Immunoglobulin M: Fixation of Human Complement by the Fc Fragment

Abstract. *The Fc fragment [$(Fc)_5\mu$, with a molecular weight of 342,000] of human immunoglobulin M from patients with Waldenström's macroglobulinemia has a complement fixing ability approximately 19 times greater on a molar basis than that of the parent immunoglobulin M. This fragment, which occurs naturally as a pentamer fixes human but not guinea pig complement, and this activity remains unchanged even after the fragment is reduced to the monomeric form.*

Antibodies of the immunoglobulin M (IgM) class are capable of fixing complement, and by analogy with IgG this property is generally attributed to the Fc portion of the molecule. No direct confirmation of this has been reported, however, probably in large part because of the difficulty of obtaining sufficient quantities of pure Fcμ. Although the minimum unit for complement fixation of IgG consists of two adjacent antibody molecules linked by an antigen bridge, fixation can occur with a single IgM antibody molecule (*1*). Immunoglobulin M (whose molecular weight is 8.5×10^5) is composed of five apparently identical 7*S* subunits linked by disulfide bonds, probably into a circular pentamer (*2*). Studies of the effect of ionizing radiation on IgM (*3*) indicate that it contains more than one complement-combining site and that such sites become available for complement component attachment when IgM antibody reacts with antigen.

Trypsin has been used for the proteolysis of IgM at 56° to 65°C, so that it is possible to isolate the intact pentameric $(Fc)_5\mu$ in good yield. Using these Fc preparations as well as other proteolytic products of IgM, we have studied the ability of these fragments to fix complement in the absence of antigen-antibody reactions. The components studied for complement fixation were as follows: (i) IgM, intact macroglobulin, with a molecular weight of 845,000, and obtained from patient Dis; (ii) $(Fc)_5\mu$, purified pentameric Fc fragment of IgM, with a molecular weight of 342,000; (iii) Fcμ, the monomer obtained after reduction (0.01*M* dithiothreitol) and alkylation of the pentamer $(Fc)_5\mu$, with a molecular weight of 34,300; (iv) Fabμ, the Fab fragment of IgM, with a molecular weight of 42,000. The prefix BDB means that the component has been aggregated by bis-diazotized benzidine according to the method of Ishizaka and Ishizaka (*4*).

Purified Waldenström IgM of λ and κ type were proteolyzed by trypsin at 65°C for 7.5 minutes (0.05*M* tris-HCl buffer, *p*H 8.1, with 0.0115*M* $CaCl_2$; the ratio of enzyme to protein being 1 : 20 by weight). The products of digestion were separated into $(Fc)_5\mu$ and Fabμ by chromatography on Sephadex G-200 in an eluting system consisting of 0.01*M* phosphate buffer and 0.15*M* NaCl, *p*H 7.3. The technical details of these experiments and the characteristics of the fragments have been previously reported (*5*).

Complement fixation reactions were performed as described (*6*) except that the procedure was modified slightly for use with human complement. A portion (0.2 ml of an appropriate dilution) of the IgM or fragment was incubated with 0.2 ml of normal human serum in the presence of 0.7 ml of triethanolamine-buffered saline (*6*) for 1 hour at 37°C. Controls included buffer blanks and Sephadex column effluents free of protein. The complement before and after fixation was titrated, and the percentage of complement fixed was calculated as the ratio of the complement fixed to the activity of the controls. In order to determine whether $(Fc)_5\mu$ had skin reactive properties, serial dilutions of this material were injected intradermally into guinea pig skin. After the injections, the animals received a 2 percent solution of pontamine sky blue intra-

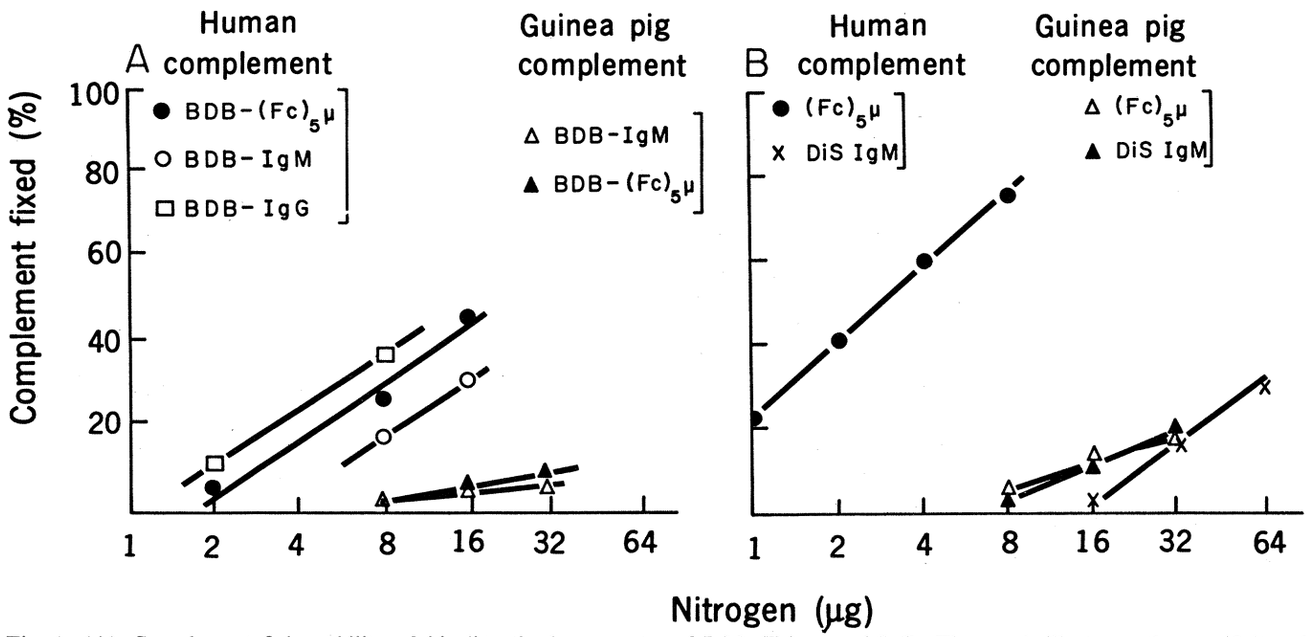

Fig. 1. (A) Complement fixing ability of bis-diazotized aggregates of IgM, $(Fc)_5\mu$, and IgG. The graph illustrates the specificity of IgM components for human complement. (B) Complement fixing ability of $(Fc)_5\mu$ with respect to intact IgM. The data illustrate significant complement fixation by the $(Fc)_5\mu$ and the specificity for human complement.

venously, either immediately or 30 minutes later. The skin sites were examined at intervals of 15 minutes for the next 2 hours for evidence of increased vascular permeability.

The ability of BDB-IgM and BDB-$(Fc)_{5}\mu$ to fix either human or guinea pig complement was compared to that of BDB-human IgG, the latter serving as a positive control. The three aggregated preparations are capable of fixing human complement to a significant degree (Fig. 1). However, exact comparisons of their complement fixing capacity could not be made because the degree of aggregation was not comparable in the various preparations. Both preparations derived from IgM fixed only human complement. Although not shown in Fig. 1A, BDB-IgG fixed guinea pig complement as well as human complement, as has been described (*7*).

Nonaggregated $(Fc)_{5}\mu$ effectively fixes human complement. Its superiority to nonaggregated IgM is seen by the parallel displacement of the respective dose response plots which, when quantiiated, indicate that $(Fc)_{5}\mu$ is 31 times as effective as intact IgM on a weight basis, and 19 times on a molar basis. These results were obtained with a λ type IgM, and they were confirmed with two IgM proteins of κ type whose corresponding $(Fc)_{5}\mu$ fragments were found to be 7 and 11 times as effective in complement fixation as the parent molecule on a molar basis. The above results could not be attributed to spontaneous aggregation of $(Fc)_{5}\mu$ preparations since analytical ultracentrifugation showed that the preparations were free of detectable aggregates when tested at a concentration of 25 mg/ml. As in the case of the BDB-aggregated components, $[(Fc)_{5}\mu]$ preparations did not fix guinea pig complement. The Fabμ did not fix detectable amounts of either human or guinea pig complement. Also a fragment (*8*) termed intermediate-L (Int-L) isolated from tryptic digestion of λ type IgM did not fix complement.

Table 1 indicates that the reductive monomers (Fcμ) of two different $(Fc)_{5}\mu$ preparations have a complement fixing capacity identical (on a weight basis) to that of the unreduced preparations. The completeness of reduction was confirmed by analytical ultracentrifugation. The preservation of activity could not be accounted for by an anticomplementary effect of residual reducing or alkylating agents, since preparations of IgG reduced and alkylated under identical conditions showed a complete loss of complement fixing ability. An unfractionated tryptic digest of IgM containing $(Fc)_{5}\mu$ and Fabμ fragments was relatively weak in complement fixing capacity, only 2.5 times as effective as intact IgM on a molar basis. We have no explanation for this finding. One possibility is that there is a weak association between Fc and Fab fragments that results in blocking of the complement fixing sites in the Fc portion by the Fab. Such blocking could occur in the intact IgM molecule.

Table 1. Capacity of varying amounts of pentameric and monomeric Fcμ fragments that fix human complement.

Amount used (μg of nitrogen)	Complement fixed (%)	
	Pentamer $(Fc)_{5}\mu$	Monomer (Fcμ)*
	Preparation A	
4	60.0	62.5
2	41.9	42.5
1	23.2	19.8
	Preparation B	
4	34.1	31.8
2	22.0	18.0
1	4.5	7.1

* Reduction of the pentamer by 0.01*M* dithiothreitol.

In one set of experiments, the skin reactive properties of the various IgM fragments studied were explored. Intradermal injections of the complement fixing $(Fc)_{5}\mu$ into guinea pigs resulted in no apparent increase in skin vascular permeability in doses as high as 1 mg. The other fragments were also negative.

These data demonstrate that nonaggregated fragments of IgM selectively fix human complement to an extent significantly greater than that of the intact IgM molecule. Guinea pig complement is not fixed by the same preparations. Selective fixation of human complement was also shown by BDB-aggregates of the various IgM fragments. These results are interesting in the light of the observation of Zvaifler (*9*) that IgM rheumatoid factor when reacting with aggregated IgG is capable of fixing human but not guinea pig complement.

The most effective fragment on a molar basis for complement fixation was $(Fc)_{5}\mu$. Although fixation did not depend on antigen-antibody reaction or aggregation, this pentamer was highly efficient on a weight basis, being roughly half that of rabbit IgG antibody reacting with antigen at equivalence. Two experimental observations are difficult to explain: (i) the ability of Fcμ monomer to fix complement and (ii) the relative inefficiency of the whole, unfractionated digest of IgM. That the complement fixing ability of $(Fc)_{5}\mu$ is unlikely to be an artifact created during its chromatographic purification is indicated by the inability of Fabμ, Int-L fragment and protein free portions of the column eluate to fix to a significant extent. Also, $(Fc)_{5}\mu$ preparations isolated by several different techniques, and from several donor sources, gave similar results. Moreover, the specificity of the observed fixation for human complement argues against the presence of contaminating anticomplementary substances in these fractions. The failure to induce skin reactions likewise argues against the presence of undetected biologically active denatured and aggregated material. The finding that Fc monomer fixes complement suggests that, unlike IgG, the Fc portion of a single subunit of IgM antibody bears a suitable complement receptor site. Insofar as $(Fc)_{5}\mu$ has 19 times the complement fixing capacity of the intact parent IgM, one may conclude that the fixing sites are inaccessible in the whole molecule and are exposed when either the $(Fc)_{5}\mu$ is isolated or IgM antibody reacts with antigen.

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References and Notes

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