Variations of immunoglobulins in disease

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The introduction of simple methods for the immunochemical quantitation of human immunoglobulins has been followed by the publication of several hundred papers during the past 10 years concerning the diagnostic significance of quantitative changes in these proteins in human body fluids. The subject was extensively reviewed in 1971 by Professor Hobbs and no fundamentally new findings can be reported at the present time. The following survey will consist mainly of a review of confirmatory data taken from more recent publications.

First the analytical methods used for quantitative estimation of immunoglobulins will be reviewed briefly, followed by a discussion of the normal ranges and the problems of interlaboratory standardization. Finally the usefulness of immunoglobulin analysis will be illustrated by a survey of some selected pathological conditions in which immunoglobulin determinations are of proven clinical value.

Analytical Techniques

Three techniques are currently in routine use for the specific immunochemical quantitation of the immunoglobulins, namely, single radial immunodiffusion (RID) (Mancini, Carbonara, and Heremans, 1965), electroimmunoassay (EIA) (Laurell, 1966), and nephelometric immunoassay (Schultz and Schwick, 1959).

RADIAL IMMUNODIFFUSION

Because of its simplicity radial immunodiffusion is the most frequently used. Agarose gel plates containing class-specific antisera to the immunoglobulins, a microlitre syringe, and a simple device for measuring the diameter of the precipitin ring are the only equipment needed. In the hands of a technician with average talents the day-to-day reproducibility should be about $\pm 5\%$. At the expense of some accuracy, time can be saved by measuring the precipitin ring diameters before the endpoint of diffusion is reached, giving a rough estimation in cases of suspected dysgammaglobulin-

aemias within approximately six hours. A more accurate evaluation can be made when diffusion is completed. Provided the plates contain low concentrations of antiserum and have large application wells, quantitation of immunoglobulins can be carried out in serum and even in cerebrospinal fluid and urine without concentration. Visible immunoprecipitates can usually be measured down to a concentration of about 10 mg/l. If intensification procedures like DOPA treatment (Madhosingh and Wood, 1971) are used, the lower limit of detection drops to about 0.5 mg/l (Sieber and Becker, 1974).

ELECTROIMMUNOASSAY

The electroimmunoassay ('rocket technique') raises special technical problems since the electrophoretic mobility of the human immunoglobulins is similar to that of the animal immunoglobulins of the antisera incorporated in the gel as the 'stationary' reactants. However, IgG, IgA, and IgM may be successfully quantitated after carbamylation (Weeke, 1968), or, if the assay is performed at pH 5, the rabbit antibodies can be used in the carbamylated form because their mean electrophoretic migration at this pH in agarose gel is zero (Bjerrum, Ingild, Løwenstein, and Weeke, 1973).

NEPHELOMETRIC ASSAY

The nephelometric assay has experienced a re-birth in conjunction with the introduction of automated equipment such as continuous flow analysers (Alper, 1969) or, very recently, centrifugal fast analysers (Tiffany, Parella, Johnson, and Burtis, 1974). The main advantage of these procedures is the rapid availability of the results which facilitates the incorporation of immunoglobulin analyses into the programme of a routine clinical laboratory.

Standardization

The results of immunoglobulin quantitation may be expressed in absolute terms (weight/volume), in terms of International Units (IU/ml), or as a percentage of the normal mean value for the population concerned. An international collabora-

tive study initiated by the WHO Immunoglobulin Reference Centre in 1970 showed that the values for a reference serum, obtained by several highly qualified laboratories using their own purified immunoglobulins as primary standards, differed widely (Rowe, Grab, and Anderson, 1972); the ratio of the highest to the lowest value obtained was 2.2 for IgG, 3.2 for IgA, and 5.0 for IgM. This may be mainly due to the heterogeneity and poor stability of 'highly purified' immunoglobulins. Also commercially available standards are not comparable so far as their declared immunoglobulin weight content is concerned, probably for the same reason.

The results of the WHO collaborative calibration study led to the decision to recommend a stable lyophilized human serum as a WHO international reference preparation for immunoglobulin quantitation (code no. 67/86, Anderson *et al*, 1971). By definition, this material contains 100 International Units of IgG, IgA, and IgM per ampoule (81.47 mg of lyophilized serum). Manufacturers of standard sera have used this WHO standard for calibrating their products so that, in principle, world-wide comparability of quantitative immunoglobulin determinations has been made possible, at least when identical analytical techniques are used.

Because of a certain reluctance to use arbitrary units instead of grams, it has recently been recommended by an official announcement that serum immunoglobulin concentration should continue to be estimated relative to the International Reference Preparation, but that the values may be expressed in terms of weight based on the best available estimates. The following weights correspond to one IU (Humphrey and Batty, 1974):

$\mu\text{g/IU}$	
IgG	80.4
IgA	14.2
IgM	8.47

It must be pointed out that the stated milligram values of commercial preparations which have been standardized in international units may not be in accordance with these WHO findings.

For clinical practice, the confusing problem of standardization may be eliminated by using as a reference preparation a pool of serum from normal adults, kept deep frozen in aliquots. Provided that the patient under investigation fits into the population for which the serum pool is representative, results can be expressed as a percentage of the mean normal adult value.

Quality Control

As with other analytical procedures standardization of immunoglobulin analysis should be supplemented by independent control of precision and accuracy, by means of unassayed and assayed control sera.

The Normal Range

It has been found that in a given normal individual the variation of serum immunoglobulins over long periods of time is very small. Mondorf and Kollmar 1969 found in 17 adults observed over a period of five months that the average coefficient of variation was $\pm 12.7\%$ for IgG, $\pm 15.9\%$ for IgA, and $\pm 16.5\%$ for IgM, including the error of the radial immunodiffusion method. Hobbs (1971) states that the

Author and Country	Number of Individuals	Age Range (yr)	IgG (IU/ml.)		IgA (IU/ml)		IgM (IU/ml)	
			Mean	Range	Mean	Range	Mean	Range
From Rowe (1972)								
England	51	20-29 (♂)	123	73-207	115	46-289	133	47-372
Germany	45	20-29 (♂)	124	86-178	108	48-244	133	59-298
Netherlands	100	20-29 (♂)	116	65-206	94	40-223	127	48-334
Sweden	94	20-29 (♂)	126	90-177	126	57-282	135	52-345
Switzerland	100	20-29 (♂)	135	87-208	136	56-334	176	81-380
Becker (1972)								
Germany and Switzerland	1385	15-80	141	90-200	132	50-250	♂ 145 ♀ 180	60-300
Šinkov <i>et al</i> (1973)								
Bulgaria	60	21-50	129	64-180	114	48-240	♂ 115 ♀ 147	60-295
Čejka <i>et al</i> (1974)								
USA	22	Adults (♂)	137	82-229	113	50-254	157	48-310
Becker <i>et al</i> (1968)								
Germany	150	15-64	144	92-207	125	54-268	♂ 144 ♀ 184	69-322

Table Immunoglobulin concentrations in terms of international units in the sera of healthy Caucasian individuals

individual variation usually remains within $\pm 20\%$ over years.

Apart from special conditions, such as obvious Ig deficiencies and familial hypogammaglobulinaemia, genetic factors have a minor influence on serum immunoglobulin levels. It is environmental challenge that makes the major contribution to immunoglobulin variation. Distinct seasonal changes have been found in Nigerians (McFarlane, 1966). In 25 subjects the mean levels for serum IgG and IgM were respectively 2.5 and 3.5 times higher in the rainy season than in the dry season; for IgA no difference was found. Hobbs (1971) has reported that IgA and IgM levels in natives of underdeveloped countries are much higher than in natives of Great Britain; however, Nigerians who have lived in London for over two years have levels approaching those of the British; the remaining difference can be regarded as racial.

It must be noted that, in contrast to the situation some years ago, the normal ranges defined for similar populations by different authors using the WHO immunoglobulin reference preparation or related standards are now in good accordance (table I).

The frequency distribution of normal values has been reported to be both Gaussian and logarithmic in the case of IgG, whereas most authors who consider this point agree upon logarithmic IgA and IgM distributions (Cegla, 1974).

Age- and sex-related differences have been studied by numerous authors, and are reviewed by Kalff (1970).

There is a statistically significant sex difference in IgM levels which, however, seems not to be constant over the whole life span. Most authors agree that in adults there is a continuous increase of IgG and IgA with age whereas IgM levels, particularly in females,

Fig 1

Figs 1 and 2 Distribution of serum IgG (fig 1) and of serum IgA (fig 2) values in normal subjects
 N = number of patients, $\bar{X}$ = arithmetic mean value

Fig 2

Fig 3 Distribution of serum IgM values in normal males and females
N = number of patients
 $\bar{X}$ = arithmetic mean value

are maximal during the fifth decade. Our own results (Becker, 1972) are graphically represented in figures 1-4. The tendency towards higher values for total immunoglobulin concentration with age, which is not unexpected in view of the greater number of antigens encountered in a long life (Jerne, 1974), is in contrast with the distinct decrease in the titre of antibodies against specific bacterial and viral antigens in the elderly, thus indicating remarkable alterations of the antibody spectrum (Schwick and Becker, 1969).

Fig 4 Normal immunoglobulin concentrations in relation to age.

Nevertheless, when old people were actively immunized with tetanus toxoid their immune response was not significantly different from that of a younger group (Solomonova and Vizev, 1973) suggesting that the humoral defence mechanism is largely intact.

In spite of the decrease of antibodies against exogenous antigens, the percentage of subjects with one or more positive autoantibody reactions has been found to increase with age (Rowley, Buchanan, and Mackay, 1968). This anomaly, as well as the reported higher frequency of M-components in old people, may contribute to the observed increase of total immunoglobulins during aging.

Immunoglobulins in Disease

SERUM
Alterations of immunoglobulins may be quantitative or qualitative.
Quantitative changes may involve more than one class of immunoglobulin. An overall decrease occurs in patients with hypogammaglobulinaemia, while some patients may have a selective deficiency of one or two of the immunoglobulin classes. Similarly an

increase in gamma globulins may be either a diffuse polyclonal increase of one or more classes, associated with an increase of both light chain types, or an isolated monoclonal increase of one molecular species homogeneous with respect to class, subclass, and light chain type.

The term 'qualitative changes' is used to refer to functional aspects. Measuring such changes means the qualitative and quantitative determination of antibody activities by which a disease may be diagnosed. Identification and isolation of appropriate antigens and studies of the distribution of their corresponding antibodies within each immunoglobulin class will be one of the interesting tasks of future research work. In the case of IgE and specific reagins considerable progress has been made during recent years but only a few diagnostic tests for studying functional abnormalities in other disease groups, eg, autoimmune disorders, are available at present.

Specific immunochemical measurement of immunoglobulin alterations in serum and other body fluids may provide valuable information in conjunction with other diagnostic criteria, as will be demonstrated by the selected examples in the following sections.

Polyclonal hyperimmunoglobulinaemia is a condition commonly arising after strong stimulation of the immune defences. The antigens involved may be known, as in infectious diseases, suspected or under dispute, as in autoimmune disorders, or difficult to define at all, as in subacute and chronic liver disease or sarcoidosis (Heremans and Masson, 1973).

Most generalized infections provide multiple antigenic challenges through many routes and therefore all immunoglobulin classes frequently show elevations in infectious diseases. Because this is so nonspecific, it is of little clinical value other than to indicate or confirm that the patient is making a broad immune response, probably to exogenous antigens (Hobbs, 1971). However, the immunoglobulin increase can be quite unbalanced and in the extreme case discrete antibody bands may appear in agarose gel electrophoresis against the diffuse background of the gamma globulin fraction. Restricted heterogeneity of the immune response may be due to various factors, some of which are discussed in a recent survey of the clinical value of specific immunoglobulin analysis by Heremans and Masson (1973).

PERINATAL INFECTIONS

Some years ago at the Center for Disease Control in Atlanta a new acronym—TORCH—was devised to focus attention on a group of microbial agents that cause infections in the embryo or fetus during

gestation or in the newborn at the time of delivery. The letters stand for Toxoplasma, Rubella virus, Cytomegalovirus, Herpes simplex viruses, the O being for Other possible candidates such as the Epstein-Barr virus, herpes virus and hepatitis viruses. Nahmias (1974) has recently reviewed the present knowledge of the TORCH complex.

All of these agents can produce long-term ill effects in the infected fetus or newborn, so that prognosis must be guarded. The infections may be clinically inapparent; when they are clinically manifest their signs and symptoms are often indistinguishable. It is estimated that infection of the newborn by the TORCH agents occurs in from 1 to 5% of all deliveries, that in the United States every year a minimum of 400 deaths results from these perinatal infections, and that at least 2000 surviving infants are left with significant psychoneurological, cardiovascular, ocular, or hearing defects. Since preventive and therapeutic measures are now possible for some of these conditions, the development or improvement of diagnostic tests is one of the urgent needs of perinatal medicine.

Numerous reports published in the last 10 years indicate that measurement of the immunoglobulins in cord or neonatal serum may be a valuable procedure to detect such infection (Alford, Schaefer, Blankenship, Straumfjord, and Cassady, 1967; Stiehm, Ammann, and Cherry, 1966; Sever, 1968; Blankenship, Cassady, Schaefer, Straumfjord, and Alford, 1969; Khan, Ali, Werthmann, and Ross, 1969; Miller, Sunshine, and Remington, 1969). Either intrauterine or neonatal infection can lead to abnormally high IgM and/or IgA levels as part of a specific humoral immune response. However, the value of IgM and IgA quantitation as a general

Fig 5 Screening scheme for TORCH agents, based on measurement of IgM and IgA in cord serum (Antoniadis and Saling, 1972).

screening procedure seems to be limited, as normal levels have been found in some cases of recognized infections (McCracken, Hardy, Chen, Hoffman, Gilkeson, and Sever, 1969; de Crousaz Baillod, 1971). This could be the result of a deficiency in the ability of the fetus to respond appropriately to antigenic stimulation, or to the fact that the infection occurred too close to the time of delivery for a measurable immunoglobulin response to have taken place.

An elevated IgM level is not always diagnostic of infection (Finkel, Dent, Emrich, Gent, and Rahim, 1974). Leakage of maternal blood across the placenta or other antigenic stimuli, such as maternal allotypic proteins, can elevate both IgM and IgA. However, such false positive results do not substantially diminish the value of IgM quantitation for screening purposes. Antoniadis and Saling (1972) have proposed the scheme shown in fig 5 as a rational diagnostic procedure. Quantitation of IgM and IgA can be performed economically on specially designed immunodiffusion plates (Antoniadis and Tischer, 1973) or even by a simple and rapid latex agglutination technique (Tympner and Neuhaus, 1972; Oehme, Pfirrmann, and Gutzeit, 1972).

Maternal blood present in a cord blood sample will lead to an elevation of both IgM and IgA levels. If, when measured after an interval of one week, the newborn's serum IgA values are higher than they were in cord serum or if abnormally high individual values of either IgM or of IgA are found in cord serum, specific diagnostic measures should follow.

It is obvious that this scheme does not solve the problem of false negative screening results. Further studies are necessary to establish the true place of IgM and IgA determinations in the diagnosis of perinatal infection and to determine whether these tests can be recommended for mass screening programmes or should only be used as confirmatory tests in suspected cases.

LIVER DISEASES

Acute and chronic liver diseases are associated with abnormal serum immunoglobulin patterns which, considered in conjunction with clinical and other laboratory findings, were found to be of great value in the differential diagnosis of these diseases. Figures 6-9 show the remarkably concordant results which have been published during the last six years. For comparison all values are expressed as a percentage of the mean of the relevant control group.

Acute hepatitis

The 'immunograms' in fig 6 can be taken as typical patterns of acute hepatitis. They are characterized by an initial dominant elevation of IgM, the levels then falling during the course of the disease,

Fig 6 Immunoglobulin patterns in acute hepatitis. The height of a column represents the mean value expressed as a percentage of the mean of the normal range. The dotted line indicates the upper limit of normal.

returning to normal after about eight weeks. IgG and IgA may be slightly elevated, but are often within the normal range in uncomplicated cases. If the IgG rises continuously as the IgM level falls, the development of chronic hepatitis must be suspected. This finding is of particular importance since other laboratory tests fail to indicate definitely the onset of chronic disease (Kienholz, Bindewald, and Stockinger, 1973).

The documented association between hepatitis B antigen (HBAG, Australia antigen) and long-incubation serum hepatitis has stimulated comparative studies of immunoglobulin patterns in HBAG-negative and HBAG-positive infective hepatitis. Iwarson and Holmgren (1972) have measured the immunoglobulins in 65 patients with demonstrable HBAG and 40 histologically verified cases of hepatitis without demonstrable HBAG in acute-phase serum. The levels of IgM in the HBAG-negative group were significantly raised during the first four weeks after the onset of jaundice whereas the levels in the HBAG-positive group remained normal during the entire period of observation. This difference between hepatitis A and hepatitis B has been confirmed by Thompson, Carter, Stokes, Geddes, and Goodall (1973). Thirty-one of 44 HBAG-

negative patients but only two of 17 HBsAg-positive patients had initial IgM levels greater than 2 standard deviations above normal. These results agree with the findings of Giles and Krugman (1969) who studied experimentally infected institutionalized children and found significant IgM elevations only in cases of short-incubation hepatitis. Kindmark and Laurell (1972) too observed only insignificant changes of the immunoglobulins during the course of long-incubation hepatitis.

Primary biliary cirrhosis

A similar immunoglobulin pattern (fig 7) has been found in primary biliary cirrhosis. However, the isolated elevation of IgM in this form of intrahepatic cholestasis is even more pronounced than in acute hepatitis.

For the differential diagnosis of jaundice the measurement of a low-density serum lipoprotein of abnormal composition and properties, called LP-X, has recently been shown to be of great diagnostic value. LP-X was positive in 277 of 280 patients with cholestasis, but could be detected in the serum of only nine out of 277 patients with liver diseases where cholestasis could be excluded (Seidel, Gretz, and Ruppert, 1973). However, it is not possible to differentiate between extrahepatic biliary obstruction and intrahepatic cholestasis since both are LP-X positive. At least one form of intrahepatic cholestasis, namely, primary biliary cirrhosis, can be distinguished from extrahepatic obstruction by combining LP-X determination and IgM quantitation. The combination of 'LP-X positive/strongly elevated IgM' seems to be characteristic of primary biliary

Fig 7 Immunoglobulin patterns in primary biliary cirrhosis.

The height of a column represents the mean value expressed as a percentage of the mean of the normal range. The dotted line indicates the upper limit of normal.

cirrhosis whereas in extrahepatic biliary obstruction the IgM usually remains within the normal range. An elevated IgA as an isolated abnormality was frequently found by Thompson *et al* (1973) in patients with extrahepatic obstruction and could serve as a further parameter for diagnostic discrimination.

Chronic hepatitis

In chronic active ('lupoid') hepatitis a predominant IgG elevation occurs (fig 8) which correlates well with the progress of the patient, falling as clinical im-

Fig 8 Immunoglobulin patterns in chronic active hepatitis.

The height of a column represents the mean value expressed as a percentage of the mean of the normal range. The dotted line indicates the upper limit of normal.

provement occurs. This disease is mainly found in women. In chronic persistent hepatitis it is again mainly IgG that is significantly elevated, usually accompanied by a positive HBsAg test.

Cirrhosis

Micronodular cirrhosis (fig 9) is characterized by a marked elevation of IgA which correlates with the severity of the toxic inflammatory liver damage. In active alcoholic cirrhosis IgG reaches mean values of twice the normal and IgA increases to about four times the normal mean value (Gleichmann and Deicher, 1968b). In cases of inactive or less active alcoholic chronic hepatitis and cirrhosis the im-

Fig 9 Immunoglobulin patterns in alcoholic liver cirrhosis.

The height of a column represents the mean value expressed as a percentage of the mean of the normal range. The dotted line indicates the upper limit of normal.

balance of the immunoglobulin classes is similar to the pattern seen in active cirrhosis, but the increase of IgG is less marked. Since classical laboratory tests such as transaminase and bilirubin estimations show considerable overlap with the normal ranges, immunoglobulin analysis has gained particular importance for the differential diagnosis and prognosis of liver cirrhosis.

Evaluating the literature available in 1970, Hobbs (1971) analysed the reliability of the serum immunoglobulin patterns for the differential diagnosis of liver diseases and concluded that, provided diseases elsewhere can be excluded, these patterns are generally more than 90% reliable. Studies performed and published during the last three years confirm this statement and underline the diagnostic importance of specific immunoglobulin analysis.

IMMUNOGLOBULINS IN CEREBROSPINAL FLUID

With the exception of very high molecular weight

proteins such as IgM and β -lipoprotein, all plasma proteins can be detected immunochemically in normal cerebrospinal fluid. However, their relative proportions differ considerably from those of plasma (Laterre, 1973).

Probably most elevations of immunoglobulins in the csf are largely the result of local synthesis, being associated as a rule with infectious diseases of the central nervous system (cns). In patients with meningitis, quantitation of the csf immunoglobulins appears to be a valuable diagnostic aid for differential diagnosis. In acute bacterial and, to a lesser extent, in tuberculous meningitis, a marked rise of IgM concentration can be found (Kaldor and Ferris, 1969; Smith, Bannister, and O'Shea, 1973). The mean value for 24 cases of purulent meningitis was 43 ± 58 mg/l whereas for 35 patients with acute viral meningitis the IgM elevation was only moderate: 5.0 ± 5.8 mg/l. It seems that IgM concentrations greater than 30 mg/l in acute meningitis exclude a viral aetiology.

Apart from these differences in IgM levels in meningitis of different aetiology, IgA and IgG levels are elevated later in viral meningitis, in purulent meningitis, and in tuberculous meningitis.

In contrast to the increase of more than one immunoglobulin class in infectious diseases of the central nervous system, multiple sclerosis and panencephalitis are usually accompanied by a selective increase in IgG (Gottesleben and Bauer, 1967; Hartley, Merrill, and Claman, 1966). Frick and Scheid-Seydel (1958) demonstrated that the greater part of csf-IgG in multiple sclerosis does not originate from the serum but is locally synthesized by immunocompetent cells within the central nervous system. Cutler, Watters, Hammerstad, and Merler (1967) found the same to hold true for patients with subacute sclerosing leucoencephalitis. These results were obtained by studying the distribution of isotope-labelled IgG between serum and cerebrospinal fluid. They accord well with observations made by Cohen and Bannister (1967) who were able to show that lymphocytes derived from the csf of a patient with multiple sclerosis synthesized IgG *in vitro*.

Ganrot and Laurell (1974) recently pointed out that a normal total IgG concentration in csf does not necessarily mean normal local IgG production, because an abnormal oligoclonal or monoclonal electrophoretic IgG pattern may sometimes be found in csf together with an IgG concentration that is still within the relatively wide 'normal range'. However, the normal range is narrower for the ratio of IgG/albumin in the csf (Tourtellotte, Tavolato, Parker, and Comiso, 1971).

An index giving even higher discrimination has

been proposed by Delpech and Lichtblau (1972) and its value was confirmed by Ganrot and Laurell (1974). This index is obtained by measuring IgG and albumin in both csf and plasma, and thus includes a correction for variations of these two proteins in plasma which may influence csf levels if there is increased permeability of the blood-csf barrier. Six out of 20 patients with multiple sclerosis showed normal IgG values in their csf, but when the csf/plasma ratios for IgG were plotted against the csf/plasma ratios for albumin, the values obtained for normal controls and for the patients were discrete. The scatter diagram for these ratios in individuals without disease of the central nervous system has been demonstrated to be a most useful reference when it is desired to differentiate between local IgG synthesis in the subarachnoid space and an increase in csf protein for other reasons.

The extensive IgG elevation in csf from patients with subacute sclerosing leucoencephalitis is mostly accompanied by the appearance of free immunoglobulin light chains of both types, kappa and lambda, probably the result of an unbalanced local synthesis of immunoglobulin H- and L-chains (Koch, Becker, and Schwick, 1970).

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