

Differences in Serum IgG Structure in Health and Rheumatoid Arthritis as Studied by Circular Dichroic Spectra. By PETER JOHNSON and JOHN WATKINS (*MRC Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks.*)

There is evidence for the presence of conformational anomalies in the serum IgG of patients with rheumatoid arthritis. Such evidence stems mainly from catabolic studies of normal IgG (from healthy volunteers) and rheumatoid IgG in man and in mouse; direct physico-chemical evidence has been lacking. Recently we have studied the circular dichroic (CD) spectra of serum IgG isolated from two healthy volunteers and from two patients with seropositive RA (volunteers E.S. and D.W.; IgG catabolic studies described previously (Watkins and Swannell, 1973)), together with IgG samples isolated from pools of normal and rheumatoid sera respectively. Some significant differences were observed between the normal and the rheumatoid IgG proteins and these observations are presented in this communication.

Circular dichroism (CD) spectra measure the unequal absorption of right and left circularly polarized light by optically active compounds. The main feature of the spectra of human IgG is a large negative maximum at 217 nm, which has been demonstrated to be characteristic of the β -structure of the protein (Doi and Jirgensons, 1970). At higher wavelengths there are smaller, but still characteristic, absorptions resulting from transitions in aromatic amino acid residues and from the asymmetric environment of disulphide linkages.

Using a Cary 61 recording spectropolarimeter, we found that the spectra of rheumatoid and normal IgG differed only in two regions, at 280 nm. and 294 nm. Rheumatoid IgG appears to have a decreased negative maximum at 280 nm, and an increased positive maximum at 294 nm. The variation in the spectra at 280 nm. is particularly interesting, since the hinge region is implicated in this transition from our parallel studies on the CD spectra of the IgG subfragments, Fab and F(ab')₂: only the latter fragment contains the hinge region.

From this it would appear that rheumatoid patients have a conformational anomaly in the hinge region of at least a proportion of their serum IgG molecules.

The observation that these spectral differences could be enhanced by multiple freezing and thawing of rheumatoid sera before fractionation of the IgG protein lends weight to a hypothesis that the structural anomaly occurs as a natural molecular ageing process *in vivo* and that abnormal quantities of these altered molecules are retained in the serum of rheumatoid patients where they may stimulate a variety of immune phenomena.

Discussion

DR. EVANS (*Bath*) Have you measured the CD spectra of normal IgG under denaturing conditions such as freezing and thawing?

DR. JOHNSON Yes. Limited denaturation by multiple freezing and thawing will produce a marked reduction in the negative absorption at 280 nm. of both normal and rheumatoid IgG. We have also studied grossly denatured

IgG samples that have been heated at 60°C. for 15 minutes. In these cases the CD spectra are drastically altered, indicating gross conformational changes.

DR. P. A. BACON (*Bath*) The thing most likely to produce structural alterations in IgG is antigen binding. Have you had a chance to look at antibody after binding with specific antigen?

DR. JOHNSON We have not looked at the spectra of specific antibody, nor of antibody dissociated *in vitro* from an antigen-antibody complex. Any conformational changes in antigen binding may depend on the antigen used. However, our previously reported catabolic studies do indicate some difference between 'normal' and 'antibody' IgG; but there is no evidence of excessive and specific IgG antibody production in the RA patient.

PROF. K. W. WALTON (*Birmingham*) In relation to the two slides comparing normal and rheumatoid IgG, you pointed out that in both there was an anomaly at 289 nm. which you interpret as being in the hinge region; I noticed that in the second of the paired comparisons there also appeared to be quite a marked anomaly in the region of 295 nm. Would you like to comment on that? I think the second of your suggested explanations was that there might be binding by another molecule. Gamma G globulin acts as a substrate for plasmin. Isolated gamma G in our laboratory does show slight alterations on storage as a result of the action upon it of traces of plasmin still present in the isolated preparation. Little is known about the site of attack of plasminogen but I think it is reasonable to suppose that it may attack in the hinge region.

DR. JOHNSON We believe that the changes in the 280 nm. region of the CD spectra are more significant than those at 294 nm. because the former represent almost a complete disappearance of a peak. The changes at 294 nm. represent small changes within a peak. We have not emphasized this part of the spectra since, during a study of the CD spectra of IgG subclass proteins, we found good reproducibility except in this region at 294 nm. To answer your second point, we have no evidence of plasmin in our IgG preparations, but in view of your comments we shall have to look for plasmin more critically. However, the changes in the CD spectra involve the whole of one peak, suggesting that most of the IgG molecules are involved, whereas the effects of plasmin should involve only a small proportion of the molecules.

References

- Doi, E., and Jirgensons, B. (1970) *Biochemistry (Wash.)*, 9, 1066
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Criteria for Classification of Systemic Lupus Erythematosus. By P. DAVIS and G. R. V. HUGHES (*Department of Rheumatology, Royal Postgraduate Medical School, London*)

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Discussion

DR. P. A. BACON (*Bath*) It seems to me that in many ways you do agree with the conclusions of Fries and Siegel