical dislocation. Then, mice were perfused with 10 ml of a 0.9% NaCl solution to wash out the blood. The liver, spleen, and kidney were removed aseptically, weighed, and homogenized mechanically in sterile saline. For homogenization, the liver was diluted (w/v) 2-fold; spleen and kidney were diluted 10-fold. The suspensions were diluted and plated out on sterile nutrient agar. After overnight incubation at 37 °C, CFU numbers were counted.

Measurement of α₁-AGP—The concentration of α₁-AGP in mouse serum was measured using a home-developed sandwich enzyme-linked immunosorbent assay. A rat monoclonal antibody was coated (0.1 μg/ml) on 96-well Maxisorb plates. After blocking with 1% BSA and PBS, samples and a murine α₁-AGP standard were titrated, after which the plates were incubated for 1 h at 37 °C. A rabbit polyclonal antiserum (1/1,000) and subsequent alkaline phosphatase-conjugated anti-rabbit antibody (1/5,000) were used as secondary and third antibody. Human α₁-AGP was measured by nephelometry using a goat anti-human α₁-AGP polyclonal antibody.

RESULTS

Protective Effect of Turpentine against a Lethal Infection with K. pneumoniae—To investigate whether turpentine conferred protection, mice were pretreated with 100 μl of turpentine subcutaneously 24 or 48 h before a lethal bacterial challenge (10⁶ CFU unless otherwise stated) of K. pneumoniae. When turpentine was given 48 h before the challenge, mice were significantly (p < 0.0001) protected versus PBS-pre-treated controls. In contrast, turpentine treatment 24 h before the challenge was unable to induce significant protection (p = 0.1137; not significant). Mice were observed for 6 days, after which no further deaths occurred (Fig. 1).

Protective Effect of IL-1 to a Lethal Infection with K. pneumoniae—To determine the time point of optimal protection by IL-1, 1 μg of IL-1 was given at 12, 24, and 48 h before the lethal challenge (Fig. 2). IL-1 protected significantly when given 48 h (p = 0.0279), 24 h (p = 0.0020), or 12 h (p < 0.0001) prior to the challenge, compared with PBS-pre-treated controls. IL-1 protected significantly better at −12 h compared with −48 h (p = 0.0455).
Protection of 1 μg of IL-1 against a lethal challenge of K. pneumoniae. IL-1 was given intraperitoneally at different time points ( ), 48 h; ( ), 24 h; and ( ), 12 h) prior to a lethal injection with 10^6 CFU of K. pneumoniae. Control mice ( ) received saline (n = 6) for all groups, except for the control group (n = 7). *, **, *** p < 0.05, p < 0.01, p < 0.001, respectively.

Induction of α1-AGP by Turpentine or IL-1—To study the kinetics of induction of α1-AGP by turpentine or IL-1, mice were injected subcutaneously with 100 μl of turpentine or intraperitoneally with 1 μg of IL-1. At different time points after the injection, mice were sacrificed, and serum was collected. The concentration of α1-AGP in the serum was measured as described under “Materials and Methods.” The α1-AGP levels in the serum were induced significantly 48 h (p < 0.01) and 72 h (p < 0.01) after turpentine injection compared with the basal levels (PBS injected mice had no increased α1-AGP at any time point) and 12 h (p < 0.01), 24 h (p < 0.01), and 48 h (p < 0.05) after IL-1 injection. The levels after 12 h were significantly higher than those after 48 h (p = 0.0455). The α1-AGP levels 144 h after turpentine or IL-1 injection had reached basal levels again (p > 0.05; not significant) (Fig. 3).

Protection of α1-AGP against a Lethal Bacterial Challenge—To test whether α1-AGP was able to protect, several doses were injected intraperitoneally 2 h before and/or 24 h after a lethal challenge with K. pneumoniae. When mice were treated with 10 mg of α1-AGP 2 h before the lethal challenge or with 10 mg of α1-AGP spread over two injections (5 mg 2 h before and 5 mg 24 h after the lethal challenge), significant protection was observed (p = 0.0094 and p = 0.0006, respectively) compared with the control mice that received PBS as pretreatment (Fig. 4). When mice were given a total dose of 5 mg, protection was only marginal, whereas a dose of 2 mg did not protect. Furthermore, pretreatment with α1-AGP proved to be necessary because mice treated with 10 mg of α1-AGP 24 h after the lethal challenge were not protected (data not shown).

In a separate experiment (Table I), we observed that 10 mg of BSA was not able to protect against 10^6 CFU of K. pneumoniae, which illustrates the specificity of the effect of α1-AGP.

To compare the serum levels of α1-AGP induced by turpentine or IL-1 on the one hand and those after injection of 10 mg of α1-AGP on the other hand, we performed the experiment shown in Table II. Mice were injected with 10 mg of human α1-AGP, and α1-AGP was measured in the blood after 2, 8, 24, 48, and 72 h. It was found that, taking into account a blood volume of 2 ml and a serum volume of 1 ml, 2 h after injection 2.3 mg of α1-AGP/ml of serum was present, which is comparable with the amounts found after injection with turpentine or IL-1 (Fig. 3).

Spread of K. pneumoniae in Blood and Different Organs—To shed light on the mechanism of protection against a lethal challenge with K. pneumoniae, we studied the number of bacteria in the blood and in different organs. Mice received either 10 mg of α1-AGP (5 mg at 2 h before and 5 mg at 24 h after the lethal challenge), 1 μg of IL-1 24 h before the lethal challenge, or 100 μl of turpentine 48 h before the lethal challenge. Mice treated with α1-AGP, IL-1, or turpentine showed significantly less bacteria in the blood (p < 0.001, p = 0.0087, and p = 0.0021, respectively), liver (p = 0.0058, p = 0.0038, and p = 0.0038, respectively), spleen (p = 0.0021, p = 0.0135, and p = 0.0010, respectively), and kidney (p = 0.0079, p = 0.0312, and p = 0.0046, respectively) compared with control mice 36 h after an intramuscular injection of 1 × 10^6 CFU of K. pneumoniae (Fig. 5). These data suggest that the mechanism of protection by all of these agents could be at the same level and that α1-AGP may mediate both the IL-1 and turpentine effects. It is also clear from Fig. 5 that turpentine, which induces higher amounts of α1-AGP than IL-1, is better in reducing the spread of bacteria.

It has already been shown that IL-1 is not directly cytotoxic to bacteria (9). We have tested the effect of α1-AGP by plating out bacteria on nutrient agar plates containing different concentrations of α1-AGP (1.5, 0.5, 0.15 mg/ml or none). We found no difference in the number of bacteria grown in the presence or absence of α1-AGP (data not shown).

Lethal Response of Rat-α1-AGP Transgenic Mice—A challenge of 10^5 bacteria results in almost 100% lethality over a period of 2 weeks. When heterozygous α1-AGP transgenic mice and wt littermates were challenged with bacteria, the transgenic mice were significantly protected compared with the wt littermates (Fig. 6). The constitutive α1-AGP levels in these heterozygous transgenic mice amount to 2 mg/ml (38). This result was reproduced four times. Control mice started dying at day 2 and transgenic mice at day 5. Eventually, 83% control mice and 39% transgenic mice succumbed (p = 0.0002). At a higher inoculum, protection by the transgenic α1-AGP was no longer observed (data not shown).

Bacterial Clearance in Rat-α1-AGP Transgenic and Control Mice—As described before, 24–48 h after challenge, bacteria are found in most organs (9), most pronounced in the spleen (39). We injected α1-AGP transgenic mice and control littermates and counted the bacteria in blood, spleen, liver, and kidney 36 h later. The numbers of CFU are expressed per g of tissue (Fig. 7). We found that in the blood, spleen, liver, and kidney, α1-AGP transgenic mice had significantly several 100-fold less invasion of bacteria (p = 0.0432, p = 0.0033, p = 0.0437, and p = 0.0256, respectively).
Necrosis in Spleens of Rat $\alpha_1$-AGP Transgenic and Control Mice—2 days after a challenge of $10^5$ K. pneumoniae, transgenic as well as control mice were sacrificed and their tissues fixed. Tissue sections revealed that mainly the spleen of control mice was heavily damaged (Fig. 8). In these mice, massive influx of bacteria was observed, along with erythrocytes. Also, large necrotic areas, apoptosis and blood clots were found. $\alpha_1$-AGP-transgenic mice were completely protected from these lesions (Fig. 8).

**DISCUSSION**

Septic shock is the result of an overwhelming Gram-negative bacterial infection (1). The bacteria and/or their lipopolysaccharides are found in the circulation, and shock is the result of the combined action of several proinflammatory cytokines induced in macrophages and other cells (40). Septic shock and sepsis, leading to cardiovascular depression and multiple organ failure, are causing more than 100,000 casualties per year in the...
Several clinical trials had the proinflammatory cytokines as a target: the IL-1 receptor antagonist, soluble TNF receptors, or TNF-neutralizing antibodies were used. These approaches and also those inhibiting platelet-activating factor or bradykinin were relatively disappointing (42). Clearly new and other approaches are needed.

The toxic and lethal effects of a variety of infections or of bacterial endotoxins can be reduced by preadministration of a small dose of endotoxin 6–48 h before (4). Several other substances of natural origin or synthetic compounds based on microbial structures also increase the natural endogenous resistance mechanisms. The protective effects are clearly nonspecific because endotoxin from Gram-negative bacteria can protect against lethality caused by an antigenically unrelated organism such as *Candida albicans* (43). Therefore the phenomenon was called induction of “nonspecific resistance to infection” (11). Administered under adequate conditions, such bacterial agents markedly increase nonspecific immunity, even against strains made resistant to antibiotics (4). However, because of their toxicity, these immunomodulatory substances have not gained acceptance as a therapy in humans. Therefore, further investigation is needed to obtain a clear insight into the mechanism of induction of nonspecific immunity. Several investigators found that IL-1 plays a crucial role in inducing nonspecific resistance to infection because it can protect mice against a lethal challenge of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *C. albicans*, and *K. pneumoniae* (7–10, 44). It was also shown that pretreatment with IL-6 or TNF could protect against a lethal bacterial challenge, although these

![FIG. 5. Counts of colonies of *K. pneumoniae* in the blood, spleen, liver, and kidney of mice 36 h after an intramuscular injection of 10^6 CFU of *K. pneumoniae*. Each bar represents the mean ± S.D. of log CFU/ml of homogenized tissue from three mice; *p* values are versus controls that received saline. *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001.](http://www.jbc.org/)

![FIG. 6. Kaplan-Meier plot of lethal response of α1-AGP transgenic mice (■) (*n* = 23) and wt littermates (○) (*n* = 35) to 10^5 CFU of *K. pneumoniae*. Lethality was scored daily for 1 month after the intramuscular inoculation. No further deaths occurred. ***, *p* < 0.001.](http://www.jbc.org/)

![FIG. 7. Bacterial counts in the tissues of wt mice (*n* = 4) and rat α1-AGP transgenic mice (*n* = 4). 36 h after an intramuscular challenge with 10^6 CFU of *K. pneumoniae*, blood and tissues were collected, homogenized, and plated, after which bacteria were counted. Logarithms of the number of bacteria are expressed per g of tissue. The ratios of control mice to transgenics for blood, liver, spleen, and kidney are 440, 324, 136, and 512, respectively. *, *p* < 0.05; **, *p* < 0.01.](http://www.jbc.org/)
cytokines were clearly less potent than IL-1 (9, 44). These cytokines all play an important role in inducing the acute phase response. The acute phase response is the answer of the organism to disturbances of homeostasis caused by infection, tissue injury, or immunological disorders. It consists of a local reaction at the site of injury characterized by a number of responses, such as platelet aggregation and activation of leukocytes, which in turn release acute phase cytokines (such as TNF, IL-1, and IL-6). In addition, activated fibroblasts and endothelial cells are able to produce cytokines such as IL-6. These cytokines act on specific receptors on different target cells leading to a systemic reaction characterized by fever, leukocytosis, increase in secretion of glucocorticoids, activation of complement, and clotting and dramatic changes in the concentration of some plasma proteins called acute phase proteins (45). The acute phase response is a fundamental protective system that has evolved (21). The expression of several proteins is augmented by the liver several hours and days after the start of an infection or trauma. Most of these proteins are supposed to have protective or healing activities. The set of acute phase proteins varies from one species to another. The most predominant acute phase proteins in the mouse are serum amyloid A, serum amyloid P, and α1-AGP (15).

α1-AGP is a glycosylated protein with a molecular mass of ~43 kDa and a pI of 2.7 (14). Human α1-AGP contains five N-glycosylation chains (46) containing sialyl Lewisx structures (47). It is produced mainly by hepatocytes as a response to IL-1 and IL-6 (22). During an acute phase condition the glycosylation pattern changes (48), and the serum concentration of α1-AGP rises up to 5-fold. Although the precise biological function of the protein is not known, most in vitro experiments suggest an anti-inflammatory role. It inhibits platelet aggregation (23), activation of neutrophils (24, 25), and the proliferative response of peripheral blood lymphocytes to phytohemagglutinin (49). In vivo, α1-AGP has been shown to protect against TNF-induced lethal shock and hepatitis (50).

In the present study, we have investigated the hypotheses that (i) induction of an acute phase reaction by turpentine would enhance nonspecific resistance to infection among others by inducing IL-1; and (ii) α1-AGP would be a mediator in turpentine- and IL-1-induced nonspecific resistance to infection in mice. This work is an extension of previous research revealing that both IL-1 and α1-AGP protect against a lethal injection of TNF in mice (28, 50).

We observed that injection of turpentine completely protected when it was given 48 h before a lethal challenge with *K. pneumoniae*. Because turpentine is a strong inducer of the acute phase response, we believe that induction of acute phase proteins could be responsible for the protection.

In agreement with others, we have shown that IL-1 was able to protect when it was given 24 h before a lethal challenge with *K. pneumoniae* (10). We observed that IL-1 still protected when it was given 12 or 48 h before the lethal challenge. Because IL-1 is a strong inducer of a subset of acute phase proteins, we believe that protection by IL-1 is also mediated by induction of acute phase proteins. Moreover, IL-1-induced protection has been reported to be blocked by coadministration of galactosamine, a specific hepatotoxin (51).

Furthermore, we found that α1-AGP, a major acute phase protein in the mouse, protects significantly when given 2 h before a lethal challenge with *K. pneumoniae*. Protection was not observed with an equal dose of another protein, BSA. To investigate whether turpentine- or IL-1-induced protection could be mediated by induction of α1-AGP, we measured the induction of α1-AGP at different time points after injection of turpentine or IL-1. Significant induction of α1-AGP was found 48–72 h after turpentine and 12–48 h in the case of IL-1 injection. Remarkably, the time interval between administration of turpentine (~48 h) or IL-1 (~12 or ~48 h) and the lethal challenge with *K. pneumoniae* corresponded with the time needed to obtain optimal induction of α1-AGP in mouse serum. Moreover, we observed that turpentine pretreatment 24 h before the lethal challenge did not protect significantly. This is in agreement with the fact that no significant induction of α1-AGP was found 24 h after turpentine injection. Protection of IL-1 at ~12 h is significantly better compared with IL-1 at ~48 h. The protection data are in line with the induction data. We believe that the data can be explained quite logically: when mice are pretreated with IL-1 and challenged with bacteria 12 h later, most of the α1-AGP is induced during the bacterial infection. When mice are pretreated with IL-1 at 48 h prior to the challenge, then most of the α1-AGP has gone by the time of bacterial challenge, and only minute amounts of α1-AGP are present during the infection, so less protection will be observed. In that view, the intermediate protection of the ~24 h group is also explained. The data suggest that a minimal exposure time to α1-AGP is required. Because it was described that induction of IL-1 reaches peak values 24–48 h after turpentine injection (52) and that the type I IL-1 receptor is responsible for the hepatic acute phase protein response following turpentine or IL-1 injection (53–55), we suggest that turpentine-induced protection is caused by the induction of IL-1 and ultimately, at least partially, by induction of α1-AGP. By studying the clearance of α1-AGP from the serum of mice, we found that injection of a protective dose of α1-AGP (10 mg) leads to serum levels at the time of challenge (2.3 mg/ml) which are comparable to those that are obtained after injection with turpentine (2.8 mg/ml) or IL-1 (2.0 mg/ml).

Because protection induced by α1-AGP was less impressive compared with IL-1 or turpentine, we surely cannot exclude a role for other acute phase proteins in IL-1- or turpentine-induced nonspecific resistance to infection.

To clarify the mechanism of protection, we studied the spread of bacteria in the blood and in different organs. The number of bacteria in the blood, liver, kidney, and spleen 36 h after infection with a lethal dose of *K. pneumoniae* was significantly lower when mice were pretreated with a protective dose of turpentine, IL-1, or α1-AGP. This result contradicts the data obtained by van der Meer et al. (10), who found no difference in the number of bacteria. A possible explanation for these contradictory results could be the fact that they looked at the...
sensitivity of bacteria 24 h after the lethal challenge, whereas we looked 36 h after the lethal challenge. Finally, van der Meer et al. (10) showed that IL-1 had no direct cytotoxic effect on K. pneumoniae. We obtained similar results using α1-AGP.

We conclude that turpentine, IL-1, and α1-AGP protect against a lethal challenge of K. pneumoniae. Because galactosamine blocks IL-1-induced protection and because the time points of optimal protection by turpentine or IL-1 and the time points of peak induction of α1-AGP after turpentine or IL-1 injection coincide, we argue that α1-AGP is a possible mediator in turpentine- and IL-1-induced protection. We also found that the number of bacteria in the blood and in different organs was reduced significantly in mice pretreated with turpentine, IL-1, or α1-AGP. Because we have shown that α1-AGP has no direct antimicrobial effect, the reduced number of bacteria in pre-treated mice has to be explained by another mechanism.

Furthermore, we demonstrate that overexpression of α1-AGP, in mice, protects against a lethal Gram-negative infection. Heterozygous transgenic mice were back-crossed to C57BL/6 mice, and the female offspring differing only in the rat α1-AGP gene were used in the experiments. We found that the transgenic mice were relatively resistant in this sepsis model. The mice were significantly (p = 0.0002) protected. The heterozygous transgenic mice used in the experiment constitutively express 2 mg/g α1-AGP/ml of serum, which is comparable with the amounts found after turpentine injection, IL-1 injection, or α1-AGP injection in wt animals. However, the transgenic mice repeatedly were not protected against a 10-fold higher inoculum. We also found that blood as well as tissues of the transgenic mice contained several 100-fold less bacteria than the wt littermate mice and that the spleen, the most important target organ of the bacteria, showed gross lesions associated with fibrin clots, necrosis, and apoptosis in wt littermates only.

In most experiments, bovine α1-AGP was used. However, human α1-AGP was also clearly active in our system (data not shown). The glycosylation of human and bovine α1-AGP consists of sialyl Lewis structures, and these are absent on the transgenic α1-AGP because it is well known that mice are unable to synthesize sialyl Lewis structures, based on their deficiency in α2-fucosyltransferase, an essential enzyme in the synthesis of sialyl Lewis structures. Clearly, transgenic α1-AGP is protective, so the protection is not mediated by sialyl Lewis structures.

We hypothesize that the effect of α1-AGP is related to its effect on the endothelial cells. It was described that α1-AGP, to a certain degree, is able to control the transport of small and large molecules through the endothelium to the subendothelial spaces. We believe that this activity works in both directions, such that the invasion of the blood vessels by bacteria is prevented (to some degree) by α1-AGP. The reduced numbers of bacteria in the tissues support the hypothesis that this could indeed be the mechanism by which α1-AGP protects against lethal Gram-negative infection. We believe that our data open new possibilities for the treatment of Gram-negative bacterial infection and sepsis.

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