

Circulating Immune Complexes and Rheumatoid Factors in Patients with Farmer's Lung

E. O. TERHO,¹ P. LINDSTRÖM,² R. MÄNTYJÄRVI,³ H. TUKIAINEN¹ and O. WAGER²

¹Dept. of Pulmonary Diseases, Kuopio University Central Hospital, Kuopio, ²Laboratory of Clinical Immunology, Helsinki, and ³Dept. of Clinical Microbiology, University of Kuopio, Kuopio, Finland

Circulating IgG immune complexes (CICs), IgM rheumatoid factors (RFs), complement level (C3 and C4), and IgG antibodies to *Aspergillus fumigatus*, *Aspergillus umbrosus*, *Thermoactinomyces vulgaris*, and *Micropolyspora faeni* were analysed in the sera of 14 patients with farmer's lung (FL), 10 in the acute and four in the subacute phase of the disease. Ten spouses of FL patients served as exposed healthy controls. C3 and C4 were measured fluoronephelometrically. C3 levels were above the normal range and C4 levels near the upper limit of the normal range in both patients and controls; no statistically significant difference between patients and controls were observed. CICs were determined by enzyme immunoassays (EIA) of conglutinin-binding (KGB) and C1q-binding (C1qB). CIC levels above the normal range were detected in 12 (KGB-EIA) and nine (C1qB-EIA) of the patients and three (KGB-EIA) and six (C1qB-EIA) of the controls. No statistically significant differences were found in the mean levels between patients and controls. In contrast, RF levels in the acute phase of FL were significantly higher in the patients ($P < 0.01$) than in the controls. CICs correlated positively with most microbial IgG antibodies, but negatively with RFs. RFs also correlated negatively with microbial IgG antibodies, as well as with both C3 and C4. In FL, the increased RF level may, in the absence of increased CIC and decreased complement levels, represent an immune response (IgM anti-IgG autoantibodies) induced by local mechanisms of IgG immune complexes in the lungs.

Key words: alveolitis, extrinsic allergic; complement; farmer's lung; immune complexes; rheumatoid factors.

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Despite intensive studies during the past 20 years, the immunopathogenesis of extrinsic allergic alveolitis (EAA) has not been indisputably clarified. The presence of precipitating antibodies in the sera of patients with EAA, and the schedule of symptoms and signs in relation to the antigen exposure, match the concept of a reaction mediated by immune complexes (Type III) (12). There are, however, many findings suggesting the involvement of other immune mechanisms (16). Recently, the immunopathogenic role of circulating precipitins in EAA has been seriously questioned (4).

Only a few studies about circulating immune

complexes (CICs) in EAA have been published (5, 6), and with respect to the immunopathogenic role of CICs the results have been contradictory.

We have analysed CICs, complement level (C3 and C4), IgM anti-IgG autoantibodies (rheumatoid factors, RFs), and IgG antibodies to microbes present in mouldy hay in a group of patients with farmer's lung (FL), a form of EAA occurring among a farming population. Our results imply the involvement of local reactions of IgG immune complexes in the lungs in the immunopathogenesis of FL.

MATERIAL AND METHODS

Patients

Sera from 14 FL patients diagnosed at the Dept. of Pulmonary Diseases, Kuopio University Central Hospital, Finland, were analysed. All patients had handled mouldy hay or straw and fulfilled rigorous criteria for FL, including the presence of compatible symptoms and signs (13), physiological findings (7), and roentgenological changes (7). According to patient history, the most recent contact with visibly mouldy hay or straw had occurred less than 2 weeks before admission in six, and within 3 months in all except two patients. Blood samples from 10 patients were taken at the first admission, in the acute phase of the disease. Serial samples from eight of these were taken during the course of the disease, 1–6 months after the first admission. In four patients serum samples had not been obtained until 1–5 months after the first admission. From one of these, follow-up samples were also taken. Thus, a total of 33 patient sera was investigated. The sera were kept at -80°C until analysed.

Controls

Ten spouses of FL patients working on farms served as exposed healthy controls. Blood samples were taken at the time of the diagnosis of their diseased spouses. No follow-up samples were obtained. The sera were treated as above.

Conglutinin-binding enzyme immunoassay (KgB-EIA)

Details of the procedure are described elsewhere (19). Briefly, isolated bovine conglutinin (Kg) was adsorbed to polyamide rings (Lab-systems, Helsinki). Immune complexes attached by immune complex-bound C3bi to solid-phase Kg were detected by alkaline phosphatase-labelled anti-IgG serum. For measurements of the optical absorbances at 405 nm the FP-9 analyser system was used (Lab-systems, Helsinki). The results were expressed as SD units (SDU) according to the formula $(X - M)/SD$, where X is

the absorbance of the test specimen, M is the mean absorbance of a basic set of 40–50 blood donor sera, and SD their standard deviation. The SD unit was adjusted daily (SD_d) according to the formula $(SD \times M_d)/M$, where M_d is the mean absorbance of nine basic blood donor sera included in the daily run.

C1q-binding enzyme immunoassay (C1qB-EIA)

Details of the procedure are described elsewhere (19). Briefly, polystyrene cuvettes (Lab-systems, Helsinki) were coated with porcine C1q, and binding of IgG to the solid phase C1q was detected by alkaline phosphatase labelled anti-IgG serum. Optical absorbances were measured and the results expressed as SD units as above for KgB-EIA.

Enzyme immunoassay for IgM rheumatoid factors (RF-EIA)

Details of the procedure are described elsewhere (19, 20). Briefly, polystyrene cuvettes (Lab-systems, Helsinki) were treated with optimized dose of tetanus toxoid (TT) and thereafter with antitoxic IgG (aT). The TT-aT-coated cuvettes were used as IgG solid phase for detection of IgM RFs by the use of the anti-IgM conjugate. The procedure was the same and the results were expressed as SD units as above for KgB-EIA.

Assay for complement

C3 and C4 levels were assayed fluoronephelometrically (Technicon A.I.P.[®]).

Assay for microbial IgG antibodies

Cellular antigens were prepared from *Aspergillus fumigatus*, *Aspergillus umbrosus*, *Thermoactinomyces vulgaris*, and *Micropolyspora faeni* by extraction procedures described elsewhere (10, 11). *A. umbrosus* was a strain isolated from Finnish mouldy hay, the strains of the other microbes were obtained from the Mycological Reference Laboratory, London, U.K. Serum samples were tested for antibodies by EIA ac-

cording to Voller et al. (23) with modifications described previously (10, 11). Briefly, antigens were adsorbed on microtiter plates and binding of antigen-specific IgG to the solid phase antigens was detected by horseradish peroxidase-conjugated antihuman IgG. Optical absorbances were measured with a Titertek photometer (Eflab, Helsinki, Finland). The titer of a serum was expressed as the negative logarithm of the serum dilution giving an absorbance of 0.5. A positive control serum as well as a serum considered negative to all antigens were included in each run. These controls were used to correct the titers for variation between experiments.

Statistical methods

The significance of the differences of means between the study groups was tested with Student's *t*-test. Pearson correlation coefficients were used in the analysis of correlations between the immunological parameters investigated.

RESULTS

The CIC levels, measured by the two methods, and the RF levels in controls and 10 patients in the acute phase of the disease are shown in Fig. 1. CIC levels above the normal range (≥ 3 SDU) were found more often in patients than in controls with the KGB-EIA method, but equally as often in patients and in controls with the C1qB-EIA method. The mean CIC levels ($\pm$ SD) with KGB-EIA were 7.6 ± 5.1 SDU in the patients and 4.0 ± 4.6 SDU in the controls, and with C1qB-EIA, 4.9 ± 4.0 SDU and 6.5 ± 6.5 SDU, respectively. There were no statistically significant differences between patients and controls in the mean CIC levels measured either by KGB-EIA or C1qB-EIA. In contrast, all except two patients had RF levels above the normal range (≥ 3 SDU), whereas only two controls had slightly elevated RF levels. The difference in the mean RF levels between patients and controls was statistically significant ($P < 0.01$).

The levels of C3 and C4 in 10 patients in the acute phase and in nine controls are shown in

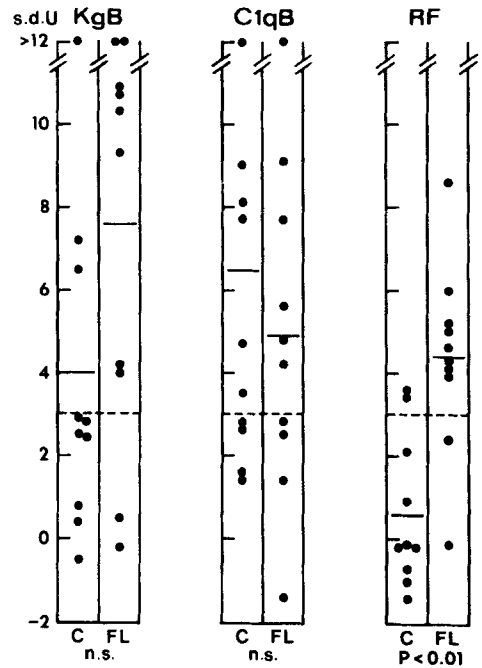


Fig. 1. Levels of circulating IgG immune complexes measured by conglutinin-binding enzyme immunoassay (KGB) and C1q-binding enzyme immunoassay (C1qB), as well as levels of IgM rheumatoid factors (RF) measured by enzyme immunoassay in 10 patients with farmer's lung in the acute phase of the disease (FL) and in 10 exposed healthy controls (C). Bars indicate the mean levels and broken lines the upper limits of the normal range. Statistical significance between patients and controls are shown; n.s. = not significant.

Fig. 2. In one control, the levels could not be determined because of the turbidity of the serum sample. The mean C3 levels were slightly above normal range (0.55–1.4 g/l) and the mean C4 levels near the upper limit of normal range (0.12–0.34 g/l) in both patients and controls. None of the C3 or C4 levels were below the normal range in any of the patients or controls. The mean levels ($\pm$ SD) for C3 were 1.74 ± 0.16 in the patients and 1.74 ± 0.50 in the controls, and for C4, 0.27 ± 0.10 and 0.27 ± 0.09 , respectively. There were no statistically significant differences between patients and controls.

The mean titers of IgG antibodies ($\pm$ SD) to *Aspergillus fumigatus* were 2.9 ± 0.65 in 10 patients in the acute phase and 2.7 ± 0.51 in the controls, to *Aspergillus umbrosus* 4.1 ± 0.54 and

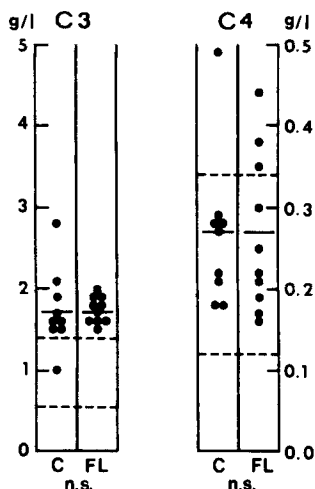


Fig. 2. Complement levels (C3 and C4) measured fluoronephelometrically in 10 patients with farmer's lung in the acute phase of the disease (FL) and in nine exposed healthy controls (C). Bars indicate the mean levels. Between the broken lines is the area of the normal range. Statistical significances are shown; n.s. = not significant.

3.5 ± 0.65 , to *Thermoactinomyces vulgaris* 3.6 ± 1.37 and 2.8 ± 0.85 , and to *Micropolyspora faeni* 3.8 ± 1.21 and 2.4 ± 0.88 , respectively. Between patients and controls, there were statistically significant differences in the levels of IgG antibodies to *A. umbrinosus* ($P < 0.05$) and *M. faeni* ($P < 0.01$), but not in the levels of IgG antibodies to the other two microbes.

The four patients whose serum samples were obtained after the acute phase of the disease, had also increased CIC levels, but only one (first serum sample, 1 month after first admission) had an increased RF level. The RF level in that patient did not return to normal within 6 months of the first admission. The C3 level was above normal range in three and the C4 level in two of these four patients.

In the group of eight patients investigated in the acute phase of the disease and again 1–6 months after, six of them had increased RF levels at first admission. In three of them, RF levels returned to normal range within 1 month and the levels of the other three showed a slight tendency towards normalization during the course of disease. In one patient, the RF level was within normal range at first admis-

sion, clearly elevated after 3 months, and normal again within another 3 months. These few follow-up samples showed no drastic changes in other immunological parameters.

All 33 patient sera were included in the correlation matrices. The CIC levels correlated positively with most microbial IgG antibodies but negatively with RFs. RFs correlated also negatively with microbial IgG antibodies, as well as with C3 and C4. There was a very poor positive correlation between CICs measured by the two methods.

In the controls, CICs correlated positively with IgG antibodies to *Thermoactinomyces vulgaris* and *Micropolyspora faeni* and again negatively with RFs. IgG antibodies to the two *Aspergilli* correlated negatively with CICs. IgG antibodies to *Aspergillus umbrinosus* correlated negatively also with RFs, C3, and C4. None of these correlations was, however, statistically significant. There was a good positive correlation between CICs measured by the two methods ($r = 0.82$; $P < 0.01$).

DISCUSSION

The results of the present study on CICs match quite well the results of Girard et al. (6), who found CICs by the "C1q binding test" in patients with EAA but also in about 26% of exposed controls. The CIC frequency in our exposed (as confirmed by titers of IgG antibodies to microbes) controls was even greater than that in the exposed controls of Girard et al. Dorval et al. (5), using a modified Raji cell technique, has also found CICs in patients with EAA. Our results on complement levels parallel the findings reported by Moore et al. (9) in pigeon breeders. The authors found that in patients with pigeon breeder's lung (another form of EAA) complement levels measured by CH_{50} were normal or slightly elevated and did not decrease after antigen challenge. Our finding on RFs confirm the findings of Banaszak et al. (3), who, using the latex-agglutination technique, found increased RF levels in four patients with "air conditioner pneumonitis", also a form of EAA.

If CICs were to have pathogenic systemic significance in EAA, one would expect that high levels of CICs and low levels of both serum C3 and C4 would be present in the patients in the acute phase of the disease. Such is the finding, e.g., in systemic lupus erythematosus, a systemic immune complex-mediated disease par excellence (18). In EAA the findings seem to be reversed: no drastically elevated CIC levels were found and complement levels, especially C3, were even higher than normal. Normal C3 and C4 levels might be explained by the fact that the time between latest mould exposure and blood sampling may have been long enough for the normalization of complement levels. In another context it has been found that C3 may behave as "acute phase reactant" (2). Increased C3 levels in the patients of the present study would support this concept but the causative agent for the elevation of C3 is uncertain. Furthermore, CIC levels in the patients were not higher than in the exposed asymptomatic controls. One should take into account, however, that the negative correlations between CICs and RFs on the one hand, and between microbial IgG antibodies and RFs on the other, may reflect the previously found (15, 19) inhibitory effects of RFs on the detection of CICs and IgG (as well as IgA) antibodies by solid phase EIA techniques. Thus, due to this inhibition, small differences in CIC levels (as well as in microbial IgG levels) between patients and controls may have remained unrecognised.

CIC and complement findings can also be considered in relation to the patients' increased RF levels, i.e. IgM anti-IgG autoantibodies, and thus, would suggest an analogy with rheumatoid arthritis (RA). The immunological profile of RA (18) resembles that of FL in our study: no high CIC levels, normal or elevated serum C3 and C4 levels, and a clearly increased serum RF level. In contrast, large amounts of immune complexes and lowered complement levels have been found in the synovial fluid in RA (22). In RA, serum RFs seem to be indicators of a local Type III reaction (mediated by IgG immune complexes) occurring at the synovial level. Analogously, in FL the increased RF level may, in the absence of increased CIC and decreased

complement levels, represent an immune response induced by local mechanisms of IgG immune complexes in the lungs.

The pathogenic role of CICs in EAA remains obscure. Our results, obtained from an admittedly small material, imply that the role may be of minor importance. Nevertheless, CICs may have been implicated in such cases of EAA in which the involvement of other organs besides the lungs has been reported (1, 8, 14, 17, 21).

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Address:

Erkki O. Terho, M.D.
Dept. of Pulmonary Diseases
Kuopio University Central Hospital
SF-71880 Tarinaharju
Finland