AGGLUTINATION OF IgG-COATED PARTICLES BY COLLAGEN

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1. Introduction

Structural similarities between C1q, one of the three subcomponents of factor C1 of complement, and collagen are now well documented [1,2]. However, it is still not established whether C1q and collagen share some common functional properties. A few data support this hypothesis with regard to interactions of collagen and C1q with immune complexes [3] and blood platelets [4,5]. We report here that polystyrene particles coated with human IgG (IgG–latex) are agglutinated by guinea-pig and human-skin collagen; the observed interaction is specific for the Fc region of IgG and for native collagen. This preliminary observation of collagen–IgG interaction was unexpected as a few other data [6,7] suggest that the C-terminal regions of the C1q subunits, and not the collagen-like segments, react with the immunoglobulins. The deposits of immune complexes in collagenous tissues [8] could perhaps be explained by the particular affinity of collagen for the Fc region of IgG.

2. Materials and methods

Most experiments were carried out with either neutral-salt-soluble or acid-soluble collagen purified [9] from guinea-pig skin. Human acid-soluble collagen was extracted from normal foetal skin kindly provided by Dr E. Eggermont (Academisch Ziekenhuis, Universiteit Leuven). The minced skin was first extracted twice for 24 h with 10 vol. 0.15 M NaCl. The residue was then extracted 3 times with 5 vol. 0.1 M acetic acid. The acid-soluble collagen was precipitated with 0.9 M NaCl in the presence of 0.1 M acetic acid and extensively dialyzed against 0.05 M Tris–HCl buffer (pH 7.5) containing 0.4 M NaCl. Bovine type I and type III collagens were isolated [10] from calf skin and bovine type IV collagen was prepared [11] from glomerular basal membranes kindly provided by Dr Ph. Mahieu (Université de Liège). Unless otherwise indicated, collagen solutions were prepared in 0.4 M NaCl.

Human IgG was isolated either as Cohn's fraction II (Institut Mérieux, Lyon) or by chromatography of normal human serum on DEAE-cellulose [12]. $F(ab')_2$ fragments of human IgG were prepared as in [13]; Fc fragments were obtained from Behringwerke (Marburg).

Polystyrene (latex) particles (0.8 μm diam.) from Dow Chem. Co. (Indianapolis) were coated with human IgG by simple adsorption [14]. The agglutination assay was carried out at room temperature by incubating equal volumes of a 0.05% (w/v) suspension of IgG–latex and of the collagen solution either in test tubes under continuous agitation or in a continuous flow system [14,15]. After appropriate dilution (2000 times) the non-agglutinated particles were counted in an AutoCounter (Technicon Instr. Corp., Tarrytown, NY) and the difference between the total number of particles and the number of free (non-agglutinated) particles was expressed as a percentage of the total number of particles.

Human serum albumin and human transferrin were from Behringwerke and bacterial collagenase (425 Units/mg) from Worthington Biochemical Corp. (Freehold, NJ). Enzymatic degradation of [^{14}C]collagen and of [^{3}H]casein was measured as in [16].

3. Results and discussion

Exploratory experiments with a manual agglutination test on a dark plate revealed a slight agglutination of IgG–latex by native salt- or acid-soluble guinea-pig-skin collagen. Far more evident and measurable agglutinations were detected by using the AutoCounter. The agglutination of IgG–latex increased linearly with increasing concentrations of native collagen over 0.1–0.7 mg/ml (fig.1). It was also dependent on the concentration of IgG used to coat the particles (fig.2). Agglutination was greatest around neutral pH.

Particles coated with human albumin or human transferrin were not agglutinated by collagen (table 1A), whereas they were agglutinated by specific antisera. IgG–latex was not agglutinated by heat-denatured (10 min at 60°C) collagen (table 1,B).

Incubation of native collagen with trypsin (22 μg/ml) for 4 h at 25°C did not abolish its agglutinating activity (table 1,C). Its trypsin-sensitive part (i.e., the non-helical telopeptides) or contaminating proteins sensitive to trypsin are thus not responsible for the agglutination.

The agglutinating capacity of collagen was progressively lost under the influence of a preparation of bacterial collagenase (fig.3) that was free of any

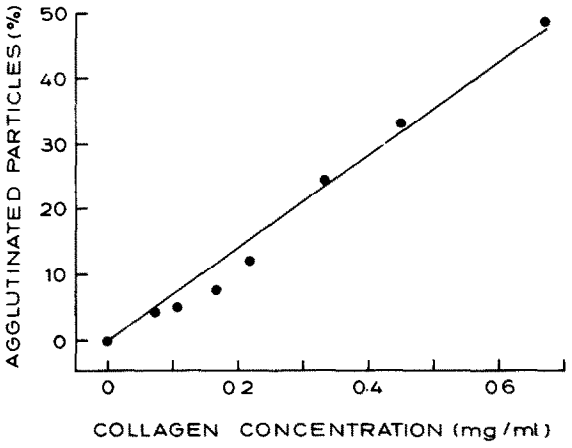

Fig.1. Influence of collagen concentration on the agglutination of IgG-coated polystyrene particles. Latex particles coated with human IgG were incubated in the presence of the indicated concentrations of acid-soluble guinea-pig collagen and assayed for agglutination.

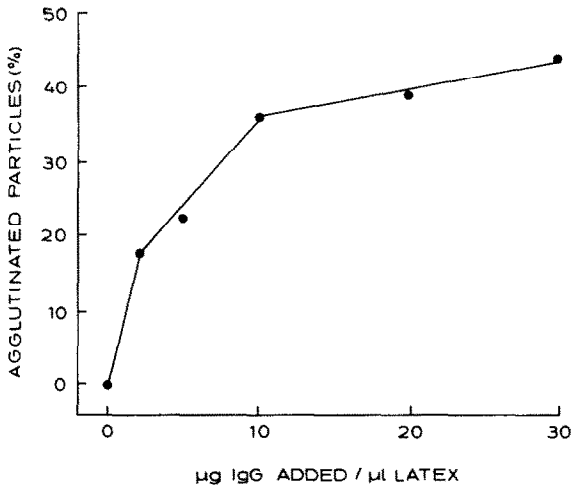

Fig.2. Influence of the amount of IgG used to coat latex on its agglutination by collagen. Coating of latex was done in the presence of the indicated amounts of human IgG. Salt-soluble guinea pig collagen (0.67 mg/ml) was used for the agglutination.

detectable caseinolytic activity at the concentrations used. The close correlation observed between the amount of degraded collagen and the loss of its agglutinating activity confirms our hypothesis that it is the triple-helical part of collagen and not another hypothetical trypsin-resistant protein that is responsible for the agglutination of IgG–latex. Even possible contaminating C1q could not account for the agglutination. Indeed C1q would have been inactivated by the treatments used for the purification of collagen, particularly the precipitation by trichloroacetic acid or ethanol.

Latex coated with the Fc fragment of human IgG instead of intact IgG was also agglutinated by collagen, while latex coated with the $F(ab')_2$ fragment of human IgG was not agglutinated (table 1,D). Agglutination can therefore not be due to the presence in the IgG preparations of either anti-collagen antibodies or fibronectin as the latter would have been degraded during the isolation of the Fc fragment [17]. Moreover fibronectin would interact with heat-denatured collagen [17].

The experiments described above were done with soluble collagen purified from guinea-pig skin. Human-skin collagen also agglutinated the IgG-

Table 1
Influence of various parameters on the agglutinating activity of collagen

Exp.	Coating of latex	Treatment of collagen	Agglutination (%)
A	Human IgG	No	57
	Human serum albumin	No	0
	Human transferrin	No	0
B	Human IgG	Replaced by solvent	0
	Human IgG	No	79
	Human IgG	Denaturation (10 min, 60°C)	0
C	Human IgG	No	35
	Human IgG	Trypsin (22 μg/ml, 4 h, 25°C)	32
	Human IgG	Trypsin + soya-bean trypsin inhibitor	28
D	Human IgG	No	48
	Fc fragment of human IgG	No	37
	$F(ab')_2$ fragment of human IgG	No	0

Variability of the controls from one experiment to another results from the use of different preparations of IgG–latex and collagen

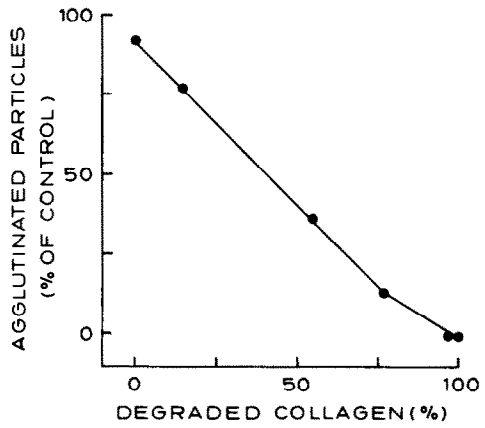

Fig.3. Influence of bacterial collagenase on the agglutinating activity of collagen. Salt-soluble guinea-pig-skin collagen (1.6 mg/ml) was incubated for 2 h at pH 7.5 and 25°C with various concentrations (0.01–4 μg/ml) of either active or, for the controls, heat-inactivated bacterial collagenase; no caseinolytic activity could be detected under these conditions. Each collagen preparation was then assayed for its agglutinating effect on IgG–latex and for the amount of degraded (non-gelifiable) collagen. Maximal agglutination (control) corresponded to 39% of agglutinated particles.

coated particles, but bovine collagen (types I, III, IV) did not. On the other hand, the amount of collagen that binds to the IgG–latex during agglutination must be very low (<1%) because we could not detect any significant diminution of the ^{14}C-labelled collagen concentration after sedimentation of the agglutinates, and the supernatants kept their initial agglutinating activity. These observations suggest that the agglutination of the IgG-coated particles is caused by a small proportion of the collagen, possibly by molecules that are in a specific conformation or state of aggregation. The influence of the concentration of collagen on the agglutination could thus reflect an equilibrium existing between these specific molecules and the non-agglutinating molecules of collagen.

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