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Catabolism of Human IgG in Mice Sensitized to Various IgG Fragments

SIMILARITIES TO THE CATABOLISM OF RHEUMATOID IgG IN MICE

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Summary. Whole body elimination studies of human serum IgG have shown that C57Bl mice are tolerant to this protein at low concentrations. The present study demonstrates that tolerance to this protein may be broken by presensitization of the mouse with the pepsin-derived fragments of human IgG ($F(ab')_2$ and pFc'), in marked contrast to the papain-derived fragments (Fab and Fc). Sensitization with $F(ab')_2$ fragments induced a distinctive elimination pattern of the intact protein which was analogous to that observed in non-sensitized mice injected with serum IgG isolated from patients with rheumatoid arthritis. Since, by circular dichroism studies, we have previously implicated a structural anomaly at or near the hinge region of the 'rheumatoid' IgG molecule, our observations are discussed in relationship to a possible immune aetiology for rheumatoid arthritis.

INTRODUCTION

The enzymes papain and pepsin hydrolyse IgG into well characterized fragments. Papain cleaves the protein into three fragments of approximately equal size, two of which, the Fab fragments, are identical. The other fragment is antigenically distinct and is known as the Fc fragment. In contrast, pepsin hydrolyses the molecule to produce the $F(ab')_2$ fragment (molecular weight 110,000), the pFc' fragment (molecular weight 27,000) and small peptides. In a previous communication we reported on the catabolism of these fragments and of the intact protein in both man and mouse (Watkins, Turner and Roberts, 1972).

The experiments showed that at least 93 per cent of the experimental animals were tolerant to serum IgG isolated from normal healthy volunteers, but that serum IgG isolated from patients suffering from rheumatoid arthritis was considerably more immunogenic in the mouse; we suggested that this might indicate a structural anomaly in 'rheumatoid IgG'. Studies of the circular dichroic spectra of normal and rheumatoid IgG have substantiated the presence of a structural anomaly in the latter protein, located at or near the hinge region of the molecule (Johnson and Watkins, 1974; Johnson, Watkins, Scopes and Tracey, 1974). These experiments suggested to us that the observed

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immunogenicity of rheumatoid IgG in the mouse may arise from determinants which are not exposed in the normal molecule. Similar determinants may also be exposed following proteolytic scission of the molecule, notably on the $F(ab')_2$ fragment which contains the hinge region. We postulated that sensitization of mice to the various human IgG fragments prior to or simultaneously with the study of the elimination of the intact normal molecule might induce the abnormalities of whole body elimination previously observed with rheumatoid IgG.

MATERIALS AND METHODS

Animals

Inbred strain C57Bl mice were used in all experiments. The animals were young adults, approximately 3 months old. The mice were given iodine water to block thyroid uptake of radioiodine before and during the experiment.

Immunoglobulin materials

The papain and peptic fragments prepared by digestion of Kabi Cohn Fraction II IgG, were generously donated by Dr M. W. Turner. This Kabi Cohn Fraction II IgG preparation was also used for challenging the mice following sensitization with IgG fragments. Previous catabolic experiments had shown that removal of dimeric IgG from Kabi preparations by gel filtration had no effect on the immunogenicity of this protein, although differences were observed between the initial rates of catabolism of the dimer-containing, and the dimer-free, preparations when measured in mice.

Radioisotope procedures and experimental protocol

The Kabi IgG preparation was labelled with ^{125}I by the iodine monochloride technique of McFarlane (1958) and filtered through a sterile Millipore filter (0·45 μm) prior to injection into mice, to ensure both sterility and the removal of aggregated protein.

Groups of six, twelve or eighteen male mice were used in the experiments. Mice were injected intraperitoneally (0·2 ml) with 0·3 mg of each fragment, or mixtures of fragments or with unlabelled IgG as a control. Five weeks later each mouse was injected intraperitoneally with 0·2 mg of ^{125}I-labelled human IgG with an activity of 0·3–0·5 μCi. In two experiments pFc′ fragments were mixed with the labelled IgG in the challenge dose and as a control the labelled IgG and pFc′ mixture was also injected into non-sensitized mice. The loss of radioactivity in the mice was followed by daily whole body counting measurements made over 15 days in a modified Panax Gamma One-Sixty scintillation counter (Panax Equipment Ltd, Surrey).

RESULTS

With or without presensitization, three basic IgG whole body elimination patterns were recognized in the mice used in these experiments. These patterns are shown in Fig. 1: (a) a normal log-linear elimination; (b) a primary immune pattern with rapid elimination of the injected material (mice showing this pattern invariably gave secondary type response curves on a subsequent challenge with human IgG); (c) patterns intermediate to these that show an initial break followed by rapid elimination of only part of the injected material. Previous catabolic experiments (Watkins *et al.*, 1972) in C57Bl mice using human

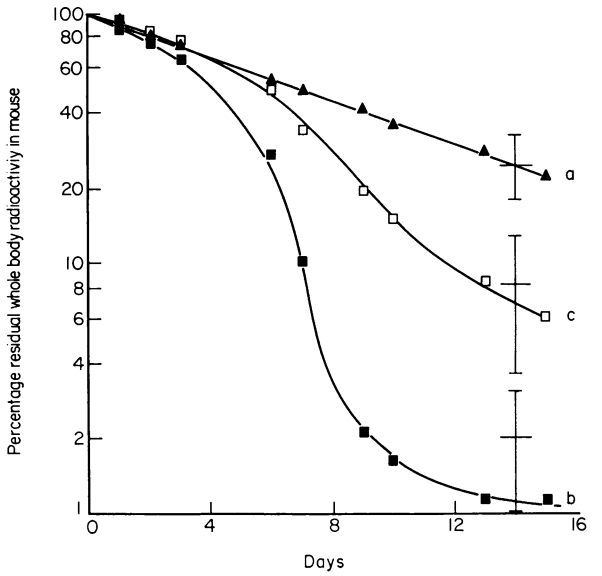

FIG. 1. Typical elimination patterns of human IgG in mice. (a) Normal. (b) Immune. (c) 'Rheumatoid-type'. These three patterns form the basis of the classification reported in Table 1. The vertical bar lines at 14 days indicate the ranges within which the elimination patterns lie. These show the mean residual human IgG measured in the mice at this point and the standard deviations from the mean. The ranges were calculated from the data of twenty examples of each elimination pattern, obtained from several experiments.

rheumatoid IgG have shown a high proportion of the latter elimination pattern (Fig. 1, pattern c), particularly among female mice, and we have come to consider this as 'rheumatoid-type' elimination.

The results of the present study on C57Bl mice presensitized to the fragments of human IgG and subsequently challenged with intact human IgG are summarized in Table 1. The results on the mode of elimination of the intact challenge IgG are expressed as normal, immune or rheumatoid-type on the basis of the classification illustrated in Fig. 1. Immune responses for the most part appeared to be a primary phenomenon, although some secondary responses, with a characteristic exponential decay apparent from the 1st day of the experiment were also observed. Both types are reported immune. Odd numbers of mice

TABLE 1

ELIMINATION PATTERNS IN PRESENSITIZED MICE

Sensitizing protein	Challenge protein	Number of C57Bl mice in the experiment	Catabolic pattern	Mean $T_{\frac{1}{2}}$ (days)
IgG	IgG	10	10/10 Normal	6·2
Fc+Fab	IgG	16	15/16 Normal	5·3
$F(ab')_2$+pFc′	IgG	12	12/12 Immune	
None	IgG+pFc′	17	13/17 Immune	
			4/17 Normal	5·8
$F(ab')_2$	IgG	11	3/11 Normal	5·4
			8/11 Rheumatoid	
pFc′	IgG	12	12/12 Immune	

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in Table 1 indicate the death of one or more of the original group of mice during the experiment.

Mice sensitized with intact, normal IgG and also those sensitized with the papain-derived fragments, Fab plus Fc, demonstrated no immune or immune-type elimination of the challenging dose of human IgG. Rheumatoid-type elimination patterns (Fig. 1, pattern c) were observed only in mice sensitized with $F(ab')_2$ fragments and not in mice sensitized with other fragments or combinations of fragments. The rheumatoid-type elimination of intact IgG was observed for eight out of the eleven mice presensitized with $F(ab')_2$, the other three mice showed a normal elimination of intact IgG. The pFc' fragment proved to be extremely immunogenic, eliciting an immune response in all of the mice sensitized with this fragment and in a high proportion of non-sensitized mice when injected as a mixture with intact IgG.

DISCUSSION

The rates of catabolism of the fragments of human IgG in the mouse have been previously reported (Watkins *et al.*, 1972). With the exception of the Fc fragment, which contains the catabolic site and has a half life similar to mouse IgG itself ($T_{\frac{1}{2}}$ = 4·8 days) the remainder are rapidly catabolized with half-lives of the order of 24 hours and would not compete with intact challenge IgG for preferential catabolism.

Presensitization of the mice to Fab and Fc fragments did not stimulate a response to intact IgG. It was surprising to discover the extreme immunogenicity of the pFc' fragment in the mouse, since previous studies (Watkins *et al.*, 1972) had indicated a very rapid clearance (within 24 hours) of the fragment from the mouse. Presensitization of mice with $F(ab')_2$ fragments gave rise to an IgG elimination pattern in eight out of eleven mice tested that was identical to that previously observed with 'rheumatoid' IgG (Fig. 1, pattern c). This proportion of mice, however, is significantly higher than that reported for male mice injected only with rheumatoid IgG (Watkins and Swannell, 1972), indicating the exposure on the $F(ab')_2$ moeity following enzymic cleavage of groupings more immunogenic than those present at the hinge region of the rheumatoid IgG molecule. The distinctive IgG elimination pattern in these mice probably results from the elimination of a soluble immune complex. Hence the system shows similarities with the presence of rheumatoid factor and its soluble complexes in man.

Sensitization of the mouse by $F(ab')_2$ fragments to produce 'rheumatoid-type' elimination patterns of intact IgG does not appear to be specific to human immunoglobulins since we have been able to demonstrate the same elimination pattern with mice sensitized to bovine $F(ab')_2$ fragments. In this case eleven out of twelve mice gave rheumatoid-type elimination curves when challenged with intact bovine IgG (Watkins and Paine, unpublished work).

We have previously reported other data indicating a conformational anomaly in the hinge region of rheumatoid IgG (Johnson *et al.*, 1974; Watkins *et al.*, 1972, Watkins and Swannell, 1972). This structural anomaly may be compatible with the appearance in rheumatoid arthritis of 19S rheumatoid factor reacting with determinants in the Fc region of human IgG (Turner, Stanworth, Normansell and Bennich, 1969) and having the same binding affinity for autologous and homologous IgG (Normansell, 1970). We suggest that conformational changes at the hinge region of IgG may be necessary

to increase the affinity of non-specific binding to B cells, which in turn allows the stimulation of those B cells that carry a specific receptor for the antigenic part of the molecule, an unaltered site in the Fc region. Hence T-cell tolerance to this self-protein could be by-passed. Indeed, we have recently obtained evidence that rheumatoid IgG and notably $F(ab')_2$ fragments, express an increased non-specific binding to B cells.

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