Serum immunoglobulin levels in farmer's lung disease

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The serum concentrations of the five major classes of immunoglobulins were measured in 27 farmer's lung patients and compared with a group of normals and a group of patients with diffuse lung disease, but no precipitins to our antigen panel. The mean IgG and IgA levels in the sera of the farmer's lung patients were found to be significantly higher than that of the normal group. In addition, the IgG level of the farmer's lung group was also significantly higher than the group with negative precipitin tests. The remaining immunoglobulin classes, IgM, IgD, and IgE, were not remarkably different from normal in either diffuse lung disease group.

Farmer's lung disease is a well-recognized example of the group of lung diseases classified as the hypersensitivity pneumonias[1] or extrinsic allergic alveolitides.[2] Patients with farmer's lung have a history of episodes of fever, cough, and dyspnea 4 to 8 hours after exposure to dust containing thermophilic actinomycetes spores and histologically have a diffuse interstitial, sometimes granulomatous change in their lungs. They also exhibit precipitins to thermophilic actinomycetes antigens in the sera. Chronic exposure to the sensitizing spores found commonly in moldy hay or grains can lead to irreversible lung changes and finally cor pulmonale.

Although it is widely accepted that this group of diseases is due primarily to an abnormal immune response in the lung, the nature of the immunologic mechanism remains unknown. A necessary step in the over-all evaluation of the immune system in persons with hypersensitivity pneumonias is the measurement of the serum concentrations of all five classes of immunoglobulins, and this is the subject of this paper.

Salvaggio and associates[3] found elevated levels of IgG and IgA and normal IgM levels in 20 patients with bagassosis, a hypersensitivity pneumonitis to thermophilic actinomycetes found in bagasse workers. Patterson and associates[4] measured the immunoglobulin levels in patients with pulmonary allergic aspergillosis and compared them with, among others, 8 patients with hyper-

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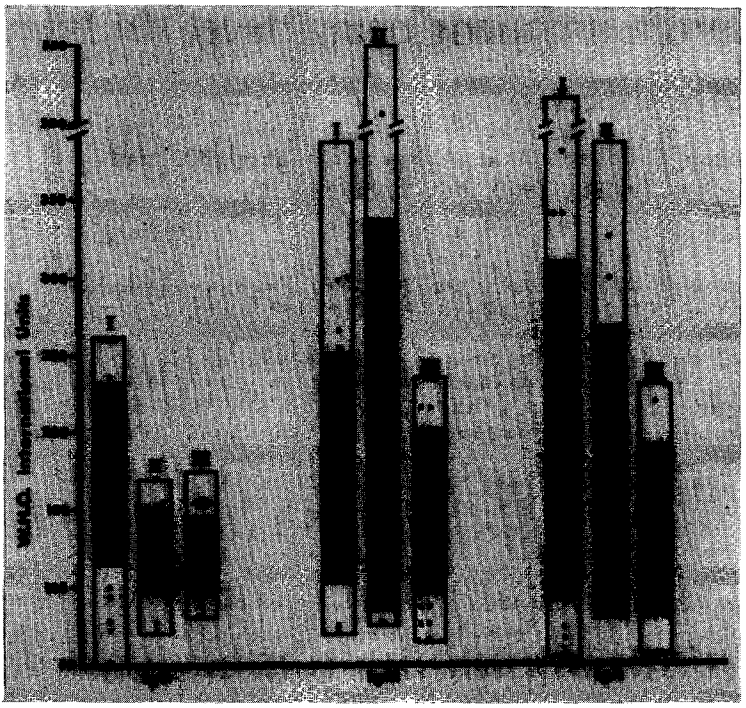

Fig. 1. The IgG, IgM, and IgA levels for (I) farmer's lung patients, (II) diffuse lung disease with negative precipitin tests, and (III) normal adults. The mean is indicated by the heavy stripped line, the gray zone represents one standard deviation, and the total length of the bar represents the range. The dots are the individual values expressed as W.H.O. International Units per milliliter.

sensitivity pneumonitis, 4 with farmer's lung, and 4 with pigeon breeder's disease. Patients with allergic aspergillosis were found to have marked elevations of the IgE class, while the hypersensitivity pneumonitis patients had normal IgE levels. No consistent abnormality in the other immunoglobulins was noted in this limited sample of hypersensitivity pneumonitis; however, a range of values higher than normal was reported for IgG.

In the study herein reported, the serum concentrations of all five classes of immunoglobulins in a group of 27 farmer's lung patients and a group of 17 patients with diffuse lung disease of undetermined etiology are compared with normal controls.

MATERIALS AND METHODS

Patients and normals

Group I—Farmer's lung patients. The sera from 27 known farmer's lung patients were selected from our collection accumulated from 1966 to early 1972. All patients had hypersensitivity pneumonitis by radiologic evidence, clinical history, and in many cases by histologic evidence of pulmonary biopsy, as well as strong precipitins to either *Micropolyspora faeni* or *Thermoactinomyces vulgaris* antigens.[5, 6] The sera had been stored frozen at -20° C. and thawed only once immediately before the assays reported here.

TABLE I. Summary of data on IgE and IgD for farmer's lung patients (Group I), negative precipitin sera (Group II), and published normal ranges

Immunoglobulin	I	II	Normal
IgE (I.U./ml.)			
Number	27	16	
Range	30-2,300	8-1,232	1-1,215*
Mean	308.8	210.8	80
Standard deviation	452.0	286.1	
Median	200	148	36
IgD (mg./100 ml.)			
Number	27	17	100†
Range	0-20	0-13.5	0-40
Mean	3.82	3.08	3.0 (median)
Standard deviation	4.15	3.76	

*Gleich, G. J., Averbeck, A. K., and Swedlund, H. A.: Measurement of IgE in normal and allergic serum by radioimmunoassay, J. Lab. Clin. Med. 77: 690, 1971.

†Rowe, D. S., and Fahey, J. L.: A new class of human immunoglobulins. II. Normal serum IgD, J. Exp. Med. 121: 185, 1965.

Group II—Patients with lung disease of undetermined etiology, thermophile precipitin negative. This group of patients had diffuse interstitial lung disease whose clinical picture prompted the attending physician to request testing for precipitins against a panel of antigens known to be involved in hypersensitivity pneumonitis. This panel consisted of antigen extracts of the thermophilic actinomycetes including *M. faeni, T. vulgaris, T. viridis;* Aspergillus species *A. fumigatus, A. sydowi, A. clavatus, A. terreus, A. niger, A. glaucus;* pigeon serum and droppings, and *Cryptostroma corticale.* The sera from Group II patients showed no precipitins against this panel when tested by double diffusion in agar. The absence of precipitins to the above panel does not rule out hypersensitivity pneumonitis but probably rules out the antigens tested as the sensitizing agents. This group thus represents diffuse pneumonitis of unexplained etiology and, therefore, represents an interesting group to compare with positively diagnosed farmer's lung disease. For conciseness, this group is referred to as the negative precipitin group.

Group III—Normals. A group of 24 consecutive blood donors at the St. Joseph's Hospital, Marshfield, Wisconsin, was chosen as normals for the IgG, IgM, and IgA determinations. The age range was 21 to 52 with the mean age and one standard deviation 32.7 ± 10.7. The sex distribution was 8 males and 16 females. (The farmer's lung group contained 20 males and 7 females with a mean age of 46.4 and a standard deviation of 12.2.) Since normal adults in these age ranges exhibit only minor differences and negligible sex differences,[7] the above normals should be an acceptable control group.

IgG, IgM, IgA, and IgD assays. These immunoglobulins were measured by the single radial immunodiffusion method (RID) first described by Mancini and associates[8] using commercially available kits (Kallestad Laboratories, Minneapolis, Minnesota). The standard curves were prepared by plotting the diameters of the diffusion rings obtained with the manufacturer's standards against the logarithm of their concentrations. Different plots were used for determining the concentrations in World Health Organization International Units (W.H.O. I.U.)[9] and as milligrams per 100 ml. except for IgD for which W.H.O. I.U. were not yet available. The coefficient of variation (standard deviation divided by the mean × 100) for replicate RID assays is of the order of 10 per cent in this laboratory.

Statistical comparisons of the means of each Ig class for each of the three groups were made by the Student's t test after the variances for the groups shown not to be statistically different by the F test.[10]

IgE assay. The IgE levels in Groups I and II were determined by radioimmunoassay using the Phadebas Kit (Pharmacia Laboratories, Piscataway, New Jersey). This assay makes use of anti-IgE antibodies covalently linked to Sephadex beads. The amount of IgE in the samples is determined by its ability to displace ^{125}I-labeled IgE from the insoluble immuno-

TABLE II. Summary of mean concentrations and ranges of IgG, IgA, and IgM in farmer's lung that samples came from same population as normals

Immunoglobulin	Group I		
	Mg./100 ml.	W.H.O. I.U.	p
IgG			
Mean (± S.D.	1,516 (± 533)	173 (± 59)	< 0.01
Range	420-2,450	49-261	
IgA			
Mean (± S.D.	354 (± 206)	200 (± 110)	< 0.01
Range	91-900	54-515	
IgM			
Mean (± S.D.	104 (± 44)	177 (± 76)	> 0.05
Range	41-225	70-388	

adsorbant. The results are expressed in W.H.O. I.U., which were equivalent to the nanograms per milliliter concentrations of the standards according to the manufacturer.

RESULTS

The IgG, IgM, and IgA levels for each individual in the three groups of samples are present in Fig. 1 in W.H.O. I.U. per milliliter. The mean and standard deviation for each set is also indicated. The IgG levels in the farmer's lung patients (Group I) covered a wider range and tended to be higher than the other two groups. The negative precipitin group (Group II) and the normals (Group III) showed nearly identical ranges and means.

The IgE data for Groups I and II are summarized in Table I. The IgE levels in both groups were within the reported normal ranges and unremarkable except for the following cases. One Group I patient had an IgE level of 2,300 I.U. per milliliter. The second highest IgE level in the farmer's lung group was 950 I.U. per milliliter, within the normal ranges. All other values were low normal as indicated by the median (200 I.U. per milliliter). Similarly, only one value was slightly elevated in Group II at 1,230 I.U. per milliliter, the second highest level in this group was 330 I.U. per milliliter, and the median was 148 I.U. per milliliter. Thus, with the exceptions of two individuals with moderate elevations, the IgE levels for both the farmer's lung group and the negative farmer's lung group were well within the normal limits.

The IgD levels for Groups I and II are also summarized in Table I. The results were unremarkable with the exception of one moderately elevated level of 20 mg. per 100 ml. in Group I. The significance of this elevation is unknown, but it is interesting that this individual also had elevated IgG (1,990 mg. per 100 ml.) and IgA (730 mg. per 100 ml.) levels. The remainder of the IgD levels for members of both groups range from nondetectable to 13.5 mg. per 100 ml. Since a number of the IgD levels were below the detectable limits for the method and all were close to the reported normal range, further statistical analysis was not considered necessary.

The numerical data and the statistical evaluation of the IgG, IgA, and IgM results are summarized in Table II. The mean, standard deviation, and range for each of the Ig classes for the three groups of samples are tabulated in both

sera (Group I), negative precipitin sera (Group II), and normals (Group III) and the probability

Group II			Group III	
Mg./100 ml.	W.H.O. I.U.	p	Mg./100 ml.	W.H.O. I.U.
1,103 (± 253) 640-1,480	124 (± 29) 72-170	> 0.05	1,068 (± 228) 710-1,370	120 (± 25) 80-154
295 (± 166) 123-675	173 (± 96) 78-388	< 0.01	246 (± 93) 130-390	135 (± 54) 78-232
118 (± 73) 43-305	210 (± 126) 76-550	> 0.05	101 (± 39) 44-169	148 (± 54) 67-234

mg. per 100 ml. and W.H.O. I.U. per milliliter. The IgG levels for Group I were significantly higher ($p < 0.01$) than either Group II or the normals (Group III). Group II and the normals did not have significantly different IgG levels. The IgA levels in Groups I and II were also significantly higher ($p < 0.01$) than the normals. No significant differences in IgM levels were found between the three groups.

DISCUSSION

While as a group, the farmer's lung patients had elevated IgG levels in their serum, individually many had levels falling within the normal range, and in one case the IgG level was well below the normal range (50 I.U.). As is often the case with serum immunoglobulin levels, it is difficult to reach a conclusion on the humoral immune status from these measurements alone. Evidence has been presented that the precipitins in the serum in hypersensitivity pneumonitis are primarily of the IgG class.[2] The possibility thus exists that the increased IgG levels are a reflection of the precipitins present. In order to test this possibility, the quantitative levels of the total precipitins present should be correlated with the total IgG levels in a series of farmer's lung patients. However, the accurate quantitation of precipitins is extremely difficult because of the heterogeneity of the antigens involved.[11]

The fact that both the farmer's lung group and Group II on the whole exhibited elevated IgA levels is also of speculative interest. Ablin[12] found elevated levels of serum IgA in patients with primary pulmonary emphysema, as did Biegel and Krumholz[13] in patients with chronic obstructive emphysema. Martinez-Tello, Braun, and Blanc[14] reported a significant increase in the IgA-producing plasma cells in the bronchial mucosa of patients with severe chronic bronchopulmonary disease. Perhaps an elevated IgA represents a general response to severe lung disease. Additional correlation between the severity of the lung involvement with serum IgA levels may be in order. Nothing is known about the levels of secretory IgA in the bronchial secretions of hypersensitivity pneumonitis patients.

The normal levels of IgE in the farmer's lung patients confirm the findings of Patterson and associates[4] and further support the differential diagnostic sig-

nificance of elevated IgE levels in pulmonary allergic aspergillosis. Normal levels of IgE also further suggest that type I hypersensitivity is not involved in this class of lung diseases, although the actual IgE antibody levels to these antigens must be measured to prove this.

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