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Non-classical binding of a polyreactive α-type anti-idiotypic antibody to B cells

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ABSTRACT

Detailed information on the immunological relevance of α-type anti-idiotypic antibodies is lacking after more than 30 years since Jerne postulated his Idiotypic Network Theory. The B7Y33 mutant is a mouse-human chimeric version of the B7 MAb, a polyreactive α-type anti-idiotypic antibody, generated against an anti-GM2 ganglioside IgM Ab1 antibody. It retained the unusual self-binding activity and multispecificity of the parental murine antibody, being able to recognize several anti-ganglioside IgM antibodies as well as non-immunoglobulin antigens. Previous work with the murine B7 MAb suggested that this antibody might have immunoregulatory properties, and therefore we investigated the possible interaction of B7Y33 with immune cells. We found that B7Y33 binds to human and murine B lymphocytes. Inhibition assays using flow cytometry indicated that this antibody is capable of binding the Fc γ receptor II (FcγRII). The recognition of FcγRII-expressing K562, Raji and Daudi human cell lines, together with the capability of inhibiting the binding of an anti-human FcγRII antibody to these cells, suggest that B7Y33 interacts with both the FcγRIIa and FcγRIIb isoforms. We evaluated the contribution to the binding of different surface-exposed residues at the top of the heavy chain variable region (VH) CDR loops through the construction of mutants with substitutions in the three conventional VH CDRs (HCDRs) and the "HCDR4", located in the framework 3 (HFR3). In addition, we assessed the involvement of the Fc region by performing key mutations in the CH2 domain. Furthermore, chimeric hybrid molecules were obtained by combining the B7Y33 heavy chain with unrelated light chains. Our results indicate that the multispecificity and self-binding properties of B7Y33 are not linked to its recognition of B lineage cells, and that this phenomenon occurs in a non-classical way with the participation of both the variable and constant regions of the antibody. Two possible models for this interaction are proposed, with B7Y33 binding to two FcγRIIb molecules through the Fc and Fv regions, or simultaneously to FcγRIIb and another unknown antigen on B cells. The FcγRIIb has recently received great attention as an attractive target for therapies directed to B lymphocytes. The recognition of peripheral B lymphocytes from B cell chronic lymphocytic leukemia (B-CLL) patients by B7Y33 suggests its potential application for the treatment of B cell malignancies.

1. Introduction

With the postulation of the Idiotypic Network Theory by Jerne in 1974 (Jerne, 1974), the knowledge about the function and structure of antibodies moved from the interaction with their antigens, to the complex relationships they can establish between them, of potential relevance for the immune system regulation (Winkler et al., 1979; Shoenfeld, 2004). The existence of β-type anti-idiotypic antibodies, having the ability of inhibiting the interaction of the Ab1 antibody with the nominal antigen and able to mimic the latter molecule, has been largely exploited for the design of vaccines (Poskitt et al., 1991; Betáková et al., 1998). However, the α-type anti-idiotypic antibodies, which do not impair the Ab1-antigen binding, have not been extensively studied.

Gangliosides are normal components of the plasma membranes of most mammalian cell types, and have been also associated with malignant transformation (Marquina et al., 1996; Malykh et al.,

Abbreviations: Ab, antibody; B-CLL, B-cell chronic lymphocytic leukemia; BCR, B cell receptor; CH, heavy chain constant domain; ELISA, enzyme linked immunosorbent assay; Fc, crystallizable fragment; FITC, fluorescein isothiocyanate; Fv, variable fragment; HCDR, heavy chain complementarity determining region; HFR, heavy chain framework; IVIg, intravenous immunoglobulin; MAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; PE, phycoerithryn; PBS, phosphate buffer saline; VH, heavy chain variable region; Vκ, light chain variable region.

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2001; Kannagi et al., 2008). Diverse strategies have been reported to elicit antibodies specific for these low immunogenic molecules (Helling et al., 1994, 1995; Livingston and Ragupathi, 1997). Our group has obtained several ganglioside-specific antibodies (Alfonso et al., 1995; Vázquez et al., 1995), some of which, in different formulations, have been able to induce a strong anti-idiotypic response in mice (Vázquez et al., 1998). We have thus isolated some monoclonal antibodies (MAbs) that behaved as β- or γ-type (those that inhibit the interaction of the Ab1 antibody with the nominal antigen but do not mimic it) anti-idiotypes depending on the species in which they were used as immunogens for the obtention of Ab3 antibodies (Vázquez et al., 1998; Hernández et al., 2005). Moreover, we have also generated, immunizing mice with the anti-ganglioside antibodies mentioned above, non-paratopic specific or α-type anti-idiotypic MAbs. One of them, the polyreactive B7 MAb, was obtained by immunizing BALB/c mice with the anti-NeuAc-GM2 ganglioside E1 MAb (Macías et al., 1999). The B7 MAb has shown an anti-tumor effect in a murine model of melanoma. Furthermore, it also exhibits *in vitro* some properties that resemble those of the intravenous immunoglobulin preparation (IVIg), such as the inhibition of the proliferation of human B and T cell lines and of human normal lymphocytes activated with different mitogens (Macías et al., 1999). These findings suggested that B7 MAb might play a similar immunoregulatory role as proposed for the IVIg pool, which has been successfully used in the treatment of autoimmune and inflammatory diseases and lymphoproliferative disorders (Kazatchkine and Kaveri, 2001; Krause and Shoenfeld, 2005). The mechanisms of action of this preparation rely on both the variable and constant regions of the IgG. The Fv region is responsible for the recognition of soluble and membrane-associated self-molecules, as well as idiotypes of soluble immunoglobulins and B cell receptors (BCR). On the other hand, the Fc portion contributes to IVIg effects through interactions with Fc γ receptors (FcγR), modulation of FcγRIIb expression and saturation of FcRn (Negi et al., 2007).

Though the reactivity of B7 MAb with antibodies and non-immunoglobulin antigens has been well documented (Macías et al., 1999), the nature of the interaction of this antibody with different cells of the immune system has not been yet elucidated.

The present work demonstrates the existence of a high avidity interaction of B7Y33, a mutated chimeric version of B7 MAb, with B lineage cells. The interaction with the low affinity receptor FcγRIIb proved to be critical for the recognition of this cell type. Our results point out a non-classical interaction of B7Y33 with B cells, which involves both the variable and constant regions of the antibody. The recognition of peripheral B lymphocytes from B cell chronic lymphocytic leukemia (B-CLL) patients by B7Y33 supports its potential application for the treatment of B cell malignancies.

2. Materials and methods

2.1. Cells

K562 (human erythroleukemia), Raji (Burkitt's lymphoma), Daudi (Burkitt's lymphoma), NS0 (murine myeloma), MB16F0 (non-metastatic C57Bl/6 murine melanoma) and F3II (murine breast cancer) cell lines as well as chimeric antibodies-expressing NS0 transfectomas, were cultured at 37 °C, 5% CO_2, in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (FCS), antibiotic mixtures of penicillin (100 U/mL) and streptomycin (100 μg/mL), and 2 mM L-glutamine. For the selection of whole antibody-producing transfectomas, DMEM-F12 containing 10% FCS and histidinol at 10 mM was used as selective medium.

2.2. Monoclonal antibodies (MAbs)

The E1 (Alfonso et al., 1995), P3 (Vázquez et al., 1998), and F6 (Alfonso et al., 1995) IgM MAbs were purified from mouse ascitic fluid by gel filtration chromatography using a Sephacryl S-300 high resolution column (Pharmacia, Uppsala, Sweden) equilibrated with phosphate-buffered saline (PBS) containing 0.5 M NaCl.

The following chimeric antibodies were used: P3 (López-Requena et al., 2003), two of its mutants (with arginine-to-serine replacements at HCDR1 Kabat position 31 and HCDR3 Kabat positions 98 and 100a, respectively) (López-Requena et al., 2007a), 1E10 (López-Requena et al., 2003), C5 (Roque-Navarro et al., 2003) and 14F7 (Roque-Navarro et al., 2008) (all human IgG1, κ). The chimeric antibodies, including those obtained during this work, were purified from transfectoma culture supernatants by Protein-A Affinity Chromatography (Pharmacia, Uppsala, Sweden) and analyzed by SDS-PAGE under reducing conditions.

The murine FcγRII/III-specific 2.4G2 antibody (rat, IgG2a) and the murine MHC-II-specific M5/114.15.2 antibody (rat, IgG2b) were purified from 2.4G2 and M5/114.15.2 hybridoma supernatants, respectively, by Protein-G Affinity Chromatography.

The specificity of the purified antibodies was confirmed by enzyme-linked immunosorbent assay (ELISA).

2.3. Vectors

The pAH4604 and pAG4622 vectors, containing the human γ1 and κ constant regions, respectively, have been described in detail (Coloma et al., 1992) and were kindly provided by Dr. Sherrie L. Morrison, Department of Microbiology and Molecular Genetics, UCLA, USA.

The pAH4604 (Ala/Ala) vector is a mutated version of pAH4604, where the leucine residues at positions 234 and 235 of the human γ1 CH2 region were replaced by alanines (Hinojosa et al., 2010). It was used for the expression of the B7Y33LALA mutant.

2.4. Variable region genes

The genes of the B7Y33 heavy chain variable region (VH) and its mutants were chemically synthesized (Geneart GmbH, Regensburg, Germany). The B7Y33 VH gene was designed from the published B7 VH MAb coding sequence (Hernández et al., 2007), with the substitution of the threonine residue at Kabat position 33 by a tyrosine. The changes contained in each mutant are detailed in Table 1. The synthetic genes were digested HincII/NheI (NEB, New England Biolabs, Ipswich, MA) from pGA4 Geneart vector and cloned into pAH4604 or pAH4604 (Ala/Ala), previously digested EcoRV/NheI (NEB).

The B7 MAb light chain variable region (Vκ) gene (Hernández et al., 2007) was digested EcoRV/SalI (NEB) and inserted into the equally digested pAG4622.

2.5. Chimeric antibody expression

NS0 cells were transfected by electroporation with 10 μg of both pAG4622 containing chimeric B7 MAb light chain, and pAH4604 or pAH4604 (Ala/Ala) bearing chimeric B7Y33 MAb heavy chain or its mutants, all linearized through PvuI (NEB) digestion.

NS0 transfectoma cells expressing chimeric P3 light chain or chimeric 1E10 light chain (López-Requena et al., 2003) were transfected as above with pAH4604 bearing chimeric B7Y33 MAb heavy chain.

The transfection method and the selection of transfectomas secreting chimeric IgG have been previously described (López-Requena et al., 2007a).