Serum IgG Aggregates in Systemic Lupus Erythematosus[1] (37544)

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Although there is evidence that immune complex deposition may be involved in the pathogenesis of the nephritis in systemic lupus erythematosus (1, 2), attempts at identifying such complexes in the circulation have yielded limited results (3, 4). The availability of a sensitive radioimmunoassay for IgG (5) has prompted a search for complexes of this immunoglobulin in serums from patients with SLE.

Materials and Methods. Serums from controls and SLE patients were titered for hemolytic complement by the method of Kent and Fife (6), then stored frozen at −20°. Three to 5 days prior to further study, they were thawed and held at 4°. Gross aggregates were then removed by centrifugation at 2000 rpm for 4 hr at 4°.

Immune diffusion assay for IgG aggregates was performed in 0.6% agarose containing 0.05 *M* barbiturate buffer, 0.01 *M* disodium EDTA (pH 7.2) by diffusion against purified C1q at a concentration of 200 μg/ml, according to the method of Agnello, Winchester and Kunkel. (3).

Discontinuous sucrose gradients were formed by the successive layering of 0.85 ml of 45, 35, 25 and 15% sucrose in phosphate buffered saline (pH 7.4, 0.05 *M* PO_4, 0.1 *M* NaCl), followed by diffusion at 4° for 24 hr. The serum to be tested was applied over the gradient followed by centrifugation for 16 hr at 40,000 rpm and 4° in a Spinco SW56 rotor. Sequential fractions were collected and tested for IgG by the solid-phase radioimmunoassay previously described (5). The bottom centimeter of the empty gradient tube was transected and any pelleted material was resuspended for similar immunoassay.

The assay employed (5) used polyaminopolystyrene particles diazotized to goat anti-human γ chain-specific antibody, radioiodinated IgG, and a standard preparation of highly purified IgG obtained by DEAE chromatography. This method has been shown to yield highly reproducible results comparable to those obtained with standard immunoprecipitation techniques (5).

Results and Discussion. The distribution of IgG in soluble fractions of the gradients revealed no differences between normal, control diseased and SLE patients that could not be attributed to differences in total serum IgG concentration alone. An analysis of IgG accumulating in the pellet, however, yielded noticeable differences (Fig. 1). Serums from 28 normal and control individuals, including patients with rheumatoid disease, chronic liver disease and tuberculosis exhibited a significant correlation ($p < 0.005$, $r = 0.536$ by least squares method) between total serum IgG, as measured by this assay, and pellet IgG. Serums from 24 patients with SLE, most of whom had active, hypocomplementemic nephritis, exhibited no such correlation. The upper limit of pellet IgG in normal serum was 6 μg/ml. As shown in Fig. 1, only two SLE serums fell beneath this value. Repeated testing of any given serum gave reproducible values. Although not all serums from patients with active SLE showed an increase of pellet IgG, this abnormality was never found in serum from patients with clinically inactive disease. The level of pelleted IgG was not influenced by the

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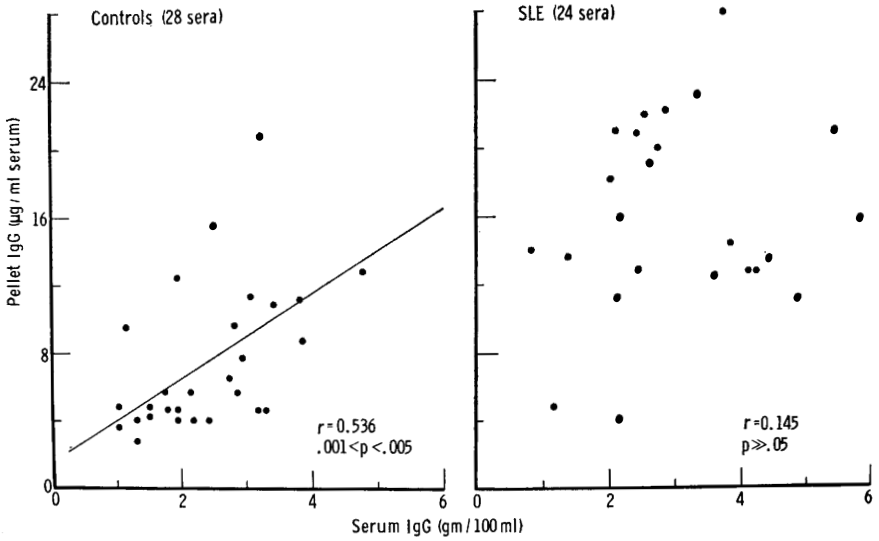

FIG. 1. Pellet IgG compared to serum IgG level in 28 normal and diseased controls and 24 patients with active and inactive SLE. Data analyzed by method of least squares.

method of gradient collection (from the top or the bottom).

Thirty-five serially obtained serums from 7 SLE patients were analyzed for pellet IgG. All gradients were aspirated from the top in this study. Figure 2 compares pellet IgG levels to the total hemolytic complement titers in the 35 serial specimens. Abnormal values for pellet IgG were not found in serum with normal complement levels. While there

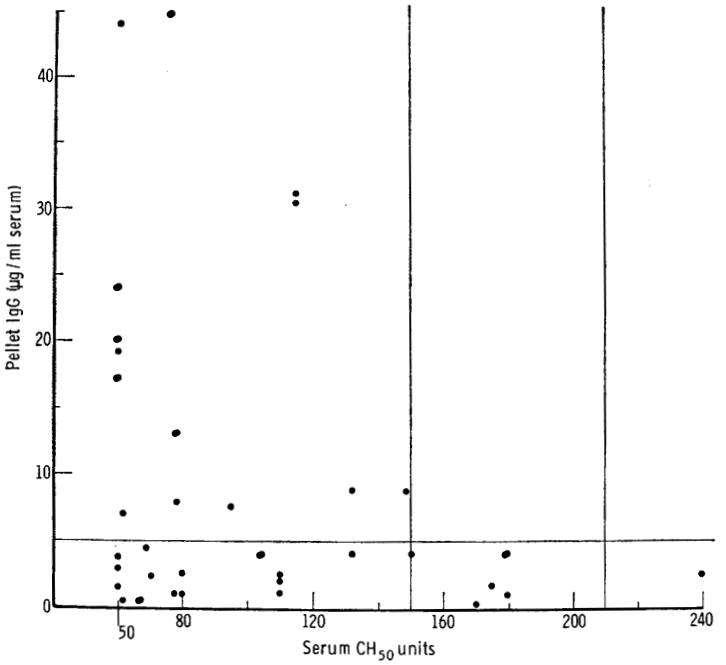

FIG. 2. Pellet IgG versus serum complement in the serial studies, showing a generally negative correlation between the two values.

was generally an inverse correlation between serum complement and pellet IgG in the seven serially studied patients, one patient, on whom samples were available over a 3-yr period, never showed abnormal quantities of pellet IgG, in spite of recurrent hypocomplementia and biopsy-proven nephritis.

Dialysis and centrifugation in isotonic pH 3.2 buffer did not alter the abnormal IgG distribution. Treatment of normal and SLE serums with DNase prior to centrifugation did not change the distribution of IgG. However, increments in pellet IgG induced by prior incubation of DNA with an SLE serum containing anti-DNA, disappeared following DNase treatment.

Comparison of the present technique with that of Agnello, Winchester and Kunkel for demonstrating IgG aggregates give similar results (Fig. 3), although the radioimmunoassay appeared somewhat more sensitive.

The significance of the above observations is not clear. There would appear to be a tendency of stored SLE serums to form high molecular weight IgG aggregates to a degree greater than that attributable to hypergammaglobulinemia. The role of storage at -20° in causing these aggregates is not known at present. However, these would appear not to be simply artifacts of storage alone, for the following reasons: (a) they were not found in stored serums obtained from SLE patients during periods of remission; (b) serums from RA patients stored for a similarly long period lacked the phenomenon; (c) one patient with periods of hypocomplementic nephritis had normal pellet IgG in sera stored at -20° for up to 3 yr; (d) of the 35 sera in the serial study, many with normal pellet IgG were obtained during periods of remission antedating by several years periods of subsequent exacerbation when pellet IgG was elevated; (e) the phenomenon has been observed in freshly obtained serum from patients with active disease.

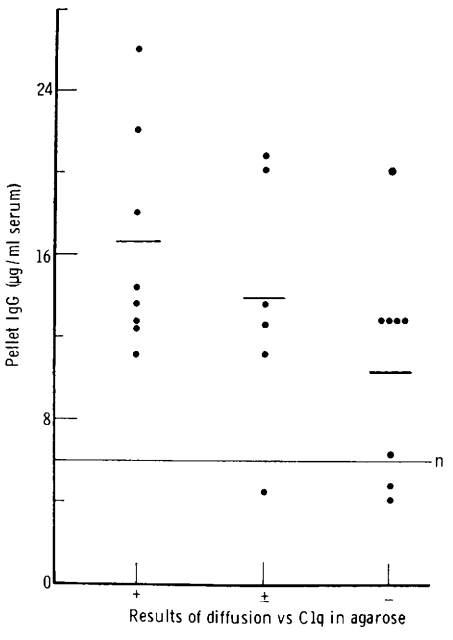

FIG. 3. Correlation between precipitation with C1q and pellet IgG levels in SLE sera. Bars represent mean pellet values for each group. n = upper limits of normal for pellet IgG.

The failure to dissociate at acid pH suggests that these do not represent classical immune complexes. In addition, the failure of DNase to alter their distribution implies DNA–anti-DNA complexes are not present. They may reflect a nonspecifically greater tendency to aggregation of the 7S IgG from clinically active patients, similar to that observed for some myeloma proteins (7). The significance of these aggregates, and the role of storage on their production remain to be elucidated.

Summary. Sera from patients with systemic lupus erythematosus were found to contain microgram amounts of aggregated IgG to a degree not seen in control sera. These aggregates were not found in sera obtained during clinical remission and were not related to the duration of storage of the sera. They were not dissociated by conditions which normally solubilize antigen–antibody complexes.

1. Koffler, D., Schur, P. H., and Kunkel, H. G., J. Exp. Med. **126**, 607 (1967).

2. Andres, G. A., Accinni, L., Beiser, S. M., Christian, C. L., Cinotti, G. A., Erlanger, E. F., Hsu, K. C., and Seegal, B. C., J. Clin. Invest. **49**, 2106 (1970).

3. Agnello, V., Winchester, R. J., and Kunkel, H. G., Immunology **19**, 909 (1970).

4. Askenase, P. W., and Leonard, E. J., J. Im-

munol. **105**, 1578 (1970).
5. Bombardieri, S., and Christian, C. L., Proc. Soc. Exp. Biol. Med. **133**, 1366 (1970).
6. Kent, J. F., and Fife, E. H., Jr., Amer. J. Trop. Med. **12**, 103 (1963).
7. Morse, J. H., J. Immunol. **95**, 722 (1965).

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