THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 63 NUMBER 5

Editorial

Circulating immune complexes and the practicing allergist

Peter F. Kohler, M.D. *Denver, Colo.*

Do allergists really need to be well informed about circulating antigen-antibody or immune complexes (CIC)? The answer to this rhetorical question is "yes" for a number of reasons.

First, over the past year four articles have appeared. Three of them were in this JOURNAL. The most recent is by Yang and colleagues on page 300 of this issue. It concerns CIC in patients with atopic respiratory disease, comparing those on hyposensitization therapy with untreated atopic controls and with normals without IgE-related disease.[1-4] In these studies, two of them from the same laboratory,[1, 4] the hypothesis was tested that long-term hyposensitization, intended to increase IgG blocking antibody, might also induce CIC composed of allergen and IgG blocking antibody. To me this is a very unlikely hypothesis when the small amounts of allergenic extracts injected and the sensitivity of the CIC detection assays are considered.

For example, Stein and associates[3] collected serum from patients 2 hr after injections of 0.5 ml of 1:100 w/v allergenic extracts when "significant antigen absorption" appeared. CIC were measured with a radiolabeled C1q-polyethylene glycol assay, which had a detection sensitivity equivalent to 5 μg/ml of human IgG.[3] The total protein content of the allergenic extract injected was approximately 5,000 PNU (0.5 mg N or 3,250 μg protein). In a 60-kg adult, the IgG distribution space is 50% intravascular and approximately 80 cc/kg or 5,400 ml; therefore, less than 1 μg allergen per cubic centimeter of plasma could be expected. If allergen were combined with IgG antibody in a soluble complex, concentrations of less than 3 μg/cc of CIC would be expected. This amount cannot even be reliably detected with any of the procedures used (Table I).

The initial study by Cano and associates,[1] using the C1q deviation assay, did find an increased prevalance of CIC in atopic patients on therapy for 5 yr or more; however, no differences related to hyposensitization have been found in the subsequent three studies (Table I).[2-4]

While direct participation of the allergens and blocking antibody in CIC is most unlikely and an increase in CIC directly related to hyposensitization therapy was not shown, these studies do not exclude the possibility that hyposensitization may induce antibody which cross-reacts with autologous antigens in highly selected patients and thereby induce pathogenic CIC. This possibility has been suggested by the onset of vasculitis during hyposensitization in 6 of the 20 consecutive patients with polyarteritis nodosa who have been evaluated by our group.[5] While the consensus opinion that hyposensitization is not associated

Reprint requests to: Peter F. Kohler, M.D., Division of Clinical Immunology, Department of Medicine, University of Colorado Medical Center, Denver, CO 80262.

0091-6749/79/050297+03$00.30/0

TABLE I. Circulating immune complexes (CIC) in atopic patients

Study	CIC		Atopics with increased CIC concentrations			
	Measured by	"Normal" range	On hyposensitization	(%)	Controls	(%)
Cano et al.[1]	Inhibition of ^{125}I C1q binding	<12% (n = 25)	19/32*	(59)	14/21	(67)
Levinson et al.[2]	^{125}I C1q binding and PEG precipitation	<25 μg†/ml (n = 28)	3/41	(7)	1/22	(14)
Stein et al.[3]	^{125}I C1q binding and PEG precipitation	<15% (n = 42)	15/49	(31)	9/42	(21)
Yang et al.[4]	IgG binding to Fc receptors of Raji cells	<20 μg†/ml (n > 100)	21/95	(22)	6/46	(13)
		Overall	58/216	(27)	30/131	(23)

PEG, polyethylene glycol.
*Number with elevated CIC/total tested.
†Equivalent of heat-aggregated human gamma globulin.

with any significant increase in CIC concentrations seems to be well established, none of the studies could have defined a 1% or smaller subgroup of patients who might be placed at risk by hyposensitization.

In three studies, the atopic patients had significantly increased CIC, unrelated to hyposensitization, compared to nonatopic controls (Table I). This observation is of great interest, but no explanations for these increases were offered and the observations need confirmation. If real, the CIC in atopics might be related to enhanced antigen absorption by atopics through respiratory and/or gastrointestinal mucosa. This phenomenon has been demonstrated in individuals with selective IgA deficiency who have precipitating antibody to milk proteins and a high prevalance of CIC which contain bovine proteins.[6] Selective IgA deficiency occurs in approximately 1 in 500 (0.2%) of the normal population and is a known predisposing factor for both atopic and autoimmune diseases in which its frequency is approximately 1% to 2%.[7]

Yang and associates[4] observed higher IgE levels in treated atopics with increased CIC (193 IU/ml compared to 103 IU/ml). If this observation is also confirmed, do the CIC concentrations vary with food ingestion and is there any correlation between CIC and IgA or IgE levels? Finally, the most relevant but difficult problem will be the identification of the causal antigens in the CIC of atopics.

I stress this because three of the assays listed in Table I depend on the binding of C1q to presumptive antigen-IgG or -IgM antibody complexes and the fourth on the attachment of antigen-IgG antibody complexes to lymphoid cell Fc receptors. None of the procedures, however, can distinguish between nonspecifically aggregated IgG and IgG antibody linked by antigen.

The C1q assays measure CIC by binding to IgG or IgM altered by interaction with antigen. They also are limited by the fact that false-positive results can occur when test sera contain deoxyribonucleic acid (DNA) or bacterial endotoxin which also react with C1q. In addition, the recently described serum C1q inhibitor protein might affect C1q assays by blocking the Ig binding sites.[8, 9]

The Raji cell is a lymphoblastoid B cell line from a patient with Burkitt's lymphoma with surface membrane receptors for C3b, C3d, C4, C1q, and IgG Fc. As originally described, the Raji assay detected CIC which were bound to the cell through its C3b receptor.[10] Others have subsequently claimed that CIC bind to Raji cells through C1q and not C3b receptors.[11] Yang and associates have modified this assay by selecting for Raji cells with a high density of IgG Fc receptors and using heated serum (which would eliminate the effect of C1q but not of C3b). Heating serum often causes IgG aggregation which may have influenced their CIC determinations. Also, since aggregated IgG_2 and IgG_4 bind only weakly to lymphocyte Fc receptors and staphylococcal protein A does not react with IgG_3, this modified Raji assay probably detects only CIC containing IgG_1 subclass molecules.[12, 13]

All these limitations need to be considered for a critical evaluation of the rapidly expanding literature on CIC in clinical medicine. Since IgG equivalents up to 25 μg/ml were detected in the healthy controls (Table I), as much as 60 mg of IgG (25 μg × 40 ml × 60) might be part of CIC in a "normal" 60-kg adult without exerting any apparent pathologic effects.

In addition to patients with immunologically induced vasculitis and glomerulonephritis, infectious processes, and the IgA-deficient patients who show no evidence of disease, CIC have been reported in

a subgroup of patients with idiopathic interstitial pneumonitis and correlated with highly cellular lung biopsies, positive lung immunofluorescence, and favorable therapeutic responses to prednisone.[14] In the other subgroup with fibrotic biopsies, CIC were not detected, lung immunofluorescence was negative, and the therapeutic response was poor. This important study raises more questions. First, assuming that relevant CIC exist, is the antigen inhaled or is it an autoantigen from lung or other tissue; also, are the CIC the cause of the inflammatory process in the alveoli or are they simply coexistent?

The immunopathogenesis of hypersensitivity pneumonitis has been attributed to inflammation induced by an Arthus type reaction occurring in alveolar walls with exogenous organic dust reacting with circulating IgG precipitating antibody. More recently, a cell-mediated immunopathogenesis has also been postulated, but confirmation of either mechanism is notably lacking. The new data on CIC in interstitial pneumonitis should prompt studies of sera from patients with hypersensitivity pneumonitis caused by inhalation of known organic antigens. Serial CIC measurements of serum during inhalation challenges and in bronchial lavage fluids would be particularly interesting.

Another group of patients with CIC are those with certain malignancies. In children with neuroblastomas, the concentrations of the CIC have been correlated with advanced metastatic disease and poor prognosis.[15] Vascular injury is not apparent and the CIC have been postulated, but again not proved, to consist of tumor antigen and antibody. The complexes could exert a suppressor effect on the tumor-specific cell-mediated immune response and thus enhance tumor growth.

In closing, I have briefly touched on several clinical situations of immediate interest to allergists in which CIC assays have been used with provocative results. Many more questions have been raised than answered. Knowledge of CIC, including the limitations and pitfalls of current assays, is most emphatically necessary for the modern-day allergist—much more so than it was for von Pirquet, who not only first suggested the pathogenic potential of immune complexes in equine serum sickness in 1904, but also introduced the term "allergy" a few years later.[16]

REFERENCES

1. Cano PO, Chow M, Jerry LM, Sladowski JP: Circulating immune complexes in patients with atopic allergy, Clin Allergy **7:**167, 1977.
2. Levinson AI, Sommers RJ, Lawley TJ, Evans R, Frank MM: Evaluation of the adverse effects of long-term hyposensitization, J ALLERGY CLIN IMMUNOL **62:**109, 1978.
3. Stein MR, Brown GL, Lima JE, Nelson HS, Carr RI: A laboratory evaluation of immune complexes in patients on inhalant immunotherapy, J ALLERGY CLIN IMMUNOL **62:**211, 1978.
4. Yang WH, Dorval G, Osterland CK, Gilmore NJ: Circulating immune complexes during immunotherapy, J ALLERGY CLIN IMMUNOL **63:**300, 1979.
5. Phanuphak P, Kohler PF: Onset of polyarteritis during hyposensitization treatment for atopic allergies. (Submitted to Ann Intern Med.)
6. Cunningham-Rundles C, Brandeis WE, Good RA, Day NK: Milk precipitins, circulating immune complexes, and IgA deficiency, Proc Natl. Acad Sci USA **75:**3387, 1978.
7. Amman AJ, Hong R: Selective IgA deficiency: Presentation of 30 cases and review of the literature, Medicine **50:**223, 1971.
8. Conradie JD, Volanakis JE, Stroud RM: Evidence for a serum inhibitor of C1q, Immunochemistry **12:**967, 1975.
9. Müller-Eberhard HJ: Complement abnormalities in human disease, Hospital Practice, **13:**65, Dec. 1968.
10. Theofilopolous AN, Wilson CB, Bokisch VA, Dixon FJ: Binding of soluble immune complexes to human lymphoblastoid cells. II. Use of Raji Cells to detect circulating immune complexes in animal and human sera, J Exp Med **140:**1230, 1974.
11. Gupta RC, McDuffie FC, Tappeiner G, Jordan RE: Binding of soluble immune complexes to Raji lymphocytes: Role of receptors for complement components, C1q and C3-C3b, Immunology **34:**751, 1978.
12. Lawrence DA, Weigle WO, Spiegelberg HL: Immunoglobulins cytophilic for human lymphocytes, monocytes and neutrophiles, J Clin Invest **55:**368, 1975.
13. Kronvall G, Frommel D: Definition of staphylococcal protein A reactivity for human immunoglobulin G fragments, Immunochemistry **7:**124, 1970.
14. Dreisan RB, Schwarz MI, Theofilopoulos AN, Stanford RE: Circulating immune complexes in the idiopathic interstitial pneumonias, N Engl J Med **298:**353, 1978.
15. Brandeis WE, Helson L, Wang Y, Good RA, Day NK: Circulating immune complexes in sera of children with neuroblastoma. Correlation with stage of disease, J Clin Invest **62:**1201, 1978.
16. Von Pirquet CE: Allergy, Arch Intern Med **7:**411, 1911.