Immunology, 1967, **13**, 483.

Evidence for the Independence of Human Immunoglobulin Class Levels

MATHEA ALLANSMITH, BARABARA MCCLELLAN AND M. BUTTERWORTH

Department of Medical Microbiology, Stanford University School of Medicine, and Department of Statistics, Stanford University, Stanford, California, U.S.A.

(*Received* 20*th February* 1967)

Summary. Rank correlation analysis of serum IgG, IgA and IgM values from a large group of subjects showed that in health the amount of one class of immunoglobulin present in an individual is largely independent of the amounts of the other classes. A similar analysis of several sets of data from the literature concerning immunoglobulin levels in disease showed that even with severe disturbances of the immunoglobulin system, the levels remained independent.

INTRODUCTION

The immunoglobulins are now known to consist of a large number of distinct types of molecules. Of these, the concentration in the serum of only the three major classes can conveniently be measured. The range over which immunoglobulin levels from different individuals occur is wide (Table 1), in contrast to about ±20 per cent (2 S.D.) within

TABLE 1

MEANS AND STANDARD DEVIATIONS OF IMMUNOGLOBULIN LEVELS (mg/100 ml) OF 315 SUBJECTS

	IgG	IgA	IgM
Mean*	1045	169	89
±2 S.D.*	711–1536	60–489	37–212

* Calculations performed on logarithms of mg/100 ml.

which a given individual's values may be found (Allansmith, Wymer and Prieto, 1966). If the factors concerned with establishing the serum levels of the various immunoglobulins in an individual were the same or interrelated, we would expect a high level of one immunoglobulin to be associated with high levels of the others. In this report we present results of statistical analyses which demonstrate that the amount of one class of immunoglobulin present in an individual is largely, perhaps entirely, independent of the concentrations of the other major classes.

MATERIALS AND METHODS

Serum immunoglobulin levels of a large group of healthy subjects were determined and compared by rank correlation. In addition, a similar analysis was performed on

several sets of immunoglobulin levels reported in the literature (West, Hinrichs and Hinkle, 1961; McKelvey and Fahey, 1965; Tobie, Abele, Wolff, Contacos and Evans, 1966; Patnode, Allin and Carpenter, 1966) in order to test whether the pattern of correlation coefficients established by our data would also occur in other sets of data representing information obtained from both healthy and diseased subjects.

Quantitation of serum immunoglobulins

IgG, IgA and IgM were quantitated by a single radial immunodiffusion method (Mancini, Carbonara and Heremans, 1965; Chandor, Allansmith and Wymer, 1965) using commercially available antibody–agar plates (Immuno-Plates, Hyland Laboratories, Los Angeles, California, U.S.A.). The plates were standardized with purified immunoglobulins, each of which, in Ouchterlony analysis, gave one precipitin line when allowed to diffuse against an antiserum to whole human serum. The protein concentrations of the purified preparations were determined by micro-Kjeldahl analysis. Standardization curves were prepared correlating concentrations of globulins with corresponding ring diameters. At least ten points were used to establish each curve, with multiple determinations per point. Values were graphed on full logarithmic paper to obtain a linear relationship between ring diameters and protein concentrations.

The error of the technique for the three immunoglobulins was ± 12 per cent (± 2 S.D.). This figure was determined from control values, two per antibody–agar plate, accumulated over a 6-month period. The controls consisted of two secondary standards of normal human serum.

Sera

Blood was collected from 315 adults of whom 255 were blood donors and sixty were laboratory personnel. The subjects, who were 21 years or older, claimed to be in good health at the time of bleeding. Total serum proteins were determined by the Biuret method on eighty-six of the sera as an assay of the 'normalness' of the group. The mean level was 7·45 g/100 ml with a standard deviation of 0·6 g/100 ml and a range of 5·7–9·4 g/100 ml.

Thirty-six of the 315 subjects were females. To determine if immunoglobulin levels differed significantly with sex a *t*-test was performed comparing the means for each globulin class of the two groups. The *P* values were greater than 0·1 for IgG and IgA and at the significance level for IgM. The two groups were combined.

Calculations

Calculations on our data were performed using the log of the mg/100 ml protein. When expressed this way, the frequency distribution of the immunoglobulin values is a normal one. This conversion was not necessary for the calculation of the rank correlation, but it was used for convenience. A Kendall tau rank correlation (Kendall, 1948) was performed for the following pairs: IgG with IgA, IgG with IgM, and IgA with IgM. A correlation was also performed between IgG and IgA plus IgM. Calculations on data from the literature were made on values for mg/100 ml.

RESULTS AND DISCUSSION

The degrees of correlation and *P* values are shown in Table 2. Small but significant

correlations were found between IgG and either IgA or IgM. No significant correlation was found between IgA and IgM. The correlation coefficient of IgG compared with IgA was 0·189 ($P<0·0001$); IgG with IgM was 0·139 ($P = 0·0004$); and IgA with IgM was 0·056 ($P>0·1$). As expected, a small but significant correlation exists between IgG levels and the sum of IgA and IgM ($\tau = 0·201$, $P<0·0001$).

From the results we conclude that some, but not many, of the factors concerned with determining the amounts of IgG and IgA in serum are shared, as are some, but not many, of the factors determining the amounts of IgG and IgM. It is unlikely that the shared factors in the two cases are the same, for if they were, a significant correlation should exist between IgA and IgM. Examination of a much larger sample might reveal a significant correlation between IgA and IgM, but this would undoubtedly be smaller than that between the other two pairs.

The degree of correlation is very small. It is apparent that most of the factors responsible for concentrations of immunoglobulins in the various classes are independent of each other.

The nature of the factors involved in determining immunoglobulin levels are not known, but may be presumed to include the quality and quantity of antigenic stimuli, the influence of heredity, or, given adequate antigenic stimulation, the main factor may be the availability of some product which is neither antigen nor antibody.

TABLE 2

CORRELATION BETWEEN IMMUNOGLOBULIN LEVELS FROM 315 SUBJECTS

Levels compared	Correlation*	*P* value (two-tailed)
IgG with IgA	0·189	<0·0001
IgG with IgM	0·139	0·0004
IgM with IgA	0·056	>0·1
IgG with (IgA+IgM)	0·201	<0·0001

* Kendall's tau rank correlation (Kendall, 1948).

Two possible explanations for the correlations observed are these: First, each of the many subclasses of immunoglobulins may have its own set of regulators. Most of the regulators would affect only one subclass or if more than one was affected, the subclasses would be within the same major class. A few of the regulators would affect subclasses of both IgG and IgA and another few subclasses of both IgG and IgM. Comparisons between subclasses not sharing regulating mechanisms would yield correlation coefficients of about zero. Comparisons between subclasses sharing regulators would give high correlation coefficients.

A second explanation would be that, within the sample population, there were unrecognized subgroups different from the main group. The effect of adding such a group would be to make a correlation. If such a subgroup exists, it is not composed of the females. The correlations were determined on the males alone and the results were approximately the same as with the entire group. Many types of subgroups could be present. For example, if certain levels increased with age, the older individuals would constitute a subgroup, etc.

The first explanation would gain credence if subclasses were found with correlation coefficients different from those seen here. Identifying these subgroups or subclasses would be a tedious task in view of the small correlation coefficients found. The second explanation

would gain merit if interior groups were found with immunoglobulin levels different from the general adult population. The main point is that no matter how many subclasses and/or subgroups exist, very little correlation exists between the levels in the different immunoglobulin classes.

A rank correlation was performed on the data presented by Tobie *et al.* (1966) (Table 3).

TABLE 3
CORRELATION BETWEEN IMMUNOGLOBULIN LEVELS OF SUBJECTS WITH EXPERIMENTALLY INDUCED MALARIA AS REPORTED BY TOBIE *et al.* (1966)

Levels compared	Correlation	*P* value (two-tailed)
A. Pre-infection (n = 17)		
IgG_1* with IgA_1	0·267	>0·1
IgG_1 with IgM_1	−0·222	>0·1
IgM_1 with IgA_1	−0·353	0·05
B. Post-infection (n = 12)		
IgG_2† with IgA_2	0·431	0·06
IgG_2 with IgM_2	0·246	>0·1
IgM_2 with IgA_2	0·154	>0·1
C. Amount of increase in each class (n = 12)‡		
(IgG_2–IgG_1) with (IgA_2–IgA_1)	0·373	0·04
(IgG_2–IgG_1) with (IgM_2–IgM_1)	0·546	0·02
(IgM_2–IgM_1) with (IgA_2–IgA_1)	0·321	>0·1

* Subscript $_1$ indicates pre-infection level.
† Subscript $_2$ indicates post-infection level.
‡ Calculations performed on mg/100 ml.

In this study, twelve healthy volunteers were experimentally infected with *Plasmodium vivax* and five with *Plasmodium cynomolgi*. The paper reported pre-infection immunoglobulin levels and maximum values observed during the course of the disease.

We determined correlations on three sets of values from this report. First, the pre-infection levels of the seven volunteers were examined; the correlation coefficients were comparable to ours. Next, the acute infection levels for the twelve volunteers infected with *P. vivax* were examined to see whether an infection that resulted in a severe alteration of the immunoglobulin levels would also show a change in the immunoglobulin relationships. No alteration was detected. Third, the amount of immunoglobulin increase in each class was compared to the amount of increase in other classes. Barely significant correlations were found when the increase in IgG was compared to the increase in IgA and when the increase in IgG was compared to the increase in IgM. This probably means that those who had the greatest antigen stimulation also had the greatest immunoglobulin level changes. Although IgM increased 357 per cent, IgA 55 per cent, and IgG 42 per cent in the subjects infected with *P. vivax*, no large change in relationships was found.

A correlation was performed between the pre-infection level and the amount of increase for each class (Table 4). A highly significant correlation was found in the case of IgA, indicating some connection between pre-infection levels and magnitude of response of this class of immunoglobulin.

Another rank correlation was performed on the data reported by Patnode *et al.* (1966) (Table 5). In this study, immunoglobulin levels of twenty-nine patients with sarcoidosis were determined and compared with levels from nineteen normal adults. Calculations were made on both sets of data. No significant correlations were found in either group.

A correlation study of the data from twenty normal individuals studied by West *et al.* (1961) was done to test whether concentration values determined by a technique different from ours would yield similar results. Individual values were given only for IgA and IgM;

TABLE 4

CORRELATION BETWEEN PRE-INFECTION LEVEL AND AMOUNT OF INCREASE FOR EACH IMMUNOGLOBULIN CLASS [FROM DATA ON TWELVE SUBJECTS WITH EXPERIMENTALLY INDUCED *P. vivax* MALARIA AS REPORTED BY TOBIE *et al.* (1966)]

Levels compared (n = 12)*	Correlation	P value (two-tailed)
IgG_1† with (IgG_2‡–IgG_1)	0·000	>0·1
IgA_1 with (IgA_2–IgA_1)	0·63	0·006
IgM_1 with (IgM_2–IgM_1)	0·03	>0·1

* Calculations performed on mg/100 ml.
† Subscript $_1$ indicates pre-infection level.
‡ Subscript $_2$ indicates post-infection level.

TABLE 5

CORRELATIONS BETWEEN IMMUNOGLOBULIN LEVELS OF SUBJECTS IN A STUDY ON SARCOIDOSIS BY PATNODE *et al.* (1966)

Levels compared	Correlation	P value (two-tailed)
A. Subjects with sarcoid (n = 29)		
IgG with IgA	0·04	>0·1
IgG with IgM	−0·01	>0·1
IgM with IgA	−0·02	>0·1
B. Normal controls (n = 19)		
IgG with IgA	0·18	>0·1
IgG with IgM	0·12	>0·1
IgM with IgA	0·02	>0·1

group value was given for IgG. The correlation coefficient comparing IgA with IgM was −0·18 with P>0·1. This result was similar to ours.

A study by McKelvey and Fahey (1965) (Table 6) on immunoglobulin levels in disease

TABLE 6

CORRELATIONS BETWEEN AVERAGE IMMUNOGLOBULIN LEVELS FOR VARIOUS TYPES OF INFECTIOUS AND LIVER DISEASES [LEVELS FROM A STUDY BY MCKELVEY AND FAHEY (1965)]

Levels compared (n = 12 groups)	Correlation	P value (two-tailed)
IgG with IgA	0·29	>0·1
IgG with IgM	0·17	>0·1
IgM with IgA	0·41	0·09

included data obtained from subjects with various infections (coccidiomycosis, leprosy, etc.) and liver diseases (cirrhosis, hepatoma, etc.). One set of immunoglobulin levels was given for each disease, each set representing the average of the levels for two to fifteen

patients. The twelve diseases examined by us were those in which secondary disturbances of the immunoglobulin system occurred. Correlations were calculated on the average levels given for each disease. No significant correlations were found. This means that a wide range of disturbances affect immunoglobulins in such a way that an increase in the level in one class has little or no association with changes in the other classes. This is the same situation that exists in an individual without such disturbances.

ACKNOWLEDGMENT

This work was supported by Research Grant E-320B from the American Cancer Society.

REFERENCES

ALLANSMITH, M., WYMER, B. and PRIETO, G. (1966). 'Repeated immunoglobulin determinations in 15 individuals over a 6 month period (abstract).' *J. Allergy*, **37**, 114.

CHANDOR, S., ALLANSMITH, M. and WYMER, B. (1965). 'Application of the ring diameter method for measuring serum immunoglobulin levels (abstract).' *Fed. Proc.*, **24**, 502.

KENDALL, M. (1948). *Rank Correlation Methods*. Griffin, London.

MANCINI, G., CARBONARA, A. O. and HEREMANS, J. F. (1965). 'Immunochemical quantitation of antigens by single radial immunodiffusion.' *Immunochemistry*, **2**, 235.

MCKELVEY, E. M. and FAHEY, J. L. (1965). 'Immunoglobulin changes in disease: Quantitation on the basis of heavy polypeptide chains, IgG (γG), IgA (γA), and IgM (γM), and of light polypeptide chains, type K (I) and type L (II).' *J. clin. Invest.*, **44**, 1778.

PATNODE, R. A., ALLIN, R. C. and CARPENTER, R. L. (1966). 'Serum immunoglobulin levels in sarcoidosis.' *Amer. J. clin. Path.*, **45**, 389.

TOBIE, J. E., ABELE, D. C., WOLFF, S. M., CONTACOS, P. G. and EVANS, C. B. (1966). 'Serum immunoglobulin levels in human malaria and their relationship to antibody production.' *J. Immunol.*, **97**, 492.

WEST, C. D., HINRICHS, V. and HINKLE, N. H. (1961). 'Quantitative determination of the serum globulins $beta_{2A}$ and $beta_{2M}$ by immunoelectrophoretic analysis.' *J. Lab. clin. Med.*, **58**, 137.