# Human X Chromosome Carries Quantitative Genes for Immunoglobulin M

Abstract. *Concentrations of immunoglobulin M in serum were one-third higher in females than in males in the Black and White populations of Virginia. In family studies, a much closer correlation was shown between boys and their mothers than between boys and their fathers. The immunoglobulin M concentrations in girls were more closely correlated with those of their fathers than with those of their mothers. The higher mean values for IgM in females and the relative magnitudes of the correlation coefficients between parents and offspring support the hypothesis that the X chromosome of man carries genes with an effect on IgM concentration. These patterns were not demonstrated for immunoglobulins A or G.*

Concentrations of isoantibodies to blood antigens A and B were found to be significantly higher in girls than in boys (*1*), and a similar sex effect was observed for immunoglobulin M (IgM) (*2, 3*). Although many of the quantitative differences between boys and girls result from hormonal differences, results of a family study have provided evidence that the X chromosome of man carries polygenes affecting the concentration of IgM. The evidence is summarized in this report.

Serum immunoglobulins were quantitated by the radial immunodiffusion technique (*4*). Specially prepared, standardized antibody agar plates, produced from the same antiserum lots, were obtained from Melpar (Springfield, Virginia). Undiluted serum (8 $\mu$l) was used for each well, and testing was done under standardized conditions. From each of six individuals, five different serum samples were obtained at equal intervals during 1 year. In an analysis of variance design, each sample was tested four times. Results were reproducible and there was no marked change in immunoglobulin levels during 1 year.

Table 1. Serum immunoglobulin concentrations according to race and sex (*N*, number of individuals).

| *N* | Immunoglobulin in serum (mg/100 ml) | | |
|---|---|---|---|
| | IgG | IgM | IgA |
| | *Black males* | | |
| 106 | 1040 (± 19.7) | 80.9 (± 2.53) | 176.8 (± 7.34) |
| | *Black females* | | |
| 136 | 1043 (± 21.3) | 106.8 (± 3.56) | 164.8 (± 6.79) |
| | *White males* | | |
| 97 | 809 (± 18.1) | 72.4 (± 2.76) | 152.5 (± 7.57) |
| | *White females* | | |
| 105 | 807 (± 18.9) | 94.1 (± 3.06) | 137.9 (± 7.16) |

A total of 444 individuals belonging to 64 families from the Richmond area were included in the immunoglobulin studies. Half of the families were Black and half White; the average size was five children per family. All families with illegitimacy, as determined by analysis of blood group antigens, were excluded from the study. The ages of the children ranged from 5 to 25 years. Age of the individual (parent or child) had no effect on IgM concentration in the present data, but the value for immunoglobulin G (IgG) increased slightly with age, and that for immunoglobulin A (IgA) increased markedly. The effects of age had been determined in detail in another population ranging in age from 10 to 95 years (*3*).

The mean concentrations of immunoglobulins according to race and sex are given in Table 1. The IgG values are significantly higher in Blacks than in Whites, and no sex effect is indicated for IgG. In each sex, the IgM and IgA values are significantly higher in Blacks than in Whites. The striking effect, however, is that IgM concentrations are higher in females than in males. In Blacks, the mean concentration of IgM in females is 32 percent higher than that in males; the corresponding value for Whites is 30 percent. Thus the effect of sex on IgM values is of similar magnitude in the two races, and in both races the difference is significant ($P <$ .001).

Higher values in females are expected if the X chromosome carries a gene or genes with effects on IgM concentration, but the means alone do not rule out a possible hormonal effect. However, additional evidence for an effect of the X chromosome is obtained from comparisons of parent and offspring, because in normal individuals the son always receives his X chromosome from the mother and never from the father, while the daughter receives the X chromosome from the father and one from the mother.

The correlation coefficients (Table 2) provide a measure for the degree of association of IgM values between child and parent. Correlations are used because they provide a more meaningful measure for comparing the degree of association than do regression coefficients, which are influenced by the effect of sex on means and variances. Correlation coefficients between sons and fathers are small, while those between sons and mothers are relatively high and differ significantly from zero. When correlation coefficients between sons and fathers are compared to those between sons and mothers, the differences are significant in Whites and for the races combined ($P <$ .05). The larger correlations between sons and mothers than between sons and fathers thus suggest that the X chromosome carries a gene or genes for IgM concentration. That is, the absence of X chromosome transmission from father to son is accompanied by low correlation of the IgM values.

Daughters receive the X chromosome of the father and one of the mother. While the X chromosome of the father is always the same, the mother can transmit either of two, or possibly a chromosome formed by crossing-over and combining parts of the two maternal chromosomes. The correlation coefficient is expected to be higher between daughters and fathers than between daughters and mothers if the X chromosome carries genes for IgM concentration, because the X chromosome transmitted from father to daughter is always the same. The correlation coefficients between daughters and fathers are about twice as large as those between daughters and mothers, and corresponding correlation coefficients in Blacks and Whites are of

Table 2. Correlation coefficients for IgM between offspring and parents; except where indicated all values are positive.

| Subjects | Race | | |
|---|---|---|---|
| | Blacks | Whites | Both |
| Son-father | .15 | −.01 | .10 |
| Son-mother | .24* | .43‡ | .36‡ |
| Daughter-father | .28† | .35† | .31‡ |
| Daughter-mother | .16 | .18 | .19* |
| Mother-father | .08 | .03 | .12 |

* $P <$ .05 for difference from zero. † $P <$ .01 for difference from zero. ‡ $P <$ .001 for difference from zero.

Table 3. IgM concentrations in sons of families with four or more sons per family.

| Family number | IgM in serum of sons (mg/100 ml) |
|---|---|
| 04 | 78, 70, 60, 72 |
| 12 | 91, 57, 60, 60, 64, 65 |
| 20 | 60, 64, 70, 56 |
| 23 | 80, 86, 60, 69, 64 |
| 35 | 62, 115, 60, 60 |
| 41 | 50, 88, 93, 59, 65 |
| 48 | 125, 104, 56, 82, 64, 98, 72, 111, 87 |
| 58 | 83, 62, 110, 115, 120 |
| 60 | 60, 80, 31, 49 |
| 62 | 62, 126, 103, 60, 73 |
| 76 | 69, 68, 62, 70 |

similar magnitude. Thus the correlation coefficients between parents and offspring support the hypothesis that the X chromosome carries genes for IgM concentration.

For IgM, the correlation coefficient between sibs (without regard to sex) was .27 for 728 pairs. The correlation coefficients between brothers and those between sisters were of similar magnitude. That is, the fact that sisters carry one X chromosome in common was not reflected in the comparisons. For IgG and IgA concentrations, neither the means nor the correlation coefficients between parents and offspring indicated an effect of the X chromosome.

If the X chromosome does carry genes for IgM concentration, are the genes clustered in a short segment or distributed over the whole chromosome? If the genes are in a short segment, a trend toward two distinct classes of IgM values is expected in the sons of some mothers. In contrast, if the genes are distributed over the whole chromosome no distinct classes are expected. Table 3 shows the IgM values for sons in families with four or more sons. The values in families 12, 35, 41, and 62 reveal a trend toward two classes; those in families 48 and 60 indicate a continuous distribution, and those in the remaining families provide no trend. These data, therefore, do not permit any conclusions about the distribution within the X chromosome of the genes that affect the IgM concentration in man. Obviously, sources other than the X chromosome also contribute to the variation in IgM values.

The higher mean IgM values in females and the relative magnitudes of the correlation coefficients between parents and offspring support the hypothesis that the X chromosome of man carries genes influencing IgM concentration. Rhodes *et al.* (*5*) observed that IgM concentration is influenced by the number of X chromosomes. They found that IgM values were highest in females with three X chromosomes, intermediate in normal females, and lowest in normal males. The IgM values in XO subjects were similar to those in normal males but lower than those in normal females (*6*). The observation that certain types of agammaglobulinemia might be sex-linked (*7*) also points to an association between immunoglobulin and the X chromosome.

If the X chromosome carries a gene or genes for IgM, the higher concentrations in females indicate that for these genes there is no or little inactivation of the second X chromosome. In this respect, these genes resemble the *Xg* locus, which is not subject to inactivation when it is carried on a structurally normal X chromosome (*8*). The increase in IgM concentration when more X chromosomes are present further suggests some form of additive gene action.

Girls are more resistant to certain types of infections than are boys (*9*). The higher concentrations of IgM in girls may explain the difference in susceptibility to infections. However, this difference in susceptibility should disappear in middle-aged individuals because the sex difference in IgM concentrations decreases with increasing age (*3*).

F. J. GRUNDBACHER
*Medical College of Virgina,*
*Health Sciences Division,*
*Virginia Commonwealth University,*
*Richmond 23219*

**References and Notes**

1. F. J. Grundbacher, *Z. Immunitaetsforsch. Allerg. Klin. Immunol.* **134**, 317 (1967).
2. M. Butterworth, B. McClellan, M. Allansmith, *Nature* **214**, 1224 (1967).
3. F. J. Grundbacher and D. C. Shreffler, *Z. Immunitaetsforsch. Allerg. Klin. Immunol.* **141**, 20 (1970).
4. ———, *Amer. J. Hum. Genet.* **22**, 194 (1970).
5. K. Rhodes, R. L. Markham, P. M. Maxwell, M. E. Monk-Jones, *Brit. Med. J.* **3**, 439 (1969).
6. C. B. S. Wood, W. Martin, M. Adinolfi, P. E. Polani, *Atti Ass. Genet. Ital.* **15**, 228 (1970).
7. J. M. Garvie and A. C. Kendall, *Brit. Med. J.* **1**, 548 (1961).
8. R. R. Race, *Int. Congr. Hum. Genet. 4th* (1971), p. 9.
9. T. C. Washburn, D. N. Medearis, B. Childs, *Pediatrics* **35**, 57 (1965).
10. I thank B. Bladen for skillful technical assistance. Supported in part by PHS grant AI 08736.

19 October 1971; revised 20 January 1972

## Immunologic Tolerance: Role of the Regional Lymph Node

Abstract. *Dansyl chloride, a skin sensitizer when injected in complete Freund's adjuvant, induced marked tolerance and no sensitization when applied to the intact skin of guinea pigs. Application of this chemical to alymphatic skin islands failed to induce tolerance or sensitization. Lymphatic connections between skin and regional lymph nodes were essential for the development of immunologic tolerance.*

Dansyl chloride (dimethylaminonaphthalenesulfonyl chloride) (*1*), a potent skin sensitizer when injected in complete Freund's adjuvant, was found to have the unusual property of inducing marked tolerance and no sensitivity when appled to the skin of guinea pigs. This observation made it possible to study the roles of lymphatic and hematogenous pathways in the induction of immunologic tolerance. Skin islands known to have no lymphatic drainage (*2, 3*) were prepared in 12 guinea pigs (Hartley strain) according to the method of Frey and Wenk (*2*), and 10 $\mu$mole of dansyl chloride in 0.1 ml of acetone was applied within a shaven area (15 to 20 mm$^2$) on the skin of each island. Each of a second group of 20 animals received the same amount of dansyl chloride on shaven intact skin, and each of a third group of 8 animals received 0.1 ml of acetone on intact skin. The sites of application and the skin islands were excised after 7 days. Viability of the islands was confirmed by growth of fur, bleeding when cut, and lack of necrosis. Lack of lymphatic drainage in skin islands prepared by this procedure was verified by the injection of Evans blue dye and India ink particles into the skin of islands, and by subsequent examination of the draining lymph nodes. Four animals in the skin island group and 8 animals receiving dansyl chloride on intact skin were skin-tested 21, 35, and 49 days (*4*) after the initial application of chemical. Twofold serial dilutions of dansyl chloride in acetone beginning with 100