

Generation of IgM and IgG1 monoclonal antibodies with identical variable regions: comparison of avidity

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Generally, IgM antibodies (Abs) produced in a primary immune response show lower affinity for an inducing antigen (Ag) compared with the corresponding IgG Abs that are major switched isotypes formed in the secondary response. An IgM molecule is a pentamer with 10 Ag-binding sites that will contribute to an increase of avidity for an Ag. To estimate the contribution of the pentameric structure to the avidity of an IgM Ab, we generated IgM and IgG1 monoclonal Abs (mAbs) with identical V regions that are specific for 4-hydroxy-3-nitrophenylacetyl (NP) by in vitro class switching of B cells followed by the cell fusion with a mouse myeloma cell line. Compared with an anti-NP IgG1 mAb, the corresponding IgM mAb showed much higher avidity for NP-conjugated bovine serum albumin, which was drastically reduced after being dissociated into monomers.

1. Introduction

During a T cell-dependent Ab response, the isotype of produced Abs is switched from IgM to other classes including IgG with time after immunization (1). Another characteristic feature is a gradual increase of Ab affinity for an inducing Ag particularly in the switched isotypes, a process termed affinity maturation (1-3). It has been shown that isotype-switching and affinity maturation are strongly dependent on germinal centers (GC) that are transiently formed from Ag-stimulated B cells and CD4⁺ T cells in the follicular region of secondary lymphoid tissues (1,2). Affinity maturation of Abs is the result of somatic hypermutation of V region genes coupled with the positive selection of B cells whose mutated B cell antigen receptors (BCR) acquire higher affinity, (1,4). In general, IgG Abs have point mutations in the V region more frequently than the corresponding IgM Abs, and the former isotype shows higher affinity for the inducing Ag than the latter (4). Recent discovery of activation-induced cytidine deaminase (AID) that is essential for both class switching and somatic hypermutation has enabled us to understand the coordinated occurrence of these two processes (5).

For estimating the binding strength of an Ab to an Ag, one has to measure not only the affinity of each Ag-binding site for an antigenic epitope, but also the binding activity taking into account the valency of the interaction, termed avidity. Therefore, avidity, instead of affinity, generally reflects the precise binding strength of an Ab.

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Although each Ag-binding site in an IgM molecule generally shows low affinity for an Ag, this is considered to be somewhat compensated by its pentameric structure that contribute to increase the avidity. Thus, class switching to IgG without somatic mutations, if it occurs, will result in the formation of useless Abs with low avidity.

To examine to what extent the pentameric structure of IgM is responsible for its avidity, we generated IgM and IgG1 mAbs that bear a common V region in the heavy chain (VH) and that in the light chain (VL), and compared these two mAbs for the binding to the relevant Ag. As a B cell source, we used the quasi-monoclonal (QM) mouse, in which one of the JH loci is replaced with the VHDJH segment (VHT) derived from an anti-NP IgG mAb, 17.2.25, with the other JH locus and both κ loci being disrupted (6). Thus, approximately 80% of QM B cells expressed VHT-encoded NP-specific BCR that bear λ chains exclusively (6,7).

2. Materials and Methods

QM mice were generously given by Dr. Marilia Cascalho (Mayo Clinic, Rochester, MN, USA). To induce class switching without causing somatic mutations, QM B cells were cultured in vitro for 5 days with 20 μ g/ml of lipopolysaccharide (LPS) from *Escherichia coli* (Sigma, St. Louis, MO, USA) and 10 ng/ml of interleukin (IL)-4 (Peprotech, Princeton, NJ, USA), followed by the cell fusion with a mouse myeloma cell line, NSO^{bcl-2} as the fusion partner (8,9). NSO^{bcl-2} is advantageous in generating hybridomas efficiently since the transfected bcl-2 gene is expected to rescue hybridomas from apoptosis (9). Among hybridomas secreting anti-NP IgM or anti-NP IgG mAbs, an IgM-secretor and an IgG1-secretor that used the VHT-encoded μ or γ 1 chain paired with the λ 2 L chain were selected by using a rat mAb, R2.438 that is specific for the idiotype of VHT-encoded H chains (a gift from Dr. T. Imanishi-Kari, Tufts University, Boston, MA) and by anti-mouse λ 2 mAb (Pharmingen, San Diego, CA). In some cases, the use of the λ 2 chain was confirmed by λ 2-specific reversetranscription-dependent PCR as described (7). We sequenced the VH and the VL genes in these two mAbs as described previously (7), and confirmed that there was no point mutation in the VH as well as the V λ 2 gene as expected (Fig. 1). The culture supernatants of the hybridomas or mAbs that were purified from the hybridoma ascites by an affinity chromatography on a NP-conjugated Sepharose 4B column were used as test samples.

For preparing NP-conjugated bovine serum albumin (NP-BSA), 4-hydroxy-3-nitrophenylacetic acid (Tokyo Kasei, Tokyo, Japan) was converted to the N-hydroxysuccinimide ester, and then reacted with BSA for 3~90 min as described previously (7). Low and high-density NP-BSA that bear 3 and 25 NP moieties per BSA molecule, designated as NP₃-BSA and NP₂₅-BSA, respectively, were prepared. The high-density NP-BSA binds low-affinity anti-NP Abs as efficiently as the high-affinity Abs, while the low-density NP-BSA reacts preferentially with the high-affinity Abs, but poorly with the low-affinity Abs (10). Thus, anti-NP Ab affinity can be estimated by comparing the binding of the Ab to NP₃-BSA and NP₂₅-BSA (11).

Varying concentrations of the anti-NP IgM and IgG1 mAbs were incubated for 2 h in the 96 well microplates (NUNC, Roskilde, Denmark) that were coated with 4 μ g/ml of NP₃-BSA or NP₂₅-BSA followed by blocking with 1 mg/ml BSA in phosphate-buffered saline (PBS). After washing thoroughly with PBS, bound anti-NP mAbs were determined by reacting with 1 μ g/ml peroxidase-conjugated goat anti-mouse IgM or IgG1 as the second Ab (Southern Biotechnology Associates, Birmingham, AL).

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VH1T  1:GAGGTT CAGCTGCAGCAGTCTGGGGCAGAGCTTGTGAAGCCAGGGGCCTCAGTCAAGTTG
          CDR1
        61:TCCTGCACAGCTTCTGGCTTCAACATTAAGACACCTATATGCACTGGGTGAAGCAGAGG
          CDR2
       121:CCTGAACAGGGCCTGGAGTGGATTGGAAGGATTGATCCTGCGAATGGTAATACTAAATAT
       181:GACCCGAAGTTCCAGGGCAAGGCCACTATAACAGCAGACACATCCTCCAACACAGCCTAC
          Dsp2.3
       241:CTGCAGCTCAGCAGCCTGACATCTGAGGACACTGCCGTCTATTACTGTGCTAGATACTAT
          JH4
       301:AGGTACCCTTACTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCAACCGTCTCCTCA

VL2  1:CAGGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCTGGTGGAACAGTCATACTC
          CDR1
        61:ACTTGTGCTCAAGTACTGGGGCTGTTACAAGTAACTATGCCAACTGGGTCCAAGAA
          CDR2
       120:AAACCAGATCATTTATTCACTGGTCTAATAGGTGGTACCAGCAACCGAGCTCCAGGTGTT
       180:CCTGTGACAGTTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTCACCATCACAGGGGCA
          Jλ2
       241:CAGACTGAGGATGATGCAATGTATTTCTGTGCTCTATGGTACAGCACCCATTATGTTTTTC
       301:GGCGGTGGAACCAAGGTCACTGTCCTAG

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Fig. 1. VH and VL nucleotide sequences in anti-NP IgM and IgG1 mAbs. The $\lambda 2L$ chain was used in either mAb.

The bound peroxidase activity was assayed using 2,2'-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid as the substrate (8). Unless otherwise stated, all incubations were carried out at 25 °C. Data were presented as mean values from triplicate experiments with standard errors, which did not usually exceed 5% of the mean, and are often invisible in the figures.

3. Results and Discussion

As shown in Fig. 2, approximately the same dose-response curves were observed in the binding of the anti-NP IgM mAb to either plates, while the anti-NP IgG1 mAb bound to NP₂₅-BSA-coated plates significantly, but not to plates coated with NP₃-BSA at the Ab concentrations tested. These results suggest that the IgM mAb showed stronger binding (higher avidity) to NP-BSA than the IgG1 counterpart. Because identical VH and VL genes encode the Ag-binding site in these two mAbs, the superiority of the IgM over the IgG1 may be due to that IgM has a pentameric structure that bear ten Ag-binding sites, while there are only two Ag-binding sites in an IgG1 molecule. The contribution of the oligomeric structure to an increase in the avidity was further analyzed in the following experiments.

A displacement ELISA was carried out to confirm the higher avidity of the anti-NP IgM mAb in the dissociation reaction. The anti-NP IgM or IgG1 mAbs were incubated at 200 ng/ml for 1 h in an NP₂₅-BSA-coated microplate, followed by washing. Then, varying concentrations of free NP- ϵ -aminocaproate were added in each well, and incubated for 1 h. After removing dissociated Abs by washing, residual mAbs were determined as described above. The anti-NP IgG1 mAb was displaced by much lower concentrations of NP- ϵ -aminocaproate than those required for the displacement of the

IgM counterpart (Fig. 3). The concentration of NP- ϵ -aminocaproate that caused 50% displacement was estimated to be 1×10^{-3} M for the anti-NP IgM mAb and 5×10^{-5} M for the anti-NP IgG1 mAb, respectively, thus showing again that the IgM has higher avidity for NP-BSA than the corresponding IgG1.

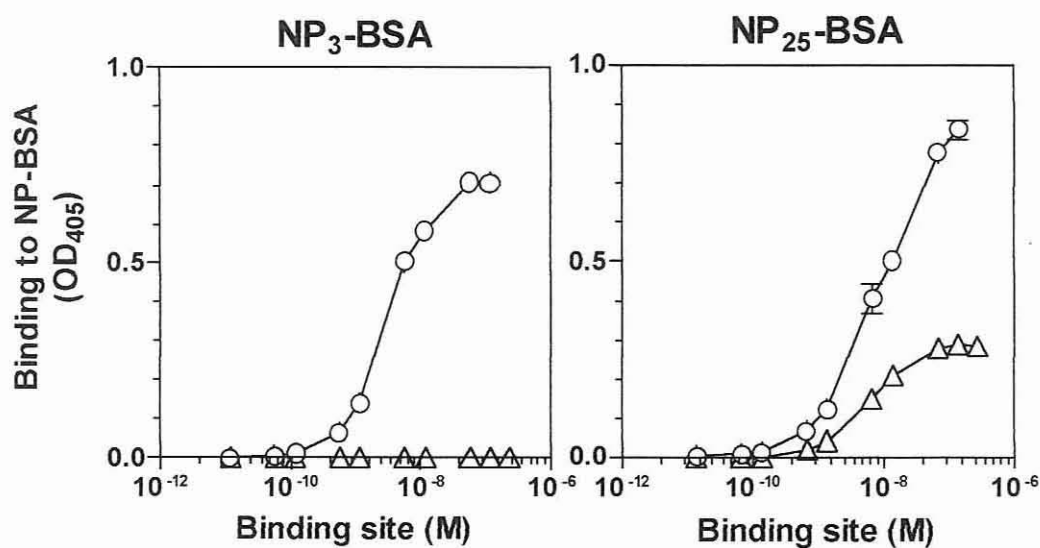


Fig. 2. Binding of the anti-NP IgM (circles) and IgG1 (triangles) mAbs to microplates that were coated with NP₃-BSA (left panel) or NP₂₅-BSA (right panel).

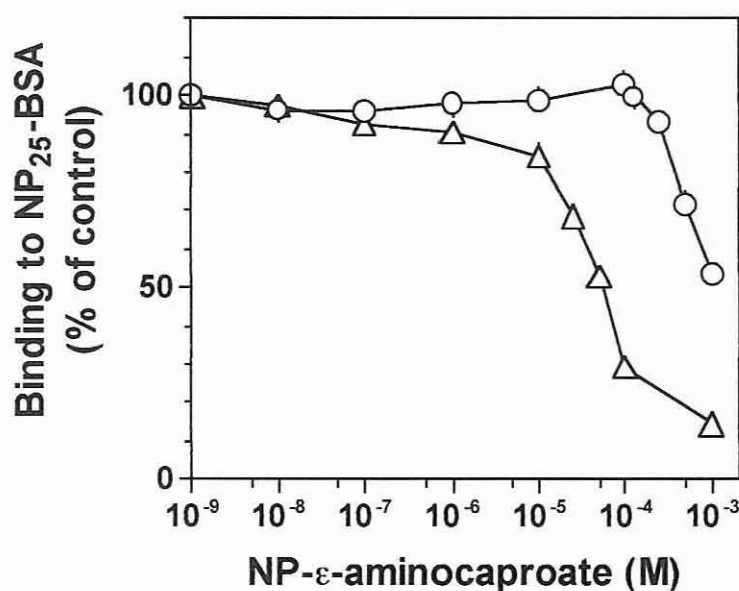


Fig. 3. Displacement by free NP of the anti-NP IgM and IgG1 mAbs that bound to NP₂₅-BSA-coated microplates. Varying concentrations of NP- ϵ -aminocaproate was added in each well to displace the bound anti-NP IgM (circles) or anti-NP IgG1 (triangles) mAbs. Data were presented as relative values, where the binding in the absence of the free NP was expressed as 100.

To estimate the role of the pentameric structure in the avidity of anti-NP IgM mAb, the IgM was dissociated into monomers by the treatment with 2-mercaptoethanol (2-ME) (10). Anti-NP IgM or IgG1 mAb (10 μ g/ml) was treated with 1 M 2-ME at 37 °C for 2 h in PBS containing 4 mM EDTA, and examined for the binding to NP₂₅-BSA-coated plates at varying concentrations (Fig. 4). The 2-ME-treatment did not significantly affect the binding of the IgG1 mAb, suggesting minimal effects of 2-ME on the conformation of the Ag-binding sites. In contrast, the binding of the anti-NP IgM mAb was drastically reduced by the 2-ME-treatment to the level that is comparable to or a little lower than that of the IgG1. The monomeric anti-NP IgM showed more than 1000-fold reduced binding to NP-BSA compared with the pentameric form. Thus, our results indicate that the pentameric structure of an IgM molecule plays an important role in the binding to an Ag by increasing avidity. A set of mAbs belonging to various isotypes that bear a common V region have been isolated from a lymphoma patient (12), or obtained as spontaneous class switch variants of hybridomas (13,14). In the former case, anti-tubulin mAbs of the IgA and IgM classes showed higher affinity than the IgG1 counterpart. The authors have suggested that not only the polyvalency but also the interaction between the V region and the Fc portion may modulate the Ag-binding capacity (12). This can not be ruled out in our experiments either because the monomeric form of the IgM mAb showed a little weaker affinity than the corresponding IgG1mAb (Fig. 4).

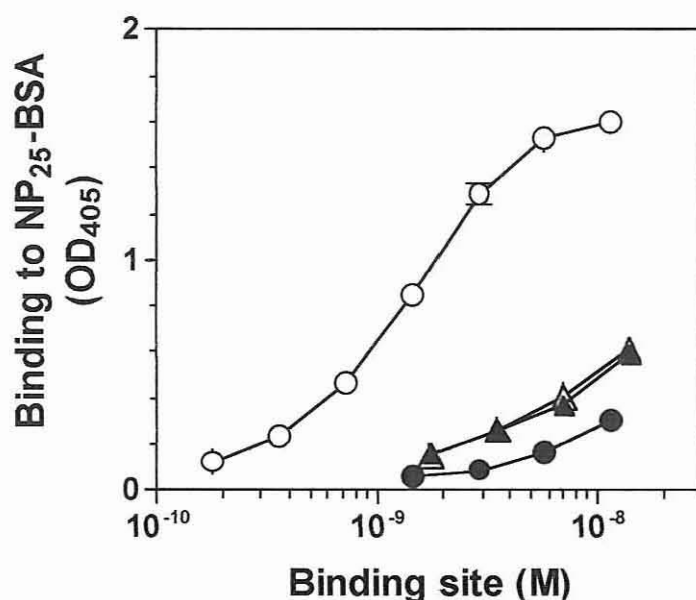


Fig. 4. Effects of 2-ME-treatment on the avidity of anti-NP mAbs. Anti-NP IgM (circles) and IgG1 (triangles) mAbs were treated with 1 M 2-ME (closed symbols) or left untreated (open symbols) as described in the text, and assayed for the binding to NP₂₅-BSA-coated plates.

In conclusion, the polyvalency in IgM Abs may play a crucial role in improving avidity by increasing the density of Ag-binding sites. Affinity maturation of IgG Abs is considered to be the process in which a great loss of avidity during class switching to

IgG is compensated by somatic hypermutation of V genes coupled with the positive selection of mutated B cells.

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