

Levels of Complement Factors in Human Serum during Immediate and Late Asthmatic Reactions and during Acute Hypersensitivity Pneumonitis

X. BAUR, W. DORSCH and T. BECKER

Pulmonologische Abteilung der Medizinischen Klinik I und Institut für Klinische Chemie des Klinikums Großhadern der Ludwig-Maximilians-Universität, Munich, W. Germany

In 16 asthmatic patients and in four subjects suspected of having hypersensitivity pneumonitis, serum levels of CH_{50} , C3, C4, C5 and factor B were measured before, between 10 and 20 min, between 5 and 7 h and, in the latter group, also 24 h after allergen challenges provoking type I bronchial reactions or acute hypersensitivity pneumonitis.

There was a significant decrease in one of the complement factors in two patients during the immediate asthmatic phase, but in no patient during the late asthmatic phase and in no patient with hypersensitivity pneumonitis. On the other hand, significant increases of C3, C4, and/or CH_{50} were seen in five patients during immediate asthmatic reactions, in seven patients during late asthmatic reactions and in all cases with hypersensitivity pneumonitis. However, with respect to the particular complement factors the vast majority of the patients showed no appreciable change. Investigations of C3 split products, which were done in seven patients gave negative results. No correlations existed between the changes in the levels of complement factors to increases of R_{aw} , decreases of DLCO, size of skin test reactions or RAST scores.

The cause and pathophysiological role of the non-uniform behaviour of serum complement levels after inhalation challenges is not yet clear; obviously both consumption and formation of complement factors take place during allergen-induced asthmatic reactions and hypersensitivity pneumonitis.

Key words: bronchial asthma; complement factors; hypersensitivity pneumonitis; immediate reaction; inhalation challenge; inhalant allergens; late reaction.

Accepted for publication 25 October 1979

The involvement of complement in local and general anaphylactic reactions has been suggested by several authors (4, 25, 28). This view has been supported especially 1) by the finding of complement activation *in vitro* by different allergens (4, 28), 2) by evidence for complement receptors on the surface of mast cells and basophils (7, 15), and 3) by complement-induced release of mediators in lung tissue as well as from mast cells and basophils *in vitro* (7, 11, 13, 15, 21).

In acute hypersensitivity pneumonitis, there is strong evidence for activation of the complement system, especially when immuno-fluorescent analysis and studies of complement consumption are considered (2, 5, 6, 9, 10, 16, 22, 29); on the other hand, lung biopsies do not always reveal a typical Arthus type reaction with deposition of antibodies and complement in alveolar capillaries (27).

Measurement of complement levels in man during immediate and late asthmatic reactions

Table 1

Clinical data and test results of 16 asthmatic patients who underwent studies of serum complement levels during immediate and/or late asthmatic reactions caused by allergen inhalation

Patient	Age (years)	Duration of asthmatic symptoms (years)	Total IgE ^a (u/ml)	Test results with allergens					Other allergens to which there were positive skin tests ^a RAST ^b and/or inhalation tests ^c
				Allergen	Intracutaneous test ^c Conc. ^b	(20 min)	RAST ^d (PRU/ml)	Inhal. challenge ^b Minimal conc. necessary for positive challenge	
1	16	5	535	Grass p.	1:5000	3	16.9	1:3000	tree pollen ^a cat fur ^{a, b, c}
2	42	4	2930	Grass p.	1:5000	3	25.7	1:1500	—
3	39	34	318	Grass p.	1:5000	2	1.0	1:70	cat fur ^b , <i>D. pteronyssinus</i> ^{a, b, c}
4	34	16	149	Grass p.	1:5000	3	0.4	1:700	house dust ^{a, c} , <i>D. pteronyssinus</i> ^{a, b, c} cat fur ^a
5	34	3	244	Grass p.	1:5000	3	6.8	1:7000	—
6	41	2	569	Bermuda grass p.	1000 NE	3	2.9	1000 NE	flower pollen ^{a, b}
7	20	3	132	Cat fur	1:200	3	33.2	1:333	dog hair ^{a, b, c}
8	33	12	722	Cat fur	1:200	3	60.7	1:666	grass p. ^{a, b}
9	36	1/2	240	Cat fur	1:200	4	39.3	1:17	—
10	25	2 1/2	512	Cat fur	1:200	3	33.5	1:17	grass p. ^{a, b}
11	27	3	490	Cat fur	1:200	3	9.5	1:170	grass p. ^{a, b} , moulds ^{a, b, c}
12	43	2	787	Dog hair	1:200	3	15.3	1:17	—
13	39	1/2	379	<i>D. pteronyssinus</i>	1 0 E	2	53.1	500 E	—
14	31	3	236	Aspergilli species	1:100	3	0	1:100	grass p. ^{a, b}
15	43	4	795	Mixed threshings	1:200	2	0	1:17	—
16	58	3	530	Penicillin	1:100	1	0	1:100	tree p. ^{a, b}

Grass p. means mixed grass pollen (B₂, Bencard)

^a Normal range: up to 150 u/ml.

^b Concentrations of allergen solutions according to the manufacturer's declaration.

^c Cutaneous reactions to allergens were related to histamine reactivity and scored from 0 to 4 according to Aas & Belin (Acta Allergol. 27, 439, 1972).

^d RAST was performed using commercially available test reagents and reference system (Pharmacia, Uppsala, Sweden); paper discs with grass pollen (B₂, Bencard), Aspergilli species and mixed threshings had been coupled in our laboratory. The results are expressed in Phadebas RAST units (PRU)/ml serum as recommended by the manufacturer.

and during hypersensitivity pneumonitis may be useful for studying the pathophysiological role of complement in these bronchopulmonary diseases. Until now there have been only a few such investigations with contradictory results (1, 12, 14, 19).

PATIENTS AND METHODS

This study was performed on 16 asthmatic

patients and four patients whose acute and chronic symptoms as well as the evidence of interstitial pulmonary changes on their roentgenograms and lung function studies gave reason to suspect hypersensitivity pneumonitis. The clinical data is summarized in Tables 1 and 2. None of the patients was on treatment during the investigation. Commercially available allergen extracts were used for skin tests and inhalation tests: House

Table 2

Clinical data and test results of four patients who underwent studies of serum complement levels during acute hypersensitivity pneumonitis

Patient	Age	Symptoms probably induced by hay dust (Nos. 17, 18, 19) or pigeon droppings (No. 20)	Duration of symptoms (years)	Total IgE (μ /ml)	Total IgG ^a (g/l)	Precipitating antibodies ^b against	Inhalation challenge	
							Substance	Minimal time of exposure or inhaled amount necessary for positive results
17	45	Cough, fever, loss of body weight, dyspnoea on exertion	9	70	18.2	<i>Micropolyspora faeni</i> <i>Cryptostroma cort.</i> <i>Aspergillus fum.</i> <i>Cephalosporium acrem.</i>	hay dust	30 min
18	47	Cough, chills, fever, dyspnoea after several hours	1/2	78	12.0	<i>Aspergillus fum.</i>	hay dust	45 min
19	46	Cough, chills, fever, loss of body weight, dypnoea on exertion	2	73	17.8	—	hay dust	60 min
20	33	Chills, fever, dyspnoea after several hours, loss of body weight	1	2613	24.2	pigeon droppings pigeon serum	pigeon serum	1/10 ml

^a Mean $\pm$ 1 s.d.: 11.78 $\pm$ 2.00 g/l.

^b Determined by Ouchterlony test with allergen extracts from Hollister Stier, Spokane, Washington, USA. All sera were routinely tested with extracts of *Thermoactinomyces vulgaris*, *Micropolyspora faeni*, *Aspergillus fumigatus*, *Pullularia pullulans*, *Cephalosporium acremonium*, *Trichoderma viride*, *Cryptostroma corticale*, *Sitophilus granarius*, pigeon serum and pigeon droppings.

dust mite (*Dermatophagoides pteronyssinus*) and Bermuda grass pollen were purchased from Hal, Haarlem, The Netherlands; Penicillin V-Kalium was from Siemensgluss, W. Germany, the other allergens were purchased from Bencard, Brentford, England.

We conducted intracutaneous tests routinely with 16 common allergens: three pollen groups (grass, tree, and flower pollen), rye pollen, three mould groups, *Merulius lacrymans*, feathers, house dust mite, house dust, cat fur, dog hair, horse hair, egg/milk and a fish group. We broadened this allergen spectrum when there was evidence of other sensitizations.

In inhalation challenges the patients were first given diluent and then allergen extracts in increasing concentrations with a nebulizer. For details see Baur et al. (3). Challenges by hay dust were performed in a small cabin: the patients strew the hay (15–30 l) they had been exposed to on their farms, inhaling the dusty content. Airway resistance (R_{aw}) was measured in a body plethysmograph before and after inhalation of diluent and at intervals of 10 to 60 min up to 7 h after challenge.

A significant asthmatic reaction is assumed if R_{aw} rises more than 100% reaching values above 0.4 kPa·l⁻¹·sec or more than 50%

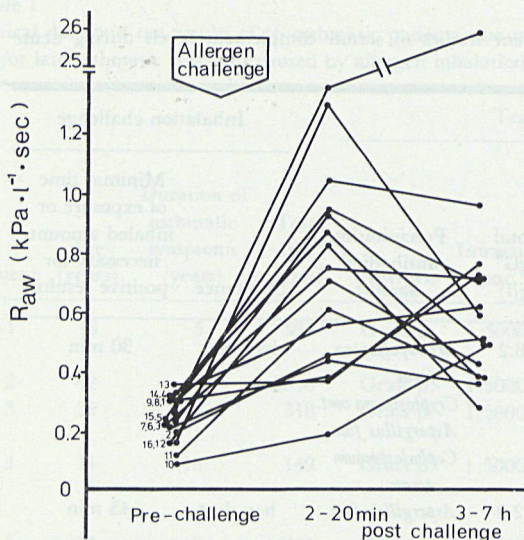


Fig. 1. Changes in airway resistance (R_{aw}) after allergen challenge in 16 asthmatic patients. Curves represent maximum values during immediate (2–20 min) and late phase (3–7 h) post allergen inhalation. Numbers correspond to the numbered patients in Table 1. Inhaled allergens see Table 1.

reaching values above $0.5 \text{ kPa} \cdot \text{l}^{-1} \cdot \text{sec}$. In addition, diffusing capacity for CO (DLCO), vital capacity (VC) and 1-sec forced expiratory volume (FEV_1) were measured in patients suspected of having hypersensitivity pneumonitis before allergen exposures, then every 1 to 2 h until the 7th and again at the 24th h.

Venous blood samples were taken before, between 10 and 20 min (immediate phase) and between 5 and 7 h (late phase) after allergen inhalation. In addition, 24-h blood samples were taken in tests with hay dust or pigeon serum. The samples were put into an ice bath immediately and centrifuged. The sera were either used at once for complement determination or frozen at -30°C for a few days pending measurement.

We measured total complement activity (CH_{50}) by determination of the point of 50% lysis of sheep erythrocytes according to the method of Mayer (17).

Concentration of C3 was determined by nephelometry (Technicon AIP Analyser) utilizing a standard curve with commercial

reference sera. C4, C5 and factor B were quantitated by radial immunodiffusion, using commercially available antibody containing agar plates as described by the manufacturers (C4, factor B: Behringwerke; C5: Meloy). The standard deviations of these methods were about 5%; changes of more than 3 s.d. ($> 15\%$) of initial values are regarded as significant.

In addition, we studied circulating C3 split products by crossed immunoelectrophoresis (Laurell Technique) in the serum of seven patients before, between 10 and 20 min and between 5 and 7 h, and in patients No. 18 and 19 also 24 h after challenge; the antisera used in this study were purchased from Behringwerke.

RESULTS

Immediate and late asthmatic reactions

As shown in Fig. 1, inhalation of allergens led to immediate asthmatic reactions in 13 patients, and also to late asthmatic reactions in eight. In three subjects there were isolated late asthmatic responses.

Prechallenge values for CH_{50} were normal in 11 of 16 patients, decreased in four and elevated in one. Five patients had decreased and 11 normal values of C3; C4 was increased in three cases, decreased in one and normal in 12. Factor B was within the normal range in all serum samples. C5 was elevated in 10 of the 16 asthmatics (Fig. 2). There was a significant decrease in complement levels during the immediate phase for CH_{50} in one case (patient No. 5) and for C5 in another (patient No. 6). Most of the other patients showed minimal reduction or increase, lying within the error of the methods used. Surprisingly, there were significant rises of CH_{50} (patient No. 16), C3 (patient No. 14), C4 (patients No. 2 and 3) and factor B (patient No. 7).

During the late phase after allergen challenge no case showed a significant reduction in any of the complement components (Fig. 2); however, in relation to

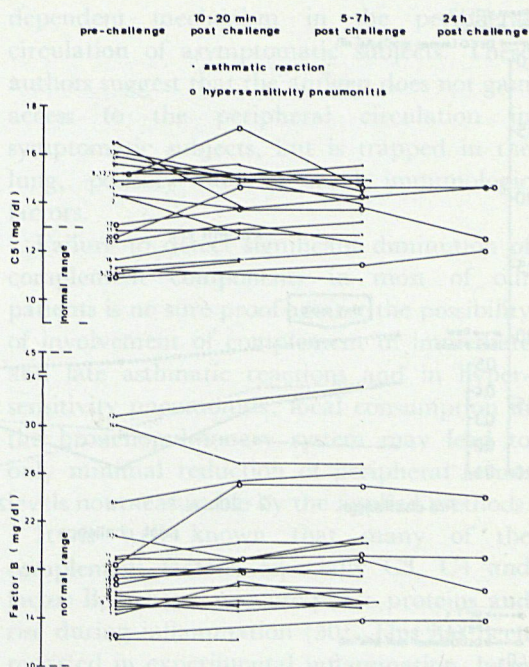
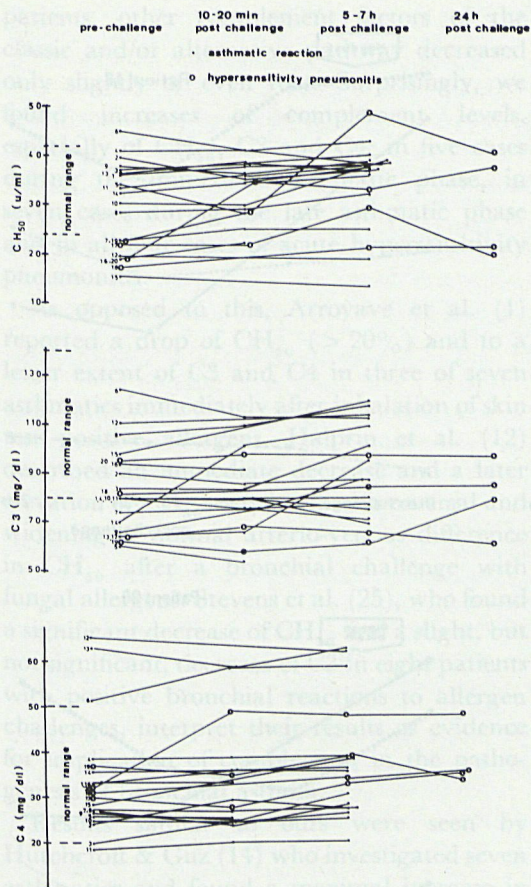


Fig. 2. a, b, c, d, e. Changes in CH₅₀, C3, C4, C5 and factor B during allergen-induced immediate and/or late asthmatic reactions (patients 1-16) and during acute hypersensitivity pneumonitis (patients 17-20). Normal ranges are given as indicated by the manufacturers. Numbers correspond to the numbered patients in Table 1 and Table 2.

pre-challenge values there was a significant elevation of CH₅₀ in three cases (patients No. 10, 11, 14), of C3 in five cases (patients No. 3, 9, 13, 14, 15) and of C4 in two cases (patients No. 3, 15). Circulating C3 split products, which were investigated in patients No. 8, 10, 11, 13, 16 during the immediate and late phase after allergen inhalation, were not found. Neither during the immediate phase nor during the late phase did there exist a correlation between the increase of R_{aw} and the changes in complement levels. There was also no correlation seen when comparing the complement values, with the RAST scores or the size of skin test reactions.

Hypersensitivity pneumonitis

Patients No. 17 to 20 showed typical changes for hypersensitivity pneumonitis between 3

and 7 h after challenges by hay dust or pigeon serum: Temperatures rose 1.6, 3.0, 3.2 and 1.1°C respectively; the peripheral leucocyte count increased 6.1, 7.0, 3.0 $\times 10^3/\text{mm}^3$ (in case No. 17 this measurement was not done). All the subjects complained of cough, fatigue, and mild dyspnoea. DLCO, VC and FEV₁ decreased; the DLCO proved to be the most sensitive method for detecting the pulmonary disturbances, whereas VC and FEV₁ did not always show significant changes (Fig. 3). No significant increase of R_{aw} was found in any of these subjects. In patient 20 there was a drop in VC and FEV₁ also immediately after inhalation of pigeon serum.

Before challenge, all four patients had decreased CH₅₀, two of them also had decreased C3 levels; C5 was increased in two of these cases. Exposure to hay dust or pigeon

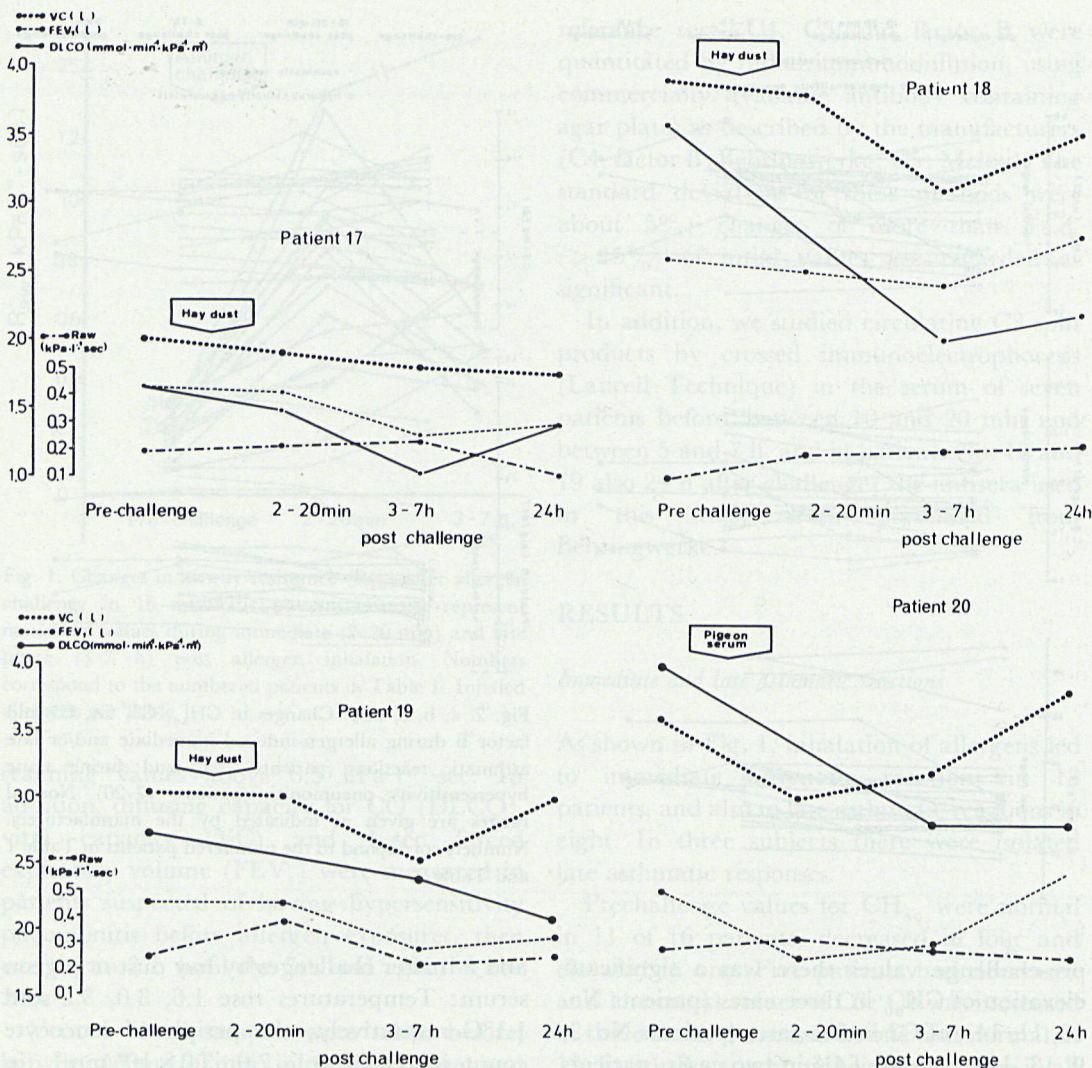


Fig. 3. a, b, c, Changes in DLCO, VC, FEV₁ and R_{aw} after inhalation challenges by hay dust or pigeon serum in four patients with hypersensitivity pneumonitis. For details see Table 2. Curves represent maximum changes during the immediate (2-20 min) and late phase (3-7 h) post challenge.

serum led to a significant increase of CH₅₀ in all four cases (32%, 28%, 117%, and 93%, respectively), and of C3 in one (patient No. 19), C4 in two (patients No. 17 and 20) and factor B and C5 in one (patient No. 20). In most cases these elevations were highest 5 to 7 h or 24 h after inhaling the allergens (Fig. 2). C3 split products could not be detected in the sera of patients No. 18 and 19.

DISCUSSION

Our results do not show uniform changes in serum complement levels following bronchial or pulmonary hypersensitivity reactions from inhalant allergens. A significant decrease of one of the complement components was observed in only two cases during immediate asthmatic reactions. However, in these

patients, other complement factors of the classic and/or alternative pathway decreased only slightly or even rose. Surprisingly, we found increases of complement levels, especially of CH_{50} , C3 and C4, in five cases during the immediate asthmatic phase, in seven cases during the late asthmatic phase and in all four cases of acute hypersensitivity pneumonitis.

As opposed to this, Arroyave et al. (1) reported a drop of CH_{50} ($> 20\%$) and to a lesser extent of C3 and C4 in three of seven asthmatics immediately after inhalation of skin test positive allergens. Halprin et al. (12) described an immediate decrease and a later elevation of CH_{50} associated with reversal and widening of normal arterio-venous difference in CH_{50} after a bronchial challenge with fungal allergens. Stevens et al. (25), who found a significant decrease of CH_{50} and a slight, but not significant, decrease of C3 in eight patients with positive bronchial reactions to allergen challenges, interpret their results as evidence for implication of complement in the pathogenesis of bronchial asthma.

Results similar to ours were seen by Hutchcroft & Guz (14) who investigated seven asthmatics and found a maximal increase in C3 of 30 mg/dl and in C4 of 10 mg/dl. On the other hand, in one of their patients with a high normal initial level, C4 halved after allergen challenge. These authors attribute their results to be evidence against significant activation of complement in allergen-induced asthma.

Normal or even increased levels of complement during active symptomatic hypersensitivity pneumonitis were reported by Moore et al. (18, 20) and Stiehlm et al. (26). In exposed asymptomatic individuals or in immunized animals there was a decrease of serum complement after aerosol challenge by allergens capable of causing hypersensitivity pneumonitis. Despite this, these subjects remained totally well. Moore et al. (19) and Schatz et al. (23) interpret this unexpected finding to a protective clearance of the allergens by antibodies and a complement-

dependent mechanism in the peripheral circulation of asymptomatic subjects. These authors suggest that the antigen does not gain access to the peripheral circulation in symptomatic subjects, but is trapped in the lung, possibly due to local immunologic factors.

Failure to detect significant diminution of complement components in most of our patients is no sure proof against the possibility of involvement of complement in immediate and late asthmatic reactions and in hypersensitivity pneumonitis; local consumption in the bronchopulmonary system may lead to only minimal reduction of peripheral serum levels not measurable by the applied methods.

It is well known that many of the complement factors, especially C3, C4 and factor B, behave as acute-phase proteins and rise during inflammation (30). This has been reported in experimental inflammation, burn trauma, bacterial infection and after injection of endotoxins (8, 24). Inflammatory responses induced by the inhaled organic materials may also be the cause of the increases of complement levels as seen in some of our patients.

In review of serum complement levels *in vivo*, it must be considered that the values observed represent the result of complement formation and complement consumption. Our results may be interpreted as evidence that common inhaled substances are capable of initiating mechanisms resulting in increases of complement factors. Under these conditions, consumption would not be detectable. Changes in the levels of the various factors may vary from one another and not be correlated. Perhaps this is the explanation of the contradictory results found in the literature. Further investigations, especially determination of complement components at the site of action, i.e. in the bronchopulmonary tissue and local blood stream, are necessary in order to study the exact role of the complement systems in the pathogenesis of bronchial and pulmonary hypersensitivity reactions.

REFERENCES

1. Arroyave, C. M., Stevenson, D. D., Vaughan, J. H. & Tan, E. H.: Plasma complement changes during bronchospasm provoked in asthmatic patients. *Clin. Allergy* 7, 173-182, 1977.
2. Barrowcliff, D. F. & Arblaster, P. G.: Farmer's lung: A study of an early fatal case. *Thorax* 23, 490-500, 1968.
3. Baur, X., Fruhmann, G. & v. Liebe, V.: Allergologische Untersuchungsmethoden (inhalativer Provokationstest, Hauttest, RAST) für die Diagnose des Asthma bronchiale. *Klin. Wochenschr.* 56, 1205-1212, 1978.
4. Berrens, L. & Van Rijswijk-Verbeek, J.: Inactivation of complement (C3) in human serum by atopic allergens. *Int. Arch. Allergy* 45, 30-39, 1973.
5. Berrens, L., Guikers, C. L. H. & Van Dijk, A.: The antigens in pigeon-breeder's disease and their interaction with human complement. *Ann. N. Y. Soc.* 221, 153-162, 1974.
6. Caldwell, J. R., Pearce, D. E., Spencer, C., Leder, R. & Waldman, R. H.: Immunologic mechanisms in hypersensitivity pneumonitis. I. Evidence for cell-mediated immunity and complement fixation in pigeon-breeder's disease. *J. Allergy Clin. Immunol.* 52, 225-230, 1973.
7. Cochrane, C. G. & Müller-Eberhard, H. J.: The derivation of two distinct anaphylatoxine activities from the third and fifth components of human complement. *J. Exp. Med.* 127, 371-386, 1968.
8. Cooper, N. R. & Fogel, B. J.: Complement in normal and disease process. *J. Pediatr.* 70, 982-996, 1967.
9. Edwards, J. H.: A quantitative study on the activation of the alternative pathway of complement by mouldy hay dust and thermophilic actinomycetes. *Clin. Allergy* 6, 19-25, 1976.
10. Ghose, T., Landrigan, P., Killeon, R. & Dill, J.: Immuno-pathological studies in patients with farmer's lung. *Clin. Allergy* 4, 119-129, 1974.
11. Grant, J. A., Settle, L., Whorton, E. B. & Dupree, E.: Complement mediated release of histamine from human basophils. II. Biochemical characterization of the reaction. *J. Immunol.* 117, 450-456, 1976.
12. Halprin, G. M., Buckley, C. E., Zitt, M. J. & McMahon, S. M.: Changes in arteriovenous complement activity induced by inhalation challenge. *Am. Rev. Respir. Dis.* 108, 343-352, 1973.
13. Hook, W. A., Siraganian, R. P. & Wahl, S. M.: Complement-induced histamine release from human basophils. I. Generation of activity in human serum. *J. Immunol.* 114, 1185-1190, 1975.
14. Hutchcroft, B. J. & Guz, A.: Levels of complement components during allergen-induced asthma. *Clin. Allergy* 8, 59-64, 1978.
15. Johnson, A. R., Hugli, T. E. & Müller-Eberhard, H. J.: Release of histamine from rat mast cell by the complement peptides C3a and C5a. *Immunology* 28, 1067-1080, 1975.
16. Marx, J. J. & Flaherty, D. K.: Activation of the complement sequence by extracts of bacteria and fungi associated with hypersensitivity pneumonitis. *J. Allergy Clin. Immunol.* 57, 328-334, 1976.
17. Mayer, M. M.: Complement and complement fixation. In Kabat, E. A. & Mayer, M. M. (eds.): *Experimental immunochemistry*, p. II. Thomas, Springfield, 1971.
18. Moore, V. L., Fink, J. N., Barboriak, J. J., Ruff, L. L. & Schlueter, D. P.: Immunologic events in pigeon-breeder's disease. *J. Allergy Clin. Immunol.* 53, 319-328, 1974.
19. Moore, V. L., Hensley, G. T. & Fink, J. N.: An animal model of hypersensitivity pneumonitis in the rabbit. *J. Clin. Invest.* 56, 937-944, 1975.
20. Moore, V. L., Boren, M. N. & Abramoff, P.: The immunologic response of patients with pigeon-breeder's disease to a purified antigen from pigeon-dropping extract (PDE). *J. Allergy Clin. Immunol.* 57, 225, 1976, Abstr.
21. Mota, I.: The mechanism of action of anaphylatoxin. Its effect on guinea pig mast cells. *Immunology* 2, 403-413 1959.
22. Olenchock, S. A. & Burrell, R.: The role of precipitins and complement activation in the etiology of allergic lung disease. *J. Allergy Clin. Immunol.* 58, 76-88, 1976.
23. Schatz, M., Patterson, R. & Fink, J.: Immunopathogenesis of hypersensitivity pneumonitis. *J. Allergy Clin. Immunol.* 60, 27-37, 1977.
24. Schur, P. H. & Austen, E. F.: Complement in human disease. *Ann. rev. Med.* 19, 1-24, 1968.
25. Stevens, E., van Durme, P., Marien, G. & Billiet, L.: Complement changes after "bronchial challenge". In Pepys, J. & Edwards, J. H. (eds.): *Mast cell: its role in health and disease*, pp. 296-300. Pitman Medical, London, 1979.
26. Stiehm, E. R., Reed, C. E. & Tooley, W. H.: Pigeon-breeder's lung in children. *Pediatrics* 39, 904-915, 1967.
27. Turner-Warwick, M., Haslam, P. & Weeks, J.: Antibodies in some chronic fibrosing lung disease. *Clin. Allergy* 1, 209-219, 1971.
28. Weemeas, C. M. R., Stoop, J. W. & Berrens, L.: Inactivation of hemolytic complement by house dust allergen in the serum of children with atopic disease. *Clin. Allergy* 7, 75-82, 1977.
29. Wenzel, F. J., Emanuel, D. A. & Gray, R. C.: Immunofluorescent studies in patients with farmer's lung. *J. Allergy Clin. Immunol.* 48, 224-229, 1971.
30. Whicher, J. T.: The value of complement assay in clinical chemistry. *Clin. Chem.* 24, 7-22, 1978.

Address:

Dr. X. Baur

Pulmonol. Abt.

Klinikum Großhadern PF: 70 12 60

D 8000 München 70

W. Germany

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.