Development of antibodies during long-term therapy with corticotrophin in rheumatoid arthritis

II. Zinc tetracosactrin (Depot Synacthen)

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Porcine corticotrophin (ACTH) is antigenic in man (Fleischer, Abe, Liddle, Orth, and Nicholson, 1967; Landon, Friedman, and Greenwood, 1967; Norman and Sanders, 1969; Ratcliffe, Pritchard, and El-Shaboury, 1969; Glass and Daly, 1971). The predominant immunologically active site on the molecule lies towards the 'C' terminal end (Imura, Sparks, Grodsky, and Forsham, 1965). The synthetic corticotrophic peptide, tetracosactrin, which contains only the 'N' terminal 24 amino acids (Fig. 1) was thought to be unlikely to lead to antibody formation, because the immunologically active site of the natural 1-39 molecule had been removed, without loss of biological potency (Landon and others, 1967; Norman and Sanders, 1969). The 1-24 peptide should therefore be a still poorer antigen than the complete ACTH molecule.

FIG. 1 Diagrammatic representation of ACTH molecule, showing fragments used in this study.

In animal experiments, Felber, Ashcroft, Villanueva, and Vannotti (1966) were unable to raise antibodies to the unconjugated synthetic 1-24 peptide, but they showed that it might combine with antibodies raised against the full 1-39 peptide.

Ratcliffe and others (1969) demonstrated that ten out of 22 adults treated with tetracosactrin had significant titres of antibodies; however, most of their subjects had previously received porcine 1-39 ACTH. As antibodies raised to tetracosactrin would be directed to the part of the amino acid sequence of ACTH which is associated with biological activity, it may be that their development would result in impairment of the peptide's therapeutic efficacy. It seemed to us, therefore, to be of importance to know whether tetracosactrin acts as an antigen in man.

This paper reports the incidence of antibodies in subjects with rheumatoid arthritis treated with long-acting tetracosactrin (Zn-tetracosactrin, Depot Synacthen-Ciba) as the only form of ACTH therapy they had received, together with an attempt to establish whether there are multiple combining sites of the antibodies to the tetracosactrin molecule.

Materials and methods

A series of 38 subjects with rheumatoid arthritis, treated with long-acting tetracosactrin (Depot Synacthen) as their only form of ACTH therapy over a period ranging from 2 months to 3 years (mean 9 months), was studied. The total dose administered ranged from 6 to 170 mg. (mean 78.4). An equal number of subjects with rheumatoid arthritis, who had never received any form of hormone therapy, served as controls.

The following peptide fragments of the ACTH molecule were used in the various experiments: Synthetic α_h^{1-16} , α_h^{1-24} , and α_h^{17-39} . The nomenclature of ACTH peptides is as previously employed (Glass and Daly, 1971).

α_h^{1-24} ACTH and α_h^{1-16} was iodinated with ^{125}I by the method of Greenwood, Hunter, and Glover (1963). The 17-39 fragment was received iodinated with ^{125}I as a gift from Professor J.-P. Felber of Lausanne.

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The antigen-binding technique used for the detection of antibodies in serum was essentially that described by Landon and others (1967), except that labelled 1-24 peptide was used for the incubation, and other modifications and the criteria for identifying the presence of antibodies were as previously described (Glass and Daly, 1971).

The following experiments, designed to determine whether there were multiple antibody-combining sites, were carried out. Antigen displacement using 1-16 fragment was attempted on all but two of the subjects with antibodies. The displacement produced by excess 1-24 fragment was compared with the displacement produced by excess 1-24 peptide. The degrees of displacement produced by each peptide were thought to be similar when the difference between the percentages of labelled peptides remaining bound did not exceed the random variations between duplicates.

In eight subjects, antigen displacement studies were performed, using increasing concentrations of both 1-24 and 1-16 peptides. Concentration of added peptide was plotted against percentage binding, and the displacement curves obtained for the 1-24 peptide was compared with the displacement curves for the 1-16 fragment.

Incubation studies to determine the extent of binding to 17-39 fragments were also performed on sera from six of the subjects.

Results

Twelve of the 38 subjects (32 per cent.) showed antibody activity in their serum when incubated with labelled 1-24 peptide, and of the six of these whose serum was incubated with the 17-39 fragment one showed slight antibody activity. When excess unlabelled 1-24 peptide was added to the incubation mixture, the percentage of the labelled peptide bound was significantly reduced in all ten sera tested in this way (see Table).

When an excess of unlabelled 1-16 fragment was added to the incubation mixture with the same ten

Table Percentages of labelled 1-24 ACTH bound, with displacement studies using excess unlabelled peptides

Percentage 1-24 ACTH ¹²⁵ I bound			
Subject	With no unlabelled peptide added	With unlabelled 1-24 added	With unlabelled 1-16 added
W.C.	37	2	5
G.C.	51	4	16
D.F.	14	2	2
R.B.	7	5	5
S.R. (Fig. 3)	31	9	11
M.P. (Fig. 2)	32	3	14
J.R.	21	9	12
M.M.	6	0	1
J.S.	8	0	0
G.K.	6	3	4

sera, displacement was obtained in all, but in two of these (G.C. and M.P.) it was markedly less than the displacement produced by 1-24 peptide (Table).

Of the eight displacement curves obtained, six showed only shallow curves with varying concentrations of 1-16 fragment tested, whereas two others showed parallel displacement curves to those obtained with unlabelled 1-24 peptide. Two examples of these displacement curves are shown in Figs 2 and 3.

The patients who developed antibodies did not differ with respect to duration of therapy, total dose, or therapeutic regimen from those in whom antibodies were not detected.

Discussion

Although it has been shown by Felber and others (1966) that antibodies raised to the full ACTH 1-39

FIG. 2 The displacement curves—subject M.P., showing relative failure of the 1-16 peptide to displace labelled 1-24 peptide.

FIG. 3 The displacement curves—subject S.R., showing similarity of degree of displacement of bound labelled 1-24 peptide by unlabelled 1-16 and 1-24 peptides.

molecule may bind 1-24 peptide, this is the first time, to our knowledge, that the antigenicity of tetracosactrin has been documented in a series of human subjects, none of whom had previously received xenogeneic 1-39 ACTH. The long-acting tetracosactrin used in this study is combined with zinc, and it may be that without combination with zinc or some other adjuvant it would not be antigenic. This is suggested by the results of animal experiments in which only conjugates of the 1-24 peptide have proved antigenic. Felber and others (1966) failed to raise antibodies to the 1-24 peptide in guinea pigs, and Gelzer (1968) likewise failed with rabbits. Gelzer however did succeed in producing an immune response in one out of four rabbits treated with a protein conjugate of the 1-24 peptide.

The displacement studies with excess peptides suggested that there may be more than one site of combination, in that two of the patients' antisera permitted greater displacement of labelled antigen by 1-24 than by 1-16 peptide. A further eight permitted equal displacement. The dose-response curves provide better evidence that there is more than one antibody-binding site to the 1-24 peptide molecule. Six sera behaved similarly to that of subject M.P. (Fig. 2), in that increasing concentrations of 1-16 fragment failed to displace labelled antigen until excess had been added, whereas increasing concentrations of 1-24 peptide displaced progressively increasing quantities of the label, giving rise to a steep displacement curve. This suggests that the antibodies in those six sera were mainly directed towards the region of the peptide molecule between amino acids 16 and 24. In two other subjects, unlabelled 1-16 and 1-24 peptide each produced increasing displacement of label giving rise to parallel dose-response curves (see Fig. 3, Subject S.R.). Such parallelism between dose-response curves suggests identity of the immunologically active sites of the two peptides used (Aubert and Felber, 1969); hence in those two subjects the antibody-combining sites were probably between amino acids 1 and 16.

The sera of subjects in which displacement studies suggested greater affinity for 1-24 peptide than 1-16 fragment were also incubated with the labelled 17-39 fragment. The finding that only one of them bound the 17-39 fragment suggested that the antibodies in the other sera required part of the amino acid sequence on the 1-16 fragment for combination. As the ACTH fragments used in this study were selected simply according to availability, it is not surprising that they do not in fact correspond exactly to the naturally-occurring antibody-combining sites on the molecule.

Our findings suggest that the 1-24 peptide, tetracosactrin, when combined with zinc to form a

long-acting preparation, is definitely, albeit weakly, antigenic in man, and that the antibodies developed may be active against more than one site on the molecule. The incidence and titres of antibodies in these patients are similar to those found in a series of rheumatoid patients treated with natural porcine ACTH (Glass and Daly, 1971). The possibility that such antibodies may interfere with the biological efficacy of tetracosactrin is presently being investigated.

Summary

The antibody response to long-acting tetracosactrin has been studied in 38 subjects with rheumatoid arthritis, using an antigen displacement technique. None of the subjects had, at any time, received treatment with any other form of ACTH. Twelve (32 per cent.) of the subjects were found to have developed antibodies. Antigen displacement using α_h^{1-16} and α_h^{17-39} fragments as well as α_h^{1-24} peptide suggested that there is more than one antibody-combining site on the tetracosactrin molecule.

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D.G. is a Maynard Jenour Research Fellow and G.N. a Ciba Clinical Research Fellow.

DISCUSSION

DR. J. H. GLYN (*London*) West (1962) described acquired resistance to ACTH, which was attributed to the development of antibodies. Can you explain why you have found no similar correlation of clinical and antibody responses in patients receiving synthetic corticotrophin.

DR. GLASS The ACTH used at that time would have been impure; while noting 'resistance' to therapy and while speculating on possible association with antibody formation, no such immunological response was looked for in Dr. West's patients. The antibodies that we have been studying were directed at Synacthen. We have studied a similar series of patients treated with porcine ACTH and they have the same incidence of antibodies. We are not as yet able to detect patients with antibodies on clinical testing in either group.

DR. A. ST. J. DIXON (*Bath*) Is Synacthen immunologically pure?

DR. GLASS It may not be immunologically pure. Synacthen Depot (Ciba) contains at least 20 per cent. of the free peptide; it is not all linked to the depot. It is unlikely to be a pure antigen being synthetic and this is worth further analysis.

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