Natural Autoreactive B Cells in Transgenic Mice Reproduce an Apparent Paradox to the Clonal Tolerance Theory

Severine Koenig-Marrony, Pauline Soulas, Sylvie Julien, Anne-Marie Knapp, Jean-Claude Garaud, Thierry Martin and Jean-Louis Pasquali

*J Immunol* 2001; 166:1463-1470; 
doi: 10.4049/jimmunol.166.3.1463

http://www.jimmunol.org/content/166/3/1463

---

**References**

This article cites 36 articles, 19 of which you can access for free at:

http://www.jimmunol.org/content/166/3/1463.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Natural Autoreactive B Cells in Transgenic Mice Reproduce an Apparent Paradox to the Clonal Tolerance Theory

Severine Koenig-Marrony, Pauline Soulas, Sylvie Julien, Anne-Marie Knapp, Jean-Claude Garaud, Thierry Martin, and Jean-Louis Pasquali

Naturally occurring autoreactive B cells are thought to be physically eliminated or rendered functionally silent through different mechanisms of tolerance. However, multireactive low affinity natural autoantibody-producing B cells seem to escape these mechanisms in normal adults and could constitute the B cell pool from which pathological autoantibodies can emerge. To analyze this apparent paradox to the clonal tolerance theory, we have made two transgenic mouse lines (μκ, μδκ) producing a natural low affinity multireactive human autoantibody. These models enable us to test both the central tolerance mechanisms (reactivity with single-stranded DNA) and the peripheral tolerance mechanisms after Ag administration. Not only are the multireactive B cells not deleted in the bone marrow, they circulate and remain in the periphery even after the prolonged administration of Ag, the presence of membrane IgD increasing the number of mature autoreactive B cells. Self-reactive B cells are shown to be autoantigen ignorant both in vivo and in vitro, but they are not anergic because they can be easily activated through both B cell receptor-dependent and -independent pathways. Thus, these mouse lines reproduce an apparent paradox to the clonal tolerance theory meriting further investigation of the biological significance of this phenomenon. The Journal of Immunology, 2001, 166: 1463–1470.

In either normal or pathological circumstances, B cells with self-reactivity can mature and produce either so called natural autoantibodies (nAAbs) or pathological autoantibodies. However, different mice models, mainly transgenic (tg), have been used to test the clonal selection hypothesis and have all shown that autoreactive B cells are deleted or rendered silent to self Ags through different processes (anergy or receptor editing) after central or peripheral Ag encounter (1). To be precise, B cell tolerance appears to be more complex than has been thought; the first distinction that could be of importance in determining the autoreactive B cell fate seems to be the mode of autoantigen presentation to the B cell membrane (mb) anchored or soluble Ag. When mb-bound Ag are presented to immature bone marrow B cells, these are usually deleted (2, 3), in some cases to various degrees (4) even when the B cell receptor (BCR) has a very low affinity for the autoantigen (5). However, the actual situation is probably more complex than previously supposed, because natural autoreactive B cells with low affinity for a mb Ag (Thy1) appear to be positively selected (6).

B cell tolerance to soluble Ags has also largely been explored in both tg and non-tg mice. It appears that a number of parameters can affect this complex process and the biology of the self-reactive B cells. For instance, in the case of high affinity BCR for an autoantigen, different outcomes of the B cells have been described depending on the site of Ag encounter. If the Ag is present in the bone marrow, newly generated B cells can be deleted (7), rendered anergic (8 –11), or subjected to receptor editing (12, 13). In contrast, if these high affinity B cells encounter the autoantigen in the periphery, they are deleted (14, 15). Murine models expressing tg BCR with moderate affinity for soluble autoantigens were also studied in the case of rheumatoid factors (RF) (16) and single-stranded DNA (11); the models indicated that anergy is the main tolerance mechanism. However, in addition to the affinity of the BCR and the geography of the Ag encounter that determine the autoreactive B cell fate, the valency of the Ag also appeared critical (17), as well as the cross-reactivity (18).

It is of note that the two last mentioned parameters (extensive cross-reactivity with repetitive determinants on soluble self-Ags) as well as low affinity are the main characteristics of nAAbs. Usually, these Abs are multireactive low affinity IgM encoded by almost unmutated germline variable region genes. They could be the main components of the primary B cell repertoire (19) and their natural escape from the known mechanisms of B cell tolerance in newborns and in adults constitutes an apparent paradox to the clonal tolerance theory. The study of nAAb-producing B cells could focus on related questions relevant both to B cell physiology and to pathologic autoimmunity: 1) how multi self-reactive B cells can escape tolerance check points, and what is their functional state when they reach the periphery; 2) what is the biological function of these nAAb B cells; and 3) are the different autoantigens able (and under what conditions) to drive B cell maturation and lead to the production of potentially pathogenic AAbs?

These questions are not easy to address in a normal polyclonal state. To precisely follow the behavior of multireactive nAAb producing B cells, we generated tg mice that were specifically designed to produce this type of AAb in experimental conditions where both central and peripheral tolerance mechanisms could be tested. The multireactive AAb is a prototype of natural human
AAb and reacts with single stranded DNA, thyroglobulin, myoglobin, and human IgG (hIgG; Ref. 20). Here we describe these models and show that nAAb producing B cells are autoantigen ignorant on a nonautoimmune background but can still be activated through BCR-dependent and -independent pathways.

Materials and Methods

DNA constructs

Two vectors, pESAC38 and AC38K (provided by K. Rajewsky and coworkers, University of Cologne, Germany), were modified. These vectors allow the expression of a murine Igκ, except restriction sites SalI and Clal were introduced in both vectors by site-directed mutagenesis on both sides of the murine-rearranged variable region (V) Ig H and L chain genes. The vectors were digested by SalI and Clal, and the murine Vκ-D-JH and Vκ-Jk regions were removed. The human rearranged V genes originating from a prototypic multireactive nAAb (SmI) bearing the G6 VH Id and the 17 109 VK Id (20) were amplified by PCR with the original vectors pRTMI and PSVG-Vk3 (20) for the light chain. After amplification, the rearranged H and L chain V genes (VH-D-JH and Vk-Jk) were subcloned into pESAC38 and AC38K, respectively. The resulting vectors BISMI (H chain) and AC38KISMI (L chain) allow the expression of nAAb SmI variable regions grafted onto murine constant regions (μκ). We modified BISMI to allow the expression of IgM and IgG. For this purpose, BISMI was digested by XhoI and EcoRI, and the resulting VDJ-Cμ fragment was subcloned into the plasmid pHelix (Boehringer-Mannheim Biochemicals, Mannheim, Germany). A 12-kb region XhoI-Smal originating from the pG113XH vector (provided by K. Rajewsky) containing the C6-C8s region was subcloned into the vector pHelix-VDI Cμ. The resulting vector was termed pHelixα6SMI. Fig. 1 shows the maps of the different vectors.

Creation of tg mice

H and L chain genes coding for the chimeric murine-human nAAb were excised from vector sequences by EcoRI digestion. The inserts were purified on a 10–50% sucrose density gradient followed by extensive dialysis against 5 mM Tris, 0.1 mM EDTA pH 7.5 buffer. The purified construct was microinjected into pronuclei of fertilized (C57BL/6 + SJL) F1 zygotes, and these were transferred to pseudopregnant females to produce the tg mice. Founder lines were then maintained by backcross mating with the C57BL/6J or SJL J1 strains (Jackson Laboratory) at 0.5 mg/ml.

Injection of Ags

Mice were i.p. injected with 2 or 4 mg of monomeric hIgG (Novartis, Basel, Switzerland), i.v. injected with 2–8 mg of aggregated (15 min at 65°C) hIgG, or i.v. injected with 8 mg of myoglobin (Novartis).

Quantification of serum IgM, IgMb, IgMa/17 109, and reactivity with the different Ags

The level of the nAAb in the serum of 5- to 8-wk-old mice was determined by ELISA. Plates were coated with goat anti-mouse IgM (Cappel Laboratories, Durham, NC) at 1 μg/ml, then washed three times. Plates were blocked for 1 h at room temperature with BSA 1%, washed, and incubated with serial dilutions of serum samples at 37°C in 100 μl wells for 1 h. Bound hIgG, hIgM, or hIgG/hIgM was determined by adding biotin-labeled anti-mouse IgM (anti-IgMa or anti-IgMb; Cappel Laboratories). After washings, streptavidin-peroxidase (Jackson ImmunoResearch, West Grove, PA) and peroxidase substrate (o-phenylenediamine dihydrochloride; Sigma, St. Louis, MO) were sequentially added. Absorbance was measured at 492 nm.

FACS analysis

Preparation and staining of cells was as follows: spleen cells were isolated by disruption of spleen between glass slides, lysis of RBC with NH4Cl-Tris, then counting. Peritoneal cells were obtained before splenectomy by washing the peritoneal cavity with 3 ml of medium followed by RBC lysis. Cells were stained for 15 min on ice in RPMI 1640 (BioWhittaker, Walkersville, MD) with 2% FCS and 10 mM Na2S. Following washes, when necessary, cells were stained for 15 min with streptavidin–PE (Jackson Laboratory) at 0.5 mg/ml.

After washes and incubation in propidium iodide, the cells were analyzed using a single laser FACSscan flow cytometer (Becton Dickinson, San Jose, CA), and data was collected using the FACS CellQuest program. Only live cells were included in further analysis by excluding propidium iodide-stained cells.

Cell phenotyping was determined using the following reagents: FITC anti-mouse B220 (PharMingen, San Diego, CA), biotin anti-mouse IgMa or IgMb (PharMingen), biotin anti-mouse IgD (PharMingen), biotin 17 109 (provided by D. A. Carson, University of San Diego, San Diego, CA), biotin G6 (provided by Roy Jefferis, University of Birmingham, Birmingham, England), biotin goat anti-mouse IgM (Jackson Immunoresearch), biotin anti-CD19 (PharMingen), biotin anti-CD23 (PharMingen), biotin AA-4.1 (provided by R. Ceredig, Basel Institute for Immunology, Basel, Switzerland), biotin goat anti-mouse κ light chain (Jackson Immunoresearch), and streptavidin-PE (Jackson Immunoresearch).

IgG binding to tg B cells was tested by incubating C57BL/6 or nAAb tg splenocytes with increasing concentrations of hIgG after CD32 binding was inhibited by an anti-CD32 Ab (2.4G2, 1 μg/ml, Fe Block; PharMingen). After washing, cells were labeled with an FITC anti-mouse IgMa (PharMingen) and PE-labeled F(ab)’2 of goat anti-hIgGFc (Jackson Immunoresearch). Immunohistochemistry

Tissues were snap-frozen in optimal cutting temperature medium (Tissue-Tek; Europemedex, Soufflèwesiersheim, France). Six-micrometer sections were prepared from the tissue blocks and stained with FITC goat anti-mouse IgMa (or IgMb) (PharMingen), FITC goat anti-mouse IgDa (PharMingen), FITC goat anti-mouse CD3 (PharMingen), and biotin 17 109 followed by streptavidin–PE (Jackson Immunoresearch). Briefly, 100 μl of medium or an optimal dilution of the reagent was added to each slide and incubated for 2 h on ice. Slides were then washed with PBS (pH 7.2) before adding 100 μl of streptavidin–PE. The slides were then washed before mounting.

Generation of nAAb-secreting hybridomas

Spleens were removed from μ/μg tk mice and then teased apart in serum-free IMDM (Irvine Scientific, Santa Ana, CA). Spleen cells (103) were combined with nonsecreting myeloma SP20 cells, washed in serum-free medium, and then pelleted. Polyethylene glycol 1500 (0.8 ml; Boehringer Mannheim) was added slowly to the pellet while stirring. After 2 min, the cells were diluted slowly with medium, washed, and then plated out in four 24-well plates at 1 ml/well in IMDM plus 10% FCS. After 24 and 48 h, the wells were fed with medium supplemented with 10% FCS, HAT (hypoxanthine, aminopterin, thymidine; Sigma), and 10 μg/ml of anti-IgMa (or IgMb). Fusion culture supernatants were tested for the presence of the nAAb between days 14 and 21. Positive wells were then cloned and positive monoclonal hybridomas were selected by ELISA on the basis of 17 109 expression. Multireactivity of nAAb-tg hybridomas was tested as previously described (22, 23), except that biotin-labeled 17 109 was used to detect the binding of the chimeric Ab and of original SmI Ab.

B cell proliferation assays

Spleens were removed from tg mice, non-tg littermates, or control C57BL/6 mice and teased apart. Cells were washed and resuspended at 2 × 106/ml in RPMI 1640 (BioWhittaker) with the addition of antibiotics, glutamine, 50 μM 2-ME, and 10% heat-inactivated FCS. Cells (2 × 105) were added to each well of a round-bottom 96-well plate in a total volume of 100 μl. Another 100 μl of medium was then added containing one of the following reagents: LPS from Salmonella typhosa (Sigma) at 50 μg/ml, goat anti-mouse IgM (Jackson Immunoresearch), and revealing by biotin-labeled monoclonal anti-idiotope 17 109 (0.05 μg/ml). The serum reactivity was tested with human IgG, thyroglobulin, myoglobin, and ssDNA as previously described (20) except that nAAb were revealed with biotin-labeled anti-IgMa.
soluble or aggregated human IgG (Novartis) at different concentrations (0–20 mg/ml). Aggregated human IgG were prepared by heating hIgG at 65°C for 15 min.

Proliferation was measured on day 3 by adding 200 µl of medium containing 10% Alamar Blue (Interchem, Paramus, NJ). After 4 h, absorbance was measured at 590 nm using the Fluorite 1000 reader (Dynatech Laboratories, Chantilly, VA).

After in vitro stimulation with anti-IgM, LPS, or hIgG, the phenotype of the cells was determined by FACS analysis. Briefly the cells were stained using FITC goat anti-mouse IgMa (PharMingen), biotin goat anti-mouse CD86, or biotin anti-MHC class II (PharMingen), washed, and labeled with streptavidin-PE. The cells were incubated in propidium iodide and analyzed.

Results

The tg mice were designed to produce a chimeric nAAb with murine constant regions and human variable regions, which confer the multireactivity with different Ags, one of which is self Ag (ssDNA). The vectors contained all the mouse regulatory sequences flanking the Ig genes (Fig. 1).

nAAb expressing B cells are not deleted

By crossing mice expressing nAAb V_H (µ or µδ) and mice expressing nAAb Vk, we obtained nAAb double-tg mice (µk and µδk), which were analyzed at 4–6 wk of age. Approximately 35% (µδk) and 32% (µk) of total splenocytes express the AAb variable regions. The tg µ-chain expression can be detected by both anti G6 Id anti-µµ reagent, which were used indifferently in subsequent experiments. Fig. 2 clearly shows that in µδk mice, both in the bone marrow and in the spleen, the vast majority of B220^+ B cells coexpresses the tg H and L chains, respectively, µa^+ and 17109^+. Similar results were obtained with µk mice (data not shown). Only 2% of the bone marrow cells and 3% of the splenocytes express endogenous µb H chains in µδk nAAb mice compared with 3% (bone marrow) and 3% (spleen) in µk nAAb mice, showing that the allelic exclusion was almost complete. The maturation of the tg µδk B cells from the bone marrow to the spleen is associated with an increased expression of δa and CD23, but a decreased expression of AA4.1 as expected. Table I describes the phenotype of bone marrow, and splenic and peritoneal B cells in these mice. Thus, it appears that nAAb-expressing B cells are not deleted and join the periphery. The precise localization of the nAAb B cells in primary follicles was determined by immunohistology (Fig. 3). They are strictly localized in the B cell zones and were never detected (µδk or µk) within the germinal centers of secondary follicles or within the T cell zones, which could have indicated Ag-driven activation and expansion.

Chimeric nAAb retains original multireactivity

Grafting Smi variable regions onto murine constant regions of the H and L chains of Ig could modify the reactivity profile of the Ab. Therefore, we checked: 1) the serum reactivity of the IgMa; 2) the light chain Id expression of these IgMa; and 3) the ability of the tg B cells to bind hIgG. Finally, we generated hybridomas from tg splenic B cells. The serum reactivity of nAAb tg mice is shown in Fig. 4. At a serum concentration of 5 µg/ml of IgMa, the nAAb tg serum reacts with hIgG and slightly with ssDNA. Considering the Id expression, the levels of serum tg IgM were very similar in nAAb tg mice when the ELISA quantifications were performed using as a second reagent an anti-IgMa or 17109, implying that the tg H chain was only associated with the tg light chain (data not shown). We also checked the ability of nAAb tg B cells to bind hIgG. After CD32 blocking, and despite the low affinity of the nAAb, a concentration-dependant discrete binding of hIgG was detectable on IgMa-bearing B cells in tg splenocytes, as shown in Fig. 5. Finally, we generated hybridomas from tg splenic B cells;
these monoclonal chimeric Ab (both G6 and 17 109 positive, data not shown) reacted with ssDNA, human thyroglobulin, human myoglobin, and human IgG in a manner similar to the original human Smi Ab (Fig. 4).

Table 1. Phenotype of lymphocyte subpopulations in bone marrow, spleen, and peritoneum of nAAb μκ and μκ tg mice determined by FACS analysis

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Bone marrow</th>
<th>Spleen</th>
<th>Peritoneum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nAAb μκ</td>
<td>nAAb μκ/κ</td>
<td>nAAb μκ</td>
</tr>
<tr>
<td>B220</td>
<td>28 (10)</td>
<td>22 (4)</td>
<td>32 (9)</td>
</tr>
<tr>
<td>B220/IgMa</td>
<td>28 (9)</td>
<td>20 (3)</td>
<td>30 (8)</td>
</tr>
<tr>
<td>B220/IgMb</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>IgMa/IgMb</td>
<td>1 (1)</td>
<td>0.5 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>IgMa/17 109</td>
<td>24 (11)</td>
<td>20 (4)</td>
<td>30 (5)</td>
</tr>
<tr>
<td>IgMa/A.A 4.1</td>
<td>22 (8)</td>
<td>17 (5)</td>
<td>8 (5)*</td>
</tr>
<tr>
<td>IgMa/IgDa</td>
<td>9 (2)</td>
<td>30 (5)</td>
<td>30 (5)</td>
</tr>
<tr>
<td>IgMa/CD23</td>
<td>2 (2)</td>
<td>4 (2)</td>
<td>20 (6)*</td>
</tr>
</tbody>
</table>

*Values are expressed as the mean percentage of total mononuclear cells (± one SD) obtained from groups of five mice. *, Statistical differences between nAAb μκ/κ mice and nAAb μκ mice (p < 0.05, Mann-Whitney test).
Expression of the H chain on nAAb tg B cells seems to favor B cell maturation

Central tolerance mechanisms operate at the B cell stage where IgM, but not IgD, is expressed. The comparison of the two tg lines, mk and m, showed that mk tg B cells were neither deleted nor arrested at the pre-B stage. However, in the periphery, the percentage of tg B cells that express an immature phenotype (AA4.1 positive, CD23 negative) is higher in mk nAAb mice compared with m mk mice despite nearly equivalent absolute numbers of total B cells (Table I). Although not significantly different, the serum levels of IgMa seem higher in m mk mice compared with mk mice (Table II).

For instance, when heat-aggregated hIgG (8 mg) was i.v. injected in mk mice (Table III) we did not observe any modification of the tg B cell populations 2 and 5 days after Ag administration. In particular, Ag administration, no matter what autoantigen, does not lead to the deletion of autoreactive B cells nor to their activation either in the bone marrow or in the spleen. It does not induce a maturation arrest in the bone marrow, the fluorescence intensity of the tg mb IgM was not modified (data not shown), and tg B cells were not activated as judged by their unchanged expression of CD54, CD86, CD44, and MHC class II molecules (Fig. 5). We observed the same negative results after myoglobin administration. The results are identical in mk mice injected with aggregated hIgG at various concentrations. Finally, even repeated hIgG injections over a 1-month period of time, maintaining serum levels of hIgG close to 2.5 mg/ml, were unable to significantly affect nAAb B cells (data not shown).

Autoantigen ignorant but not anergic

There is no general consensus on the exact phenotype of anergic B cells, but a down-regulation of mb IgM was previously reported in the HEL-anti-HEL tg model (8). The density of mb IgMa and mb IgDa was equivalent in tg nAAb B cells, in non-tg IgMb-bearing B cells, in single tg μ and μ B cells, and in control IgMa allotype BALB/c mice (data not shown). Peripheral administration of human IgG or myoglobin did not modify the density of mb IgMa on the surface of tg B cells. The levels of secretion of IgMa (13 ±

Table II. Serum nAAb levels in 4- to 5-wk-old mice (ELISA)*

<table>
<thead>
<tr>
<th>nAAb μk Mice</th>
<th>nAAb μk Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgMa μg/ml</td>
</tr>
<tr>
<td>Mean</td>
<td>13</td>
</tr>
<tr>
<td>SD</td>
<td>8</td>
</tr>
</tbody>
</table>

*Each value originates from groups of 10 mice ± SD.
8 μg/ml in μk mice; 32 ± 20 μg/ml in μdk mice) were not modified 2 and 5 days after in vivo Ag administration. These results were identical in μk and μdk mice.

In vitro μg B cells were equally Ag ignorant. Considering that heat-aggregated human IgG were able to cross-react with FcγRII on tg B cells and, by cross-linking the BCR, inhibit B cell activation (24), we also tested various concentrations of monomeric human IgG and myoglobin. They were also unable to activate the tg B cells (data not shown). However, when stimulated with bacterial LPS (50 μg/ml) or anti-IgM (10 mg/ml) splenic μk and μdk B cells were readily able to proliferate and to be activated showing that BCR-dependent and -independent pathways of B cell activation were functional (Figs. 6 and 7).

Discussion

In this paper, we describe μg models that express a nAAb against soluble Ags on a majority of B cells. The variable regions of the nAAb are of human origin; they confer a reactivity with ssDNA as well as with human Ags. This reactivity was not only seen in μdk and μk mice.

TABLE III. Lymphocyte subpopulations in bone marrow, spleen, and peritoneum from nAAb μdk mice after IV hIgG or PBS (control) injection

<table>
<thead>
<tr>
<th>nAAb Tg Mice μdk</th>
<th>Controls</th>
<th>2 days after Ag injection</th>
<th>5 days after Ag injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B220</td>
<td>22 (4)</td>
<td>18 (6)</td>
<td>21 (4)</td>
</tr>
<tr>
<td>B220/IgMa</td>
<td>20 (3)</td>
<td>17 (6)</td>
<td>20 (4)</td>
</tr>
<tr>
<td>B220/IgMb</td>
<td>2 (1)</td>
<td>1 (0)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>IgMa/17 109</td>
<td>20 (4)</td>
<td>17 (5)</td>
<td>21 (3)</td>
</tr>
<tr>
<td>IgMa/AA 4.1</td>
<td>17 (5)</td>
<td>15 (7)</td>
<td>20 (6)</td>
</tr>
<tr>
<td>IgMa/IgDa</td>
<td>9 (2)</td>
<td>8 (4)</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B220</td>
<td>32 (5)</td>
<td>29 (6)</td>
<td>28 (3)</td>
</tr>
<tr>
<td>B220/IgMa</td>
<td>31 (4)</td>
<td>28 (4)</td>
<td>26 (5)</td>
</tr>
<tr>
<td>B220/IgMb</td>
<td>2 (1)</td>
<td>2 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>IgMa/17 109</td>
<td>29 (5)</td>
<td>27 (4)</td>
<td>25 (4)</td>
</tr>
<tr>
<td>IgMa/AA 4.1</td>
<td>2 (1)</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>IgMa/IgDa</td>
<td>30 (5)</td>
<td>27 (3)</td>
<td>25 (6)</td>
</tr>
<tr>
<td>IgMa/CD23</td>
<td>26 (3)</td>
<td>26 (6)</td>
<td>22 (4)</td>
</tr>
<tr>
<td>IgMa/CD5</td>
<td>3 (1)</td>
<td>3 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B220/IgMa</td>
<td>20 (2)</td>
<td>15 (3)</td>
<td>17 (3)</td>
</tr>
<tr>
<td>IgMa/17 109</td>
<td>18 (3)</td>
<td>15 (3)</td>
<td>13 (3)</td>
</tr>
</tbody>
</table>

* Two or 5 days after injection, cells were harvested and analyzed by FACS. Data from five animals per group are presented as the mean percentage of total mononuclear cells (± SD).

FIGURE 6. MHC class II, CD44, CD54, and CD86 expressions in nAAb μdk tg mice after i.v. hIgG injection. Mice were injected with 8 mg of aggregated hIgG or PBS. Two or 5 days after injection, spleen cells were harvested, and IgMa-positive cells were analyzed by FACS for CD86, CD44, MHC class II, and CD54 expression.

The role of mb IgD in B lymphocyte development was previously studied in different models. Tg mice expressing mb IgD and IgM have an increased mature B cell pool (29) compared with tg mice expressing only mb IgM. Our results, even though originating from single IgM and IgM/IgD tg lines, are consistent with these data and those of others (30). nAAb B cells exit from the bone marrow whether they express IgD or not, but the proportion of peripheral immature B cells (AA 4.1 positive, CD23 negative) is significantly higher in tg mice that express only IgM. The autoreactive nature of the tg BCR in our mice is probably not responsible for the increased maturation of IgD-positive B cells because the same phenomenon was observed in mice lacking the light chain tg and, consequently, having a diverse B cell repertoire.

Other tg models have tested the history of autoreactive B cells against soluble Ag. Each of these models seems peculiar to itself and each could answer different questions. Tg mice expressing high affinity autoantibodies (AAb) were described and did not always confirm the results originating from the initial HEL-anti HEL model (2). Anti dsDNA B cells and RF B cells were either deleted (7, 31), rendered anergic (10), or subjected to receptor editing (32) during their bone marrow maturation. Our nAAb being a prototype of natural G67/17 109” RF, it is interesting to compare our data with the other RF tg models. Monoreactive high affinity human RF that did not encounter the Ag during ontogeny were deleted when they did encounter their Ag (hIgG) after i.p. injection (15). Mouse intermediate affinity RF B cells on a nonautoimmune background...
cells. They can also be activated through a non-BCR pathway, showing that the BCR-dependent pathway is normal in these B

human IgG (monomeric and multimeric) or myoglobin. However, they can be activated after anti-IgM treatment in vitro, clearly

show fairly similar behavior to our nAAb models; these B cells are not deleted but their functional state seems to be related to a certain

degree of Ag-specific activation as judged by spontaneous RF secretion (16). In our models, we do not think that the nAAb secretion

is linked to Ag-specific B cell activation because we also observed IgMa secretion in tg mice lacking the tg light chain. Even

large quantities of Ag, delivered in periphery, were unable to activate these B cells and to induce nAAb secretion. Considering our

in vitro results, which show that nAAb B cells are not activated after human IgG or myoglobin contact, these models are close to,

but somewhat different from, tg anti ssDNA where B cells are functionally inactivated, have a decreased total surface Ig, are distributed

throughout the B cell follicle, are phenotypically mature, and are responsive to LPS and anti-IgM stimulations (33, 34).

The mechanism by which nAAb B cells escape tolerance (deletion or anergy) is probably related to the affinity of the BCR. It

was previously shown that most B cells that can bind an Ag can be neither stimulated nor tolerated by this Ag, even when the former

is highly concentrated (35). Thus, it seems that only B cells bearing BCR with a relatively high affinity for a self-Ag would be

eliminated. B cells with a very low affinity for self-Ags will not reach the affinity threshold for tolerance induction. The recent

description of the different fates of anti-dsDNA B cells in a single tg line is in accord with the notion of a molecular threshold for tol-

erance induction (36). This notion also questions the potential roles of these nAAb B cells in general, and of natural RF B cells in

particular, in adult mice. These cells are autoantigen ignorant because both in vivo and in vitro they are unable to be activated by human IgG (monomeric and multimeric) or myoglobin. However, they can be activated after anti-IgM treatment in vitro, clearly showing that the BCR-dependent pathway is normal in these B cells. They can also be activated through a non-BCR pathway, after LPS stimulation. We could hypothesize that lymph node nAAb B cells, through their ability to cross-react with foreign Ag on different microorganisms with higher affinities, may be acti-

vated to secrete Ab and undergo H chain switch and variable region mutations. This mechanism can be considered a first line of defense against pathogens, but could also be responsible for the generation of high affinity pathogenic AAb in the presence of T cell help.

Acknowledgments

We thank Marianne Lemeur for transgenic mice, K. Rajewsky for the vec-
tors, and R. Jefferis, D. Carson, and R. Ceredig for the gift of Abs. We thank R. Ceredig for helpful discussions and E. Losay for technical assistance.

References


reactive bone marrow B cells. J. Exp. Med. 177:1009.


22. Crozier, R., T. Martin, and J. L. Pasquali. 1995. Heavy chain variable region, light chain variable region, and heavy chain CDRI3 influences on the mono-

