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Variation of serum IgG subclass concentrations with disease activity in juvenile chronic arthritis

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SUMMARY Nineteen patients with juvenile chronic arthritis were followed up and serum IgG subclass concentrations measured at different stages of disease activity. Patients were divided into three groups according to clinical activity of the disease: active disease, partial remission, and remission. The results were compared with normal values obtained in 448 healthy children aged 6 months to 18 years with a homogeneous distribution for each year of age. Serum IgG subclass concentrations of each child were first log transformed and then age corrected, taking the deviation of the log transformed value from that expected for a child of the same age. It was found that patients with partial remission had increased concentrations of IgG2 and decreased concentrations of IgG1 compared with patients with active disease. This suggests that the remission inducing process, at least in juvenile chronic arthritis, is accompanied by a switch of IgG subclass production.

In a previous study we investigated the serum concentrations of IgG subclasses in juvenile chronic arthritis and found a significant increase of IgG3 ($p<0.0001$), IgG1 ($p<0.002$), and IgG2 ($p<0.035$) compared with age matched controls.[1] When patients were divided according to clinical activity of the disease a significant increase of IgG2 was observed in patients with partial remission compared with those with active disease ($p<0.05$) or with those with remission ($p<0.025$). This suggested the presence of a differential increase of IgG subclasses in relation to the course of juvenile chronic arthritis.[1]

To study this phenomenon further we followed up 19 patients with juvenile chronic arthritis and measured serum IgG subclass concentrations at different stages of disease activity. We found that patients with partial remission have higher concentrations of IgG2 ($p=0.032$) and lower concentrations of IgG1 ($p=0.002$) than patients with active disease. This longitudinal study confirms and extends, at the individual patient level, our previous results and strongly suggests that the remission inducing process is accompanied by a switch of IgG subclass production.

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Patients and methods

PATIENTS

Nineteen patients (six boys, 13 girls), ranging in age from 15 months to 15 years, were included in the study. All fulfilled the criteria for diagnosis of juvenile chronic arthritis and were classified according to type of onset by the criteria of the juvenile rheumatoid arthritis criteria subcommittee of the American Rheumatism Association.[2] Eight patients presented a systemic form, one a polyarticular form, and 10 a pauciarticular form. Patients positive for HLA-B27 or for rheumatoid factor or with IgA deficiency were excluded from the study. Patients were divided according to disease activity into those with active disease, partial remission, and remission. Disease was defined as active if there was evidence of synovitis on examination. Remission was classified according to the American Rheumatism Association criteria for rheumatoid arthritis[3]; those patients without active joint disease, but not meeting criteria for remission, were classified as having partial remission. Patients with active iridocyclitis but without synovitis were excluded from the study.

All children were studied during a single disease flare, in active disease, and in partial remission; seven patients were also tested while in remission. The interval between active disease and partial remission ranged from three to 16 months.

Twelve patients were receiving non-steroidal anti-inflammatory drugs (NSAIDs) alone, four NSAIDs and auranofin, two NSAIDs, auranofin, and prednisone, and one NSAIDs, prednisone, and methotrexate. Fifteen of the 19 patients were following the same drug regimen when studied in active disease and in partial remission.

The results were compared with normal values obtained in 448 healthy children (265 boys, 183 girls) aged 6 months to 18 years with a homogeneous distribution for each year of age (Plebani *et al*, unpublished data).

MEASUREMENT OF SERUM IgG SUBCLASSES

IgG subclasses were measured by radial immunodiffusion in 1–4% agarose in 0·1 M barbitone buffer pH 8·6 containing 6% polyethylene glycol (PEG 3000) (Fluka). Monoclonal antisera to IgG1 (JL 512), IgG2 (GOM 1), IgG3 (ZG4), and IgG4 (RJ4) (Unipath, Bedford, UK) were diluted in the agarose at concentrations of 0·4% for IgG1, 0·6% for IgG2, 0·25% for IgG3, and 0·8% for IgG4.[4] Test sera were diluted 1/40 for IgG1, 1/10 for IgG2, and 1/5 for IgG3 and IgG4 in barbitone buffer. Plates were left at 4°C for 48 hours, washed for 24 hours in barbitone buffer containing 1% glutaraldehyde, and stained with Coomassie brilliant blue. Results were calculated using a standard serum whose IgG subclass concentrations were calculated from WHO 67/97 serum.

STATISTICAL ANALYSIS

Serum IgG subclass concentrations of each child were first log transformed and then age corrected, taking the deviation of the log transformed value from that expected for a child of the same age. As previously mentioned the reference curves for IgG subclasses were obtained in our laboratory on a sample of 448 apparently normal children.

Scatter diagrams were drawn with standardised variables obtained at different stages of the disease. Because of the variation of IgG4 concentrations with age, which prevented construction of reference curves, analysis was not performed on the data of this subclass.

Binomial probabilities were calculated to verify the statistical significance of the proportion of children showing a decrease (or increase) in serum concentrations from active disease to partial remission. The number of patients in remission was too small to allow statistical analysis.

Results

A significant proportion of patients had lower serum concentrations of IgG1 during partial remission than during active disease ($p=0·002$). Of the 11 children above the 95th centile during active disease, five fell below it during partial remission; only one child showed the reverse pattern (Fig. 1). All seven

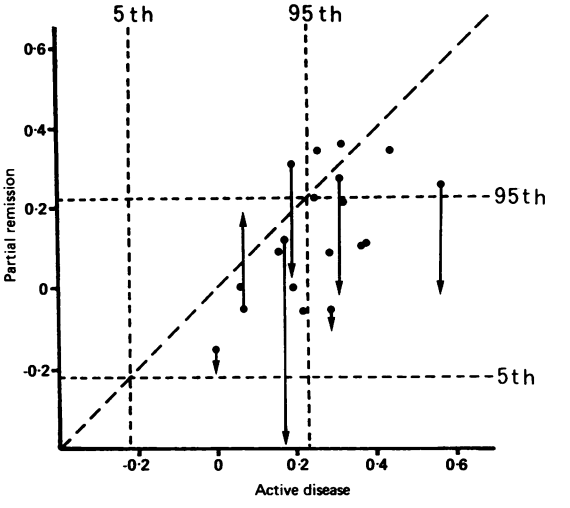

Fig. 1 *Scatter plot of serum IgG1 concentrations measured during partial remission v those measured during active disease. The arrow heads show the values reached in remission. The 5th and 95th centile values are shown in the figure beside the bisecting line.*

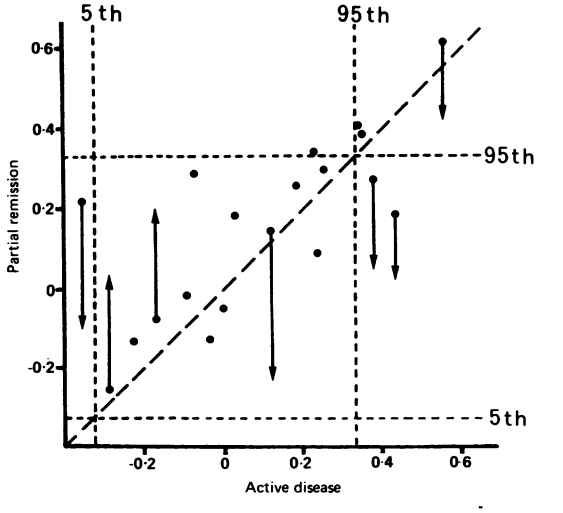

Fig. 2 *Scatter plot of serum IgG2 concentrations (see Fig. 1).*

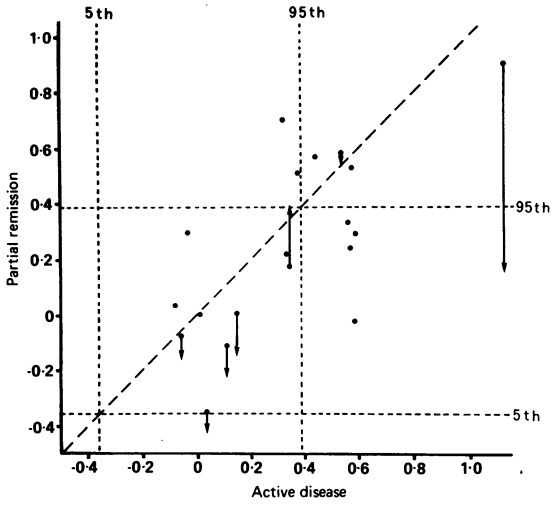

Fig. 3 *Scatter plot of serum IgG3 concentrations (see Fig. 1).*

children tested in remission had IgG1 concentrations below the 95th centile.

Fourteen of 19 patients showed an increase of serum IgG2 concentrations during partial remission compared with during active disease ($p=0·032$); in most (10/14) values were below the 95th centile (Fig. 2).

Although patients with partial remission tended to have lower concentrations of IgG3 than those with active disease, the difference was not significant ($p=0·082$) (Fig. 3).

The results obtained for IgG4 could not be evaluated because of the lack of normal reference values; indeed when we expanded our sample of healthy children from the 164 subjects used as reference in our previous study[1] to the 448 used in this study the wide dispersion of IgG4 values did not permit calculation of reference curves. A similar observation was also reported by Bird *et al.*[5]

Discussion

In this follow up study we have shown that patients with juvenile chronic arthritis show a significant increase of IgG2 ($p=0·032$) when the disease passes from active disease to partial remission; these findings confirm at the level of the individual patient the results obtained in our previous study.[1] Moreover, we have shown that this increase is accompanied by a significant decrease of IgG1 ($p=0·002$) and by a trend towards a decrease of IgG3, which is, however, not significant. As discussed in our previous paper[1] the IgG subclass response may depend on the nature of the eliciting antigen. Moreover, recent data suggest that in mice different subsets of T helper lymphocytes, expressing quite distinct sets of lymphokines, regulate IgG subclass restriction and very different sets of immune response.[6] In particular, it has recently been shown in mice that a small amount of interferon gamma stimulates the expression of immunoglobulin of the IgG2a isotype and inhibits the production of IgG1, whereas interleukin 4, another T cell derived lymphokine, promotes switching to the expression of IgG1. This increase of IgG2 together with a decrease of IgG1 when patients pass from active disease to partial remission suggests a switch in IgG subclass production during the remission inducing process; this switch could be of biological relevance as IgG2 have less inflammatory properties than IgG1 and IgG3.[1]

The possibility that IgG subclass response may depend also on the status of the inflammatory process must be taken into account in studies of serum IgG subclass concentrations or IgG subclass composition of antibodies in various autoimmune diseases, or both. Munthe and Natvig showed that IgG subclasses in rheumatoid synovial plasma cells of adult patients were present in varying quantities in individual patients, with a predominance of the same subclass in different joints of a given patient.[7] This may be explained as a difference in disease activity between patients rather than as a peculiar individual immunological reaction.

In conclusion, although the number of patients studied is limited, our findings suggest that the remission inducing process of the inflammatory reaction is accompanied, at least in juvenile chronic arthritis, by a switch in IgG subclass production from IgG1, and possibly IgG3, to IgG2. Whether this switch is directly involved in lessening inflammation or represents an indirect marker of the remission inducing process remains to be established. Further work is needed to determine whether the serum IgG subclass concentrations in the individual patient might be a useful index for predicting remission and monitoring drug efficacy in juvenile chronic arthritis, and possibly in other inflammatory disorders.

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