

Observations on the levels of γ G, γ A, and γ M globulins, anti-A and anti-B agglutinins, and antibodies to *Escherichia coli* in Down's anomaly

M. ADINOLFI, BRIGITTE GARDNER, AND WENDY MARTIN

From the Paediatric Research Unit, Guy's Hospital Medical School, London

SYNOPSIS The levels of γ G, γ A, and γ M globulins were estimated in a group of patients with Down's anomaly and in groups of mentally retarded children and normal children, matched for age and sex, using an immunological method.

Higher levels of γ G globulin were observed in patients with Down's syndrome, in association with a small but significant lower concentration of γ M globulin.

The titres of anti-A and anti-B agglutinins and antibodies to *Escherichia coli*, estimated before and after treatment of serum with 2-mercaptoethanol, were found to be within normal levels.

These data seem to suggest that patients with Down's syndrome do not produce 'faulty' immunoglobulins as has been previously postulated. It is suggested that the abnormal levels of immunoglobulins found in Down's anomaly are not peculiar to patients with Down's syndrome but occur in other disorders in which the reticuloendothelial system or the lymphocytes are involved.

Investigations on the electrophoretic pattern of serum proteins in patients with Down's syndrome have revealed higher levels of immunoglobulins than in mentally defective children (Stern and Lewis, 1957; Sobel, Strazzulla, Sherman, Elkan, Morgenstern, Marius, and Meisel, 1958; Skanse and Laurell, 1962; Pritham, Appleton, and Fluck, 1963; Appleton and Pritham, 1963). These observations might suggest that patients with Down's syndrome respond to antigenic stimuli with an excessive and persistent formation of antibodies and therefore immunoglobulins (Stern, 1965). On the other hand, there is evidence that patients with Down's syndrome have a high rate of infections (Engler, 1949; Donner, 1954; Benda, 1960), whereas atopic diseases are seldom observed (Coghlan and Evans, 1964). These findings suggest the possibility that these patients have a deficiency in the formation of one or other class of immunoglobulins.

In view of these discrepancies and since no attempt has been made to assay the concentration of γ G, γ A, and γ M globulins in these patients, the problem was further investigated (*a*) by estimating the amounts of γ G, γ A, and γ M globulins using antibodies made

specific to the heavy chains (γ , α , and μ) of each class of immunoglobulin; (*b*) by studying the levels of λ and κ light chains common to the three classes of immunoglobulins; and (*c*) by estimating the titre of the 'naturally' occurring anti-A and anti-B agglutinins and the titre of the antibodies against *Esch. coli* W 96.

MATERIALS AND METHODS

HUMAN SERA Blood samples were collected by venipuncture from a group of 34 patients with Down's syndrome; these patients were recovered in two institutions and were free from infectious diseases when the samples of blood were taken.

In the present study only 20 patients were investigated; these patients had primary trisomy of an autosome of the G-group; their age ranged from 4 to 16 years. The other 12 patients were discarded either because they had not standard trisomy or because the amount of serum collected was not sufficient to carry out the planned investigation. As a control 20 mentally retarded children were used; these patients were recovered in the same two institutions and were matched for age and sex with the 20 patients with Down's syndrome.

Sera were also collected from 12 normal children between 4 and 15 years of age.

In studying levels of γ G, γ A and γ M globulins, sera from 15 patients with Down's syndrome, 15 mentally

retarded, and 12 normal children were tested. The age of these patients ranged from $4\frac{1}{2}$ to 14 years; eight patients with Down's syndrome, seven mentally retarded patients, and six normal children were boys.

ESTIMATION OF IMMUNOGLOBULINS The concentration of γG , γA , and γM globulins was estimated by a radial diffusion plate method (Mancini, Carbonara, and Heremans, 1965) using specific antibodies incorporated in agar gel, as described by McKelvey and Fahey (1965). Each class of human immunoglobulins was identified by antisera reacting against the specific determinants of each heavy chain (anti- γ , anti- α , anti- μ). These antisera also permitted a quantitative immunological estimation of each class of immunoglobulins.

The levels of the three classes of immunoglobulins were estimated using three 'standards' for each class of immunoglobulin; the plates containing anti- γ antibody were incubated at room temperature for six hours; the plates containing anti- α and anti- μ antibodies were incubated for 16 hours. The diameter of the precipitin ring was then measured to the nearest 0.1 mm. A standard curve was prepared for each series of estimations by plotting the diameter of the precipitin rings vs. the log concentration of the three samples containing standard amounts of γG , γA , and γM globulins. In this paper values of γG , γA , and γM are expressed as mg./100 ml. of serum.

The estimates of κ and λ chains were obtained by the radial plate diffusion technique with specific anti- κ and anti- λ antibodies incorporated in agar gel; in this paper the levels of the two light chains are expressed as a percentage of the amount of κ and λ chains present in a normal adult subject (M.A.).

SEROLOGICAL TEST Anti-A and anti-B agglutinins were titrated by making serial doubling dilutions of serum in buffered saline; to each tube an equal volume of 5% suspension of red cells was added.

The mixtures were left at room temperature for 90 minutes and the deposited red cells were then examined microscopically and the degree of agglutination was scored. Antibodies against *Esch. coli* WF 96 (Colindale strain E 458/64/serotype 07 H6 K1) were tested by using the indirect haemagglutination method (Neter, 1956). Suspensions of bacteria in buffered saline were heated for one hour at 100°C. in a water bath and the supernatant was recovered by centrifugation at 15,000 r.p.m. for 30 minutes. One volume of the supernatant was mixed with 1 volume of a 10% suspension of group O red cells, previously washed with buffered saline, and incubated at 37°C. for one hour. The 'modified' red cells were washed three times and resuspended in buffered saline to make a final 5% suspension. The bacterial agglutinins were then tested using the same procedure as for anti-A and anti-B agglutinin.

TREATMENT OF SERA WITH 2-MERCAPTOETHANOL (2-ME) One volume of undiluted serum was mixed with an equal volume of 0.1 M 2-me in phosphate buffer pH 7.4 and incubated at 37°C. for two hours; the mixture was then dialysed against frequent changes of buffered saline. As a control, undiluted serum was treated with phosphate buffer.

ESTIMATION OF THE DEGREE OF AGGLUTINATION In the present paper the results of titrating antibodies are expressed as a 'score', using the method suggested by Race and Sanger (1958).

RESULTS

ESTIMATION OF γ , α , AND μ 'HEAVY' CHAINS AND κ AND λ 'LIGHT' CHAINS As mentioned in Methods, the levels of γG , γA , and γM globulins were estimated by using antisera made specific against γ , α , and μ chains.

The mean value of γG globulin present in 15 patients with Down's syndrome was 1,498 mg./100 ml. of serum; that of 15 mentally defective children was 1,227 mg./100 ml., and that of 12 normal children was 1,180 mg./100 ml. (see Table I and Figs. 1 and 2). The levels of γG globulin were significantly higher in patients with Down's syndrome than in the mentally retarded patients ($0.05 > P > 0.02$).

TABLE I

LEVELS OF γG , γA , AND γM GLOBULINS IN SERA OF PATIENTS WITH DOWN'S SYNDROME AND IN A GROUP OF MENTALLY DEFECTIVE CHILDREN (CONTROL) MATCHED FOR AGE AND SEX

| Subject | No. Tested | Immunoglobulin Levels (mg./100 ml.) | | | | | |
|-----------------|------------|-------------------------------------|-------|------------|------|------------|------|
| | | γG | | γA | | γM | |
| | | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| Control | 15 | 1,227 | 66.3 | 217 | 20.5 | 135 | 19.3 |
| Down's syndrome | 15 | 1,498 | 87.5 | 225 | 23 | 82 | 10.1 |
| Differences | | 271 | 108.2 | | | 53 | 21.3 |

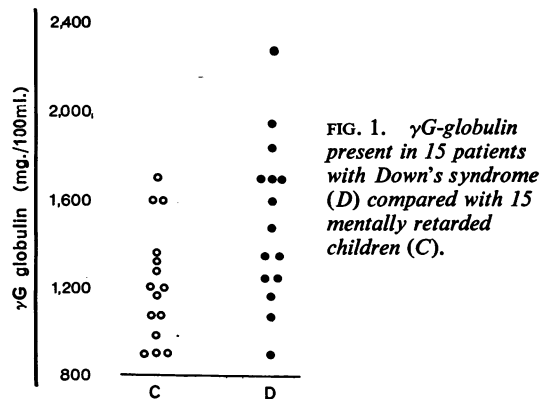


FIG. 1. γG -globulin present in 15 patients with Down's syndrome (D) compared with 15 mentally retarded children (C).

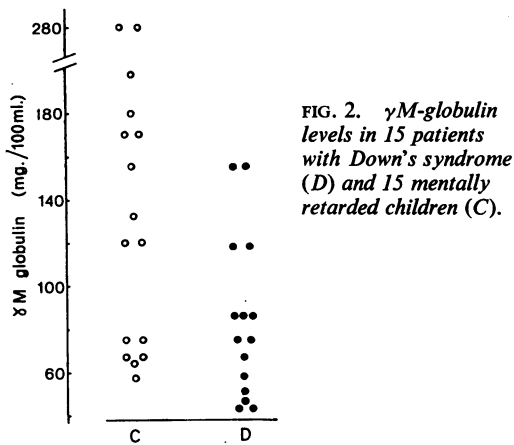


FIG. 2. γ M-globulin levels in 15 patients with Down's syndrome (D) and 15 mentally retarded children (C).

γ A globulin was estimated in 15 patients with Down's syndrome and 15 mentally defective children and the mean values were found to be 217 mg./100 ml. and 225 mg./100 ml. respectively.

The mean value of γ M globulin in the first group was 82 mg./100 ml. and that in the second group of patients 135 mg./100 ml. The difference of levels of γ M globulin in the two groups was statistically significant ($0.05 > P > 0.025$). Since the variances of the two samples were significantly different, the test recommended by Snedecor (1956) was used.

Two mentally defective children (L.S. and T.K.) showed high levels of γ M globulin (280 mg./100 ml.); one of these patients (L.S.) had a high concentration of γ A (570 mg./100 ml.) in the presence of normal levels of γ G globulin. Inspection of Figure 1 shows

that the increase of γ G globulin was not due to a bimodal distribution of this type of immunoglobulins.

There was no relation between the age and the sex of the patients and the levels of immunoglobulins (see Fig. 3).

The levels of λ chain in 15 patients with Down's syndrome ranged between 50 and 100% of the levels present in a normal subject (M.A.); the levels of κ chain ranged between 80 and 120% of the level present in the same normal subject.

It has been shown that the levels of light chains determined by the radial plate diffusion technique are lower than the values obtained using the isotopic inhibition method, and that these values reflect predominantly the levels of κ and λ chain associated with γ G globulins (McKelvey and Fahey, 1965).

As mentioned in Methods, the levels of κ and λ chains were calculated as a percentage of the levels of light chains present in a normal subject; the present data, therefore, only indicate that patients with Down's syndrome have not a marked deficiency in the synthesis of these chains.

ANTI-A AND ANTI-B AGGLUTININS AND ANTI-*Esch. coli* W9 ANTIBODY Anti-A and anti-B antibodies were tested in the serum of 19 patients with Down's syndrome and in the serum of 14 mentally defective children selected for being group O or B.

In all sera the expected isoagglutinins were present; the titre of the antibodies before and after treatment with 2-me is shown in Table II.

Treatment with 2-me completely inhibited the anti-B agglutinin in two out of nine sera from the group of A patients used as a control, and the anti-B agglu-

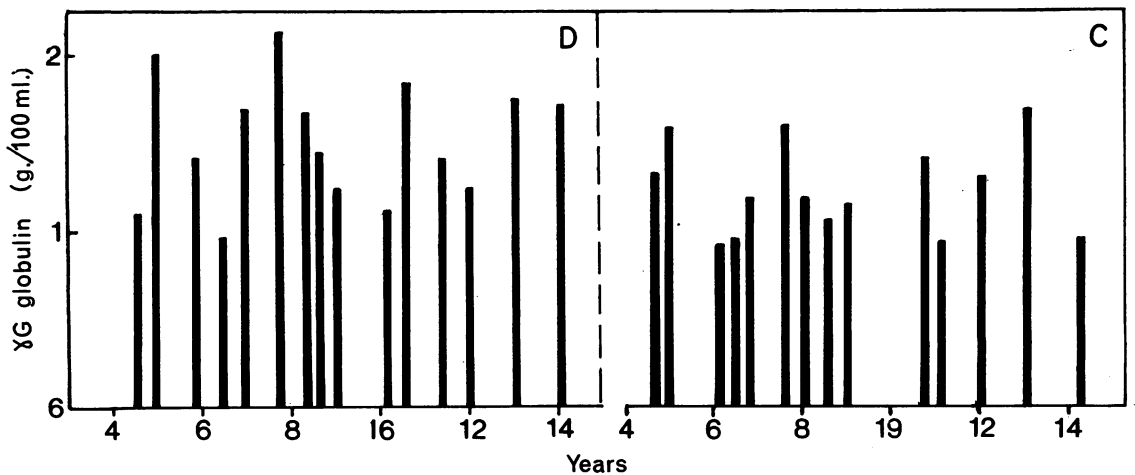


FIG. 3. Levels of γ G-globulin in a group of patients with Down's syndrome (D) and in a group of mentally retarded children (C). No relation between the age of the patients and the levels of immunoglobulins is observed.

TABLE II

TITRE (EXPRESSED AS A 'SCORE') OF ANTI-A AND ANTI-B AGGLUTININS BEFORE (BUFFER) AND AFTER TREATMENT WITH 2-MERCAPTOETHANOL (2-ME)

| Subject | ABO Blood Group | No. Tested | Anti-A | | Anti-B | |
|-----------------|-----------------|------------|--------|------|--------|------|
| | | | Buffer | 2-me | Buffer | 2-me |
| Down's syndrome | O | 8 | 61.7 | 15.2 | 48.9 | 17.5 |
| Control | O | 5 | 66.7 | 15.1 | 51.0 | 12.7 |
| Down's syndrome | A | 11 | — | — | 44.5 | 17.6 |
| Control | A | 9 | — | — | 64.4 | 11.6 |

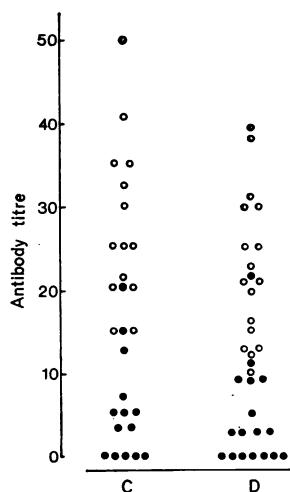


FIG. 4. Levels of anti-*Esch. coli* antibodies before (open circle) and after (closed circle) treated with 2-mercaptoethanol in 15 patients with Down's syndrome (D) and 15 mentally retarded children (C).

tinin in seven out of 11 sera from the patients with Down's syndrome (group A).

The sera of 15 patients with Down's syndrome and of 15 mentally defective children were tested for the presence of anti-*Esch. coli* antibodies before and after treatment with 2-me (Fig. 4). The mean values were 22.7 and 28.6 respectively before treatment with 2-me; after treatment with 2-me the mean values were 7.4 and 8.6.

DISCUSSION

As mentioned in the introduction, the concentration of immunoglobulins in patients with Down's syndrome was repeatedly found to be increased, using paper electrophoresis. So far no studies have been published on the levels of each class of immunoglobulin. The present data, while confirming that these patients have higher levels of immunoglobulins, show that the increase is mainly due to a higher concentration of γG -globulin.

Until more is known about the genetic and the environmental factors regulating the rate at which each class of immunoglobulin is synthesized, it is not possible to state the reasons for the abnormal behaviour of immunoglobulins in patients with Down's syndrome.

The normal levels of haptoglobin observed in patients with Down's syndrome (Skanse and Laurell, 1962; Hutton and Smith, 1964) seem to suggest that the higher values of γG -globulin are not the consequence of chronic infection. Furthermore, since bacterial antibodies are associated with γG , γA , and γM -globulins (Heremans, Vaerman, Carbonara, Rodhain and Heremans 1963; Adinolfi, Glynn, Lindsay, and Milne, 1966), bacterial infections should increase the level of all classes of immunoglobulins.

The present findings, that the titres of anti-A, anti-B, and anti-*Esch. coli* W 96 antibodies are similar to the titres observed in the groups of patients used as controls, appear to exclude the possibility that the higher levels of immunoglobulins found in patients with Down's syndrome may be due to the production of 'faulty' immunoglobulins, as postulated by Appleton and Pritham (1963) and Fluck and Pritham (1964).

Of interest is the observation that differences in the levels of immunoglobulins similar to those found in patients with Down's syndrome, have been observed in patients with diseases in which the reticuloendothelial system or the lymphocytes were involved (McKelvey and Fahey, 1965; Ngu, McFarlane, Osunkoya, and Udeozo, 1966). It may be postulated that a common dysfunction of the cells producing immunoglobulins is involved in this group of disorders, which have already been shown to have other characteristics in common, as, for example, the synthesis of the new serum protein variant 'Au' (Blumberg, 1966).

We are grateful to Dr. B. H. Kirman (Queen Mary's Hospital, Carshalton) and to Dr. Philip Benson (Paediatric Research Unit) for obtaining blood samples from some of the patients studied.

This investigation was supported by the Spastics Society.

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