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Complement Components (C1, C2, C3, C4) in Bronchial Secretions After Intranasal Infection of Guinea Pigs with *Mycoplasma pneumoniae*: Dissociation of Unspecific and Specific Defense Mechanisms

MICHAEL LOOS[1]* AND HELMUT BRUNNER[2]

Institut für Medizinische Mikrobiologie, Johannes Gutenberg Universität, D-6500 Mainz,[1] and Medizinische Mikrobiologie und Virologie der Universität Düsseldorf,[2] West Germany

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Shortly after intranasal infection of guinea pigs with *Mycoplasma pneumoniae*, the titers of the complement components increased significantly in bronchial secretions by the following amounts, compared with the titer of a control group: C1, about 2-fold; C2, 1.6-fold; C3, 17-fold; and C4, 942-fold. Histopathological signs of inflammation were not apparent at this time. At 2 weeks after infection, when the titers of complement components in the bronchial secretions were at the level of control values or lower, the serum antibody titer increased, and it reached the highest level at 6 weeks after infection. Therefore, one can distinguish two phases of reaction of the macroorganism to intranasal inoculation. The increase in complement components shortly after infection may represent an early, unspecific defense mechanism of the host before the specific immune response becomes effective, since the complement system can be activated by *M. pneumoniae* via the classical as well as the alternative pathway in the absence of antibodies.

Mycoplasma pneumoniae is an important etiological agent of human respiratory tract infections. Although substantial advances have been made during recent years in the cellular biology of mycoplasmas, the pathogenesis of the disease caused by these organisms is still poorly understood. Recently, Bredt and Bitter-Suermann have shown that living cells of *M. pneumoniae* are able to activate guinea pig complement via the alternative pathway leading to an activation of C3 (4). However, the ability of fresh guinea pig serum to kill *M. pneumoniae* cells efficiently appeared to depend on the intact classical pathway of complement C1, C4, and C2 (5). It was shown that *M. pneumoniae* cells which were rounded and killed by fresh guinea pig serum not containing detectable amounts of antibody interacted directly with the first component of complement. From these results it was concluded that an antibody-independent interaction between some components of the membrane surface of *M. pneumoniae* and C1 may result in an activation of the complement system leading to the killing of the mycoplasma cells (5).

To elucidate further the pathogenetic mechanism of these microorganisms, guinea pigs were used in the present study because it had been shown previously that these animals are highly sensitive to *M. pneumoniae* infection (6, 7). The response of guinea pigs to intranasal infection was studied, and the presence of complement components in bronchial secretions was tested during the course of the infection.

MATERIALS AND METHODS

Randomly bred guinea pigs under pentobarbital anesthesia were infected intranasally with 4×10^6 colony-forming units of the Pl 1428 strain of *M. pneumoniae* (6, 7). A control group of animals received sterile broth medium. At 2 h and then in weekly intervals after infection 10 animals in the infected group and 5 animals in the control group were studied. Blood was collected by cardiac puncture, and serum antibodies were determined by the metabolism inhibition test and the staphylococcal radioimmunoassay (8). With the animals under pentobarbital anesthesia, lung secretions were washed out with 10 ml of ice-cold Veronal-buffered saline; the wash fluids were tested for the presence of the complement components C1, C2, C3, and C4. Afterwards the lungs were removed, homogenized, and cultured to quantitate *M. pneumoniae* (6).

Samples of guinea pig serum were kept at −70°C. Buffers (Veronal-buffered saline with ethylenediaminetetraacetate or sucrose), the preparation of cells and cell intermediates (E, EA, EAC-1, EAC-14, etc.), and molecular titration of C1, C2, and C4 have been described by Rapp and Borsos (14, 15). Functional

assays of C3 were performed as described by Nelson et al. (13), using the modification recommended by Cordis Corp. The results of the titrations were expressed as effective molecules per milliliter. C1 was purified by zonal ultracentrifugation by the method of Colten et al. (9). Functionally pure human and guinea pig complement components were purchased from Cordis Corp., Miami, Fla.

RESULTS

The serum antibody titers of infected animals were at the level of the control animals during the first 2 weeks after infection (Table 1). After this time, they started to increase and reached the highest level at 6 weeks after infection, with titers of 1:469 in the metabolism inhibition test and approximately 1:1,500 in the staphylococcal radioimmunoassay.

The recovery of living organisms from lung homogenates and bronchial secretions failed in most animals. This was probably due to the fact that the lungs were washed out with Veronal-buffered saline instead of sterile broth medium. The use of Veronal-buffered saline was necessary for the detection of the individual complement components (C1, C2, C3, and C4) in the wash fluids of both infected and control animals. Table 2 shows the ratios of complement component titers in infected animals to complement component titers in control animals. It can be seen that shortly after intranasal infection the titers of the complement components tested increased by the following amounts, compared with titers in the control group: C1, about 2-fold; C2, 1.6-fold; C3, 17-fold; and C4, 942-fold. During the time course of the infection the complement titers decreased to the control values. At 2 weeks after infection, when the titers of complement components in the bronchial secretions were at the level of control values or lower, serum antibody titers increased as shown in Table 1.

One can therefore distinguish two phases of the reaction of the host to intranasal infection with *M. pneumoniae*. First, shortly after infection there was an increase in the titer of the complement components in bronchial secretions, and secondly, 2 to 3 weeks after infection, specific antibodies to *M. pneumoniae* appeared in the serum.

TABLE 1. *Metabolism-inhibiting serum antibody titer in guinea pigs after intranasal inoculation*

Time after inoculation	Geometric mean metabolism inhibition (reciprocal)	
	Infected animals	Controls
2 h	45.7	33.5
7 days	29.5	16.0
14 days	17.8	32.0
28 days	117.4	8.0
42 days	469.5	64.0
70 days	332.0	32.0

TABLE 2. *Determination of C1, C2, C3, and C4 in bronchial secretions after intranasal infection of guinea pigs with M. pneumoniae*

Time after inoculation	Titer in infected animals/titer in control animals			
	C1	C2	C3	C4
2 h	1.9	1.6	16.7	942
7 days	1.2	0.9	3.0	36.7
14 days	0.6	0.8	0.6	17.5
28 days	0.3	0.8	1.0	2.1
42 days	1.1	2.6	1.1	ND[a]

[a] ND, Not determined.

DISCUSSION

Recently it was shown that young guinea pigs experimentally infected with *M. pneumoniae* developed mycoplasmacidal antibodies 2 to 3 weeks after infection and that the histopathology of the affected lungs was similar to that observed in infected hamsters (6, 7). The guinea pig model was used in this study to elucidate further the pathogenesis of *M. pneumoniae* disease after internasal infection of guinea pigs. It was found that shortly after infection of the lungs with the microorganism the titers of the complement components C1, C2, C3, and C4 increased in comparison to the control groups. Although the syntheses of the individual complement components are independent (3, 12), there is no explanation for the marked increase in the C4 activity in comparison with the other complement components tested. Histopathological signs of inflammation are not detectable at that time (6, 7). The failure to isolate living microorganisms in most animals during the course of the infection was probably due to the buffers used for washing out the respiratory tract of the animals and not to the increase in complement components, since it has been shown that under appropriate conditions living organisms can isolated (6). The titers of the individual complement components decreased to control values or lower before metabolism inhibition antibodies were detectable in serum.

Since it is well established that macrophages (peritoneal) synthesize the complement components C1, C2, C3, and C4 (3, 11, 12, 16), our findings may indicate that alveolar macrophages, which so far have been shown to synthesize C2 and C4, become attracted as a response to intranasal infection with *M. pneumoniae* (1, 2); this local accumulation of cells able to synthesize complement components could lead to a local increase in individual complement

components. This interpretation is supported by experiments recently described by Barber and Burkholder (2); these authors found that the number of alveolar macrophages and exudate macrophage-like free alveolar cells competent for C4 production increased after transnasal infection of guinea pigs with *Listeria monocytogenes*, after generation of a pulmonary granulomatous reaction by intravenous infection of heat-killed BCG or after aerosol infection of nonvaccinated animals with *Mycobacterium tuberculosis* (2). Furthermore, we have previously demonstrated that *M. pneumoniae* is able to interact directly with C1 in the absence of antibodies and that *M. pneumoniae* cells are rounded and killed by fresh guinea pig serum not containing detectable amounts of antibody (5). Gross et al. have shown that an intact complement system is required for optimal pulmonary clearance of some bacteria since, under complement depletion in cobra venom factor-(CoF) treated animals, the susceptibility to pulmonary infection with *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* is increased (10). Therefore, we conclude that the increased amounts of C1, C2, C3, and C4 in the bronchial fluids as a response to intranasal infection may represent an early, unspecific defense mechanism of the host before the immune response becomes effective. A possible activation of the complement system either via the classical or via the alternative pathway not only may lead to rounding, killing, or phagocytosis of *M. pneumoniae*, but it may also stimulate and therefore amplify these unspecific defense systems, which may explain the fact that most human infections with *M. pneumoniae* have a subclinical course.

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