

The Apparent Monovalency of Human IgG4 Is Due to Bispecificity

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Key Words

IgG4 · Non-precipitating antibody · Bispecific antibody · Chimeric antibody

Abstract

A hypothesis is put forward to explain the apparent monovalency of human IgG4. It is based upon the known instability of the IgG4 hinge. IgG4 is secreted as a regular bivalent antibody, but after secretion interacts with another IgG4 molecule. This interaction results in the exchange of half molecules (a combination of one heavy chain and one light chain) between the two IgG4 molecules. The postulated bispecific IgG4 antibodies can indeed be found in selected human sera following repeated immunization with two non-crossreacting antigens.

Introduction

IgG4 is a remarkable IgG subclass [1]: it does not activate complement (and may even inhibit complement activation [2, 3]), it binds poorly to the classical Fc- γ receptors and it behaves like a monovalent, non-precipitating antibody [4,5]. This latter feature is the topic of this communication.

Non-Precipitating (or Non-Crosslinking) Antibody

A recent review on this topic by Margni and Borel [6] summarizes much of what is currently known about non-precipitating antibodies. Steric interference between Fab's (caused e.g. by a rigid hinge) has been mentioned as a possible explanation, but the most likely explanation is structural asymmetry: one Fab being different from another Fab. In principle, this can be achieved in different ways: (1) lack of allelic exclusion; (2) post-translational modification, and (3) post-secretional modification.

Margni and Borel [6] favor the second explanation. The proposed mechanism is asymmetrical glycosylation of one of the two antigen-binding regions. There is indeed good evidence that glycosylation sites may occur in, or close to, antigen-binding loops and that these sites may be differentially glycosylated [6].

Since this effect reflects the primary amino acid sequence of the VH or VL, such a process would most likely result in asymmetric antibodies of different isotypes; more importantly, not all antibodies of these isotypes would be asymmetric.

In the case of IgG4, the information currently available indicates that most if not all natural IgG4 antibodies are non-crosslinking. This would argue against posttranslational modification of VH or VL as the mechanism. It would also argue against another mechanism mentioned: lack of allelic exclusion. Since allelic exclusion acts at early stages of

B-cell development before the isotype switch, this process would not be likely to occur in an isotype-restricted manner, unless the class switch to IgG4 would occur preferentially in non-allelically excluded B cells. Apart from the highly speculative nature of this explanation, some experimental data discussed below do not support this hypothesis.

There is, however, another option, which is more in line with the current state of knowledge. The hypothesis is that IgG4 is secreted by the plasma cell as a normal symmetric antibody, but subsequently interacts with another IgG molecule and that these two IgG4 molecules exchange half molecules (one heavy chain plus one light chain) [7]. This process results in IgG4 molecules with two different antigen-binding sites. Formally, this is a bispecific antibody, but in most instances this IgG4 would act as a monovalent antibody.

What is the experimental support for such a process.

(1) The interaction between two IgG4 half molecules in the hinge is known to be labile. This can be most convincingly shown by performing an SDS-PAGE analysis of IgG4 under non-reducing conditions. This has been described by others and confirmed by us, approximately 30% of IgG4 does not have a covalent H–H chain interaction; this is true both for normal as well as chimeric monoclonal IgG4 [8]. The structural basis for this ‘defect’ has been shown to be a single amino acid substitution in the hinge between the two cysteines involved in the interaction: in human IgG1 this sequence in the hinge is Cys–Pro–Pro–Cys, whereas in IgG4 this is Cys–Pro–Ser–Cys. Mutation in chimeric IgG4 of the Ser to Pro results in a molecule that is as stable as IgG1 in this test system [7, 9].

(2) Chimeric IgG4 is secreted as a symmetric, bivalent antibody [7].

(3) Polyclonal IgG4 in serum from selected donors can be shown to be bispecific. The test system used was: antigen-1 (coupled to Sepharose) is incubated with IgG4 antibody; after washing the complex is incubated with radiolabelled antigen-2. A positive result in such an assay system obviously could also be explained by crossreactivity between antigen-1 and antigen-2. For this reason we tested the hypothesis with a wide variety of combinations of antigens without an obvious relation, such as antigens from grass pollen, mite, honeybee venom, cat, tetanus, diphtheria, egg white, cow’s milk and banana.

(4) To exclude that polymeric antibodies were responsible for the bispecific reactivity we performed size exclusion chromatography. Bispecific reactivity co-eluted with monomeric IgG.

(5) Treatment of serum with bispecific reactivity with Sepharose-coupled monoclonal anti-IgG4 removed bispecific reactivity.

(6) On a quantitative level, the amount of bispecific reactivity closely corresponded to the level to be expected when assuming a fully random reassociation of plasma IgG4. This quantitative aspect is hard to reconcile with the alternative hypothesis: the allelic-exclusion breakdown.

However, in vitro mixing of two IgG4 antibodies did not result in exchange under physiological conditions. This was not surprising in view of the known strong non-covalent interaction between the CH3 domains of IgG. Our current working hypothesis is that the neonatal Fc receptor is involved. This MHC-like receptor has been found to be important to protect IgG from breakdown in a pinocytotic acidic compartment [10–12]. In this acidic environment the non-covalent interaction between the heavy chains is weaker, thus allowing the exchange reaction to occur.

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