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Heterogeneous antigen recognition behavior of induced polyspecific antibodies

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ABSTRACT

Polyspecific antibodies represent a significant fraction of the antibody repertoires in healthy animals and humans. Interestingly, certain antibodies only acquire a polyspecific antigen-binding behavior after exposure to protein-modifying conditions, such as those found at inflammation sites, or used in small- and large-scale immunoglobulin purification. This phenomenon is referred to as "criptic polyspecificity". In the present study, we compare the potential of different chemical agents to induce IgG polyspecificity. Depending on the treatment used, quantitative and qualitative differences in the recognition of individual antigens from a standard panel were observed. Antibodies with cryptic polyspecificity utilized common mechanisms for the recognition of structurally unrelated antigens when exposed to a particular inductor of polyspecificity. Our study contributes to the understanding of the mechanisms underlying the cryptic polyspecificity.

1. Introduction

A large proportion of circulating antibodies (Abs) in healthy individuals as well as in animals are polyspecific, i.e. single Ab molecules are able to recognize specifically various structurally unrelated antigens [1,2]. Such promiscuous antigen-binding was suggested to represent a mechanism of diversification of Ab repertoires, additional to that achieved by genetic processes of V-gene rearrangement and somatic mutation [3]. The pool of circulating Abs also includes Abs that are not polyspecific in their native form but acquire a promiscuous antigen-binding potential only after exposure to factors that affect protein stability [4]. Thus, a transient exposure of certain immunoglobulins (Igs) to low pH (pH $\leqslant$4), chaotropic agents or high salt concentration buffers has been shown to induce Ab polyspecificity [4–8]. Interestingly, the exposure of Ig's to the above-mentioned protein-destabilizing conditions is part of the Ig purification processes, both in routine research laboratory practice and during the industrial production of several licensed therapeutic immunoglobulin preparations for intravenous administration (IVIg) [9]. This may explain, at least in part, the observation that the antigen-binding reactivity of the native IgG antibodies in serum is considerably lower than that of the purified IgG fraction, isolated from the same serum [10–12]. The contact of the sensitive fraction of Igs with aggressive agents that are released *in vivo* in sites of inflammation or of tissue damage has also been shown to induce polyspecificity. Thus, exposure of certain Abs to reactive oxygen species, to iron ions or to heme as well as other oxidants results in the appearance of novel antigen-binding specificities [13–17].

Studies on the mechanisms of induced Ab polyspecificity have indicated that diversification of Ab specificity is accompanied by changes in the structural flexibility of the antigen-binding sites [7,15,18]. Little is known, however, on the effects of protein-destabilizing and redox agents at the level of antigen recognition. It thus remains unexplored whether the induction of polyspecificity by chemically different agents (redox, acidic pH, chaotropic) results in similar changes in the antigen-binding or whether treatment by each individual agent induces an unique set of new antigen-binding specificities. Moreover, it is not known whether the molecular mechanisms of recognition of structurally unrelated antigens by induced polyspecific Abs is unique or whether it depends on the antigen recognized.

In the present study, we compared the effects of different conditions known to induce *in vitro* Ab polyspecificity. It has been found that depending on the chemical nature of the treatment of the pooled IgG preparations, the recognized array of proteins by

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polyspecific Abs differs. Thermodynamic analyses have demonstrated that the recognition of structurally unrelated proteins by polyspecific ferrous ions-exposed IgG is governed by similar molecular mechanisms. Deciphering the mechanisms of acquired polyspecific antigen recognition by Abs should provide important insights towards the understanding of the biological relevance of this phenomenon in physiology as well as in pathological conditions.

2. Materials and methods

2.1. Ig preparations

Human therapeutic intravenous Ig preparations (IVIg) Intraglobin F and Intratect (Biotest AG, Dreieich, Germany) were used throughout the study. Intraglobin F and Intratect are produced without the use of an acidic pH blood-protein fractionation step. IVIg preparations were dialyzed against phosphate buffer saline pH 7.4 before use.

2.2. Treatment of Ig preparations

2.2.1. Exposure to ferrous ions

Iron (II) sulfate (Sigma–Aldrich) was dissolved in deionized water to 10 mmol/L. IVIg (50 μmol/L) was exposed to 500 μmol/L final concentration of Fe^{2+} at 4 °C for 30 min.

2.2.2. Exposure to heme

The oxidized form of heme (Hemin, Fluka), was dissolved to 1 mmol/L in dimethyl sulfoxide. IVIg (10 μmol/L in PBS) was exposed to 20 μmol/L final concentration of hemin at 4 °C for 30 min.

2.2.3. Exposure to hypochlorite

Sodium hypochlorite solution (Sigma–Aldrich) was diluted in deionized water to 16.7 mmol/L. IVIg (10 μmol/L in PBS) was exposed to 167 μmol/L of hypochlorite at 4 °C for 30 min before being used in the analyses.

2.2.4. Exposure to pH 2.8

IVIg was diluted to 50 μmol/L in 0.2 mol/L glycine, pH 2.8. The preparation was incubated for 5 min at room temperature before adjusting the pH to 7.2 by adding aliquots of 3 mol/L solution of Tris pH 9. The acid buffer-exposed Igs were then dialyzed against PBS.

2.2.5. Exposure to urea

IVIg (50 μmol/L in PBS) was dialyzed against excess of urea (6 mol/L in PBS) for 3 h at 4 °C. IVIg was then exhaustively dialyzed against PBS.

2.3. Enzyme-linked immunosorbent assays

Ninety-six well polystyrene plates (Nunc, Roskilde, Denmark) were coated for 2 h at 25 °C with 20 μg/mL of human factor IX (FIX, Wyeth, La Défense, France), human factor VIII (FVIII, Helixate®, BayerPharma, Lille, France), human von Willebrand factor (VWF, LFB, Les Ulis, France), human C-reactive protein (CRP), human C3 (both from Calbiochem), human factor H (FH), human factor B (FB, both from Complement Technology Inc, TX, USA), porcine thyroglobuline (Tg), rabbit tubulin, bovine myelin basic protein (MBP) and human hemoglobin (Hb, all from Sigma–Aldrich) in PBS. Plates were blocked for 1 h with 0.25 % v/v tween 20 in PBS at 25 °C. The native and the treated IVIg preparations were diluted to 1 μmol/L (150 μg/mL) and incubated for 2 h at 25 °C. After washing with PBS 0.05% tween 20, mouse anti-human IgG antibody (heavy-chain specific) coupled to horseradish peroxidase (Southern-Biotech, Birmingham, AL) was added and incubated for 1 h at 25 °C. Immunoreactivities were determined by using the *o*-phenylenediamine substrate (Sigma–Aldrich).

2.4. Kinetic and thermodynamic analyses

The kinetic parameters characterizing the binding of ferrous ions-exposed IVIg to VWF, tubulin and myelin basic protein (MBP) were determined by surface plasmon resonance (BIAcore 2000, GE Biacore, Sweden). Proteins were immobilized on CM5 chips by amino-coupling (Biacore). In brief, proteins were diluted in 5 mmol/L maleic acid, pH 5, to a final concentrations of 100, 40 and 5 μg/mL, respectively, and injected on pre-activated sensor surfaces. The experiments were performed by using HBS-P (0.01 mol/L Hepes, pH 7.2, 0.15 mol/L NaCl, and 0.005% v/v tween 20). Ferrous ion-exposed IVIg were injected over the sensor surface at 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 μmol/L with a flow rate of 10 μl/min for 4 min. The dissociation phase of the interaction was then monitored for 5 min. Regeneration was performed after each injection by exposing the sensor surface for 30 s to a solution containing 50 mmol/L of NaOH and 500 mmol/L of NaCl. The BIAevaluation software (version 4.1, Biacore) was used for the calculation of the kinetic rate constants. Evaluation of the thermodynamic parameters of the interactions between Fe(II)-exposed IVIg and proteins was performed as described before [7]. Briefly, all kinetic measurements were performed at 10, 15, 20, 25, 30 and 35 °C. Kinetic rate constants obtained at various temperatures were used to build Arrhenius plots. The values of slopes of the plots were calculated by linear regression analysis using GraphPad Prism v.4 software (GraphPad Prism Inc.) and subsequently substituted in the Arrhenius equation:

$$Ea = -slope\ R,$$

where the "*slope*" = $\partial \ln k_{on/off}/\partial(1/T)$, and *Ea* is the activation energy and *R* is the universal gas constant ($R = 8.3144\ J\ mol^{-1}\ K^{-1}$). The enthalpy, entropy and Gibbs free energy changes characterizing the association or dissociation phases were calculated using the following equations:

$$\Delta H = Ea - RT$$
$$\ln(k_{on/off}/T) = -\Delta H/RT + \Delta S/R + \ln(k'/h)$$
$$\Delta G = \Delta H - T\Delta S,$$

where *T* is the temperature in the Kelvin scale, k' is the Boltzman constant and *h* is the Planck's constant.

3. Results and discussion

3.1. Preferential antigen recognition by IgG antibodies with cryptic polyspecificity

The exposure of certain Abs to protein-modifying agents of different chemical nature has been reported before to induce a diversification of their antigen-binding specificities. We compared the effects of exposure of human pooled IgG (IVIg) to various agents on its binding to a panel of 11 structurally unrelated protein antigens. The latter included proteins from the coagulation cascade (FVIII, FIX, VWF), from the complement system (FB, FH, C3), from the acute phase response (CRP) and cellular proteins (Tg, tubulin, MBP and Hb). IVIg was exposed to three classes of chemical agents – redox agents (heme, ferrous ions and hypochlorite), chaotropic molecules (urea) as well as to pH 2.8 buffer. Igs may be exposed to any of these chemical agents and conditions at sites of inflammation or as a part of their purification process.

Exposure of IVIg to heme resulted in a considerable increase of the binding to all studied proteins (Fig. 1A). In contrast, other bio-