Degradation of IgG3 Monoclonal Proteins During Storage and in the Presence of a Thiol (DL-Penicillamine)

Isolated IgG is known to undergo degradation into Fab- and Fc-like fragments after prolonged storage at 4 °C[1,2]. Similar changes are observed in isolated monoclonal proteins, irrespective of the heavy chain subclass. IgG in whole serum is reported, however, to remain stable during storage[2]. Nevertheless IgG3 monoclonal proteins frequently become degraded, even when stored in serum, whereas only 3 out of 40 monoclonal proteins of other subclasses showed such changes. Degradation of IgG3 proteins in whole serum appears to be potentiated or accelerated by treatment with DL-penicillamine[3] and the present paper examines the possible use of this effect for their identification.

Material and methods. 62 IgG monoclonal proteins were investigated either after isolation or in whole serum. 26 were typed as IgG1 (17 K, 9 L); 15 as IgG2 (5 K, 10 L); 14 as IgG3 (6 K, 8 L); and 7 as IgG4 (5 K, 2 L).

Isolation was by batch chromatography on DEAE (DE 52, Whatmann), according to STANWORTH[4], in some cases after preliminary precipitation in 1/3 saturated ammonium sulphate. IgG was quantitated by radial immunodiffusion[5].

Antisera. Anti-gamma, lambda and kappa chain sera, and anti-whole IgG serum were raised in sheep, in the Department of Experimental Pathology, University of Birmingham. Antisera to the heavy chain subclasses were raised usually by immunizing animals with one or a pool of several isolated proteins of a given subclass and absorbing the resulting antisera with proteins of the other subclasses. Sheep anti-whole IgG was rendered specific for IgG1 by absorption with normal Fab and monoclonal proteins of the other subclasses. Details of the whole procedure are given elsewhere[6].

Penicillamine treatment. 28 IgG monoclonal proteins, either isolated or in whole serum, were incubated at room temperature for 6–18 h (usually overnight) with 0.05*M* DL-Penicillamine (Sigma), a concentration which has been found to affect polymeric forms of immunoglobulins but not usually monomeric proteins[3,7,8]. After incubation they were examined by immunoelectrophoresis in 1% (w/v) agar (Difco's Noble Agar; Oxoid Ionagar No. 2) in pH 8.6 buffer containing 0.08*M* Boric acid, 0.005*M* Barbitone; 0.025*M* Barbitone sodium and 0.02*M* NaOH.

Results. Immunoelectrophoresis may reveal three different stages in the degradation of IgG monoclonal proteins during storage: a) Anodal shift of the apparently unaffected protein. b) Cathodal split of the IgG precipitin arc, due to the release of light chain-rich fragments (often associated with a). c) Complete split of the protein into Fab- and Fc-like fragments.

These changes were detected in proteins of all subclasses among a randomly examined population. However, IgG3 proteins appear to be more often affected as shown in Table I, and this subclass included the only protein detected in the most advanced stage of denaturation.

A sequence for the degradative events could be established in the case of an isolated IgG3 protein. A sample stored at 37 °C showed a shift of the precipitin arc towards the anode after 11 days. 4 days later the protein was completely split into Fab- and Fc-like fragments. In a sample kept at 4 °C, a cathodal split was detected after 28 days. 10 days later the bulk of the monoclonal protein had shifted to the anode, and the split was more evident. At day 47, the protein was completely split into Fab- and Fc-like fragments.

Table I. Degradation of IgG monoclonal proteins stored in whole serum

	Apparently undegraded	%	Evidence of degradation	%
IgG1	20	91	2	9
IgG2	11	91.5	1	8.5
IgG4	6	100	0	0
IgG1 + IgG2 + IgG4	37	92.5	3	7.5
IgG3	8	61.5	5	38.5

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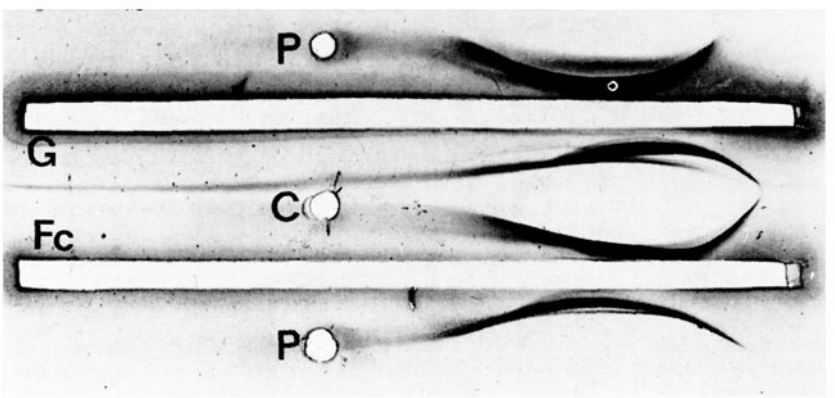

Fig. 1. Immunoelectrophoretic study of serum MG29 (IgG3 L), illustrating the increase in electrophoretic mobility induced by penicillamine. C, control samples; P, penicillamine-treated samples. Anti-whole IgG (G) and anti-IgG Fc (Fc) sera were used to fill the troughs. Anode to the left.

The changes induced by penicillamine in monoclonal proteins were found to be similar to those observed during storage. It also became apparent that their extent depended on the degree of degradation of the protein prior to treatment. As shown in Figures 1 and 2, although no changes were apparent in the control solution, an increase in the electrophoretic mobility was induced by penicillamine treatment. In one of the illustrated cases, a cathodal split also resulted. When a cathodal split was obvious prior to treatment, as illustrated by Figure 3, the result was a complete breakdown into Fab- and Fc-like fragments.

The usual effect of penicillamine treatment on isolated IgG1, IgG2 and IgG4 proteins was the increase in electrophoretical mobility, sometimes with tailing. 10 out of 15 proteins showed such changes without predominance of any subclass.

IgG3 proteins were always affected by penicillamine, whether isolated or not. However, when penicillamine treatment was carried out in whole sera, proteins of subclasses other than IgG3 were not affected, as shown in Table I.

Discussion. The spontaneous degradation of IgG into Fab- and Fc-like fragments during storage has been extensively studied[1,2,9-11]. It has been suggested that plasmin is the enzyme responsible for this degradation[2,10], and whole serum seems to contain a protective factor[1]. The actual split of normal IgG molecules appears to be preceded by dimerization of the molecule[1,11].

IgG3 proteins are undoubtedly more sensitive to denaturation changes during storage than proteins of any other subclass. This observation is consistent with their high sensitivity to enzymatic splitting[12-15] and faster catabolic rate[16,17].

The effects of penicillamine on IgG proteins could be explained in several ways. Increases in electrophoretic mobility might reflect the exposure of acidic radicals as a consequence of the scission of –S–S– bonds determining the tertiary configuration of the molecule[18]. Simultaneously, the molecule could become unfolded and more susceptible to proteolytic enzymes, explaining the release of light chain-rich fragments. Alternatively, penicillamine could potentiate a proteolytic enzyme, leading to the same result.

Table II. Effect of penicillamine when treatment was carried out in serum containing apparently undegraded monoclonal proteins

	No. of sera studied	Effect of penicillamine		
		No effect	Shift	Shift and split
IgG1	9	9	–	–
IgG2	2	2	–	–
IgG4	1	1	–	–
IgG1 + IgG2 + IgG4	12	12	–	–
IgG3	6	0	4	2

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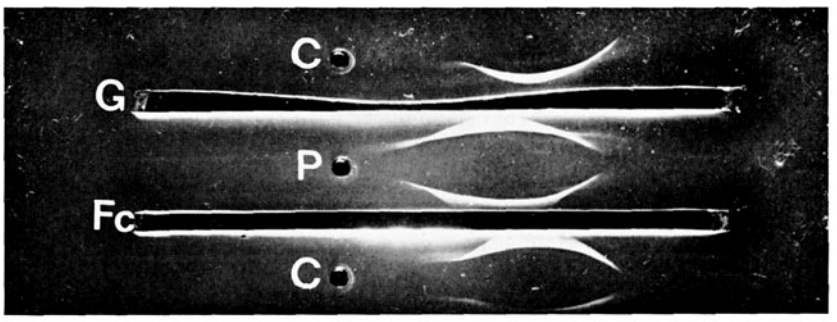

Fig. 2. Immunoelectrophoretic study of serum MG30 (IgG3 K) showing the association of anodal shift and cathodal split resulting from penicillamine treatment. C, control samples; P, penicillamine-treated samples. The troughs were filled with anti-whole IgG (G) and anti-IgG (Fc) sera. Anode to the left.

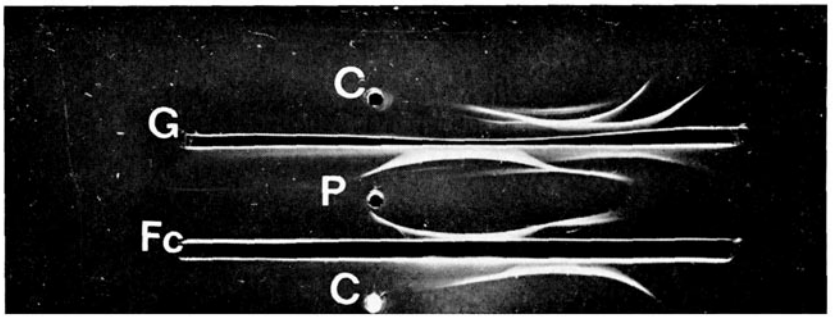

Fig. 3. Immunoelectrophoretic study of serum MG29 (IgG3 L). The control sample, kept at 4 °C during 4 weeks, shows a very evident cathodal split. Penicillamine induced a complete breakdown into Fab- and Fc-like fragments. C, control samples; P, penicillamine-treated samples. Anti-whole IgG (G) and anti-IgG Fc (Fc) were used to fill the troughs. Anode to the left.

The accentuation of previously existing changes, as illustrated by Figure 3, could be better explained if penicillamine acted by reducing residual –S–S– bonds holding together the fragments resulting from previous proteolysis, as suggested in relation to papain digestion of IgG[19].

The high susceptibility of IgG3 proteins to penicillamine treatment and to denaturation during storage could be a result of a loose tertiary structure. Under those circumstances the protein molecules would be more easily unfoldable and would become more readily susceptible to degradation.

Penicillamine treatment, by revealing these differences, appears as a promising system for the identification of IgG3 monoclonal proteins[20,21].

Résumé. Il est bien connu que les immunoglobulines IgG maintenues longtemps à des températures voisines de 0 °C sont dénaturées. L'addition de pénicillamine au sérum peut accentuer cette dénaturation. Dans ces conditions, les immunoglobulines du type IgG3 sont les plus sensibles et le traitement par la pénicillamine peut être utilisé pour les identifier.

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Immunological Adjuvants IV. Relationship between Adjuvant Activity and Antigenicity in Mycobacterial Adjuvant

BRAUN et al.[1,2] postulate that an antigenicity of the lipopolysaccharide (endotoxin) of Gram-negative bacteria may play an important role in its adjuvant activity. They also inferred a similar mechanism operating in relation to mycobacterial adjuvants[2]. SCHIERMAN and MCBRIDE[3] reported the necessity of antigenicity for an adjuvant located in a chicken erythrocytes.

Lipopolysaccharide of tubercle bacilli designated as wax D[4] behaves as an adjuvant[5]. Endotoxin and wax D have many properties in common[4,6]. We now report, however, that antigenicity of wax D appears not to be correlated with its adjuvant activity.

Materials and methods. We have recently isolated a 'pure' wax D containing little or no tuberculin-sensitizing antigen from H37Ra strain cultivated 4 weeks on Sauton medium. A subfraction AD6 was prepared by acetylation from the 'pure' wax D as described previously[7].

Guinea-pigs were sensitized with a single injection into the right hind foot pad of 0.2 ml of the water-in-oil emulsion containing 1 mg of usual wax D isolated from the strain H37Ra with or without 1 mg of twice-recrystallized ovalbumin (Sigma Chemical Co. USA) or 3 mg of the AD6 subfraction with or without 1 mg of the ovalbumin. The emulsion was prepared by mixing 0.7 ml of a phosphate buffered saline solution, 0.1 ml of Arlacel A and 0.6 ml of Drackeol. Zero, 5, 10, 15, 21 and 28 days after the sensitization, animals were bled for the assay of antibody titers and 35 days after the sensitization skin and corneal tests were performed. Antibody titers were measured by passive hemagglutinations using sheep erythrocytes sensitized either by ovalbumin or tuberculo-protein π^8 according to Persellin's technique[9], or by the water-soluble portion of wax D, as described previously[10]. Figure 2 shows data obtained on day 35 only.

Results and discussion. As shown in Figures 1, 2 and Table, the group I which had received usual wax D alone showed positive corneal and skin reactions to the tuberculo-protein and produced antibodies against the tuberculo-protein as well as to the water-soluble portion of wax D, indicating that usual wax D has antigenicities. When the antigen ovalbumin was added to this wax D in the sensitizing emulsion, animals exhibited high levels of corneal and delayed type skin reactions to the tuberculo-protein and ovalbumin and produced high titers of antibodies to ovalbumin, as well as to tuberculo-protein and the water-soluble portion (group II).

On the contrary, as shown in Figures 1, 2 and Table, corneal and skin reactions of the group III injected with only AD6 were completely negative and no antibody production occurred. Addition of ovalbumin to this AD6 in the sensitizing emulsion resulted in a marked antibody production to ovalbumin and in high levels of corneal and delayed type skin reactions to ovalbumin (group IV). Again, no immunological response was detected to the antigens associated with the usual wax D or tubercle bacilli. Guinea-pigs in group V which had received ovalbumin alone exhibited the positive but only immediate skin reaction.

In other experiments guinea-pigs which had received even greater amounts of AD6 (5 and 10 mg per animal)

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