Low affinity, antibody binding of an *Escherichia coli*-derived component

Mats Ohlin *, Carl A.K. Borrebaeck

Department of Immunotechnology, Lund University, P.O. Box 7031, S-220 07 Lund, Sweden

Received 21 September 1995; revised 18 November 1995; accepted 1 December 1995

Abstract

This investigation describes the detection of a component in *Escherichia coli* capable of binding a large proportion of human antibody variable domains including otherwise highly monospecific antibodies induced by an in vivo antibody response. This interaction is of low affinity, but cross-linking of IgG molecules by, e.g. anti-immunoglobulin preparations, provides a sufficient degree of multivalency to promote a high avidity interaction. This binding which occurs both with \( \kappa \) and \( \lambda \) light chain-containing antibodies, appears to involve the variable region of human antibodies making it a superantigen-like activity. This is proposed based on the facts that: (i) different human antibodies of IgG1 isotype appear to bind to different extents suggesting that variable domain differences determine the binding activity; and (ii) addition of soluble antigen abrogates the interaction with the *E. coli*-derived molecule. Future studies of the nature and possible in vivo consequences of these interactions are warranted since any superantigen activity associated with this binding might affect the human immune response occurring as a consequence of *E. coli* infections.

Keywords: Antibody-binding molecule; Antibody variable domain; *Escherichia coli*; Superantigen

1. Introduction

The immune system has, for a considerable length of time, been known to specifically interact with foreign matter (antigen) including pathogenic organisms. This interaction is characterized by phenomena such as clonal selection and affinity maturation. In particular the selected antibody repertoire is known to not only expand clones interacting well with the antigen in question but also to improve the antibody binding affinity/rate constants through a process involving somatic mutation. Recently, however, it has become clear that both viruses and bacteria produce molecules capable of interacting with a large fraction of all T and B cell antigen receptors irrespective of the antigen specificity of those receptors. Such molecules, also termed superantigens, include e.g. staphylococcal enterotoxins and mouse mammary tumor virus antigens (T cell superantigens) [1,2] and staphylococcal protein A or HIV-1 gp120 (B cell superantigens) [3,4]. These superantigen and T or B cell receptor interactions modify the immune responses of the host by inducing non-antigen-specific clonal expansion, deletion or anergy and may thus have important consequences for the host.

In this study, we have identified the presence of
molecules in extracts of *Escherichia coli* which possess broad human antibody-binding capacity. This activity displays a low affinity for antibodies but upon cross-linking the avidity is sufficient to demonstrate substantial binding activity. Furthermore, this bacterial moiety appears to be specific for variable domains of human antibodies giving it superantigen characteristics.

2. Materials and methods

2.1. Antibodies

Human monoclonal antibodies specific for cytomegalovirus (CMV) pp65 and glycoprotein B (gB), tetanus toxoid and *Neisseria meningitidis opc* (summarized in Table 1) were prepared from human × mouse heterohybridomas as tissue culture supernatants. All of them have proved to be highly monospecific in ELISA when investigated against a variety of antigens including those which frequently are recognized by polyspecific antibodies ([7,12]; unpublished data). Affinity-purified, sheep anti-human IgG (heavy chain specific) (The Binding Site, Birmingham, UK) was conjugated to ruthenium (II) Tris-bipyridine chelate (TAG) (IGEN Inc., Rockville, MD, USA) according to the recommendations of the manufacturer. A coupling density of approximately 5 TAG molecules/IgG molecule was used.

Table 1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>VH subgroup</th>
<th>Isotype/light chain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7 15A2</td>
<td>tetanus toxoid</td>
<td>3</td>
<td>IgG1/κ</td>
<td>[5]</td>
</tr>
<tr>
<td>ITC33</td>
<td>CMV gB, epitope AD-1</td>
<td>5</td>
<td>IgG1/κ</td>
<td>[6,7]</td>
</tr>
<tr>
<td>ITC39</td>
<td>CMV gB, epitope AD-1</td>
<td>3</td>
<td>IgG1/λ</td>
<td>[6,7]</td>
</tr>
<tr>
<td>ITC48</td>
<td>CMV gB, epitope AD-1</td>
<td>4</td>
<td>IgG1/κ</td>
<td>[6,7]</td>
</tr>
<tr>
<td>ITC52</td>
<td>CMV gB, epitope AD-1</td>
<td>5</td>
<td>IgG1/κ</td>
<td>[6,7]</td>
</tr>
<tr>
<td>ITC63B</td>
<td>CMV gB, epitope AD-1</td>
<td>5</td>
<td>IgG1/κ</td>
<td>[6,7]</td>
</tr>
<tr>
<td>ITC88</td>
<td>CMV gB, epitope AD-2</td>
<td>3</td>
<td>IgG1/κ</td>
<td>[6,8]</td>
</tr>
<tr>
<td>LuNm03</td>
<td><em>Neisseria meningitidis opc</em></td>
<td>1</td>
<td>IgG3/κ</td>
<td>[9,10]</td>
</tr>
<tr>
<td>MO53</td>
<td>CMV pp65</td>
<td>3</td>
<td>IgG1/κ</td>
<td>[11,12]</td>
</tr>
<tr>
<td>MO58</td>
<td>CMV pp65</td>
<td>1</td>
<td>IgG1/κ</td>
<td>[11,12]</td>
</tr>
<tr>
<td>MO61</td>
<td>CMV pp65</td>
<td>3</td>
<td>IgG1/κ</td>
<td>[11,12]</td>
</tr>
<tr>
<td>MO81</td>
<td>CMV pp65</td>
<td>4</td>
<td>IgG1/κ</td>
<td>[12]</td>
</tr>
</tbody>
</table>

2.2. Antigens

Bovine serum albumin (BSA) and tetanus toxoid were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and the Swedish Institute for Infectious Disease Control (Stockholm, Sweden), respectively. Recombinant proteins Mbg58 (recombinant CMV gB, epitope AD-1) and HM90-5 (recombinant CMV gB, epitope AD-2) [13] were generously provided by Dr. M. Mach (der Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany). Recombinant UL57/2-GST (CMV UL57 (residues 1144-1196) fused to glutathione-S-transferase) [14] and Pex/Stu (expressing an unfused β-galactosidase fragment) [15] were kindly provided by Dr. R. Vornhagen (Biotest AG, Dreieich, Germany) and Dr. W. Lindenmaier (GBF, Braunschweig, Germany), respectively. These recombinant proteins and BSA were coupled to tosyl activated Dynabeads M 280 (Dynal A/S, Oslo, Norway) according to the manufacturer’s description. Furthermore, soluble biotinylated antigen NRD1-BCCP [16] kindly provided by M. Silvestri was also used in these investigations. This *E. coli* derived recombinant protein expresses the AD-1 epitope of CMV fused to a fragment of the bacterial biotin carboxyl carrier protein (BCCP) subunit of acetyl-CoA carboxylase [17]. The protein is directly biotinylated by *E. coli*; thus providing a product which is efficiently captured by streptavidin-coated magnetic beads in the absence of further in vitro modifications.
Antigen-antibody interactions were investigated using a recently developed electrochemiluminescence (ECL) technique [18] using the ORIGEN Analyzer (IGEN Inc.). In summary, this technology permits detection of interacting molecules (of which one is labelled with a luminescent moiety) following capture of the complex at the surface of an electrode. The chemiluminescent reaction is initiated electrochemically at this surface following removal of excess reagents through an automated washing procedure. In practice, antigen-coated Dynabeads (final dilution 1/30–1/60) were incubated for 30 min with human antibodies and affinity-purified, TAG-labelled sheep-anti human IgG (final concentration approximately 0.3 µg/ml) in assay diluent containing gelatin (IGEN Inc.) with continuous mixing. Human antibody concentrations were determined by an isotype-specific ELISA [11]. In some experiments, non-bound antibodies were removed by washing with phosphate buffered saline containing 0.1% BSA prior to incubation with the TAG-labelled sheep-anti human IgG preparation. Samples were assessed in duplicates. Following addition of assay buffer the amount of antibody bound to the beads was estimated on the ORIGEN Analyzer using the ORIOS software (version 2.00; IGEN Inc.) with settings including bead capture mode, assay gain = 1000, ramp wave form (final voltage: 2800 mV), ramp rate = 4800 mV/s and no pre-operative potential.

SDS-PAGE (12.5%) semi-dry transfer to nitrocellulose and immunodetection was performed essentially as described elsewhere [6]. In some cases, the primary (human monoclonal) and secondary (peroxidase-labelled goat anti-human IgG (Zymed Labs Inc., San Francisco, CA, USA)) antibodies were incubated together on the nitrocellulose to provide cross-linking of the detecting antibody system. Bound antibodies were detected using the enhanced chemiluminescence (Amersham, Little Chalfont, Buckinghamshire, UK) technique.

Statistical analysis of the relation of V-region usage and binding capacity was performed by ANOVA using Excel 5.0 software (Microsoft, Redmond, WA, USA) running on a Macintosh LC630 computer.

3. Results and discussion

When initially investigating the specific binding of human monoclonal antibodies to E. coli-derived
Fig. 2. Ability of antibodies to non-specifically bind immobilized, *E. coli* derived Mbg58 (CMV gB, epitope AD-1) when the antibody is incubated with the antigen in the presence of a secondary, cross-linking, TAG-labelled antibody (filled columns) but not when excess free antibody is removed by washing prior to addition of this detection reagent (hatched columns). The specific binding of AD-1 specific antibodies ITC48 and ITC52 is, however, retained even if excess antibody is removed prior to addition of the detection antibody. Error bars indicate the CV of the assay.

Fig. 3. Reactivity of human antibodies with a range of immobilized *E. coli* derived (Mbg58, UL57/2-GST and Pex/Stu) as well as other (streptavidin and BSA) antigens. Error bars indicate the CV of the assay. HM90-5 (CMV gB, epitope AD-2) immobilized to Dynabeads also mediated similar non-specific reactions (data not shown).
antigen Mbg58 (CMV gB, epitope AD-1 fused to β-galactosidase) immobilized to Dynabeads using the ORIGEN Analyzer surprising binding of highly monospecific human IgG antibodies of other specificities were observed. When further investigating this phenomenon it was observed that virtually every human antibody interacted to some extent with the antigen-coated beads in the presence of the TAG-labelled, affinity-purified, cross-linking antiserum (Fig. 1). The fact that the non-antigen-specific binding (but not the antigen-specific interaction) was not observed if excess human antibody was removed prior to addition of the cross-linking antibody (Fig. 2) strongly suggested that the interaction was of low affinity and thus dependent on multivalency to provide high avidity.

To determine whether this antibody-binding activity of immobilized Mbg58 preparation was related to the CMV-derived cloned antigen, the β-galactosidase moiety of the fusion or to some other E. coli-derived components, a number of other proteins were immobilized on Dynabeads and assayed for their antibody binding capacity. It was evident that all the investigated E. coli-derived preparations obtained from different laboratories contained the antibody-binding properties while immobilized bovine serum albumin did not (Fig. 3). It was thus concluded that the low-affinity interaction of antibodies of these recombinant proteins was a property not of the recombinant proteins as such or the immobilization procedure but rather of some other E. coli-derived component frequently found in such recombinant antigen preparations. To eliminate the possibility that the binding was entirely related to an affinity for the cross-linking sheep anti-human IgG immunoglobulin preparation we investigated whether aggregated purified human IgG would by itself bind the immobilized Mbg58 preparation. It was found that purified, lightly heat-aggregated (56°C, 30 min) antibody MO61 (anti-CMV pp65) would indeed bind the antigen to some extent even if excess antibody was removed prior to addition of the cross-linking TAG-labelled sheep immunoglobulin preparation (data not shown).

Since the level of interaction with different human antibodies varied over more than one order of magnitude it might be anticipated that the affinity of the E. coli-derived component was directed towards the variable domain of the human antibodies. In order to investigate this assumption we tried to block this interaction by addition of soluble antigen. It was evident that antigen specific for the human antibod-

![Inhibition of Reactivity](https://academic.oup.com/femspd/article-abstract/13/2/161/583004/165)

**Antibody (specificity)**

Fig. 4. The superantigen-related binding of antibodies to immobilized HM90-5 is inhibited in the presence of soluble antigen specifically recognizing the antibody under study but not by irrelevant antigen. The soluble antigens represent a negative control preparation (filled columns) and tetanus toxoid (hatched columns). Non-specific binding to Mbg58 was similarly affected by soluble antigen (data not shown).
The biological significance of this antibody-binding capacity of E. coli is not yet defined. However, irrespective of whether this component is actually expressed on the surface of the bacteria or only is found as an intracellular component in the live bacteria it may, apart from its possible interference in certain in vitro analytical procedures, have in vivo biological significance. Surface expressed or released (following bacterial lysis), aggregated substance may interact with and cross-link immunoglobulin surface receptors on B cells, thus mediating a B cell superantigen activity. Such signalling may provide a basis for clonal deletion/expansion/anergy associated with E. coli-related infections depending on the co-stimulatory signals that are available to the B cells, in analogy to similar HIV-1 gp120-related activity [19]. Such activities might affect the outcome of diseases associated with E. coli-related infections.

Analytical approaches to avoid problems associated with immunoglobulin-binding immobilized E. coli-derived products were also investigated. Such non-antigen-specific interactions might, for example, be expected to occur if the antibody preparation contains significant amounts of antibody aggregates, which frequently accumulate following antibody purification. This reactivity may also create problems.
Fig. 6. Specificity of reactivity of human antibodies to biotinylated NRD1 (CMV gB epitope AD-1)-BCCP fusion protein immobilized on streptavidin-coated Dynabeads at two different antibody concentrations. Error bars indicate the CV of the assay.

whenever the analyte contains cross-linking components such as rheumatoid factors which are found in many serum samples. It could also be suspected that naturally multivalent antibodies, in particular IgM may be particularly prone to such reactivities. The use of BCCP-antigen fusion proteins provides an efficient means to directly biotinylate recombinant proteins in vivo in E. coli [17]. This approach eliminates the need for in vitro biotinylation steps that may also label for example protein contaminants found in the antigen preparation. Such a protein, NRD1-BCCP, which just as Mbg58 expresses the AD-1 epitope of CMV gB, was shown to specifically bind human AD-1-specific antibodies when immobilized on streptavidin-coated Dynabeads (Fig. 6) without displaying general variable domain reactivity. This approach thus provides an alternative to in vitro-labelled antigens when using recombinant proteins in immunochemical analysis whenever variable domain binding activity of E. coli-derived preparations would otherwise interfere.

In conclusion, we have shown that E. coli produces a component which has the capacity to specifically interact with human immunoglobulin variable domains. This interaction is of low affinity but a substantial avidity can be achieved under cross-linking conditions. Consequently, the compound could have the ability to act as a superantigen.

Acknowledgements

We thank IGEN Inc. for providing the ORIGEN Analyzer necessary for this investigation. Furthermore we are grateful to Michel Silvestri and Drs. M. Mach, R. Vornhagen and W. Lindenmaier for providing recombinant antigens.

References


