Serum Immunoglobulin A and Antibody to M-Associated Protein in Patients with Acute Glomerulonephritis or Rheumatic Fever

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Serum immunoglobulin A (IgA) was markedly increased in 80% of 50 patients with acute rheumatic fever in Trinidad in contrast to 20% of 63 patients with acute glomerulonephritis, whereas serum IgG was increased in nearly all of both groups. Since total antibody to M-associated protein (MAP) (assessed by complement fixation) is the only antibody as yet consistently found to be of higher titer in patients with acute rheumatic fever than in patients with acute glomerulonephritis in Trinidad, it was measured and the titers were related to serum levels of IgA. The titers of total antibody to MAP were ≥40 in 58% of the rheumatic fever patients, 43% of the nephritis patients, and 8% of well school-children. However, its presence in rheumatic fever patients did not correlate directly with amounts of serum IgA present ($r = 0.0507$). Moreover, titers of total antibody to MAP related equally well to enzyme-linked immunosorbent assay indicated amounts of IgG antibody ($r = 0.3939$) and IgA antibody ($r = 0.3054$) to MAP in rheumatic fever patients but not in nephritis patients ($r = 0.0301$ for IgA antibody and 0.6909 for IgG antibody to MAP), whereas they related best to IgM antibody to MAP in the school-children ($r = 0.4204$).

For many years we have studied all patients admitted to the General Hospital in San Fernando, Trinidad, with acute glomerulonephritis (AGN) or acute rheumatic fever (ARF) to learn about the pathogenesis of these very different sequelae of group A streptococcal infections. Although different types of streptococci have been associated with the two diseases, we have found little difference in the titers of antibodies to streptolysin O, hyaluronidase, DNase B, or group A carbohydrate (5, 6). However, investigators at Colindale Laboratories have described higher titers of antibody to streptococcal M-associated protein (MAP) in patients with ARF than in patients with AGN in Trinidad as well as elsewhere (13, 14). In the present study, we found that serum immunoglobulin A (IgA) also was more increased in patients with ARF than in patients with AGN whereas serum IgG was increased in both groups of patients. Therefore, we looked for correlation between serum IgA levels and antibodies to MAP. We also tried to identify the antibodies to MAP as IgA antibodies by applying the enzyme-linked immunosorbent assay (ELISA) technique to their assay.

MATERIALS AND METHODS

Clinical material. AGN was diagnosed when patients had sudden appearance of hematuria or edema or both, proteinuria, and decreased serum $B1_c$ globulin plus evidence of recent streptococcal infection. ARF was diagnosed when patients had at least two major Jones criteria (2) or one major and two minor Jones criteria, plus evidence of recent streptococcal infection. Twenty AGN patients with scabies, 33 AGN patients with skin infections but no scabies, and 10 AGN patients without either were selected for study along with 50 patients with ARF, none of whom had skin infections. Acute-phase sera were obtained from all patients within 1 to 3 days of admission to the hospital, and follow-up sera were obtained from 24 of the AGN patients 3 to 12 weeks after admission. Sera also were obtained from 50 well school-children 5 to 8 years old for control values. All sera were frozen and stored over dry ice or at −70°C until studied.

Measurement of serum immunoglobulins. IgG, IgA, and IgM were measured in serum samples by radial immunodiffusion in agar gel, using kits from Calbiochem-Behring Corp. (La Jolla, Calif.).

Measurement of anti-type 12 antibodies. Bactericidal tests for type-specific antibodies (4) were performed on 40 AGN sera, 40 ARF sera, and 30 control sera to be tested for antibodies to MAP. The M-type 12
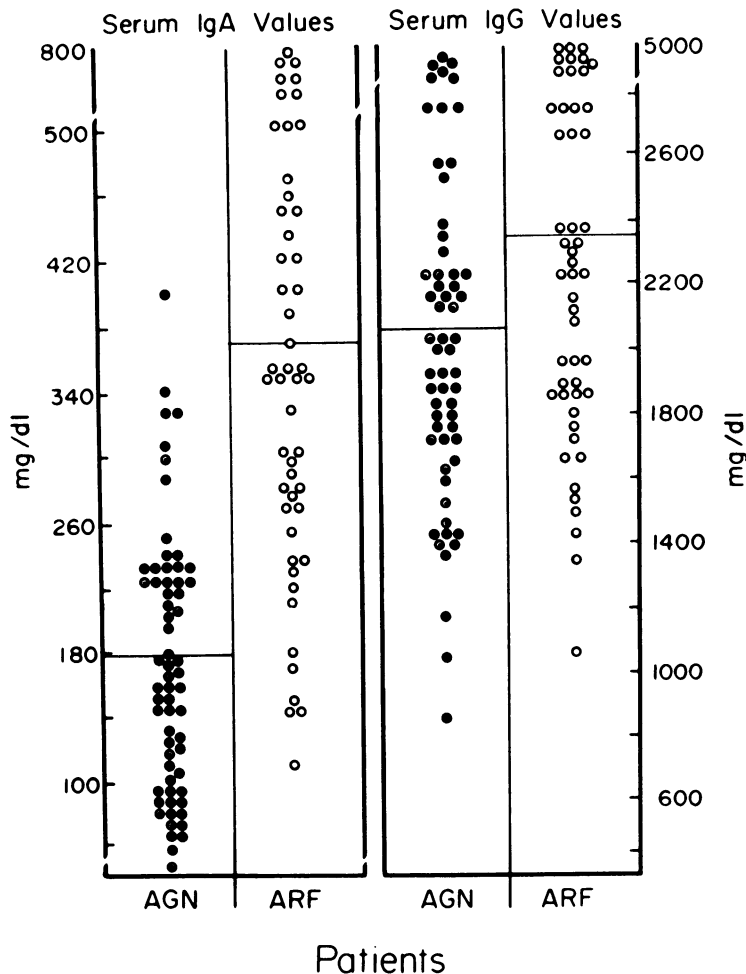
FIG. 1. Serum IgA and IgG values in AGN and ARF patients. The mean for each study group is indicated by a solid line. Symbols: ●, AGN patients with skin infections; ⊙, AGN patients without skin infections.

streptococcal strain used for these tests was the same one used to prepare our MAP antigen, as described below. Penicillinase (Riker Laboratories, Northridge, Calif.) was added to each serum (4,000 U per ml of serum) tested.

Measurement of TA-MAP by complement fixation. Total antibodies (IgG, IgA, and IgM) to MAP (TA-MAP) were assayed according to Widdowson et al. (13), using a complement fixation technique described by Bradstreet and Taylor (1). Semipurified M-type 12 protein from a streptococcal strain which had lost its group A antigen during lyophilization or animal passage in our laboratory (4) was prepared from 0.2 N acid extracts by precipitation at pH 2, RNase treatment, and ammonium sulfate fractionation (3) for use as MAP antigen since only one strain of M-type 12 streptococcus has been found in Trinidad during the past 16 years. Briefly, 0.025 ml of the antigen (1.77 μg/ml) was added to 0.025 ml of the heat-inactivated serum to be tested (at various dilutions) and 0.025 ml of guinea pig complement (containing 3 hemolytic units). After incubation overnight at 4°C for comple-

ment fixation, 0.025 ml of sensitized sheep erythrocytes was added, and the mixture was incubated for 30 min at 37°C. Results were read as that dilution of serum with which 50% hemolysis occurred.

Measurement of IgG, IgA, and IgM antibodies to MAP by ELISA. ELISA for IgG, IgA, and IgM antibodies to MAP were performed according to Voller et al. (11) and Sepulveda et al. (7) as follows. The wells of disposable Immulon Polystyrene Microelisa plates (Dynatech Laboratories, Inc., Alexandria Va.) were filled with 200 μl of the semipurified M-protein plus MAP antigen (2.85 μg of protein per ml) in carbonate-bicarbonate buffer, pH 9.6. The plates were incubated overnight at 4°C in a moist chamber. The MAP antigen solution was aspirated from the wells, and the wells were filled with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, Mo.) and 0.02% sodium azide. The plates were allowed to stand for 3 min and then emptied by inverting and shaking. This washing procedure was repeated two more times. Then, 200 μl of sera to be tested, diluted as found in preliminary assays to be
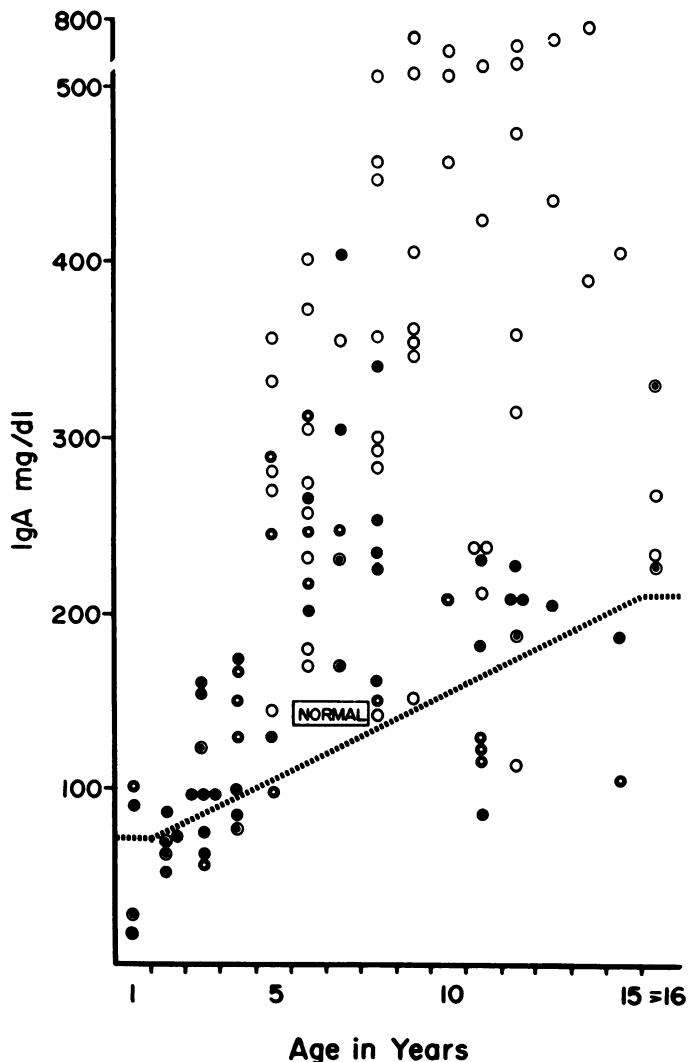
FIG. 2. Serum IgA values in AGN and ARF patients plotted according to age. Symbols: ●, AGN patients with scabies; ⊙, AGN patients with other skin infections; ⊙, AGN patients with no skin infections; ○, ARF patients. Normal means according to age (J. R. Hobbs, Assoc. Clin. Pathol. Broadsheet no. 68, 1970) are indicated by the heavy dotted line. The mean of 50 normal Trinidad school-children 5 to 8 years old is indicated by the box labeled "normal."

satisfactory for these tests (1/1,600, 1/3,200, and 1/6,400 for IgG; 1/40, 1/80, and 1/160 for IgA; and 1/20, 1/40, and 1/80 for IgM), was added to the appropriate wells, the plates were incubated for 2 h at room temperature, and the washing procedure was repeated.

A 200-μl portion of a previously determined dilution of rabbit antiserum to human IgG, IgA, or IgM (Cal-biochem-Behring Corp.) in phosphate-buffered saline-Tween was added to the appropriate wells. The plate was incubated at 4°C overnight and washed as before. Finally, alkaline phosphatase-conjugated goat antirab-bit IgG (Sigma Chemical Co.) appropriately diluted in phosphate-buffered saline-Tween was added to all test wells in 200-μl amounts. After incubation for 3 h at room temperature and washing, 200 μl of p-nitro-

phenyl phosphate (1 mg/ml; Sigma Chemical Co.) in 10% diethanolamine buffer, pH 9.8, was added to each well. After 60 min at room temperature, 50 μl of 3 M NaOH was added to each well to stop the reaction. The absorbance of each well was read at 410 nm on a Microelisa Minireader, MR 590 (Dynatech Instruments Inc., Santa Monica, Calif.). Negative and positive serum controls as well as appropriate reagent controls were included in each run.

RESULTS

Serum immunoglobulins. The mean serum IgA value for 63 patients with AGN (179 mg/dl) was less than that for normal adults (288 mg/dl) (Fig. 1). In contrast, serum IgA values were increased
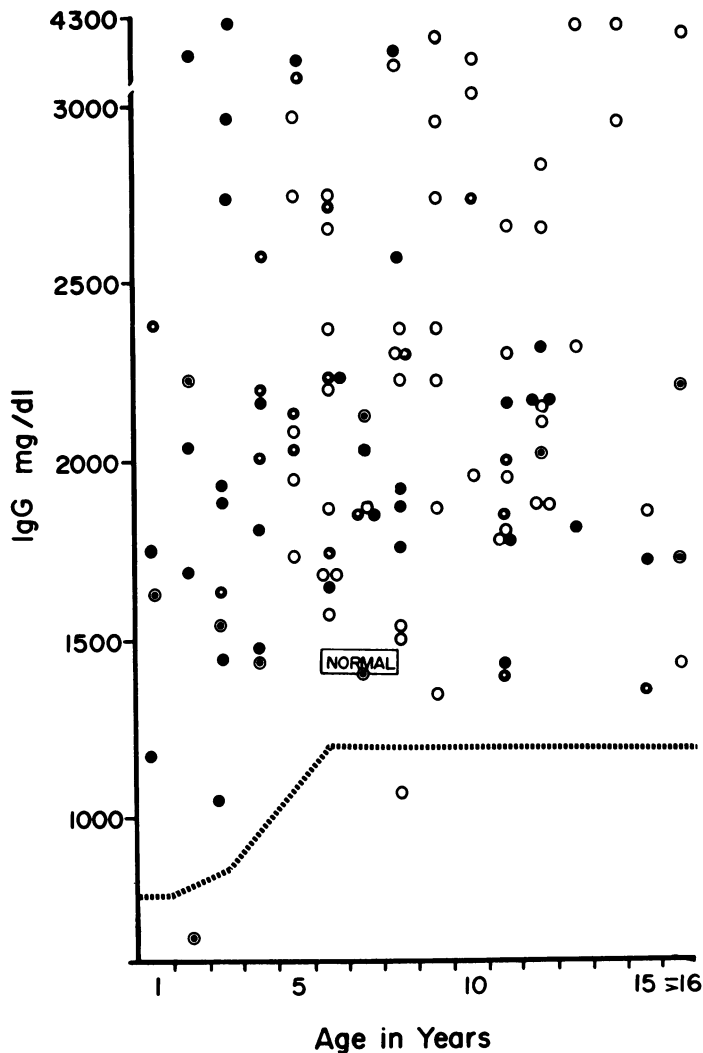
FIG. 3. Serum IgG values in AGN patients and ARF patients according to age. Symbols are the same as those used for Fig. 2. Normal means according to age are indicated by the heavy dotted line and are from the same source as those for IgA. The mean of 50 normal Trinidad school-children 5 to 8 years old is indicated by the box labeled "normal."

in most of 50 patients with ARF, with a higher mean value (372 mg/dl) than that for normal adults. The difference in IgA values in AGN and ARF patients was highly significant ($P <$ 0.0005). Serum IgA is slow to reach adult values, however, and when our values were plotted according to age (Fig. 2), they were found to be near the normal mean rather than below it in most of the children with AGN in contrast to being markedly increased in those with ARF. Exceptions to this observation were 12 AGN patients 5 to 7 years old who had increased values and 10 patients of scattered ages with ARF who had near-normal values. No relation

of these values to the presence of infected scabies versus other skin infections or to the presence of any skin infections versus no skin infections was noted (Fig. 1).

In contrast to IgA, IgG values were increased in both AGN (mean, 2,080 mg/dl) and ARF (mean, 2,345 mg/dl) patients (Fig. 1) when compared with normal adult values (mean, 1,200 mg/dl), and this remained so when plotted according to age (Fig. 3). The difference in IgG values in AGN and ARF patients was not significant ($P =$ 0.06). Amounts of serum IgG correlated overall with amounts of serum IgA in the ARF patients ($P < 0.001$; $r = 0.6278$) but not at all in the AGN
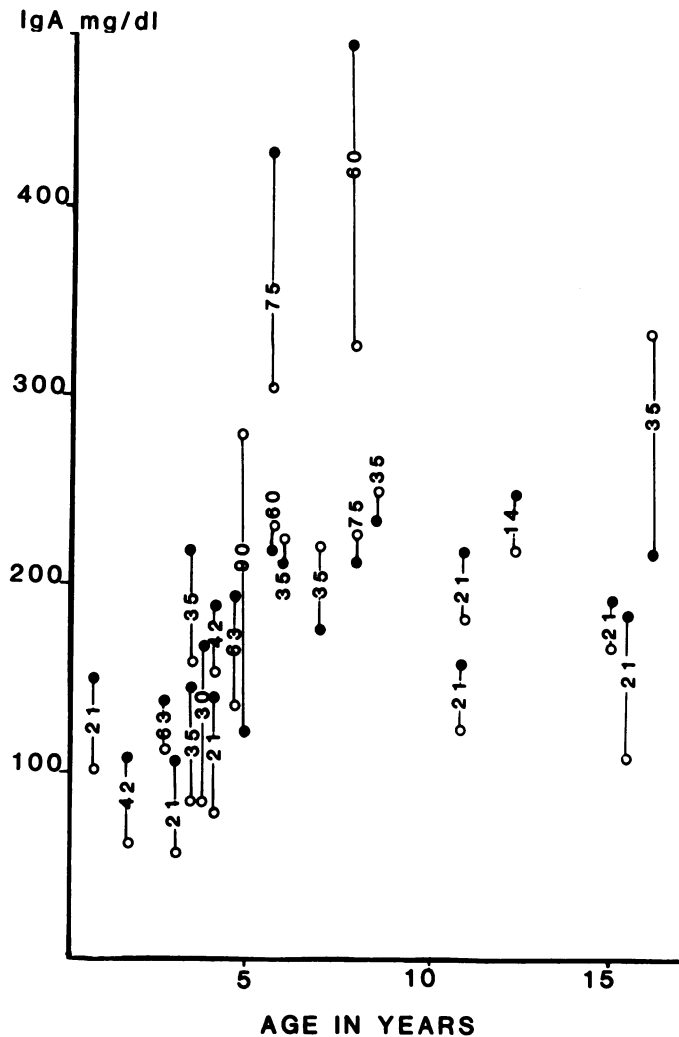
FIG. 4. IgA values in acute (○) and follow-up (●) sera of AGN patients. Days of follow-up are indicated between the two sera from each patient.

patients ($P < 0.1$; $r = 0.0434$). Again, this was due at least in part to the younger ages of many AGN patients and the normally delayed appearance of adult IgA values.

Follow-up serum IgA and IgG values (3 to 12 weeks after the onset of AGN) showed little increase or decrease (Fig. 4). Only 9 of the 24 sera studied differed more than 50 mg/dl in either globulin from the acute-phase serum.

Anti-M-type 12 antibodies. The bactericidal tests for M-type 12 antibodies gave indices of less than 3 for all sera tested except one AGN serum. Therefore, with one exception, there was no evidence of antibodies to the M-protein present in our preparation of MAP. One Chicago serum, known to contain antibody to M-type 12 protein, was used as a positive control. This serum gave a titer of 40 for TA-MAP and mean

ELISA readings (×100) of 185 for IgG, 189 for IgA, and 71 for IgM. In contrast, a Chicago serum known not to have antibody to M-type 12 protein used as a negative control gave a titer of 10 for TA-MAP and ELISA readings (×100) of 43 for IgG, 52 for IgA, and 41 for IgM.

TA-MAP by complement fixation assay. TA-MAP were increased (titers, ≥ 40) in more ARF patients (58% of 40 studied) than AGN patients (43% of 39 studied) ($P < 0.05$) and in more AGN patients than normal school-children (8% of 27 studied) ($P < 0.002$). Mean titers also were higher in ARF patients (42.8) than in AGN patients (31.9) ($P < 0.05$) and in AGN patients than in school-children (23.1) ($P < 0.002$). Cumulative titers of these antibodies in ARF patients, AGN patients, and school-children are shown in Fig. 5. Attempts also were made to
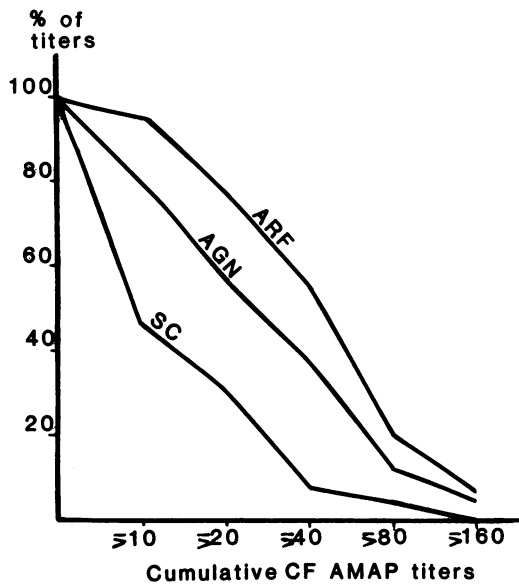
FIG. 5. Comparison of cumulative titers of TA-MAP in sera of ARF patients, AGN patients, and school-children (SC). CF, Complement fixation.

FIG. 6. Mean serum ELISA values for IgG, IgA, and IgM antibodies to MAP and ARF patients, AGN patients, and school-children. A_{410}, Absorbance at 410 nm.

correlate total amounts of serum IgG, IgA, and IgM with TA-MAP in ARF patients. These showed little correlation, which was, however, better for IgM ($r = 0.1841$) and IgG ($r = 0.1775$) than for IgA ($r = 0.0507$).

IgG, IgA, and IgM antibodies to MAP by ELISA. The dilutions showing the greatest differences in values between positive and negative sera were 1/1,600 for IgG antibodies, 1/40 for IgA antibodies, and 1/20 for IgM antibodies so these values were used for analyses of the data. Thus, there appeared to be more IgG than IgA antibody in all of the sera. However, there was not necessarily 40 times as much IgG as IgA antibody (as suggested by the optimal dilutions of 1/1,600 for IgG antibody assays and 1/40 for IgA antibody assays) since sera diluted 1/3,200 for the former had more than half as much IgG antibody (as indicated by readings at 410 nm) as sera diluted 1/1,600, whereas sera diluted 1/6,400 (a fourfold dilution of 1/1,600) had approximately one-half the readings at 410 nm of the 1/1,600 dilutions. Fortunately, we were able to compare readings at 410 nm of the same dilutions of sera from patients with ARF and AGN and normal school-children since these dilutions were optimal for each class of antibody in all study groups.

Respective amounts of IgG, IgA, and IgM antibodies bound by the MAP antigen from the sera of ARF patients were slightly higher than those bound from the sera of AGN patients which, in turn, were higher than those bound
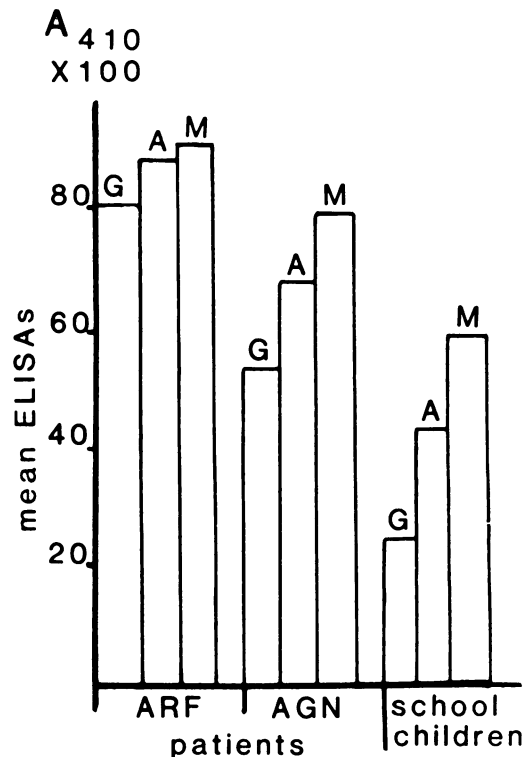
from the sera of school children (Fig. 6). Thus, these observations agree with those of the TA-MAP assays by complement fixation. More IgM than IgA and more IgA than IgG were bound at these dilutions from all of the sera studied, but these figures must be corrected for the dilutions of sera used for assay of respective classes of antibody, as discussed above.

Attempts were made to correlate the TA-MAP titers with IgG, IgA, or IgM antibodies by plotting them against each other (Fig. 7). Amounts of IgG antibody were greatest in the six ARF or AGN patients' sera with TA-MAP titers of 160, whereas this correlation was absent in the school-children: those with the highest TA-MAP titers (40 and 80) had increased IgM antibody rather than IgG antibody. IgA antibody titers increased with increase in TA-MAP titers in ARF patients ($P < 0.05$; $r = 0.3954$) but so did the IgG antibody titers ($P < 0.05$; $r = 0.3929$). IgA antibody titers showed no correlation with TA-MAP titers in AGN patients ($P > 0.1$; $r = 0.0301$), whereas IgG antibodies did ($P < 0.001$; $r = 0.6909$).

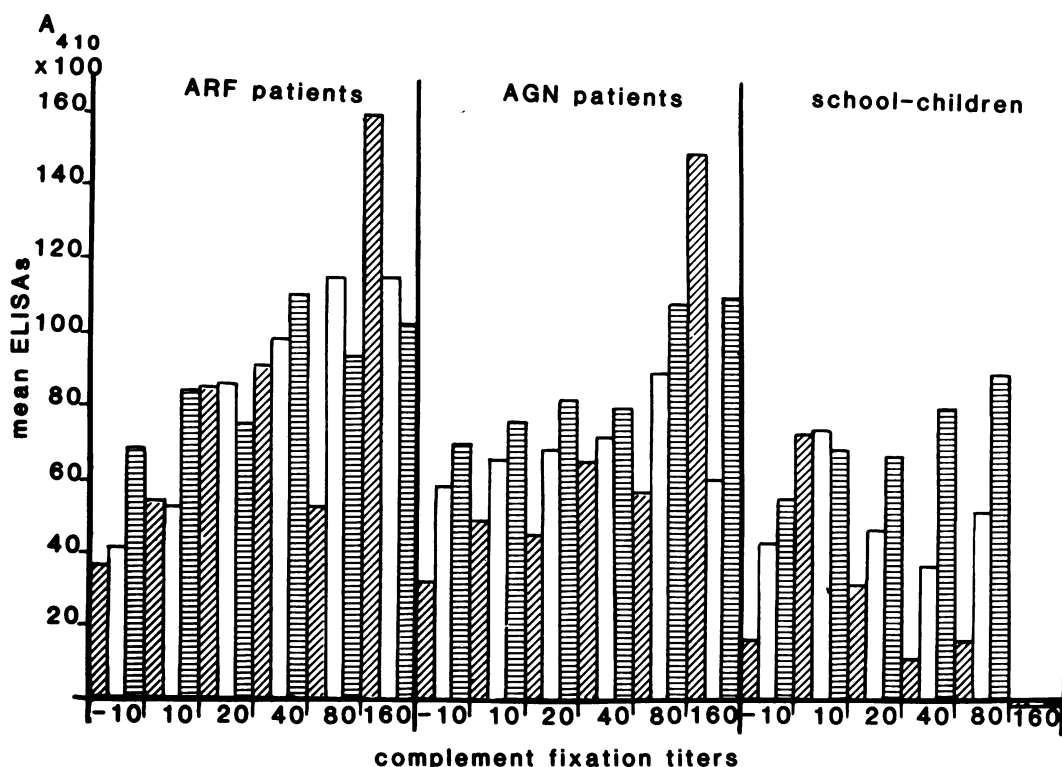Total amounts of antibody by ELISA (IgG plus IgA plus IgM) correlated generally with TA-

FIG. 7. Relationship to TA-MAP titers by complement fixation to IgG (□), IgA (□), and IgM (□) antibodies to MAP by ELISA. A_{410}, Absorbance at 410 nm.

MAP titers by complement fixation (Fig. 8). However, this correlation was due more to the IgG antibody than to the IgA antibody bound.

DISCUSSION

The main differences noted to date between patients with AGN and patients with ARF in Trinidad have been: a large number of children less than 5 years old with AGN versus very few with ARF; many skin infections in AGN patients versus very few in ARF patients; and different streptococcal types isolated from the two groups of patients and their families. Other investigators have described higher anti-streptolysin O (9, 12) and anti-group A carbohydrate (8) titers in ARF patients than in AGN patients, but we have failed to find these antibodies impressively or consistently different among the patients with AGN and ARF in Trinidad. Antihyaluronidase and anti-DNase B titers also have been similarly increased in the patients with AGN and ARF in our studies (5, 6). Therefore, we were happy to learn that Widdowson et al. (13, 14) found definite differences between TA-MAP titers in AGN and ARF patients, some of whom were patients in Trinidad. These observations led us to measure TA-MAP titers in additional Trinidad

patients in the present study so that we might examine the relationship of these titers to the increased levels of serum IgA we were finding in patients with ARF in contrast to patients with AGN. The TA-MAP titers were higher in ARF patients than in AGN patients, although the difference was less than that found by Widdowson et al. (13, 14).

When total amounts of serum IgA were compared with TA-MAP titers in individual ARF patients, no relationship was evident. The correlation coefficient was much lower than those for amounts of IgG or IgM, which also were not statistically significant. IgA antibody does not fix complement by the classical pathway, however, so that one might not expect correlation of IgA globulins with TA-MAP titers, which are measured by complement fixation. Aggregated IgA does activate complement by the alternate pathway, however, and thus may give a positive complement fixation test. Furthermore, the inability of IgA to activate complement by the classical pathway should not affect the ELISA, and the results of these tests also did not point to IgA as the major class of antibody reacting with the MAP. Indeed, there was probably 20 to 40 times as much IgG as IgA fixed by the MAP antigen in the ELISAs (based on optimal serum
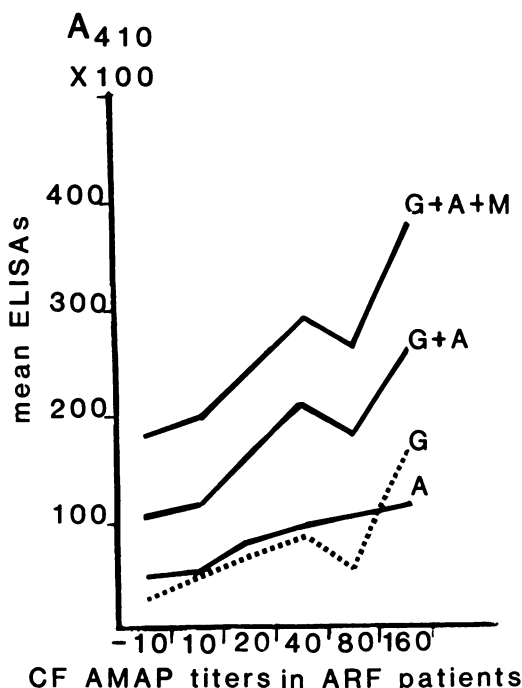
FIG. 8. Relationship of TA-MAP titers by complement fixation (CF) to TA-MAP by ELISA (IgG plus IgA plus IgM). A_{410}, Absorbance at 410 nm.

dilutions for these tests), whereas there is only five to six times as much total IgG as IgA in normal adult serum and an average of seven times as much in the ARF patients' sera in this study. In sera from the younger AGN patients, however, there was up to 52 times as much total IgG as IgA (3,540 to 67 mg/dl in one).

In other studies (E. V. Potter, J. B. Vincente, R. T. Mayon-White, M. A. Shaughnessy, T. Poon-King, and D. P. Earle, Am. J. Epidemiol., in press) we have examined secretory IgA in similar patients when they had recovered from AGN or ARF to look for basic deficits in this immunoglobulin. No decrease was found in the serum or saliva IgA of the recovered ARF and AGN patients and no difference in values was found between them. Furthermore, IgA did not appear to be secreted into the patients' sweat or into the sweat of normal siblings. In earlier work (10), we found no difference in amounts of IgA in saliva of acutely ill ARF patients and normal controls. Therefore, although IgA was less increased in the serum of AGN patients compared with ARF patients presently studied, it was not

deficient in the sweat, serum, or saliva of patients recovered from AGN nor does it seem to be specifically related to the more increased titers of TA-MAP found in the ARF patients.

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