Immunoglobulin Concentrations in Sera of Normal Children: Quantitation against an International Reference Preparation

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Immunoglobulins G, A, and M were quantitated in sera from healthy children of various ages by using a standard related to the international reference preparation for human serum immunoglobulins. The results, expressed in International Units per milliliter, provide a basis for the establishment of normal immunoglobulin values in different laboratories.

Additional Keyphrases: normal values • inter-laboratory comparisons • IgG, IgA, IgM • pediatric chemistry • radial immunodiffusion • sex- and age-related changes

The quantitation of immunoglobulins in human sera is a common procedure in clinical laboratories and is useful both for clinical and research purposes. Several papers have been published on the subject. Because significant age-related changes occur before adult life, most reports deal with immunoglobulin values in normal children (1–4). However, the data from different laboratories are difficult to compare because a wide variety of immunoglobulin preparations, obtained from different sources, have been used as standards for the quantitation; thus, data expressed in mg/100 ml and based on local immunoglobulin standards are of limited use for comparative studies. With the object of improving the uniformity of immunoglobulin quantitations in different laboratories, an international reference preparation for human serum immunoglobulins has been established (5) by the WHO International Center for Immunoglobulins. It has been recommended (6) that the concentration of the individual immunoglobulins be estimated against this reference preparation and expressed in International Units (I.U.) per milliliter.

Our aim was to establish normal serum immunoglobulin values from birth to adulthood, in terms of I.U./ml; this approach should help in unifying immunoglobulin quantitations on the basis of the international standard, facilitate valid comparisons between different laboratories, and lead to full appreciation of the clinical significance of immunoglobulin determinations.

Materials and Methods

Cord-blood sera were obtained from 29 full-term, uncomplicated deliveries, chosen without conscious bias. A total of 207 sera were collected by venipuncture from apparently healthy children, 0.5 month to 16 years old; the children were either attending public schools or had come to our hospital for minor surgery. Complete history and physical examination excluded possible allergic episodes or infections in these children. The group included 106 boys and 101 girls, 96 of whom were Caucasians and 68 Negroes; the race of the blood donors who provided the remaining 43 sera was not established. Sera were also obtained from 22 healthy men who were in apparent good health. The race of these adult donors is unknown.

The immunoglobulins were quantitated within 24 h of collection, except in cases in which samples had been stored at −80 °C until the day of use. IgG, IgA, and IgM were measured by single radial immunodiffusion (7), on immunodiffusion plates (both “high-level” and “low-level”) purchased from Meloy Laboratories, Springfield, Va. 22151. Each serum was measured in duplicate and the two results were averaged. Immunoglobulin concentrations of a local serum reference standard were determined on Meloy plates, as well as on immunodiffusion plates obtained from Hyland Laboratories, Costa Mesa, Calif. 92626. The standard was a pool of 25 normal human sera from adults, and it was calibrated by multiple measurements on different plates and on different days according to the international reference preparation No. 67/95 (NCI Immunoglobulin Reference Center, Springfield, Va. 22151), which was stored in small aliquots at −80 °C and used throughout the whole study. Twofold dilutions of the standard, as well as of serum samples whose immunoglobulin values were found to be outside the range of the calibration curve, were made with human serum albumin, 50 g/liter (Grade A; Calbiochem, La Jolla, Calif. 92037). At least three points were used in preparing each standard curve. Wells were filled and all other procedures were done according to the vendors’ instructions. After staining (10 g of Amido Black per liter of acetic acid:water, 7:93 by vol), the diameters (d) of the precipitin rings were measured under 10-
fold magnification and plotted vs. concentration (c) on semilogarithmic paper [(log c)/d].

Serum immunoglobulin values were converted to natural logarithms for statistical analysis. Means and 95% confidence intervals were computed for each age group for each immunoglobulin. We used a two-factor analysis of variance (8) to evaluate the overall relationship of the sex and race of donors and their effect on IgG, IgA, and IgM values. The Wayne State University version of the University of Miami's Multivariate Analysis of Variance (MANOVA) program, with suitable corrections for unequal sample sizes, was used. In addition, where sample sizes within an age group were sufficiently large to allow further analysis, t tests, as well as Mann–Whitney U tests, a nonparametric analog of the t test (9), were used to evaluate differences attributable to sex and race.

Results and Discussion

For our local serum standard, the average values obtained for IgG, IgA, and IgM were 135, 131, and 141 I.U./ml, respectively. The coefficient of variation ascribable to the use of different plates, different positions of the antigen within a single plate, and day-to-day variation during a two-week period was 3.5% of the mean for IgG, 2.5% for IgA, and 4.5% for IgM on Meloy plates. The results obtained from Hyland plates were within the limits of experimental error found for Meloy plates. All subsequent immunoglobulin determinations on cord-blood sera, sera from the group of healthy children, and sera from healthy men were done on Meloy immunodiffusion plates.

Race- and sex-related differences. The separate and joint effects of race and sex on the values for individual immunoglobulins were determined on samples pooled over the age span of 0.5 month to 16 years, and separately for each of the age groups studied. A two-factor analysis of variance was performed on each of the three immunoglobulins. Examination of the results (Table 1) indicates that, in general, race and sex of donors are unrelated to their individual immunoglobulin values. In addition, we detected no interactions between the two factors. In the one instance in which a significant effect of race was observed (IgA), Caucasian donors had a significantly higher average immunoglobulin value than did Negroes. This trend was evident, but not statistically significant, for IgG and IgM as well; probably it results from the fact that there was a significant difference in the average age of the two groups of donors (74 months for the Caucasians, 58 months for the Negroes; t = 2.40, P < .02).

When racial differences were examined separately for each age grouping, we found no statistically sig-

Table 1. Analysis of Variance in IgG, IgA, and IgM Concentrations as a Function of Race and Sex of Donors

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>df</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td>1</td>
<td>1.49</td>
<td>6.67*</td>
<td>0.35</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.06</td>
<td>0.26</td>
<td>3.48</td>
</tr>
<tr>
<td>Race by sex</td>
<td>1</td>
<td>1.51</td>
<td>0.22</td>
<td>1.66</td>
</tr>
<tr>
<td>Within cells</td>
<td>148</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P <.05.

nificant differences except for the age groups 2–3 years and 9–12 years, in which the mean IgA and IgM values, respectively, were significantly higher (P < .05) for Negroes than for Caucasians. However, the differences were observed in groups with small sample sizes in one or both subgroups (seven Caucasians, six Negroes in the 2–3 year group; 15 Caucasians, three Negroes in the 9–12 year group) and followed no consistent pattern. In fact, these results were in the opposite direction of the overall trend. Buckley et al. (2, 10, 11) and others (12, 13) found significant race-related differences, mainly in immunoglobulin G; however, these differences were observed only in adolescents and adults. Our data did not confirm the IgA differences related to race found by Buckley and Dorsey (10, 11) for the 0- to 13-year age group.

By the same type of analyses we examined sex-related differences for the three immunoglobulins in the individual age groups, but found no statistically significant differences for IgG and IgA in any of the age groups. As for IgM, only in the three- to six-year age group was the IgM mean value of females (geometric mean 147.5) found to be significantly (P = .02) higher than that of males (geometric mean 121.4). As shown in Table 1, analyzing the data over the age span 0.5 month to 16 years, no significant sex-related differences were found for any of the three immunoglobulins. Sex-related differences were found by Buckley and Dorsey (11) in older age groups and especially in adulthood for all immunoglobulins, except for IgG, which was not statistically different in white males and females. Differences related to sex were also reported by Lichtman et al. (12) for normal individuals over 15 years of age. The results of the two groups of investigators are, however, mostly contradictory. Stoop et al. (14) found slight differences in IgG and IgM concentrations only in certain age groups of children, both values being higher for girls than for boys; they found no sex-related differences in IgA.

From the analyses of the present data we conclude that there are no significant sex- and race-related differences for the three immunoglobulins in the age span 0.5 month to 16 years. Only scattered exceptions were found in certain age groups, and these can

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1 To preserve continuity with previous practice, the following factors can be used to convert I.U./ml into mg/dl: 1 I.U. of IgG = 85 mg of IgG, 1 I.U. of IgA = 16.5 mg of IgA, 1 I.U. of IgM = 8.3 mg of IgM.
be largely ascribed to limited sample sizes in the particular groups. Age contrasts between all age groups, however, were typically highly significant when individual age-group means for each of the three immunoglobulins were compared (Table 2).

**Immunoglobulin G.** We found the mean IgG value in cord-blood sera to be the same as in healthy adults. The results showed the usual physiological postnatal decrease in IgG, the minimum IgG concentration occurring at about four months of age. Other investigators similarly reported the lowest IgG concentrations at three to six months (1), 4.5 months (2, 15) or one and a half to six months (16). The most marked increase in IgG occurred between four months and six years of age, after which the rate of increase was reduced. Adult values for IgG were attained at about 9 years. These findings agree well with those reported in the literature.

**Immunoglobulin A.** In only five of the 29 cord-blood sera studied were precipitin rings detectable with “low-level” IgA immunodiffusion plates (lower limit of detection, 1 I.U./ml); in the remaining 24, no IgA was detected. The five positive samples had IgA concentrations ranging from 1.3 to 7.4 I.U./ml. The value of 7.4 found for one sample of cord-blood serum might have been abnormally high because of a possible chronic infection in the mother during pregnancy; the IgM concentration in this serum was within the normal range. The finding of very low percentage of cord-blood sera with detectable IgA is confirmed by the results of other investigators (3, 15, 16). IgA concentrations increase gradually during infancy, reaching 50% of the adult value at about four and a half years of age. A slower increase was observed after the age of five years and adult levels were reached at about 16 years. The data reported in the literature differ considerably in this respect: adult values for IgA are reportedly attained by six to seven years (2), 12 years (3), 12 to 13 years (4), and after 16 years of age (1).

**Immunoglobulin M.** Concentrations of IgM were low in every sample of cord-blood serum tested; this is consistent with the findings of most other investigators (1-4, 15-17). Rather rapid increase of IgM was observed within the first two years of life and 50% of the adult value was reached at some point between six and 12 months of age. Essentially the same results have been reported by others (1-3). We found that after about two years of age, IgM increases much slower but continues to increase over the period of early adulthood. Most authors claim that the adult IgM values are reached in early childhood (2-4); our data are consistent with those of Stiehm and Fudenberg (1), who showed that adult IgM values are attained by sixteen years of age.

Serum immunoglobulin concentrations of healthy

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**Table 2. Serum Immunoglobulin Concentrations for Normal Subjects at Different Ages**

<table>
<thead>
<tr>
<th>Age</th>
<th>No. subjects</th>
<th>IgG I.U./mla (range)</th>
<th>% of adult level</th>
<th>IgA I.U./mla (range)</th>
<th>% of adult level</th>
<th>IgM I.U./mla (range)</th>
<th>% of adult level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood</td>
<td>29</td>
<td>135.9 (92.7-199.2)</td>
<td>99</td>
<td>0.4 (0.03-5.3)</td>
<td>0.4</td>
<td>12.5 (5.0-31.1)</td>
<td>8</td>
</tr>
<tr>
<td>1/2-3 mo</td>
<td>7</td>
<td>60.5 (36.5-100.2)</td>
<td>44</td>
<td>9.2 (2.1-40.2)</td>
<td>8</td>
<td>60.5 (18.9-193.9)</td>
<td>39</td>
</tr>
<tr>
<td>3-6 mo</td>
<td>9</td>
<td>44.8 (17.3-116.2)</td>
<td>33</td>
<td>12.9 (3.0-54.8)</td>
<td>11</td>
<td>58.0 (23.7-141.7)</td>
<td>37</td>
</tr>
<tr>
<td>6-12 mo</td>
<td>13</td>
<td>82.8 (51.0-134.4)</td>
<td>61</td>
<td>22.8 (9.1-57.6)</td>
<td>20</td>
<td>123.0 (56.1-268.9)</td>
<td>78</td>
</tr>
<tr>
<td>1-2 yr</td>
<td>22</td>
<td>78.4 (43.4-141.7)</td>
<td>57</td>
<td>25.0 (8.8-71.7)</td>
<td>22</td>
<td>121.4 (47.5-270.2)</td>
<td>77</td>
</tr>
<tr>
<td>2-3 yr</td>
<td>16</td>
<td>94.6 (60.0-149.3)</td>
<td>69</td>
<td>35.7 (15.4-82.8)</td>
<td>32</td>
<td>125.2 (63.7-246.2)</td>
<td>80</td>
</tr>
<tr>
<td>3-6 yr</td>
<td>74</td>
<td>105.7 (68.8-162.5)</td>
<td>77</td>
<td>54.2 (23.2-126.7)</td>
<td>48</td>
<td>131.0 (66.5-257.9)</td>
<td>83</td>
</tr>
<tr>
<td>6-9 yr</td>
<td>32</td>
<td>120.4 (80.3-180.5)</td>
<td>88</td>
<td>70.8 (19.6-156.1)</td>
<td>63</td>
<td>133.6 (65.1-274.3)</td>
<td>85</td>
</tr>
<tr>
<td>9-12 yr</td>
<td>20</td>
<td>119.7 (76.2-188.0)</td>
<td>88</td>
<td>85.1 (40.3-180.0)</td>
<td>75</td>
<td>166.7 (82.9-335.1)</td>
<td>106</td>
</tr>
<tr>
<td>12-16 yr</td>
<td>14</td>
<td>123.0 (83.0-182.1)</td>
<td>90</td>
<td>90.4 (53.9-154.4)</td>
<td>80</td>
<td>133.6 (57.9-308.4)</td>
<td>85</td>
</tr>
<tr>
<td>Adults</td>
<td>22</td>
<td>136.7 (81.8-228.5)</td>
<td>113.0 (50.3-253.8)</td>
<td>116</td>
<td>157.0 (47.5-310.2)</td>
<td>141</td>
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</tr>
<tr>
<td>Adultsb</td>
<td>390</td>
<td>125</td>
<td>116</td>
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</tr>
</tbody>
</table>

*a Geometric mean ±1.96 SD, converted to antilogs, are presented for each immunoglobulin at every age.

b From Rowe (18).
men (Table 2) have been compared with the average mean values for IgG, IgA, and IgM in men from studies done in five different European countries (18); agreement is fairly good for all three immunoglobulins. However, abnormally high IgG and IgM values found in the sera of West African adults and low IgM values in a normal population of Mexico City (18) suggest that regional differences exist, and call for further study of such differences among different populations.

This work was supported by a research grant from the Children's Hospital of Michigan. We thank Drs. A. J. Brough and F. Cohen for supplying blood samples for this study.

References