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# Immunoglobulin M Antibody Response Against *Mycoplasma pneumoniae* Lipid Antigen in Patients with Acute Pancreatitis

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Serial serum samples from patients with acute pancreatitis showed a significant increase in antibodies against methanol-chloroform-extracted lipid antigen from *Mycoplasma pneumoniae* when tested by complement fixation. The antibodies did not react with antigens prepared from other human mycoplasmas or from pancreatic tissue by lipid extraction. The antibodies were predominantly immunoglobulin M (IgM). No correlation with cold agglutinins or cardiolipid complement-fixing antibodies was found. The IgM antibody response seemed to be prolonged: after 3 to 4 weeks the antibodies were still in many cases exclusively IgM. Similar IgM responses were also found in certain cases of acute meningoencephalitis. We postulate that during the disease antigenic components identical or very similar to major determinants in the *M. pneumoniae* lipid antigen are revealed and elicit the IgM antibody response. Their resemblance to natural antibodies and their possible biological role is discussed.

In an earlier study on a possible microbiological etiology of acute pancreatitis, a high proportion of patients showed a significant increase in complement-fixing (CF) antibodies against *Mycoplasma pneumoniae* (MP) lipid antigen during the course of illness (4). Although MP has been incriminated as causing hepatic and pancreatic lesions in experimental animals after intraperitoneal inoculation (6), epidemiological and immunological features argue against the concept that MP would have any etiological role in this devastating disease (4). We concluded that this antibody response was instead directed against some endogenous antigenic structure exposed during the acute phase of the disease. Preliminary results indicated that the antibody response was prolonged and involved immunoglobulin M (IgM) antibodies (3). This prompted us to collect new study material with more frequent serum samples. The present study confirms the earlier observations about the occurrence of the antibody response against MP. It also shows that the response is almost exclusively due to IgM antibodies and is specific to the lipid antigen of MP. The response can be demonstrated in a majority of patients irrespective of the clinical etiology of the disease. The antibodies resemble natural antibodies, but differ from cold agglutinins.

## MATERIALS AND METHODS

**Clinical material.** A total of 60 patients admitted consecutively to the University Central Hospital of Helsinki with a clinical diagnosis of acute pancreatitis were included in this study. The diagnostic criteria and the outlines of treatment have been published earlier (8).

Serum samples were collected at 3- to 4-day intervals, and the sera were stored frozen at −20°C until used. The first serum sample was obtained within 48 h of the onset of the disease. For a control series, paired serum samples were taken from 30 adult patients admitted to the same hospital for acute appendicitis. The first sample was taken on admission and the second 2 weeks later.

In addition, paired sera from patients suffering from different diseases and in whom a significant increase of MP CF antibodies had been shown were available in the laboratory.

**Antibody assays.** The preparation of the MP lipid antigen has been described earlier (2, 4). A standard micro-CF antibody technique was used (14), employing 4 U of antigen and 2 U of complement. Class-specific antibodies were studied by separating the serum fractions by sucrose gradient ultracentrifugation: 0.1 ml of serum was layered on the top of a 5-ml linear 12.5 to 37.5% (wt/vol) sucrose gradient in Dulbecco saline with 0.2% bovine serum albumin and centrifuged in a Spinco SW 50.1 rotor at 33,000 rpm for 17 h at 5°C. Twenty fractions were collected through the bottom of the centrifuge tube, inactivated at 56°C for 30 min, and assayed for CF antibody activity. In each centrifugation, a control serum with rubella IgM antibodies was included, and the fractions were assayed for rubella hemagglutination-inhibiting antibodies.

Cold agglutinin (12) studies were performed by the Municipal Bacterological Laboratory, Aurora Hospital, Helsinki. Platelet aggregation studies for the presence of immune complexes (9) were performed in the

Laboratory of Viral Immunopathology, Department of Virology, University of Helsinki.

## RESULTS

**Frequency and kinetics of the antibody response.** Most patients with acute pancreatitis showed a significant increase (fourfold or greater) in CF antibodies against MP lipid antigen between samples taken at the onset of the disease and those taken 10 to 14 days later (Fig. 1). Almost half of them (25/60) showed a seroconversion, turning from seronegative to seropositive during the illness. Among the control patients with acute appendicitis, only one patient showed a significant increase in antibody titer (Fig. 2). The distribution of titers in the initial samples was comparable in both groups.

When serial serum samples from patients with acute pancreatitis were studied, it was found that the antibodies reached their maximum levels about a week after the onset of the disease (Fig. 3). Interestingly, nine patients who were known to have a recurrent episode showed kinetic antibody responses similar to those of the other patients (Fig. 3).

**Specificity of the antibody response.** To test the specificity of the observed antibody responses, acute- and convalescent-phase sera from 10 patients who showed a significant increase in MP CF antibodies were tested using antigens prepared from other *Mycoplasma* species. Antigens were prepared from *Mycoplasma fermentans, M. salivarium, M. orale,* and *M. hominis* in a way similar to that used for the propagation of MP antigen. The potency of these antigens was tested by using hyperimmune sera. None of the patients' sera tested showed antibody activity in a 1:8 dilution against these antigens.

An attempt was also made to extract an antigenic structure from frozen human pancreas. Five grams of pancreatic tissue was homogenized with a Dounce homogenizer, and the material was centrifuged and washed twice with cold physiological saline. The pellet was incubated overnight in an ice bath and extracted with a mixture of methanol-chloroform (2:1). After vigorous shaking, 0.1 M KCl was added, and the chloroform layer was separated and evaporated. The antigen was then dissolved into ethanol. No

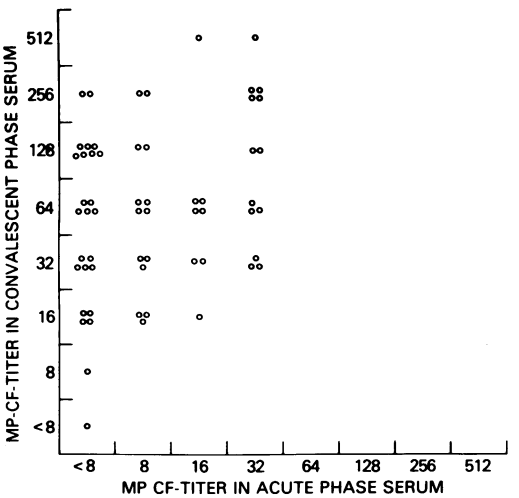


FIG. 1. *CF antibodies against MP lipid antigen in acute- and convalescent-phase serum samples from 60 patients with acute pancreatitis.*

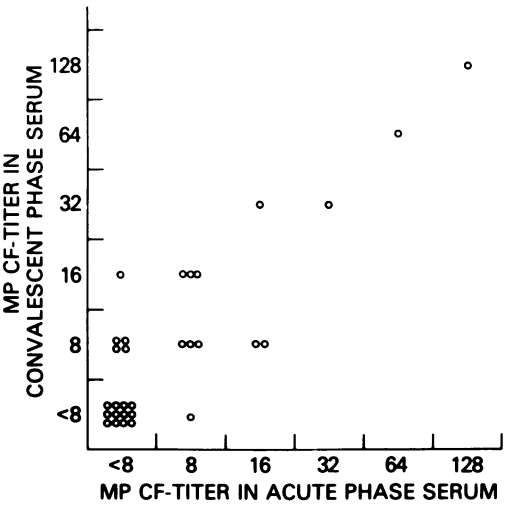


FIG. 2. *CF antibodies against MP lipid antigen in acute- and convalescent-phase serum samples from 30 patients with acute appendicitis.*

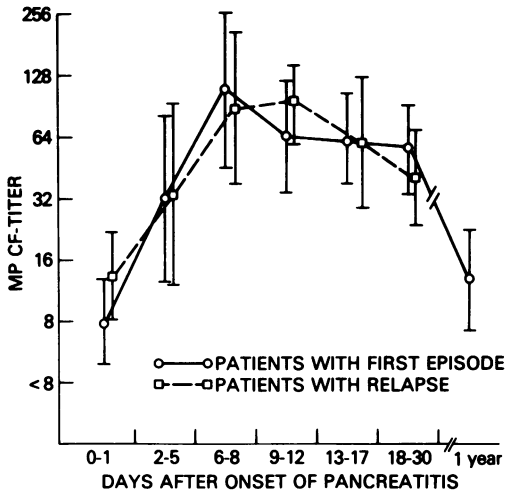


FIG. 3. *Mean CF titers in serial samples from patients with acute pancreatitis.*

antigenic activity could be demonstrated in the preparation by CF using a pool of high-titered convalescent-phase sera from pancreatitis patients. This antigen was also ineffective in adsorbing MP-reactive antibodies from convalescent-phase sera, while MP antigen was able to reduce the titers significantly (Table 1).

**IgM class antibodies.** Sucrose gradient centrifugation and subsequent CF titration of the fractions revealed that most of the MP CF antibody activity was due to IgM class antibodies. An analysis of 80 serum samples from 32 patients showed strong IgM antibody activity in all patients who had had a significant increase in CF antibody levels. This was usually most marked in the convalescent-phase samples but sometimes could already be seen after a few days from the onset of the disease. Results shown in Table 2 exemplify the incidence and magnitude of this response in six patients from whom several samples were studied.

The highest titer within the IgM peak was often as high as the CF titer of the whole serum, whereas IgG titers were regularly much lower. This was partially due to the anticomplementary activity often seen in the 19S region. However, the anticomplementary titer was much lower than the specific titers (Fig. 4). To test whether this anticomplementary activity was due to immune complexes, a platelet aggregation test was performed on the positive fractions. No direct activity could be found, indicating that complexes able to trigger the aggregation of platelets in vitro were not present in these fractions.

Treatment of a few sera with kaolin (by the standard procedure) did not always abolish the anticomplementary activity, but did reduce the titers both in IgG and IgM fractions (Fig. 5).

A convalescent-phase serum from each patient was assayed for cold agglutinins. Six of 60 were positive, all in low titers (1:4 to 1:8).

The IgM antibody often was very prominent as late as 3 weeks after the onset of the disease (Fig. 6; Table 2, cases 2 to 6). This seemed to be characteristic for acute pancreatitis; convalescent-phase sera from nine patients with pneumonia who had a significant increase in MP antibody titers during the course of the disease showed a significant IgG activity 1 to 2 weeks after the disease (Fig. 7a). One sample from a neonate was tested and showed exclusively IgG antibodies. A few more samples from MP-related acute respiratory infections (12 patients)

TABLE 1. *Adsorption of paired sera from patients with an antibody response to MP lipid antigen during acute pancreatitis, with MP lipid and an antigen prepared from pancreas tissue*

| Serum | Adsorbent | | |
|---|---|---|---|
| | None | MP[a] | Pancreas[b] |
| Acute | 8[c] | <8 | 8 |
| Convalescent | 256 | 64 | 128 |
| Acute | 8 | <8 | 8 |
| Convalescent | 64 | 16 | 128 |
| Acute | <8 | <8 | <8 |
| Convalescent | 256 | 64 | 128 |

[a] MP lipid antigen (128 antigenic units in CF) mixed with serum (0.5 ml + 0.5 ml) and incubated overnight at 4°C.

[b] Pancreas antigen was prepared by chloroform-methanol extraction from pancreas tissue, and mixed and incubated with serum as above.

[c] CF titers.

TABLE 2. *Class-specific anti-MP antibodies in patients with acute pancreatitis*

| Patient | Days from onset | CF titers | | |
|---|---|---|---|---|
| | | Whole serum | IgG fraction[a] | IgM fraction[a] |
| A.H. | 2 | <8 | Neg. | Neg. |
| | 6 | 64 | Neg. | 32 |
| | 8 | 256 | Neg. | 16 |
| | 15 | 128 | Neg. | 32 |
| G.R. | 1 | <8 | Neg. | Neg. |
| | 7 | 64 | Neg. | 64 |
| | 20 | 128 | Neg. | 64 |
| E.U. | 2 | 8 | Neg. | 32 |
| | 9 | 128 | 4 | 64 |
| | 21 | 128 | 4 | 64 |
| E.K. | 1 | <8 | Neg. | 32 |
| | 9 | 256 | 4 | 64 |
| | 19 | 64 | 4 | 64 |
| E.J. | 1 | <8 | Neg. | Neg. |
| | 7 | 128 | 2 | 32 |
| | 28 | 64 | 2 | 64 |
| K.P. | 7 | <8 | Neg. | Neg. |
| | 11 | 64 | Neg. | 16 |
| | 23 | 64 | 8 | 32 |

[a] Highest titer observed in any of the 7S or 19S fractions, respectively.

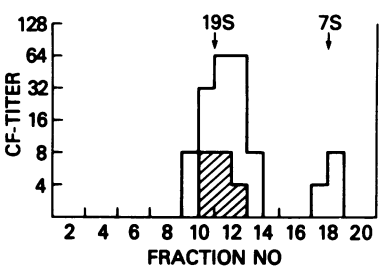


FIG. 4. *Reactivity of 19S and 7S fractions of a serum with MP lipid antigen. Shaded area = anticomplementary activity.*

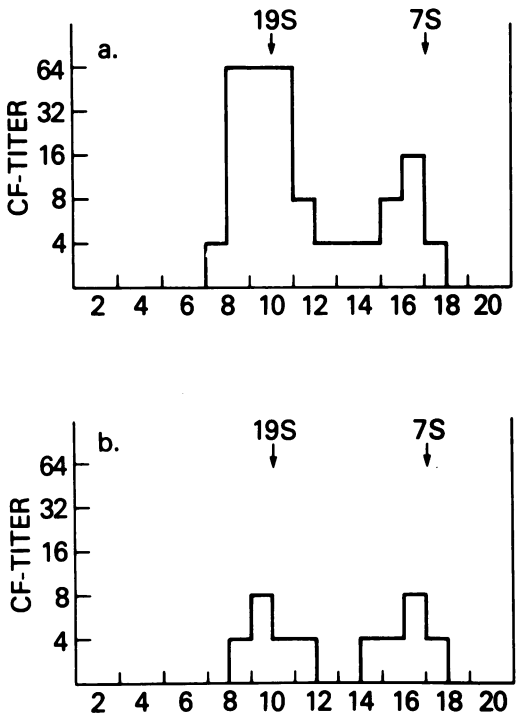


FIG. 5. *Effect of kaolin absorption on the reactivity of 19S and 7S serum fractions with MP antigen. (a) Before; (b) after kaolin treatment.*

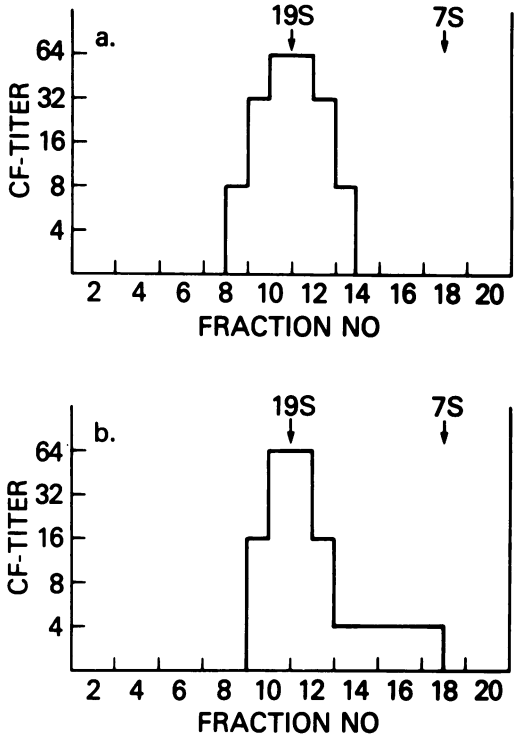


FIG. 6. *Reactivity of 19S and 7S fractions from serum samples taken 7 days (a) and 24 days (b) after the onset of acute pancreatitis against MP lipid antigen.*

also showed an IgG response. Seven patients with acute meningoencephalitis, who showed an increase in MP CF antibody titers, were also included in the control group. Unexpectedly, five showed exclusively IgM responses in convalescent-phase samples collected 14 to 24 days after the onset of the disease (Fig. 7b), whereas the two others and one patient with acute meningitis showed a mixed IgG and IgM response.

## DISCUSSION

In our previous study of patients with acute pancreatitis, we found that approximately one-third of the patients showed a significant increase in antibody levels against the MP lipid antigen (4). No fluctuation in the overall incidence of acute pancreatitis or of the "MP-positive" cases of the disease was observed during an outbreak of MP infections in the area. This and other features led us to conclude that the observed antibody response is either due to a related mycoplasma cross-reacting serologically with MP and causing the disease, or to a nonspecific phenomenon, possibly triggered by tissue destruction during the disease.

No activity could be demonstrated with several other human mycoplasma species. The specificity of the MP lipid antigen was further emphasized by the observation that the "lipid antigen" prepared from pancreatic tissue itself was inefficient compared to MP antigen in absorbing antibodies from convalescent-phase sera.

The fact that the antibody response was predominantly IgM suggests that it resembles the occurrence of natural antibodies, i.e., anti-I. Cold agglutinins were tested for in most of these sera, and, although occasionally positive, they did not seem to correlate with the antibodies developed against MP during acute pancreatitis. Selected sera were also tested with Wasserman cardiolipid antigen, which has been shown to cross-react with some MP lipid antigens (10), and, again, no correlation was found. This seems to indicate that the described antibody response reflects a specific reaction against an antigenic structure which is revealed during the process of acute pancreatitis. A similar or identical structure is probably also activated during some cases of acute meningoencephalitis. Whether this activation and the subsequent immunological reaction actually are able to cause immunopathological injuries of the affected organs remains to be shown.

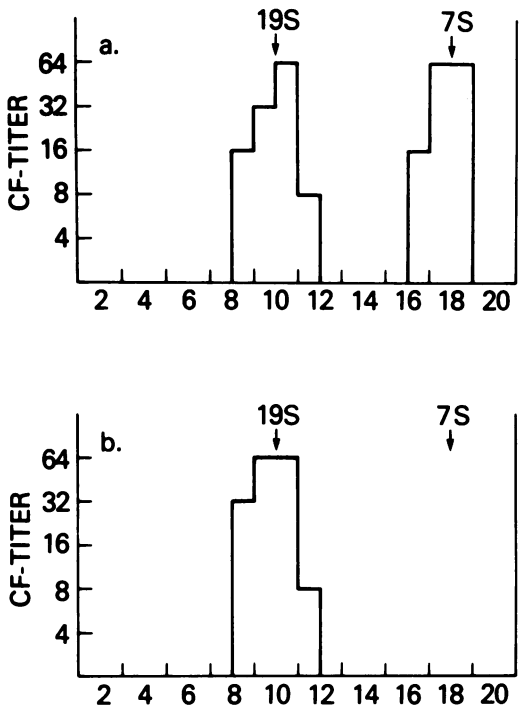


FIG. 7. *Reactivity of 19S and 7S fractions of serum samples taken 12 days after onset of pneumonia (a) and 24 days after onset of meningoencephalitis (b) against MP lipid antigen.*

In the previous study, as well as in the present study, no clinical difference could be detected either by physical or biochemical tests between those patients who did and those who did not show an antibody response against MP. This indicates that probably all patients have the response but in some cases it is too weak to be detected by CF. Although different factors such as gall stones or abuse of alcohol contribute to the etiology of the disease, the pathophysiological events are thought to be similar in all cases. The immunological event, although less important in the early steps of the disease, may prove to be important in determining the extent of tissue destruction and the later changes which take place in the pancreas and other parts of the body.

The antigen used in this and the previous study is similar to the antigen used in most laboratories in diagnostic tests for MP infections (12). Acute pancreatitis and very probably also acute meningoencephalitis, by triggering the antibody response, can erroneously be interpreted as being related to an MP infection because of the significant increase in CF titers. The lipid antigen is the dominant antigen not only in CF but also in the metabolic inhibition test and, when complexed with protein, in the indirect hemagglutination inhibition test as well (1, 12), leading to similar misleading test results. Indirect immunofluorescence (16) or the enzyme-linked immunosorbent assay using specific anti-IgG conjugate could be useful, since in patients with respiratory infections the IgG antibody response was often much more marked than in the patients with acute pancreatitis. Other tests less dependent on the lipid antigen (7, 12; K. E. Jensen, Bacteriol. Proc., p. 70–71, 1964) could also be useful.

Several antigenic components have been isolated from the lipid extract of MP. The crude lipid antigen seems to contain practically all of the antigenic material of MP (2), and the phospholipid and glycolipid constituents seem to carry the antigenic determinants (5, 10, 13, 15). Both the crude lipid antigen and the purified glycolipid fractions act as haptens since they are not capable of eliciting an antibody response in vivo (11). In an attempt to further define the nature of the antigen, we analyzed purified neutral, phospholipid, and glycolipid fractions of the antigen using high-titered serum from a patient with acute pancreatitis. Only the glycolipid fraction showed some antigenic activity (about 10% of the original activity), but, when mixed together with the other fractions, a full recovery of the antigen activity was obtained (P. Leinikki and O. Renkonen, unpublished data). It seems probable that the antigenic component that reveals the nonspecific antibody response in acute pancreatitis is the glycolipid part of the antigen complex. It is feasible to assume that the antigenic structure is either identical or very similar to that eliciting the response during the course of acute pancreatitis.

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