Local Antibody Response in Peyer’s Patches to the Orally Administered Dietary Protein Antigen

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To understand local antibody production to dietary protein antigens in the gut, the reactivity of the monoclonal antibodies (mAbs) from Peyer’s patches of BALB/c mice raised against orally administered hen egg lysozyme (HEL) was studied. These mAbs were of IgG1 (7 clones), IgA (5 clones) and IgM (13 clones) isotypes. Some of the HEL-binding mAbs preferentially reacted with reduced, carboxymethylated HEL, rather than with native HEL. mAbs of the IgA and IgM isotypes had cross-reactivity with other unrelated environmental antigens such as E. coli, single-strand DNA, and soluble components of mouse food. In contrast, the IgG1 mAbs did not cross-react with these antigens. The average of the $K_a$ values for HEL of these mAbs was in the order of $10^{-6}$ M, which is moderately higher than those of mAbs from the preimmune repertoire. These results suggest that, under normal physiological conditions, orally administered dietary proteins predominantly induce the local production of polyreactive IgA/IgM antibodies cross-reacting with environmental luminal antigens.

Key words: Peyer’s patches; polyreactive antibody; mucosal IgA response; lysozyme

Orally administered antigens, including dietary protein antigens, induce local antibody production in the gut dominated by the IgA isotype.11 As the inductive site, Peyer’s patches (PP) constitutively develop the germinal centers necessary for generating the humoral memory response to T-dependent antigens.12-15 PP differ from other lymph nodes and lack afferent lymphatics. Thus, the uptake of antigens takes place only through the follicle-associated epithelial of PP, mainly by M cells in the follicle-associated epithelium layer.16 M cells have been shown to take up and transport antigens, including bacteria,17 from the lumen of the intestine either in an intact form or as immune complexes.5,9,18 These antigens then activate B cells in PP to induce specific antibody production. This has been confirmed by studies in which monoclonal IgA antibodies specific to the infectious bacteria, Salmonella typhimurium and Vibrio cholerae, were generated from PP of mice orally administered with these pathogenic bacteria.19,20 Antigen-specific B cells activated in PP finally home to the lami-

na propria of the intestine where they secrete specific immunoglobulins into the gut lumen.5

The characteristics of antibodies locally produced in PP to orally administered antigens are considered to be different from those induced systemically in the serum in several respects. First, most B cells in PP undergo class switching to the IgA isotype,2,4,11,21,22 and the produced IgA antibodies are secreted into the gut.4,23 Second, the majority of oral antigens would lose their native conformational epitopes by digestion before reaching PP to activate B cells. Third, the abundance of environmental antigens such as dietary food components and luminal bacteria may affect the activation and selection of the B cell repertoire specific to oral antigens. Relating to this last point, we and others have previously shown that some of the IgA antibodies produced in Peyer’s patches5 and in the mucosal secrete50 were polyreactive antibodies that could bind to various antigens of different structures. The local antibody response to orally administered antigens has been studied at the polyclonal level by measuring specific antibodies in mucosal secrete such as the fecal extract. The response to protein antigens is generally accompanied by weak mucosal IgA production in the absence of an adjuvant, which makes it difficult to examine the specificity or affinity of locally produced antibodies to dietary protein antigens.

In this present study, we prepare a series of hybridomas secreting monoclonal antibodies from PP of mice orally administered with hen egg lysozyme (HEL), and examine the specificity of each by ELISA. Although the monoclonal antibodies may not always mirror the whole specificity of antibody response in the gut, the use of monoclonal antibodies still has an advantage to gain insight into the unique features of locally produced antibodies to single dietary protein antigens. Our findings would be helpful to understand the local antibody response in the gut to dietary antigens.

Materials and Methods

Materials. Hen egg lysozyme (HEL), reduced carboxymethylated HEL (MEL), turkey egg lysozyme (TEL), human lysozyme (HML), ovalbumin (OVA), calf thymus DNA, bovine serum albumin (BSA), and gelatin were purchased from Sigma Chemicals (St.

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Abbreviations: PP, Peyer’s patches; mAb, monoclonal antibody; HEL, hen egg lysozyme; MEL, carboxymethylated hen egg lysozyme; TEL, turkey egg lysozyme; HML, human lysozyme; OVA, ovalbumin; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; PBS-Tween, PBS containing 0.05% Tween 20
Louis, MO, U.S.A.). MP20 mouse food was purchased from Oriental Yeast Co. Ltd (Tokyo, Japan). E. coli K99 was kindly provided by Dr. Y. Sugita-Konishi (National Institute of Infectious Diseases, Tokyo, Japan).

**Oral administration of HEL, PP cell preparation and hybridoma production.** Female 6-week-old BALB/c mice (specific pathogen-free) were obtained from Charles River Japan (Kanagawa, Japan) and maintained in a conventional animal room. The mice were orally administered with 1 mg of HEL on days 0, 14 and 21. Sera and feces were collected on days 0, 14, 21 and 24 and stored at −20°C before use. A fecal extract was prepared as described.17 Feces were homogenized in PBS containing 0.01% sodium azide (200 mg of feces/ml) by vortexing, and the resulting supernatant was recovered by centrifugation (15,000 × g at 4°C for 10 min). The anti-HEL antibody titer in the serum and fecal extract was measured by ELISA as described later. On day 24, PP from these six mice were pooled, and a cell suspension was prepared. The cells were then fused with myeloma X63-Ag8-6.5.3 as described.18 HEL-specific clones were screened by ELISA and cloned by serial limiting dilution. The clonality was confirmed under a microscope so that single wells each contained a single cell when being plated.

**Enzyme-linked immunosorbent assay (ELISA).** The anti-HEL antibody in the serum, fecal extract, and culture supernatant was measured. An ELISA plate was coated overnight with HEL (10 μg/ml in a borate buffer at pH 8.5) at 4°C and then blocked with 1% gelatin in PBS at 37°C for 2 h. A serial dilution of the serum and fecal extract with PBS containing 0.05% Tween 20 (PBS-Tween) was applied to the plate, before incubating at 37°C for 2 h. The plate was washed four times with PBS-Tween after each incubation step. Detection was performed with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgA (Sigma), HRP-conjugated sheep anti-mouse IgG (Jackson Immuno Research, U.S.A.), HRP-conjugated sheep anti-mouse IgM (Cappel, U.S.A.) and o-phenylenediamine (OPD, Wako Pure Chemical Industries, Osaka, Japan). The subclass of the IgG monoclonal antibodies and the L chain usage were determined by using an Isotyping kit (GIBCO-BRL, Gaithersburg, MD, U.S.A.).

The concentration of IgA, IgG and IgM in supernatants of the cell cultures was determined by sandwich ELISA. ELISA plates (Immuno-plate 1, Nunc, Denmark) were coated overnight with anti-mouse Ig (5 μg/ml in PBS) at 4°C. The wells were then blocked for 2 h with 1% gelatin in PBS before incubating the supernatants for 2 h. Detection was performed with HRP-conjugated goat anti-mouse Ig specific for each isotype or with HRP-conjugated goat anti-mouse κ chain (Southern Biotechnology Associates, Birmingham, AL, U.S.A.) and OPD. Standard curves were obtained by using the controls, mouse IgA (ICN, CA, U.S.A.), IgG (Chemicon International Inc., CA, U.S.A.) and IgM (Zymed, CA, U.S.A.).

The reactivity of each mAb in the culture supernatants was examined as described.19 Briefly, ELISA plates were coated overnight at 4°C with 100 μl/well of one of the following in PBS: 10 μg/ml of HEL, carboxy-methylated HEL (MEL), turkey lysozyme (TEL), human lysozyme (HML) or OVA; 100 μg/ml of a soluble fraction of MF20 homogenized in PBS. The plates were coated with heat-denatured single-stranded calf thymus DNA (2.5 μg/ml) by air-drying at 37°C overnight.19 The unfilled sites of the wells were blocked with 1% gelatin in PBS, before being incubated with the supernatants. The binding of mAbs to Ags was detected with HRP-conjugated goat anti-mouse Ig specific to each isotype. Binding is considered to have been positive when giving an OD value at 492 nm of >0.1+background.

**Competitive ELISA and estimation of the Kd value.** Competitive inhibition studies on mAbs were performed by measuring the binding of mAbs to the antigen absorbed on a detection plate in the presence of a soluble antigen.15 mAbs were purified by gel permeation HPLC with a Superose 12 column (Pharmacia, Uppsala, Sweden). A hundred micro liters of PBS-Tween and mAbs (1–5 μg) was mixed with 100 μl of PBS-Tween containing various amounts (0–2 mg) of a soluble antigen. After 18 h of incubation at 4°C, each mixture was transferred to an ELISA plate that had been pre-coated with the corresponding antigen. After 1 h of incubation and subsequent washing with PBS-Tween, the mAb bound to the solid-phase antigen was measured as already described. The apparent Kd values were calculated from the amount of antigen that produced 50% inhibition in this assay.20

**Results**

The systemic and mucosal antibody responses of mice given HEL were assessed with ELISA by measuring the systemic and fecal antibody production specific to HEL. Only a little increase in the anti-HEL IgA and IgG antibody response in the serum was apparent by day 24, but there was no significant increase in the IgM isotype (Fig. 1(A)). A slightly elevated anti-HEL IgA response in the fecal extract was observed on day 24 (P < 0.01, Student’s t test with unequal variance; Fig. 1(B)). Therefore, although not being strong, the local anti-HEL antibody response is considered to have been induced in these mice.

The isotopes of the antibodies from hybridomas generated from PP of the mice orally administered with HEL were analyzed. Of the original 24 seeded wells, 16 and 18 wells contained detectable amounts of IgA and IgM antibodies, respectively. IgG was also detected in 4 wells. These hybridomas were further cloned by limiting dilution and resulted in 25 stable clones consisting of 7 IgG1, 5 IgA and 13 IgM isotopes. All of these antibodies used a κ light chain.

To study the specificity of HEL-binding mAbs from PP, the reactivity of the mAbs with lysozymes (chicken, HEL; turkey, TEL; and human, HML) and with denatured HEL (reduced, carboxy-methylated: MEL) was examined by ELISA. It has been shown that anti-HEL
mAbs cross-reacted with lysozymes from other species, in some cases with a higher affinity than for HEL. According to the primary structure, HEL shares 95% homology with, TEL, and 60% with HML. MEL was used as a model for denatured HEL. HEL has a structure with four disulfide bonds that should be lost in MEL by reduction and carboxy-methylation. Our previous study has shown that some of the IgA antibodies produced in Peyer's patches were polyreactive. To test if anti-HEL mAbs of the IgA and IgM isotypes might have polyreactive features, the reactivity with several other antigens (denatured single-strand (ss) DNA, BSA, OVA, and the soluble fraction of MF20 mouse food) and the bacterial agglutination activity of these mAbs were studied.

Table 1 summarizes the binding specificity of the mAbs to lysozymes and to various antigens. The reactivity of four HEL-binding IgA mAbs derived from preimmune PP is also shown. Based on the reactivity evaluated by ELISA, the mAbs were grouped as follows (Table 1): 7 IgG1 mAbs into two groups, GI and GII; 5 IgA mAbs into two groups, AI and AII; and 13 IgM mAbs into four groups, MI–MIV. The reactivity of a typical mAb in each group is shown in Fig. 2. The binding to these antigens was in a dose-dependent manner. IgG1 mAb HG1 (GI) reacted with HEL and MEL, but not with any other antigens, including TEL and HML. Similarly, IgG1 mAbs in GII reacted only with lysozymes, and not with the other antigens. IgA mAbs in AI and AII reacted with various antigens as well as with lysozymes, but did not agglutinate E. coli. All IgM mAbs agglutinated live E. coli. These IgM mAbs also bound to various antigens, except that the mAbs in MII were specific to lysozymes.

The results of the reactivity assessment indicate the following two features: 1) most of the anti-HEL mAbs (21/25 clones), except for the mAbs in GI and MIV, preferentially reacted with denatured lysozyme (MEL) rather than with native lysozyme (HEL); 2) IgG1 mAbs bound only to lysozymes, while IgA/IgM mAbs in the other groups showed cross-reactivity with one or more of the antigens, including putative environmental antigens in the gut (i.e., MF20, DNA and E. coli). Thus, many of these IgA/IgM mAbs can be considered to be

### Table 1. Reactivity of the Anti-HEL MAbs from PP with Various Antigens

<table>
<thead>
<tr>
<th>Group</th>
<th>HEL</th>
<th>MEL</th>
<th>TEL</th>
<th>HML</th>
<th>E. coli&lt;sup&gt;b&lt;/sup&gt;</th>
<th>BSA</th>
<th>MF20</th>
<th>OVA</th>
<th>DNA</th>
<th>anti-HEL mAb</th>
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<tbody>
<tr>
<td>GI</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>HG1</td>
</tr>
<tr>
<td>GII</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
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<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>HA1, 2, 5</td>
</tr>
<tr>
<td>AII</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>HA3, 4</td>
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<td>++</td>
<td>++</td>
<td>+</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>HM2, 3, 4, 6, 7, 14</td>
</tr>
<tr>
<td>MII</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>HM5, 11, 13</td>
</tr>
<tr>
<td>MIII</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>HM15</td>
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<tr>
<td>MIV</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>HM16, 17, 19</td>
</tr>
<tr>
<td>A1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>+</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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<td>+</td>
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<tr>
<td>A8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<td>A14&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ND</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> The reactivity of each mAb (10 μg/ml) was studied by ELISA. The reactivity was scored as follows according to the value of absorbance at 492 nm: <0.15, −; 0.15–0.4, +; 0.4–0.8, ++; >0.8, ++++. mAbs were grouped based on their cross-reactivity.

<sup>b</sup> Reactivity with E. coli was examined by agglutination activity as described in Methods. Positive and negative activity at 5 μg/ml is shown as + and −, respectively.

<sup>c</sup> The reactivity of preimmune IgA mAbs are shown as controls. MAbs A1, A6, A8, A11 and A14 were IgA mAbs reacting with HEL derived from PP of unimmunized BALB/c mice.

<sup>d</sup> Not determined.
polyreactive antibodies.

Although the reactivity estimated by ELISA is useful to characterize mAbs, it was not possible to compare the reactivity of mAbs of different isotype with the various antigens whose binding ability to an ELISA plate would be different. To further study the binding properties of mAbs, competitive ELISA was performed to estimate the $K_d$ values for eight representative clones of each isotype, HG1 and HG2 (IgG1), HA1 and HA4 (IgA), and HM4, HM5, HM15 and HM19 (IgM) (Fig. 3). Increasing the amount of liquid-phase antigen resulted in decreased binding of each mAb to the solid-phase antigen. However, the maximum binding inhibition of some of the IgA and IgM mAbs was no more than 50% for HEL, as well as for BSA and OVA (Fig. 2).

The $K_d$ values for HEL, MEL and the other Ags estimated by this competitive ELISA are shown in Table 2. Three mAbs, HG1, HM15 and HM19, showed higher or similar affinity/avidity to HEL than to MEL, while the other mAbs had higher affinity/avidity to denatured HEL (MEL) than to native HEL. The avidity of the mAbs to HEL/MEL was in the order of $10^{-5}$ M to $10^{-6}$ M, while that to BSA and to OVA was in the order of $10^{-7}$ M. The avidity of mAbs HA1, HA4 and HM15 to ssDNA was even higher than that to HEL/MEL, being in the order of $10^{-7}$ M and $10^{-8}$ M, respectively. In comparison, the $K_d$ values of preimmune IgA mAbs for HEL and MEL were below the detection limit ($>7.0 \times 10^{-5}$ M) (Table 2).

**Discussion**

The uptake of digested protein is considered to induce several immunological responses. One of these responses in the gut is local antibody production that is dominant in IgA antibodies against oral antigens. These locally produced IgA/IgM antibodies are secreted into

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**Table 2.** $K_d$ Values Estimated for HEL, MEL and Other Antigens

<table>
<thead>
<tr>
<th>mAb</th>
<th>HEL</th>
<th>MEL</th>
<th>BSA</th>
<th>OVA</th>
<th>ssDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG1</td>
<td>$4.34 \times 10^{-6}$</td>
<td>$8.87 \times 10^{-6}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HG2</td>
<td>$3.36 \times 10^{-5}$</td>
<td>$7.34 \times 10^{-6}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HA1</td>
<td>$&gt;7.00 \times 10^{-5}$</td>
<td>$6.29 \times 10^{-6}$</td>
<td>$&gt;1.50 \times 10^{-5}$</td>
<td>$&gt;2.30 \times 10^{-3}$</td>
<td>$5.75 \times 10^{-7}$</td>
</tr>
<tr>
<td>HA4</td>
<td>$&gt;7.00 \times 10^{-5}$</td>
<td>$9.10 \times 10^{-6}$</td>
<td>$1.50 \times 10^{-5}$</td>
<td>$2.00 \times 10^{-7}$</td>
<td>$2.32 \times 10^{-1}$</td>
</tr>
<tr>
<td>HM4</td>
<td>$1.26 \times 10^{-3}$</td>
<td>$4.97 \times 10^{-6}$</td>
<td>$&gt;1.50 \times 10^{-5}$</td>
<td>$&gt;2.30 \times 10^{-3}$</td>
<td>—</td>
</tr>
<tr>
<td>HM5</td>
<td>$&gt;7.00 \times 10^{-5}$</td>
<td>$5.95 \times 10^{-6}$</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>HM15</td>
<td>$6.40 \times 10^{-6}$</td>
<td>$5.61 \times 10^{-6}$</td>
<td>$1.19 \times 10^{-5}$</td>
<td>$&gt;2.30 \times 10^{-3}$</td>
<td>$8.51 \times 10^{-9}$</td>
</tr>
<tr>
<td>HM19</td>
<td>$2.5 \times 10^{-6}$</td>
<td>$&gt;7.00 \times 10^{-3}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A6</td>
<td>$&gt;7.00 \times 10^{-5}$</td>
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<td>—</td>
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<td>—</td>
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<tr>
<td>A11</td>
<td>$&gt;7.00 \times 10^{-5}$</td>
<td>$7.00 \times 10^{-3}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

—: not determined.
the lumen of the intestine and may act as a mucosal barrier to prevent further uptake of antigenic digested peptides that may cause an unfavorable immunological response such as food allergy. However, such local antibody production against a protein dietary antigen is generally weak, which limits a profound study on locally produced antibodies. By using a series of mAbs from PP raised against orally administered HEL, we attempted to study the features of locally produced antibodies to a single protein antigen. Although the specificity of mAbs may not always reflect those of locally produced antibodies in vivo, this approach would allow tracking some of the features of locally produced antibodies in the gut.

Protein dietary antigens are supposed to be denatured and digested in the gut. Thus, some of these locally produced antibodies would be specific to the epitopes available on denatured antigens. By using reduced, carboxymethylated HEL (MEL) as the model for a denatured form of HEL, we studied the binding preference of the HEL-binding mAbs. MEL is considered to have an unfolded flexible structure because of the loss of internal disulfide bonds, which would mimic denatured HEL. At least four groups of mAbs represented by mAb HG1, HA1, HA2 and HM19 preferentially reacted with MEL. The $K_d$ values for MEL were about ten times higher than those for HEL in these mAbs (Table 2). Thus, the specificity to denatured epitopes may reflect a feature of some of the locally produced antibodies in the gut against dietary protein antigens. It should be noted, however, that monoclonal antibodies preferentially bound to a denatured antigen could be raised by conventional peritoneal immunization with an adjuvant, as has been reported in the case of anti-$\beta$-lactoglobulin mAbs.240

Some mAbs, e.g., IgG1 mAb HG1 and IgM mAb HM19, preferentially bound to intact HEL (Fig. 2), indicating that these mAbs were likely to recognize the conformational epitopes available on native HEL. HEL with an intact epitope might be absorbed through the intestinal wall or transported by M cells into PP to elicit antibody production. Indeed, remarkable amounts of intact dietary proteins have been detected in serum after meals.1,25,26 In this connection, the specificity of locally produced antibodies in PP may depend on the presence of food additives or food components such as capsaicin27 which modulate intestinal permeability. The presence of such substances may cause an influx of large peptides or partially denatured proteins into PP without further digestion by peptidases in microvilli, which may result in profound antibody production towards native epitopes.

The local antibody response in the gut to dietary protein antigens is considered to be weak. In accordance with this, the avidity of the mAbs to HEL/MEL analyzed in this study was in the order of $10^{-5}$-10^{-6}$ M (Table 2). These values are moderately higher than those for the preimmune IgA mAbs that bound to HEL, but are significantly lower than the previously reported $K_d$ values ($10^{-8}$ M) for some of the anti-HEL mAbs raised from the spleen by conventional intraperitoneal immunization with an adjuvant.28 In the physiological condition, a natural antibody is known to take a major role in activating the complement system, which in turn enhances the affinity maturation of antigen-specific B cells. However, this enhancement is generally not as effective as that by an adjuvant at least in the systemic humoral response against non-pathogenic protein antigens. Our affinity measurement of the mAbs suggests that, in spite of the predominant appearance of natural polyclonal antibodies in pre-immune PP,19 which is different from the response to replicating pathogenic antigens, a
mucosal adjuvant would be required for efficient affinity maturation of locally induced antibodies in PP to dietary protein antigens. The majority of the IgA/IgM anti-HEL antibodies recovered from Peyer’s patches in this study showed cross-reactivity with other antigens. Moreover, five IgA mAbs and one IgM mAb had self-reactivity to ssDNA with considerable avidity, which may reflect the prevalence of anti-DNA specificity in PP.13 The polyreactive feature is generally associated with natural antibodies of the IgM/IgA isotype.29 These polyreactive natural antibodies react with several structurally unrelated antigens, including self-components such as DNA, which are considered to perform a defense function against invasive microorganisms. Natural polyreactive IgA antibodies have been found in human milk and other secretions.30 We have recently characterized B cells in mouse PP secreting polyreactive IgA antibodies,15 possibly establishing the first defense function at the site of sampling luminal antigens. Polyreactive natural antibodies are produced by B-1 B cells that express CD5 and/or Mac-1 as surface markers.11 Since about 10% of the total population of B cells in PP is B-1 B cells,32,33 it is possible that some of the polyreactive IgA/IgM antibodies in PP are secreted by activated B-1 B cells. Recent studies have revealed that B-1 B cells have distinct signaling activity through the B cell receptor and co-receptors that characterize their intrinsic proliferative behavior by antigenic stimulation.34-36 Therefore, although the population in PP is small, B-1 B cells can be readily activated to secrete polyreactive antibodies upon this exposure to cross-reactive dietary protein antigens (e.g. HEL) transported from the lumen into PP.

A molecular basis for the polyreactive features of IgA/IgM mAbs can be related to the valency of the immunoglobulin molecules. IgA and IgM form dimeric and pentameric structures, respectively. Although the individual antigen-binding sites may be of low affinity, the avidity increases greatly as a consequence of polymerization. This would be a good reason for low-affinity IgA/IgM mAbs being efficiently bound to the antigens adsorbed to the ELISA plate that offer dense antibody binding sites for these multivalent mAbs (Fig. 2 and Table 2).

The polyreactive features may also have a great advantage for the physiological functions of secretory IgA/IgM antibodies. One of the most important physiological functions of secretory antibodies is to prevent invasion by antigens from the gut.16 The variety of luminal antigens is enormous, including dietary food contents and enterobacteria. The production of polyreactive secretory antibodies with redundant specificity may be a more effective way to maximally cover the antigenic specificity in the gut than making a series of monospecific antibodies.

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References


