The relationship of race, sex, and age to concentrations of serum immunoglobulins expressed in international units in healthy adults in the USA*

S. E. MADDISON,[1] C. C. STEWART,[2] C. E. FARSHY,[3] & C. B. REIMER [4]

Only a few investigations have been made to obtain human serum immunoglobulin values in units compatible with those used by the WHO International Reference Preparation for the Human Immunoglobulins IgG, IgA, and IgM. We report our summary statistics of serum IgG, IgA, and IgM, in international units (IU), for some 800 healthy American adults grouped by age, sex, and race. Our findings are in general agreement with some, but not with all, published data. We found that the mean IgG concentration is markedly higher and the mean IgA concentration is slightly higher in blacks than in whites. Except for white females, there was a significant increase in mean IgA with age for both races. In the younger adults of both races, mean IgM values were markedly higher in females than in males. Statistically significant interactions between race, age, and sex factors were seen for all three immunoglobulin classes. Although we have attempted to estimate the normal population means and variances for the serum concentration of IgG, IgA, and IgM the process we used to select specimens may have resulted in some bias; much larger, truly randomized, and fully documented studies in different geographic areas and in different socioeconomic and racial groups are needed to provide accurate acceptable limits for human immunoglobulins.

Serum immunoglobulin (Ig) determinations are used extensively, both as diagnostic indicators of disease and to monitor therapy in a variety of hypo- and hypergammaglobulinaemic states. Concentrations of the immunoglobulins have commonly been expressed in terms of mass concentration, such as mg/ml. However, comparable results between laboratories were not obtained until a common reference standard was used (21). The WHO International Reference Preparation of Human Immunoglobulins IgG, IgA, and IgM is available as a primary standard. Each vial contains 100 international units (IU) each of IgG, IgA, and IgM. The problems resulting from the use of the unfamiliar IU, however, are well recognized and have resulted in the authorization (12) of conversion factors obtained from published data to relate the two systems of measurement: 80.4 μg of IgG, 14.2 μg of IgA, and 8.47 μg of IgM are equivalent to 1.00 IU.

Only a limited number of investigations have been made to obtain values related to the WHO preparation. An extensive investigation was carried out by Buckley & Dorsey (1) who determined the IgG, IgA, and IgM serum levels in IU in sera of 800 subjects ranging in age from 1 to 92 years. Buckley et al. (1, 2) developed a biomathematical model to aid the study of the effects of age, sex, and race on immunoglobulin levels. Rowe (22) has reported the IgG, IgA, and IgM serum concentrations in IU for healthy young adult males from 11 different countries. Cejka et al. (4) reported similar data in IU for cord sera, and sera from some normal children and a few normal adults.

* From the Bureau of Laboratories, Center for Disease Control, US Public Health Service, US Department of Health, Education, and Welfare, Atlanta, GA 30333, USA.

[1] Chief, Parasitic Immunochemistry Branch.

[2] Mathematical Statistician.

[3] Microbiologist.

[4] Chief, Immunological Products Branch.

Numerous reports have indicated that statistically significant differences in the levels of serum IgG, IgA, and IgM are related to race, age, or sex. Although many of these studies are undoubtedly consistent internally, comparison between the values reported are uncertain because a common reference preparation was not used.

Accuracy as well as precision is required if a diagnostic decision is to be made concerning the "normalcy" of a given serum sample. Here we report our findings of baseline data for serum IgG, IgA, and IgM in IU for some US adults grouped by age, sex, and race.

MATERIALS AND METHODS

All sera were obtained from the Center for Disease Control serum bank. The specimens had been collected from healthy people between 1959 and 1969 during epidemiological surveys for specific antibodies following viral epidemics in the states of Alabama, Florida, Illinois, Louisiana, Mississippi, and Texas. The sera were stored at −20°C and were not thawed more than twice during the investigation.

The study was initially designed to involve a total of 600 normal sera, with 150 from each of the following categories: white male, white female, black male, and black female. Within each of these categories, the plan was to obtain 50 sera from individuals in each of the following age groups: 20–40 years, 41–60 years, and 61–80 years. After the initial study had been completed we decided that more reliable estimates of population parameters were needed for IgA and IgM levels of white males between 61 and 80 years of age and for IgA levels of white females in the same age group. An additional 100 sera were therefore tested for each of these groups.

Concentrations of IgG, IgA, and IgM were estimated by the single-radial-immunodiffusion technique of Mancini et al. (15). Goat antisera prepared in our laboratories were shown to be monospecific by immunoelectrophoresis with whole human serum and also by inverse single-radial-immunodiffusion with purified Ig.

Fifteen antigen wells in each plate were charged with appropriate dilutions of sera or reference preparations by using a 10-μl Eppendorf [a] pipette. Four wells contained dilutions of a secondary standard [b] that we had previously calibrated (in IU per ml) after a direct comparison with the International Reference Preparation: 67/97. At least one but usually two wells on each plate contained control samples (1 and 2) from each of two distinct pools of normal human serum. The remaining nine wells on each plate contained appropriate dilutions of unknowns. Diffusion was allowed to proceed in a humid chamber at 20°C for 4 days, followed by overnight drying and then staining of the plates. The diameters of precipitation disks (generally 6–9 mm) were measured to the nearest 0.1 mm. A calibration curve was constructed for each plate by using the least squares technique (19) to fit a linear line relationship between concentrations (in IU) of the dilution of the secondary standard and the corresponding areas of precipitate calculated from the measured diameter. These calibration curves were then used to estimate concentrations of all other samples contained on each plate.

Further studies, including cellulose acetate electrophoresis, immunoelectrophoresis, and gel filtration chromatography on Sephadex G 200, were carried out on sera with high IgG, IgA, or IgM levels.

RESULTS

Estimates of imprecision (lack of reproducibility) in our procedures for quantitating immunoglobulins were obtained from the values observed on two controls included in each plate. The coefficients of variation found were: for IgG, 10.9% and 11.5%; for IgA, 7.8% and 7.2%; and for IgM, 7.4% and 5.7%.

Estimates of inaccuracy (lack of agreement with an accepted standard) in our procedure for quantitating immunoglobulins were obtained by comparing our results with results reported by others using coded (identical) serum samples in a completely blind performance evaluation of laboratories conducted by the Licensure and Proficiency Testing Division of the Bureau of Laboratories (27). Only 10 of the 125 laboratories participating in this study reported their results in IU. Results from these 10 laboratories gave coefficients of variation of 10.3%

[a] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the US Department of Health, Education, and Welfare.

[b] The value assigned to the secondary standard is the mean of at least 29 determinations with some determinations performed on each of at least 6 different plates. The standard error is no greater than 1 IU for any assigned values; that is, one can state with 95% confidence that the limiting mean value is within 2 IU (twice the standard error) of the assigned value.

Table 1. Average values in International Units per ml of coded control sera used in a completely blind evaluation accuracy among 10 participating referee laboratories

Immunoglobulin class	Mean for 10 participating laboratories (IU/ml) [a]	SD of mean (IU/ml)	Coefficient of variation (%)	CDC mean [b] (IU/ml)
IgG	143.5	14.8	10.3	146.2
IgA	91.4	11.0	12.0	87.3
IgM	218.8	32.5	14.8	212.3

[a] Average of duplicate determinations.
[b] Average of 3 determinations.

for IgG, 12.0% for IgA, and 14.8% for IgM. Our results were in good agreement with the mean results reported by these 10 laboratories (Table 1).

Descriptive statistics for the concentrations of IgG, IgA, and IgM (in IU per ml) for the samples studied are listed in Table 2 according to race, sex, and age. The distribution of values showed somewhat greater spread for IgA and IgM than for IgG. Both very low and very high levels of the three immunoglobulins are found in all groups. Distributions for all categories were skewed towards higher values.

Results from a three-factor analysis of variance technique (5, 8), used to investigate joint effects of race, sex, and age on the mean immunoglobulin concentrations, are shown in Table 3. A separate analysis was performed for each immunoglobulin class.

We found that the mean IgG concentration is markedly higher in blacks than in whites ($P<0.01$).

Table 2. Median and mean concentrations and confidence intervals of the mean, in IU per ml,[a] for IgG, IgA, and IgM according to race, age, and sex

	White male			White female			Black male			Black female		
	20–40	41–60	61–80	20–40	41–60	61–80	20–40	41–60	61–80	20–40	41–60	61–80
IgG												
No. of subjects	50	50	49	49	50	50	49	47	49	49	50	48
median	122	135	123	131	126	112	162	186	186	186	178	185
mean	119	135	126	131	132	121	168	192	187	192	180	187
standard deviation	25	36	40	30	33	41	43	40	39	53	40	46
5th empirical percentile	72	78	60	68	81	68	100	126	124	112	114	126
95th empirical percentile	166	204	215	183	202	204	250	270	258	298	263	282
IgA												
No. of subjects	50	50	146	50	50	148	47	47	49	50	50	49
median	111	143	147	99	139	119	134	147	195	141	168	186
mean	117	145	157	117	143	130	147	164	210	143	178	199
standard deviation	42	61	69	51	71	61	61	87	95	46	71	70
5th empirical percentile	59	56	64	59	44	47	53	45	82	76	72	94
95th empirical percentile	209	271	294	231	264	244	274	383	432	230	310	348
IgM												
No. of subjects	50	50	148	49	50	50	50	47	49	50	50	49
median	139	107	128	212	200	120	145	128	92	246	132	98
mean	152	129	144	220	207	130	169	141	118	256	152	137
standard deviation	68	66	90	95	114	77	75	69	63	95	80	108
5th empirical percentile	58	37	44	75	60	34	79	40	44	110	54	49
95th empirical percentile	299	279	321	455	475	284	321	303	244	456	333	477

[a] To convert to mg/litre multiply these values by 0.804 for IgG, 0.142 for IgA, and 0.0847 for IgM; to convert to mg/dl multiply by 8.04, 1.42, and 0.847, respectively. (See ref. 5.)

Table 3. Summary of analysis of variance results

Source of variation	IgG		IgA		IgM	
	degrees of freedom	F-ratio	degrees of freedom	F-ratio	degrees of freedom	F-ratio
Race	1	303.94[a]	1	55.33[a]	1	0.07
Sex	1	0.66	1	0.98	1	36.93[a]
Age	2	1.65	2	24.32[a]	2	33.24[a]
Race by sex	1	0.30	1	0.76	1	0.12
Race by age	2	1.03	2	5.08[a]	2	4.20[b]
Sex by age	2	5.51[a]	2	2.31	2	10.13[a]
Race by sex by age	2	0.85	2	0.32	2	5.02[a]
Within cells	578		774		680	

[a] $P = < 0.01$. [b] $P = < 0.05$.

One cannot conclude that the mean IgG levels are affected by age if sex is ignored, or by sex if age is ignored. However, depending on how the data are grouped, one can conclude that the change in IgG concentration with age is somewhat different in the two sexes, indicating that a statistically significant ($P<0.01$) interaction exists between sex and age factors independent of race.

Mean IgA levels were also significantly higher in blacks than in whites ($P<0.01$). Except for white females, there was a significant ($P<0.01$) increase in mean IgA with age for both races; the exception indicates the presence of a significant interaction between race and age factors for IgA in white females.

In the younger adults (20–40 years) of both races, mean IgM values were markedly higher ($P<0.01$) in females than in males. Statistically significant interactions between race, age, and sex factors for IgM (Table 3) were seen as a pronounced decrease in IgM levels with age, particularly in females of both races; the decrease was less pronounced in black males and did not occur at all in white males.

Further tests to determine if high immunoglobulin levels observed in some sera reflected monoclonal proteins were carried out on 29 sera; in 22 of these the abnormally high immunoglobulins were shown to be polyclonal (IgA : 8; IgM : 11; IgG : 3). These data are included in the results of Table 2. The remaining 7 proteins studied were monoclonal. Data from these sera were not included in Table 2.

DISCUSSION

The importance of the WHO International Reference Preparation of Human Immunoglobulins IgG, IgA, and IgM lies in its potential diagnostic usefulness for comparing observations carefully made at any time in any laboratory on the serum of any individual patient with observations of a normal population, once this baseline data in IU is well established and made generally available. For valid comparison both precision and accuracy are required. Both the methodology and the reagents used can influence final results. Obviously, results will be biased if the antisera used are not specific, particularly if they react with light chains to bind other Ig classes into the same precipitation lattice with the Ig being measured. Nonspecificity of this type has been found in commercial kits sold for diagnostic use (20). Subclass selectivity of the antisera may also bias the results.

The quality of the secondary standard calibrated in terms of the WHO preparation is of utmost importance. Purity and retention of the antigenic and conformational characteristics of the native molecule are essential.

Methodologically, imprecision when filling wells can be a major source of error (7). By using an Eppendorf pipette we believe that our error from this source is less than 1.0%. Following the essential characteristics of the original Mancini procedure (15), we have allowed 4 days at room temperature for the precipitation reaction to reach quasi-equilibrium instead of using the kinetic modification of Fahey & McKelvey (6). Recently, Heremans et al. (10) have emphasized that the kinetic measurements can only be made at the expense of accuracy and information.

As assessed in our laboratory by the coefficient of

variation of internal controls included in every radial diffusion plate, our imprecision of measurement was much less than the spread of Ig values in healthy populations. Our reproducibility is similar to that reported by others (1, 13). The higher coefficient of variation of our IgG determinations in comparison with our IgA and IgM determinations probably reflects the two dilution steps we used to obtain an appropriate dilution for IgG as opposed to the single dilution step used for the IgA and IgM determinations.

The sera we studied had been stored at −20°C for 4–14 years before testing. Veys & Wieme (28) reported that IgG levels were not influenced by relatively short-term storage of samples at −20°C, but that IgA and IgM concentrations declined when sera were stored for 3 months. In the present study the long period of storage at −20°C before testing may have affected the immunoglobulin levels reported here, but we have no evidence of this. We consider it unlikely that the storage contributed to or detracted from the statistically significant differences we observed in the immunoglobulin concentrations within the various groups. We have not detected significant changes in IgM levels following repetitive freezing and thawing of sera (unpublished data).

The number of monoclonal proteins detected here (0.75% in 800) may well be an underestimate, because IgG values were not determined on 200 sera from the older age groups. Carrell et al. (3) observed 0.5% of monoclonal proteins in the population of 2 192 adults in a New Zealand town. The occurrence of the monoclonal gammopathies in our study in the older age groups agrees with previous findings.

Previous studies of levels of immunoglobulins in normal adults, for the most part reported in milligram values or in percentages of normal adult levels, have shown variation in Ig levels with race, age, and sex. This line of investigation has been comprehensively reviewed by Hobbs (11). In trying to establish normal adult values for immunoglobulin levels in terms of IU we found, as have others, that race, age, and sex have a significant effect on the mean values of IgG, IgA, and IgM in the various groups of individuals. Although the published variations reported are not in complete accord, they do stress the importance of taking these variables into account. Grundbacher (9) has presented evidence that in humans the X chromosome carries genes with an effect on IgM levels, thus accounting for the elevated levels observed in younger adult females, both black and white, compared with the IgM levels of males of the same age group. In contrast, Stiehm & Fudenberg (25) reported no racial differences. Buckley & Dorsey (1) reported that white males had less IgG and IgA than black males, but IgM concentrations in these two groups were not significantly different. Karayalcin et al. (14) observed that blacks, both male and female, had higher levels of IgG, IgA, and IgM than whites.

Our studies were carried out on a larger number of samples than were used in most reported investigations, and the sera in our study were drawn from surveys of healthy populations fairly representative of a cross-section of each area sampled. We recognize that the sample was not randomly selected and that this may result in some bias in our findings. Unfortunately, in no study yet published has the population sample been fully documented and randomly selected. In a number of previous studies the subjects investigated were drawn partly or entirely from hospital personnel. The size as well as the heterogeneity of our population sample may account for the wide range of immunoglobulin concentrations observed in all the groups we studied.

Our data seem to be in general agreement with that reported in IU for adults by Rowe (22), Sinkov et al. (24), and Cejka et al. (4), and with IU data reported by 10 reference laboratories participating in a completely blind laboratory proficiency testing study conducted by the US Center for Disease Control (27) (Table 1). The basis of disagreement between our data and the IU data of Buckley et al. (1, 2) is unknown, but we note that the conversion factors suggested by Buckley & Dorsey (1) are quite different from those recently recommended by Humphrey & Batty (12).

In attempting a comparative analysis of the results of previous studies of immunoglobulin levels carried out in various geographic areas and in different racial groups (1, 14, 16, 17, 18, 22, 23, 26, 29, 30) it becomes apparent that sex, race, age, climate, nutrition, and possibly as yet unrecognized factors may influence serum immunoglobulin levels. Although we have attempted to estimate accurately the normal US human population means and variance for the serum concentrations of IgG, IgA, and IgM, a much larger, randomly selected, and well documented population study is needed to provide true normative limits.

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RÉSUMÉ

LA RELATION ENTRE LA RACE, LE SEXE ET L'ÂGE ET LES CONCENTRATIONS D'IMMUNOGLOBULINES SÉRIQUES EXPRIMÉES EN UNITÉS INTERNATIONALES CHEZ DES ADULTES EN BONNE SANTÉ AUX ÉTATS-UNIS D'AMÉRIQUE

L'emploi de la préparation internationale de référence OMS pour les concentrations d'immunoglobulines a permis aux laboratoires d'effectuer des déterminations comparables. Toutefois, rares sont les recherches qui ont été faites en vue d'obtenir des titres d'immunoglobuline sérique humaine en unités susceptibles d'être exprimées dans les unités internationales (UI) utilisées pour les titres désignés de IgG, IgA et IgM dans la préparation de référence de l'OMS. Cet article contient une récapitulation statistique concernant les IgG, IgA et IgM sériques exprimées en UI pour quelque 800 adultes en bonne santé résidant aux Etats-Unis groupés par âge, sexe et race.

Les spécimens ont été recueillis chez des gens en bonne santé entre 1959 et 1969 à l'occasion de prospections épidémiologiques effectuées pour trouver des anticorps spécifiques à la suite d'épidémies virales dans les Etats de l'Alabama, de la Floride, de l'Illinois, de la Louisiane, du Mississipi et du Texas, et ils ont été stockés à $-20°C$. De même que pour les autres études de titres d'immunoglobuline dont les résultats ont été publiés, l'échantillon de population n'a pas fait l'objet d'un sondage aléatoire, de sorte qu'il se peut qu'il reflète quelque erreur systématique méconnue. Dans l'ensemble, nos conclusions concordent avec quelques-unes des données — mais non la totalité — communiquées en UI pour les adultes, ainsi qu'avec les données en UI signalées par dix laboratoires de référence participant à une étude effectuée par le Center for Disease Control des Etats-Unis en vue de vérifier la qualité du travail dans les laboratoires.

La concentration moyenne d'IgG était nettement plus élevée chez les noirs que chez les blancs ($P < 0{,}01$). On ne peut conclure que les niveaux moyens d'IgG sont affectés par l'âge si l'on ne tient pas compte du sexe, ni par le sexe si l'on ne tient pas compte de l'âge. Cependant, selon la façon dont les données sont groupées, on peut conclure que la modification de la concentration d'IgG avec l'âge diffère quelque peu chez les deux sexes, ce qui indique qu'il existe une interaction statistiquement appréciable ($P < 0{,}01$) entre le facteur sexe et le facteur âge indépendamment de la race.

Les niveaux moyens d'IgA étaient, eux aussi, nettement plus élevés chez les noirs que chez les blancs ($P < 0{,}01$). Sauf chez les femmes blanches, l'IgA moyenne augmentait de façon appréciable ($P < 0{,}01$) avec l'âge chez les deux races; l'exception indique la présence d'une interaction notable entre le facteur race et le facteur âge pour l'IgA chez les femmes blanches.

Chez les adultes plus jeunes (de 20 à 40 ans) des deux races, les titres moyens d'IgM étaient nettement plus élevés ($P < 0{,}01$) chez les femmes que chez les hommes. Des interactions statistiquement appréciables entre les facteurs race, âge et sexe pour l'IgM ont été observées comme correspondant à une diminution prononcée des titres d'IgM avec l'âge, en particulier chez les femmes des deux races; la diminution était moins accentuée chez les hommes de race noire et nulle chez les hommes de race blanche.

Bien que nous ayons cherché à obtenir une estimation des moyennes et des variations normales chez la population des Etats-Unis pour les concentrations d'IgG, d'IgA et d'IgM sériques, il est nécessaire, pour obtenir les limites exactes, de procéder à une étude beaucoup plus vaste, véritablement fondée sur un sondage aléatoire, et parfaitement documentée. Il ressort des enquêtes antérieures qu'il faudrait effectuer des études analogues dans différentes régions du monde et chez différents groupes socio-économiques et raciaux afin d'évaluer complètement l'influence de ces facteurs sur les titres d'immunoglobuline.

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