Immunopotentiating properties of a multispecific α-anti-idiotype antibody

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Abbreviations: ChAb, chimeric antibody; DC, dendritic cells; IC, immune complexes; mAb, monoclonal antibody

Multispecificity is not a well-understood property of some antibodies. Different functions have been attributed to multispecific natural antibodies, commonly associated with the neutralization and clearance of antigens. Much less is known about the role of antibodies like these, based on their idiotypic connectivity. B7Y33 is a chimeric IgG1 version of a polyreactive α anti-idiotype antibody that is able to interact with different immunoglobulin and non-immunoglobulin antigens. Here we report the capacity of this antibody to enhance the immunogenicity of several autologous IgMs in adjuvant-free conditions. Our results suggest that the formation of immune complexes seems to be necessary, but not sufficient, to this activity. The potential involvement of the interaction of B7Y33 with the FcγRIIb is discussed.

Introduction

Although antibodies are known as highly specific molecules, immunological and biological evidence point to the existence of immunoglobulin multireactivity. Various terms have been used to define protein binding to multiple ligands; most of these are also valid for antibodies. Cross-reactivity by molecular mimicry, the most reported in the literature, refers to the binding of antibodies to molecules that are very similar or overlapping the original antigen. Alternative to this, and less well-characterized, multispecificity refers to binding to unrelated ligands. Different mechanisms have been proposed to explain it, including the existence of several isoforms with distinct recognition profile or the use of different sets of residues of the binding site.

Multispecificity characterizes a major fraction of the antibodies of the natural repertoire, which recognize a collection of autologous and heterologous molecules. Nevertheless, some antibodies resulting from antigen-driven selection have been shown to be polyreactive. That property enables the formation of several different immune complexes (IC) by these antibodies, which could have a significant impact in their biological role. Indeed, the IC formed by natural antibodies with auto-antigens released after a tissue damage or remodeling contribute to the clearance of cellular antigens. IC can also represent a mechanism of amplification of the immune response. On the other hand, IC are potent activators of dendritic cells and mediate an efficient FcyR internalization for processing and presentation of the antigens. Beyond their physiological role, the immunogenic potential of IC have been exploited to enhance the production of antibodies (patent5084396). B7Y33 is a chimeric version of B7 mAb, a multispecific α anti-idiotype antibody generated against the anti-NeuAc GM2 ganglioside E1 mAb. It reacts with antibodies of different specificities and several non-immunoglobulin antigens, and is able to bind to B lymphocytes. It was previously shown that FcγRIIb on B cells is critical for this interaction, which occurs in a non-conventional way involving both the variable and constant regions of this antibody. In the present work, we demonstrate the capacity of B7Y33 to potentiate the immunogenicity in the syngeneic model of some IgMs to which it binds, in the absence of any adjuvant. The potential application and the physiological role of this phenomenon are discussed.

Results

To determine whether B7Y33 could modify the immunogenicity of autologous IgMs we evaluated, by ELISA, the antibody response of BALB/c mice, induced by the subcutaneously administration of a mix of P3, E1 or F6 mAbs and B7Y33, without adjuvant.

The frequency of responder animals is shown in Table 1. All mice receiving P3 and B7Y33 developed antibodies against the former after the first dose (Fig. 1). Although P3, in contrast to the other IgMs, is known to be highly immunogenic in the syngeneic model, only two mice out of five inoculated with the mixture P3/ChC5 had a measurable response. For the other two IgMs, the antibody response was observed from the first dose in three out of five animals inoculated also with B7Y33, while control ChC5 did not show this effect. Except for one animal...
Discussion

IC are potent activators of dendritic cells and able to induce stronger immune responses than antigens alone. This is mainly due to the FcγR-dependent IC internalization, which leads to MHC-class-II-restricted antigen presentation and cross-presentation on MHC class I molecules. Thus, we evaluated whether the multispecific B7Y33, had the capability to increase the immunogenicity of some autologous IgMs it recognizes.

The experiments performed in BALB/c mice evidenced that the subcutaneously co-inoculation of B7Y33 and two non-immunogenic IgMs induced the production of IgG specific for the latter, even in the absence of adjuvant. The impossibility to generate this response when the antibodies were administered separately, suggests the role of IC in this effect. In contrast, the hybrid VHB7Y33/VKP3 antibody, despite recognizing E1 mAb, did not show this activity. This evidence indicates that the formation of IC could be necessary, but not enough for the occurrence of this phenomenon. Noteworthy, unlike B7Y33, VHB7Y33/VKP3 does not bind to B lymphocytes, which suggests that the interaction with the FcγRIIb, main target of B7Y33 on these cells, might be important for the immunopotentiating activity of this antibody. The result with both the hybrid variant and an isotype-matched control rules out any contribution of the human region of B7Y33 for this effect.

Table 1. Reactivity of the sera of BALB/c mice (IgG antibodies) against the IgMs used in each case

| Activity of the sera of BALB/c mice (IgG antibodies) against the IgMs used in each case |
|-----------------------------------------------|---------|---------|---------|---------|---------|---------|
| P3/B7Y33 | P3/ChC5 | E1/B7Y33 | E1/ChC5 | F6/B7Y33 | F6/ChC5 |
| Responders frequency | 1st dose | 5/5 | 2/5 | 3/5 | 0/5 | 3/5 |
| | 2nd dose | 5/5 | 5/5 | 5/5 | 0/5 | 4/5 |
| Table 2. Frequency of responders is referred to as the number of mice with positive serum reactivity against the IgM inoculated (2-fold increase of hyperimmune over pre-immune signal at a serum dilution of 1:50) over the total number of injected mice.

To define the importance of IC in the immunopotentiating property of B7Y33, mice were administered with E1 mAb and B7Y33 mixed or separated, in different flanks each. Seven out of ten animals inoculated with the mixture developed IgG antibodies against E1 mAb, while only two out of ten receiving the antibodies separately (Table 2). Moreover, the former group had the highest antibody levels (Fig. 2A).

We next determined whether the hybrid antibody VHB7Y33/VKP3 had the same effect. This variant of B7Y33, which bears the chimeric light chain of P3 mAb and the heavy chain of B7Y33, recognizes E1 mAb, but is unable to bind to B cells. Interestingly, the magnitude of the response for the hybrid antibody/E1 co-administration was affected in comparison with the combination E1/B7Y33 (Fig. 2B), along with a diminished frequency of responder animals (Table 2).

Finally, the influence of the dose of the E1/B7Y33 mixture on the induction of antibodies against the IgM was tested. In contrast to previous experiments, mice received the mixture once, while in the second dose only E1 mAb was inoculated. It lead to reduced IgG levels specific for E1 mAb (Dunn’s, p < 0.05) (Fig. 2C), with only two out of ten responder mice (Table 2).
Figure 2. IgG antibody response against the E1 mAb. BALB/c mice were inoculated subcutaneously with P3, E1 and F6 mAbs mixed with ChAbs, in PBS. Sera (1:50) (taken seven days after the second dose) were added to plates coated with 10 μg/ml of E1. The reactivity was measured using a polyclonal serum anti-mouse IgG alkaline phosphatase conjugate. The absorbance values of the replicates of each sample from two independent experiments are shown in the graphs. (A) Two doses of E1 mAb and B7Y33, given mixed or independently, in different flanks (DF) each were administered. (B) Two doses of E1 mAb and B7Y33 or VHB7Y33/VκP3 were administered. (C) One group (E1/B7Y33 1X) received in the first dose a mix of E1 mAb and B7Y33, and in the second one, the E1 mAb alone. The group E1/B7Y33 2X was inoculated twice with a mix of E1 mAb and B7Y33. Pre-immune sera. Dunn’s test, p < 0.05. Different letters indicate statistical differences.

Table 2. Reactivity of sera of BALB/c mice inoculated with E1MAb and the ChAbs B7Y33, VHB7Y33/VκP3 or C5

| Administration way | Immunogen | E1/B7Y33 | E1/ChC5 | E1/VHB7Y33/VκP3 | E1/B7Y33 1x |
|--------------------|-----------|----------|---------|-----------------|----------|
| Mixture            | 7/10      | 0/10     | -       | -               | -        |
| Different flanks   | 2/10      | 0/10     | -       | -               | -        |

Responders frequency

| Administration way | Immunogen | E1/B7Y33 | E1/ChC5 | E1/VHB7Y33/VκP3 | E1/B7Y33 1x |
|--------------------|-----------|----------|---------|-----------------|----------|
| Mixture            | 8/10      | 0/10     | 2/10    | -               | -        |

*Mixture* Frequency of responders is referred to as the number of mice with positive serum reactivity against the IgM inoculated (2-fold increase of hyperimmune over pre-immune signal at a serum dilution of 1:50) over the total number of injected mice. *BALB/c mice were inoculated subcutaneously with E1 mAb and the ChAbs, in PBS. These were administered together (mixture) or separated (different flanks). Sera were obtained seven days after the second dose. In all cases two doses of IgM/ChAbs were administered, except the group E1/B7Y33 1x, that received the mix of antibodies in the first dose while only the E1 mAb in the second one.*

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**Figure 3.**
Although our previous work had pointed out to the participation of FcyRIIib in the interaction of B7Y33 with B cells,16 with the present data we cannot determine which immune population expressing this receptor could be involved in the immunopotentiating activity of this antibody. Indeed, FcyRIIib is widely expressed in the hematopoietic system. It has been shown that blocking the IC binding to FcyRIIib on dendritic cells (DC) with a monoclonal antibody (mAb) led to their maturation.19 Considering that the mix of IgM and B7Y33 does not guarantee that all the B7Y33 is engaged in the IC, any free molecules of this antibody could additionally block FcyRIIib, which, in turn could influence the activation state of DC and the magnitude of the immune response. On the other hand, it would be interesting to evaluate whether the interaction of B7Y33 with the FcyRIIib on DC favors the capture and presentation of the IC B7Y33/IgM through a non-degradative way mediated by this receptor. This mechanism is described to allow the recirculation of antigens to the cell surface, the direct interaction with the BCR and, therefore, the activation of B cells, resulting in the amplification of B cell responses against microbial antigens and autoantigens.20

B7 mAb displays some properties of the so-called natural antibodies, as multispecificity and idiotypic connectivity.15 However, this antibody seems to be the result of an antigen-driven maturation process,9 for which a physiological role of this immunopotentiating activity is difficult to postulate. Whether this property also operates for some of the immunoglobulins of natural repertoire is still a question to be addressed. Nevertheless, it would be interesting to determine whether this effect is exerted only over other immunoglobulins or also over non-immunoglobulin antigens that B7Y33 recognizes.16 Also, the contribution of B7Y33/FcyRIIib interaction deserves further clarification.

Materials and Methods

Animals. Female BALB/c mice, 6 to 8 weeks old, were purchased from the Center for Laboratory Animal Production (CENPALAB). Animals were housed and bred in a barrier maintained room according to the guidelines stipulated by the Animal Subject Committee Reviews Board at the Center of Molecular Immunology (CIM). Animal studies were performed with approval from CIM’s Institutional Animal Care and Use committees.

Monoclonal antibodies. The E1,22 P3 (anti-β-glycolylated gangliosides),21 and F6 (anti-NeuAcGM1 ganglioside),22 mAbs (IgM, κ) were purified from ascitic fluid by gel filtration chromatography using a Sephacryl S-300 high resolution column (Pharmacia) equilibrated with phosphate-buffered saline (PBS) containing 0.5 M NaCl.

The chimeric antibodies (ChAbs; human IgG1, κ) B7Y33,16 and VHB7Y33/VkP3 (hybrid antibody),16 and the anti-mucin C5,23 were purified from transfectoma culture supernatants by Protein-A affinity Chromatography (Amersham Biosciences, 17-5280-02) and analyzed by SDS-PAGE under reducing conditions.

The specificity of the purified antibodies was confirmed by enzyme-linked immunosorbent assay (ELISA) and protein concentration estimated by optical density (OD) at 280 nm.

Mouse inoculation. Mice were co-administered subcutaneously, with 50 μg of the anti-ganglioside IgMs (P3, E1 and F6 mAbs) and 80 μg of ChAb B7Y33 or the hybrid VHB7Y33/VkP3 in PBS, two times, every two weeks.

The IgMs and ChAbs were mixed before the inoculation or given separately, in different animal flanks each. In one of the experiments, animals received one dose of the mix, while the second dose consisted exclusively of the murine IgM. Serum samples were obtained before and seven days after each inoculation. ChC5 was used as isotype-matched control.

Measurement of IgG response. The presence of IgG antibodies specific for the inoculated IgMs was measured by ELISA. Microtiter plates were coated with 10 μg/ml of the corresponding IgM, overnight at 4°C. After washing with PBS-T (phosphate-buffered saline containing 0.05% Tween 20, pH 7.5), the plates were blocked with PBS-T-BSA (1% bovine serum albumin in PBS-T) for 1 h at 37°C. Then, diluted (1:50) pre and hyper-immune serum samples were incubated for 2 h at 37°C. The alkaline phosphatase conjugated anti-mouse IgG (Jackson Immunoresearch, 115-005-008) was used to reveal binding, by adding the substrate solution consisting of 1 mg/ml of p-nitrophenylphosphate. Measurements were performed in triplicate and the standard deviation (SD) was less than 10%. Sera were considered positive when absorbance values were at least twice the pre-immune signal.

Statistical analysis. Each experiment was repeated at least twice. Kruskal-Wallis and Dunn’s test were used for data analysis, using the GraphPad Prism Software, version 5.03, 2010, for Windows.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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