DIFFERENT ANTIGENIC DETERMINANTS IN THE POLYPEPTIDE CHAINS OF HUMAN COLLAGEN

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1. Introduction

Antibodies should provide a valuable tool to compare human collagen from different tissues as well as collagen obtained from normal or pathologic sources. To draw reasonable conclusions from such studies a detailed knowledge is required on the sites which react with the antibodies. The antigenic properties of soluble human collagen have already been the subject of several reports [1–3]. Expect for the investigation of Lindsley et al. [3] there was as yet no attempt to characterize the immunologic activity at the molecular level. Recently, distinct groups of antigenic determinants could be localized in the polypeptide chains of various collagens [4–7]. Such attempts became feasible by studying separated polypeptide chains of collagen or even smaller peptides as obtained after cyanogen bromide cleavage.

This approach was now applied to collagen from human dura mater. The present study demonstrated an immune response in rabbits to the α1- as well as the α2-chain with an appreciable heterogeneity of the antibody specificities involved. The major antigenic site is localized on the *C*-terminal cyanogen bromide peptide, α1-CB6. Additional antigenic determinants were found in *N*-terminal or middle regions of the molecule which, however, are recognized either less frequently or only with a low antibody response.

2. Experimental

Human dura mater was obtained from donors a few days to five years old at autopsy and was immediately extracted at 4° with 0.5 M sodium acetate containing 0.1 % EDTA. Subsequently the acid-soluble collagen was extracted either by 0.05 M citrate buffer pH 3.7 or 1% acetic acid and was purified by two precipitations with KCl [8]. The α-chains were separated by CM-cellulose chromatography [9] and the α2-chains further purified by molecular sieve chromatography [10]. Since the yields of acid-soluble collagen from human tissues are very low, the cyanogen bromide peptides (CB-peptides) were prepared from the insoluble collagen not dissolved by the extractions described above. One gram was suspended in 100 ml 70% formic acid and incubated together with 1 g CNBr for 4 hr at room temperature which resulted in a complete dissolution of the collagen. The reagents were removed on Bio-Gel P-2, equilibrated with 0.1 M acetic acid and the peptides were isolated by lyophilization. The single peptides used in this study were purified by conventional chromatographic techniques [11, 12] and were characterized by their molecular weight [13], by amino acid analysis and by electron microscopy [14]. Their designations followed the suggestions of Click and Bornstein [15] and a detailed report will appear elsewhere.

Antisera against the acid-soluble collagen were prepared in rabbits by injecting 1 mg antigen, incorporated into complete Freund's adjuvant at multiple subcutaneous sites, followed by an intraperitoneal injection of 2 mg antigen without adjuvant 14 days later. This immunization course was usually repeated once or twice. Blood was collected 7 days after the last injection. For the serological studies the antisera were fractionated into two antibody solutions by immunoadsorption on a column prepared from dena-

Table 1
Agglutinating activity for red cells coated with different α-chains of antibodies to human collagen.

Antibody fraction	Titer (− log 2) for red cells coated with[a]		
	Collagen	α1-chain	α2-chain
Without affinity for rabbit collagen	5.9 (4–8)	5.8 (3–8)	1.2 (< 1–3)
With affinity for rabbit collagen[b]	6.2 (4–9)	6.8 (4–10)	4.5 (1–9)

[a] Average results from 21 different antisera, the range is given in the brackets.
[b] Tenfold concentrated.

tured rabbit collagen [16]. The antibodies not bound onto the immunoadsorbent (antibodies without affinity for rabbit collagen) were concentrated by ammonium sulfate precipitation to the applied serum volume. The bound antibodies (antibodies with affinity for rabbit collagen) were eluted with calf collagen peptides and were usually studied after a tenfold concentration with respect to the antiserum volume applied.

The specificity of the antibodies was characterized by the passive hemagglutination method as previously adapted for collagen [17]. Tanned human red cells were coated either with denatured collagen, the single α-chains or different CB-peptides and the antibody solutions were titrated against such cells (agglutination test). In another approach (inhibition tests) the single α-chains or CB-peptides were added to the antibody solutions prior to the coated red cells and the titer reduction observed was recorded.

3. Results and discussion

3.1. *Polypeptide chain specificity of the rabbit antibodies*

Rabbit antisera to acid-soluble collagen from human dura mater contained antibodies which were specifically directed to the polypeptide chains of collagen as demonstrated by a comparable reactivity against red cells coated either with collagen or the α1-chain. The separation of the antibodies on a rabbit collagen immunoadsorbent into a major non-bound and a minor bound fraction allowed the differentiation of the speci-

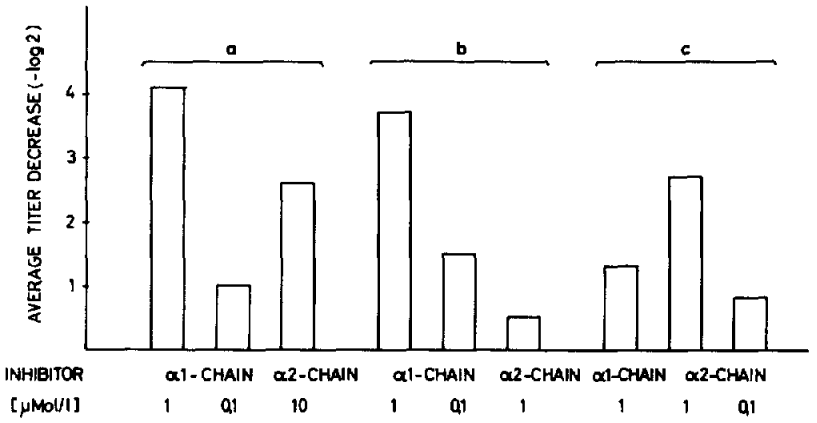

Fig. 1. Hemagglutination inhibition with α1- and α2-chains. Antibodies without affinity for rabbit collagen titrated vs. red cells coated with α1-chains (a), and antibodies with affinity for rabbit collagen titrated vs. red cells coated either with α1-chains (b) or α2-chains (c). Average results from 6 antisera in each group.

Table 2

Agglutinating activity of antibodies to human collagen for red cells coated with different CB peptides from the human α1-chain.

Antibody solution	Serum no.	Reciprocal titer vs. red cells coated with					
		Collagen	α1-chain	α1-CB3	α1-CB6	α1-CB7	α1-CB8
Without affinity for rabbit collagen	300	64	128	4	512	<2	<2
	301	128	128	4	512	<2	<2
	313	128	256	<2	4	<2	<2
	340	128	128	8	128	<2	<2
With affinity for rabbit collagen[a]	300	512	1024	16	128	1024	64
	301	128	128	8	64	256	64
	313	128	128	128	256	<2	8
	340	128	128	4	32	128	8

[a] Tenfold concentrated.

ficities concerned (table 1).

The antibodies which do not show affinity for rabbit collagen revealed high agglutination titers for α1-chain-coated red cells, but reacted not or only weakly with the α2-chain (table 1). Accordingly, α1-chains strongly inhibited the agglutination of red cells coated with α1-chains, whereas α2-chains proved to be more than tenfold less active (fig. 1a).

The antibodies with affinity for rabbit collagen reacted in the agglutination tests with both chains, although the activity was reduced for the α2-chain (table 1). Inhibition studies demonstrated heterogeneity of these antibodies (fig. 1b, c). The agglutination of the α1-chain or the α2-chain-coated red cells could be better inhibited by the corresponding than by the heterologous chain. The major part of the antibodies in a single solution seems, therefore, to be adapted to the α1-chain although some antibodies occurred which have a higher affinity for the α2-chain.

In gel diffusion it was demonstrated that the α1-chain is solely responsible for the antigenic activity of human collagen in rabbits [3]. The lower sensitivity of this serologic method when compared with the passive hemagglutination technique may, however, explain why no reaction with the α2-chain was observed.

3.2. *Localization of antigenic determinants on cyanogen bromide peptides*

Definitive proof for the diversity and heterogeneity of the individual immune response was obtained by studies with purified CB-peptides from the α1-chain. Some representative agglutination patterns for the two antibody fractions are given in table 2.

From 15 different antibody solutions which exhibit no affinity for rabbit collagen, 13 showed comparable titers for red cells coated with the α1-chains or the corresponding *C*-terminal peptide, α1-CB6 (cf. serum 300, 301, 340). This result was consistent with the finding, that α1-CB6 could effectively inhibit the agglutination of cells coated either with the α1-chain or α1-CB6. Since neither in the agglutination nor in the inhibition test a strong reaction was found for the other large peptides from the middle region of the α1-chain (α1-CB3, α1-CB7 and α1-CB8), it seems likely that α1-CB6 carries very specifically one of the major antigenic determinants of human collagen. This is in accordance with results obtained for calf skin [5] and rat skin collagen [6]. Likewise, the human antigenic determinant is very susceptible to the action of pronase and should therefore be located very close to the *C*-terminal end of the about 200 amino acids long sequence of α1-CB6.

One antibody solution (no. 313), however, revealed strong agglutinating activity for the α1-chain but not for α1-CB6. This reaction could be inhibited only by the *N*-terminal peptide, α1-CB1, but not by any other CB-peptide. Preliminary studies with this antibody solution showed complete cross reaction between

the peptides α1-CB1 of human and calf origin [18], which contain 20 and 19 amino acid residues, respectively, and differ only in one glycyl residue [12, 19]. These results indicated a very similar amino acid sequence for both peptides. As yet no evidence was obtained for an antigenic determinant in the *N*-terminal site of the human α2-chain, which seems immunologically important in guinea pig and rat skin collagen [4, 18].

The antibodies with affinity for rabbit collagen must differ in specificity from the other, since they react mainly with peptides derived from the triple-helical part of the polypeptide chain (α1-CB3, α1-CB7 and α1-CB8), but also with the terminal peptide, α1-CB6. Inhibition studies suggested that these reaction patterns may be best explained by assuming a heterogeneous antibody population which recognizes several different antigenic determinants. There are also considerable individual variations (cf. serum 300 and 313). Both results correspond to recent experience [7] on similar antibodies obtained from rabbit antisera to rat skin and calf skin collagen.

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