mg) (*20*), and 1 μg was used for reverse transcription with primers A6103 (GCTATCAGCCGGTTCATCCACTGC) or A9413 (CAGGATGGCCTATTGGCCTGGAG) and the Expand Reverse Transcriptase System (Boehringer Mannheim, Germany). PCR was performed with the Expand Long Template System (Boehringer Mannheim) in buffer containing 2% dimethylsulfoxide. After 1 hour at 42°C, one-eighth of the mixture was used for the first PCR with primers A6103 and S59 (TGTCTTCACGCAGAAAGCGTCTAG) or A9413 and S4542 (GATGAGCTCGCCGCGAAGCTGTCC). After 40 cycles, one-tenth was used for the second PCR with primers S59 and A4919 (AGCACAGCCCGCGTCATAGCACTCG) or S4542 and A9386 (TTAGCTCCCCGTTCATCGGTTGG). After 30 cycles, the PCR products were purified by preparative agarose gel electrophoresis, and eluted fragments were ligated into vector pCR2.1 (Invitrogen) or pBSK II (Stratagene). Four clones of each fragment were analyzed and a consensus sequence was established. To resolve ambiguities, we amplified shorter PCR fragments covering the corresponding region and sequenced multiple clones. The 3′ NTR was obtained by conventional PCR with an antisense primer covering the last 24 nt of the genome (*4*). The authentic 5′ NTR downstream of the T7 promoter was generated by PCR with an oligonucleotide corresponding to a truncated T7 promoter (TAATACGACTCACTATAG) and the first 88 nt of HCV and a plasmid carrying one of the 5′ fragments of the genome. The complete genome was assembled from subgenomic fragments carrying the least numbers of nonconsensus nucleotide changes and inserted into a modified pBR322 vector. Nonconsensus changes were removed by site-directed mutagenesis. To generate run-off transcripts with an authentic 3′ end, we modified the 3′ NTR of our isolate (terminating with TGT) to match the sequence of genotype 3 [clone WS; A. A. Kolykhalov, S. M. Feinstone, C. M. Rice, *J. Virol.* **70**, 3363 (1996)] terminating with AGT, which allowed us to introduce a recognition sequence for the restriction enzyme Sca I (AGTACT) at the end of the 3′ NTR. A guanine was replaced with an adenine nucleotide at position 8180 of the genome to remove an internal Sca I site. After assembly of the full-length genome with appropriate 5′ and 3′ NTRs, the complete HCV sequence [European Molecular Biology Laboratory (EMBL) accession number AJ238799] was verified.

8. Plasmid DNA was linearized with Sca I and used for in vitro transcription reactions containing 80 mM Hepes (pH 7.5), 12.5 mM $MgCl_2$, 2 mM spermidine, 40 mM dithiothreitol, 2 mM of each nucleoside triphosphate, RNasin (1 U/ml), DNA template (50 μg/ml), and T7 RNA polymerase (~2 U/μl). To increase the yields, after 2 hours at 37°C an extra 1 U of T7 RNA polymerase was added per microliter, and the reaction was incubated for an additional 2 hours. DNA was removed by extraction with acid phenol [W. Kedzierski and J. C. Porter, *BioTechniques* **10**, 210 (1991)] and treatment with 2 U of deoxyribonuclease (DNase) per microgram of DNA for 60 min at 37°C. RNA was purified and analyzed by denaturing agarose gel electrophoresis.
9. Purified in vitro transcripts corresponding to the parental or the inactivated HCV genome were used for transfection of human hepatoma cell lines and primary human hepatocytes. Cell lines were maintained in a medium as described [B. J. Yoo *et al.*, *J. Virol.* **69**, 32 (1995)] and passaged once a week. Total RNA was prepared from transfected cells, and serial dilutions were used for RT-PCR amplification of the 5′ NTR or an NS5B sequence covering the 10–amino acid deletion. This allowed discrimination between the parental and the inactivated genome carrying the in-frame deletion. We monitored RNA replication by comparing the amounts of HCV RNA found in cells transfected with the wild-type or the inactivated genome. Input RNA was detected for up to three passages, with similar amounts seen for both genomes.
10. A. A. Khromykh and E. G. Westaway, *J. Virol.* **71**, 1497 (1997).
11. S.-E. Behrens, C. W. Grassmann, H.-J. Thiel, G. Meyers, N. Tautz, *ibid.* **72**, 2364 (1998).
12. On the basis of mapping data of the 3′ boundary of the IRES [J. E. Reynolds *et al.*, *EMBO J.* **14**, 6010 (1995); R. Rijnbrand *et al.*, *FEBS Lett.* **365**, 115 (1995)], various portions of the 5′ NTR were fused to the *neo* gene and cotransfected with a plasmid encoding the T7 RNA polymerase. The maximum number of colonies was obtained with HCV nt 1 to 377 and 1 to 389. Because the AUG codon of the HCV polyprotein is at nt 342, this results in a fusion of 12 or 16 amino acids, respectively, of the core protein to the neomycin phosphotransferase. The IRES of the encephalomyocarditis virus was amplified by PCR. A Nco I site was introduced at the 3′ end and used for insertion of HCV NS proteins. Translation of the NS2-3′ replicons initiates with the authentic methionine at amino acid position 810; translation of the NS3-3′ replicons initiates at an engineered start codon, adding an extra methionine to the NH_2-terminus of NS3. The nucleotide sequences of the four replicons have been deposited in the EMBL database with the accession numbers AJ242651 (I_{377}/NS2-3′), AJ242653 (I_{389}/NS2-3′), AJ242652 (I_{377}/NS3-3′), and AJ242654 (I_{389}/NS3-3′).
13. After in vitro transcription and DNase treatment (*8*), RNA was extracted with acid phenol, acid phenol–chloroform, and chloroform and analyzed by formaldehyde agarose gel electrophoresis.
14. H. Nakabayashi, K. Taketa, K. Miyano, T. Yamane, J. Sato, *Cancer Res.* **42**, 3858 (1982).
15. RNA (15 μg) was electroporated into 8×10^6 Huh-7 cells, which were then seeded into a 10-cm-diameter dish. After 24 hours, G418 was added to 1 mg/ml, and the medium was changed twice per week. Small colonies appeared after 3 to 5 weeks and were isolated and passaged under the same conditions.
16. S.-E. Behrens, L. Tomei, R. De Francesco, *EMBO J.* **15**, 12 (1996); V. Lohmann, F. Körner, U. Herian, R. Bartenschlager, *J. Virol.* **71**, 8416 (1997).
17. Y. Gong *et al.*, *J. Gen. Virol.* **77**, 2729 (1996).
18. As will be reported elsewhere (V. Lohmann and R. Bartenschlager, in preparation), we recloned HCV replicons from 1 μg of total RNA by RT-PCR using primers S59 and A9413 (*7*). For amplification of 5′ and 3′ NTRs, we used an RNA ligation approach before PCR. Among 10 sequenced replicons, no converging mutations were found. Each replicon contained 6 to 12 amino acid substitutions scattered throughout the HCV ORF. The NTRs were highly conserved, and only sporadic nucleotide changes were observed.
19. HCV RNA contained in total RNA of cell clones 5-15 and 9-13 was quantified by Northern blot, and 20 μg of total RNA were used for transfection (*15*). An equivalent number of in vitro–transcribed replicon molecules was supplemented with total RNA from naïve Huh-7 cells to the same concentration and transfected in parallel. Cotransfection of a construct directing the expression of firefly luciferase was used to correct for transfection efficiency. No significant difference in the number of G418-resistant colonies was found between total RNA isolated from the two cell clones and the in vitro RNA mixture.
20. P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987).
21. R. Bartenschlager, V. Lohmann, T. Wilkinson, J. O. Koch, *J. Virol.* **69**, 7519 (1995).
22. T. R. Fuerst, E. G. Niles, F. W. Studier, B. Moss, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8122 (1986).

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Positive Selection of Natural Autoreactive B Cells

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Lymphocyte development is critically influenced by self-antigens. T cells are subject to both positive and negative selection, depending on their degree of self-reactivity. Although B cells are subject to negative selection, it has been difficult to test whether self-antigen plays any positive role in B cell development. A murine model system of naturally generated autoreactive B cells with a germ line gene–encoded specificity for the Thy-1 (CD90) glycoprotein was developed, in which the presence of self-antigen promotes B cell accumulation and serum autoantibody secretion. Thus, B cells can be subject to positive selection, generated, and maintained on the basis of their autoreactivity.

Although it is widely accepted that B cells with self-reactivity are deleted or rendered functionally inactive (*1*), autoantibodies can be found in the serum of healthy animals, referred to as "natural autoantibodies," in an apparent paradox to the clonal tolerance theory (*2, 3*). In contrast with disease-associated hypermutated immunoglobulin G (IgG) antibodies, these natural autoantibodies are predominantly IgM, encoded by mostly unmutated germ line variable (V) region genes, and are independent of T cell help for secretion. Natural autoantibody constitutes a large fraction of serum Ig, and the B cells that produce natural autoantibodies frequently express CD5, a phenotype rare in spleen, but more common in the peritoneal cavity of mice (*4,*

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5). The significance of such natural autoantibody is not yet clear. However, these antibodies often cross-react with antigens on bacteria or tumors, and mice deficient in serum Ig are susceptible to bacterial infection, suggesting that these Igs participate in innate immunity (*3, 5, 6*).

SM6C10 is an anti-thymocyte autoantibody (ATA) produced by a hybridoma derived from $CD5^+$ B cells (B-1 cells) of SM/J mice, a strain with elevated serum ATA. SM6C10 binds a murine-specific carbohydrate epitope of the Thy-1 glycoprotein that is expressed on thymocytes, a fraction of mature T cells, and T cell tumors, as well as on the soluble form of Thy-1 (*7*). Expression of this epitope is Thy-1–dependent and thus is absent from Thy-1 gene-targeted mice ($Thy\text{-}1^-$) (Fig. 1). Immunoglobulin V gene sequence analysis showed a V_H3609 heavy chain combined with a $V_\kappa21C$ light chain in the SM6C10 hybridoma, both unmutated from the germ line (GenBank X53097 and M21522; $V_H3609\text{-}D_HQ52\text{-}J_H2$, $V_\kappa21C\text{-}J_\kappa2$).

To understand why ATA B cells develop, we generated V_H3609 μ^a heavy chain transgenic mouse lines on the C.B17 (μ^b) background (*8*) and found that, despite introducing only the μ heavy chain transgene (Tg), these animals had high IgM ATA serum titers detectable from 1 to 2 weeks after birth onward (Fig. 2A). Most splenic B cells from transgenic mice expressed the Tgμ without endogenous μ and lacked CD5 expression ($CD5^-Tg\mu^+Endo\mu^-$), and did not secrete ATA upon lipopolysaccharide (LPS) stimulation in vitro (Fig. 2B). In contrast, there was high ATA secretion from a $CD5^+$ B cell subset enriched in the peritoneal cavity (PerC) that expressed the Tgμ alone ($CD5^+Tg\mu^+Endo\mu^-$), but not from $CD5^+$ cells expressing both Tgμ and Endoμ nor from $CD5^-$ B cells in the PerC (Fig. 2, B and C). $CD5^+Tg\mu^+Endo\mu^-$ cells were also present in spleen as a minor fraction, contributing some ATA secretion from this sample. To determine the relation between ATA specificity and light chain usage, we generated hybridomas from sorted B cell fractions. More than half (8/13) of the hybridomas established from the $CD5^+Tg\mu^+$ $Endo\mu^-$B cell fraction had ATA specificity and identical $V_\kappa21C\text{-}J_\kappa2$ light chains, with a V-J junction that lacked nucleotide addition and was identical to the sequence found in the SM6C10 hybridoma. In contrast, $CD5^-$B cell–derived hybridomas had a more diverse light chain usage, none expressed $V_\kappa21C$, and all lacked detectable ATA activity (Table 1). The predominance of identical $V_\kappa21C\text{-}J_\kappa2$ light chains by $CD5^+Tg\mu^+Endo\mu^-$ cells in contrast to $CD5^-$B cells was confirmed by sequencing mRNA from individual cells (*9*). Therefore, high ATA serum titer in the $V_H3609\mu$ transgenic mice resulted from an increase of autoreactive ATA B cells bearing a canonical $V_\kappa21C$ light chain, and these ATA B cells were exclusively $CD5^+$; that is, introduction of a $V_H3609\mu$ heavy chain had a positive effect on the generation of ATA B cells.

This $CD5^+$ ATA B cell subset was not

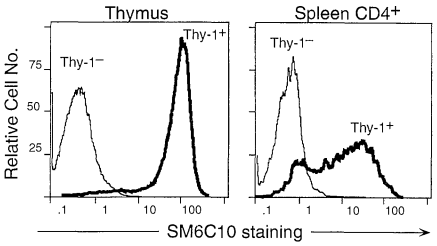

Fig. 1. Immunofluorescence staining of T cells by SM6C10 (ATA). Thymocytes and splenic $CD4^+$ T cells from wild-type C57/BL(B6) (thick line, $Thy\text{-}1^+$) and B6.$Thy\text{-}1^{-/-}$ (thin line, $Thy\text{-}1^-$) mice were stained with FL-SM6C10. Staining was negative for all cells in the thymus, liver, bone marrow, spleen, and peritoneal cavity of $Thy\text{-}1^-$ mice.

Table 1. Summary of V_κ gene usage in transgenic $V_H3609\mu$-expressing hybridomas. V_κ gene family nomenclature is according to the IMGT/LIGM database and Potter *et al.* (*26*). Designations a to d are arbitrary to distinguish individual germ line gene sequences. Only sequence data from functional V_κ genes are shown. All hybridomas secreted Tgμ. Three $CD5^-$-derived hybridomas expressed λ light chain and were ATA^-. No hybridoma from the $CD5^+Tg\mu^+Endo\mu^-$ cells were λ^+. Generation of the hybridomas (25 from $CD5^-Tg\mu^+Endo\mu^-$ and 13 from $D5^+Tg\mu^+Endo\mu^-$) is described in (*27*).

V_κ group	J_κ	Number of hybridomas	ATA
$CD5^-Tg\mu^+Endo\mu^-$ cell origin			
1	4	1	–
4/5[a]	4	3	–
4/5[b]	5	3	–
4/5[c]	4	2	–
4/5[d]	1	1	–
9B	5	2	–
12/13[a]	2	1	–
12/13[a]	5	1	–
19/28[a]	4	2	–
19/28[a]	2	1	–
19/28[b]	4	1	–
19/28[c]	5	1	–
20	4	1	–
23[a]	4	1	–
23[b]	5	3	–
23[b]	1	1	–
$CD5^+Tg\mu^+Endo\mu^-$ cell origin			
9B	2	1	–
12/13[b]	1	1	–
21C	2	8	++
21E[a]	1	1	+/–
21E[a]	2	1	+/–
23[b]	5	1	–

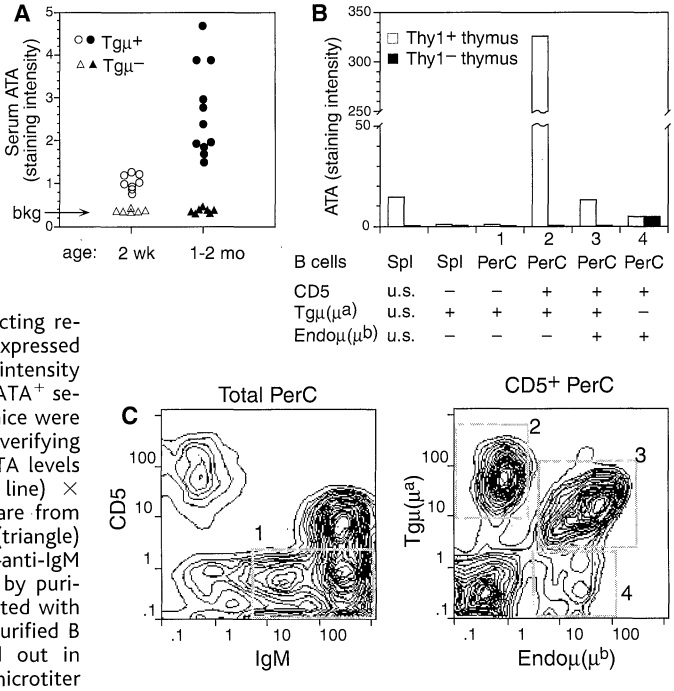

Fig. 2. High serum ATA levels in $V_H3609\mu$ transgenic mice and an analysis of B cell subsets. Serum and culture supernatant ATA levels were assayed by flow cytometry analysis with C.B17 or B6 thymocytes by using fluorescein (FL)-conjugated antibody to murine IgM (FL–anti-IgM) (antibody 331.12) as a detecting reagent. Serum levels are expressed as thymocyte staining intensity with 1:10 diluted serum. ATA^+ serum samples from Tg^+ mice were also tested with anti-μ^a, verifying Tgμ origin. (**A**) Serum ATA levels of $V_H3609\mu$ Tg (ATA1 line) × C.B17 littermates. Data are from $Tg^{+/-}$ (circle) and $Tg^{-/-}$ (triangle) individuals. Bkg, FL–anti-IgM alone. (**B**) ATA secretion by purified B cell subsets incubated with LPS. LPS stimulation of purified B cell subsets was carried out in U-bottomed, 96-well microtiter plates with 4×10^4 cells in 0.15 ml of LPS (1 μg/ml) culture medium. Levels are staining intensity by 50 μl of 3-day culture supernatant using wild-type $Thy\text{-}1^+$ thymocytes (white bars) and mutant $Thy\text{-}1^-$ thymocytes (black bars) (u.s., unseparated for CD5/μ allotype expression). PerC subsets used for purification are shown in (C) with corresponding numbers. (**C**) Peritoneal B cell subsets in the ATA1 mouse line. Right panel shows $Tg\mu(\mu^a)$ and $End\mu(\mu^b)$ expression among $CD5^+$ cells in the PerC. The percent of B cell fractions among total spleen cells or PerC are as follows: spleen, $CD5^-$ $Tg\mu^+End\mu^-$ (52.0%); PerC, $CD5^-$ $Tg\mu^+End\mu^-$ (37.8%), $CD5^+Tg\mu^+End\mu^-$ (5.0%), $CD5^+Tg\mu^+End\mu^+$ (8.2%), and $CD5^+Tg\mu^-End\mu^+$ (1.6%). These correspond to 98% of splenic B cells and 71, 9, 15, and 3% of PerC B cells, respectively.

discernible at the newborn stage (<6 days old), at which time all B cells showed exclusive Tgμ expression, but was detected at 2 weeks and increased during subsequent development. This age-dependent ATA B cell accumulation suggested that their generation might be the result of cellular selection. We therefore crossed $V_H3609\mu$ transgenic mice with antigen-deficient Thy-1^- mice. Lymphocyte development appeared normal in Thy-1^- mice (*10*). However, accumulation of the ATA B cell subset and concomitant high ATA serum titer only occurred in $Tg\mu^+$ mice that were Thy-1^+ (Fig. 3). The difference of serum ATA titer held for 2 week neonates as well but became more evident as animals aged (Fig. 3A, 7 months). LPS stimulation of total B cells purified from PerC (or spleen) of $Tg\mu^+$ mice consistently yielded much higher ATA secretion from a Thy-1^+ background compared with a Thy-1^- background (*11*), consistent with an increased frequency of $CD5^+$ ATA B cells in Thy-1^+ mice.

Therefore, in the presence of Thy-1 antigen, ATA B cells accumulate and secrete autoantibody into the serum, whereas in the absence of Thy-1 neither occurs. This absence of ATA B cell accumulation in Thy-1^- transgenic mice could be rescued by providing a Thy-1^+ environment, as revealed by stem cell cotransfer of newborn liver from Thy-1^- transgenic mice (Thy-1^-Tg^+) and bone marrow from Thy-1^+ nontransgenic (Thy-1^+Tg^-) mice into immunodeficient SCID (severe combined immunodeficiency disease) mice (Fig. 4). The $\mu^{a+}\mu^{b-}$ fraction of B cells derived from stem cells of transgenic origin contained $CD5^+$ cells (the ATA B cell fraction) when Thy-1^+ bone marrow was cotransferred (Fig. 4C), but not when Thy-1^- bone marrow was cotransferred (Fig. 4D). The same correlation held true for serum ATA. Thus, ATA B cell accumulation and serum ATA production were dependent on self-antigen.

During B cell development, immature B cells are highly sensitive to B cell receptor–mediated signaling, resulting in tolerance (*12*). To test whether ATA B cells might be generated because they were sequestered from antigen at an immature stage, we injected Thy-1^+ thymocytes into the PerC of newborn or adult mice, assuring direct antigenic exposure of developing or established ATA B cells (*13*). In both cases, thymocyte treatment failed to eliminate or inactivate ATA B cells in the PerC, and the ATA serum titer was not significantly affected. Injected thymocytes were present at significant levels in the PerC for at least 2 days after each treatment. Thus, ATA B cells have not simply escaped deletion, in contrast with disease-associated autoreactive B cell models described previously (*14*).

For most of T cell development, some level of antigen receptor–mediated signaling by interaction with self-antigen is known to be essential for survival and maturation, a phenomenon termed positive selection. In contrast, strong self-reactivity leads to deletion or inactivation of these cells (negative selection) (*15*). Although negative selection has been clearly demonstrated for B cells in several transgenic model systems, it has been unclear whether there is any positive role for self-reactivity in B cell development (*1, 16*). Previous studies showed that functionally competent B cells can be generated in the presence of self-antigen (*17*), and a certain degree of signaling involving the B cell receptor may be important in B cell maintenance (*18*). A study of V-gene usage previously suggested that self- and environmental antigens appear to influence peripheral B cell maintenance (*19*). By using a germ line gene–encoded specificity, our data provide a clear demonstration that self-antigen can positively influence B cell fate, selecting B cells bearing an appropriate light chain partner and generating a B cell pool with an autoreactive specificity.

It remains unclear, however, whether all B cells require positive selection to mature and persist. Antigens such as the carbohydrate ATA determinant may signal differently from high-affinity or high-valence antigens that normally have a negative effect on newly generated B cells (*20*), resulting in CD5 upregulation and continued maturation. Alternatively, it is possible that the positive selection we observed reflects a difference in the responder B cells. Tolerance susceptibility and B cell receptor signaling thresholds can be modulated by coreceptors (*20, 21*) whose expression on immature B cells may change with ontogeny, similar to the known difference between immature versus mature developmental stages. Autoreactive B cells (and $CD5^+$ B cells) appear to be preferentially generated early in ontogeny (*5, 22*). Thus, it is possible that positive selection is a relatively common feature of B cells generated early in ontogeny, but only rarely occurs with immature B cells generated in the adult.

The presence of natural autoantibodies in serum and their increase with age have been long recognized. Although antigenic involvement has been suggested (*5, 23*), direct evaluation of the role of self-antigen in their development has been hampered by the difficulty of experimentally altering the expression of "natural" antigens in live animals.

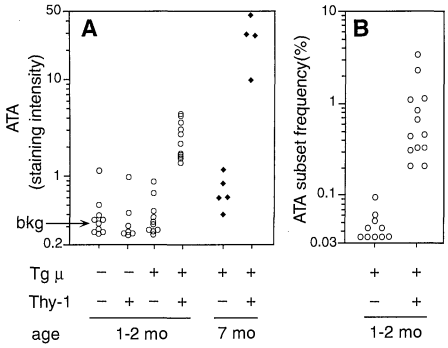

Fig. 3. Self-antigen–dependent increase of serum ATA and ATA B cells. To generate Thy-1^- Tg^+ mice, we crossed ATA1 mice with C57BL/6 (B6) Thy-$1^{-/-}$ mice, and $Tg^{+/-}$ offspring were backcrossed with Thy-$1^{-/-}$ mice. Tg, Thy-1, and H-2 typing were carried out by PCR assay and peripheral blood lymphocyte flow cytometry analysis. Individual data are from analysis of six and two litters of 1- to 2-month-old and 7-month-old mice, respectively. (**A**) Serum ATA levels of 1- to 2-month-old littermates and 7-month-old Tg^+ littermates. Serum levels are staining intensity values with 1:10 diluted serum. (**B**) PerC ATA B cell subset frequency in Tg^+ 1- to 2-month-old littermates. Percentages of $CD5^+Tg\mu^+End\mu^-$ cells among total PerC cells (1×10^6 to 3×10^6 cells per mouse in both groups) are shown.

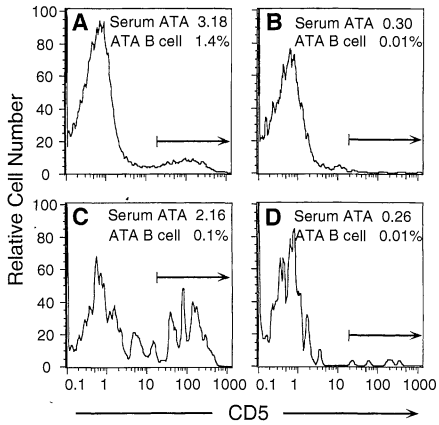

Fig. 4. ATA B cell generation from Thy-$1^-Tg\mu^+$ stem cells in a self-antigen–positive environment. Flow cytometry analysis of PerC cells gated for $\mu^{a+}\mu^{b-}$ cells in 4-week-old (**A**) Thy-1^+ compared with (**B**) Thy-$1^-Tg\mu^+$ littermates (H-$2^{b/b}$), and (**C** and **D**) in SCID mice reconstituted with stem cells 4 weeks after transfer. Cells from a pool of newborn (d0) liver from a litter between $Tg\mu^{+/-}$ and $Tg\mu^{-/-}$ of Thy-$1^{-/-}$H-$2^{b/b}$ mice (50% of newborns were $Tg\mu^+$) were divided into two groups. Cells from each of the groups were mixed with adult (3 month) bone marrow (BM) cells from either wild-type B6 (C) or B6.Thy-$1^{-/-}$ (D) mice, then intravenously injected into B6.scid mice (2×10^7 newborn liver cells mixed with 1×10^7 BM cells per mouse). The ATA serum level and the ATA B cell subset were analyzed 1 month after injection. Representative data of four mice per group are shown. Percentages of ATA B cells correspond to $CD5^+\mu^{a+}\mu^{b-}$ cell frequency (arrow marked) among total PerC cells (similar PerC recovery in all groups). Both transferred groups showed a similar predominance of $\mu^{a-}\mu^{b+}$ cell generation from BM stem cells and also from the presence of 50% $Tg\mu^-$ stem cells in the newborn liver used for cell transfer. $CD5^-\mu^{a+}\mu^{b-}$ cell frequencies were comparable. Serum ATA levels are staining intensity values 1.67 ± 0.59 and 0.29 ± 0.028, in (C) and (D), respectively ($n = 4$).

Such antigens are typically ubiquitous in distribution, representing important cellular components, and are often glycolipids or carbohydrate constituents shared among diverse glycoproteins and glycolipids (*24*). By taking advantage of the V_H3609 ATA specificity restricted to a determinant on the Thy-1 glycoprotein, we demonstrate here that self-antigen can indeed promote B cell accumulation and suggest that a significant proportion of natural autoantibody in serum is the product of such a self-antigen–dependent process. Carbohydrates expressed on glycolipids or glycoproteins, some commonly up-regulated on tumors or components of pathogenic bacteria, are the frequent targets of natural autoantibodies (*24*). In turn, the $CD5^+$ phenotype along with autoreactivity is a common feature of chronic leukemic B cells (*25*). Thus, it is intriguing to speculate that self-antigens play a positive role in recruiting B cells for immunologic surveillance as a part of innate immunity, but that this process carries a risk for potential dysregulated growth. It will be important to assess whether the positive selection responsible for generating such cells demonstrated here is the property of a distinct subset of B cells.

References and Notes

1. C. C. Goodnow *et al.*, *Nature* **334**, 676 (1988); D. A. Nemazee and K. Bürki, *ibid.* **337**, 562 (1989); J. Erikson *et al.*, *ibid.* **349**, 331 (1991).
2. S. Boyden, *ibid.* **201**, 200 (1964); M. Schlesinger, *ibid.* **207**, 429 (1965); A. J. Cunningham, *ibid.* **252**, 749 (1974).
3. W. J. Martin, S. E. Martin, *ibid.* **249**, 564 (1974).
4. L. A. Herzenberg *et al.*, *Immunol. Rev.* **93**, 81 (1986); K. Hayakawa and R. R. Hardy, *Annu. Rev. Immunol.* **6**, 197 (1988); R. R. Hardy, C. E. Carmack, Y. S. Li, K. Hayakawa, *Immunol. Rev.* **137**, 91 (1994).
5. R. R. Hardy and K. Hayakawa, *Adv. Immunol.* **55**, 297 (1994).
6. D. E. Briles *et al.*, *J. Exp. Med.* **153**, 694 (1981); M. C. Carroll, *Annu. Rev. Immunol.* **16**, 545 (1998).
7. K. Hayakawa, C. E. Carmack, R. Hyman, R. R. Hardy, *J. Exp. Med.* **172**, 869 (1990); M. Gui *et al.*, in preparation.
8. The $V_H3609\mu$ construct was prepared by first ligating a 5-kb EcoR I VDJ fragment from the SM6C10 hybridoma cell line (containing the promoter and regulatory motifs) into the pICEm vector that contains a μ constant region. This gene was next cotransfected with a neomycin resistance (*neo*^{R}) gene–containing plasmid by electroporation into the J558L variant plasmacytoma line, characterized by the lack of heavy chain, to verify expression. Purified V_H3609-μ insert was microinjected into fertilized murine eggs (FvB strain). The transgene was detected by polymerase chain reaction (PCR) as a V_H3609 leader–J_H2 fragment (5′ primer, sense CCCTCCCAGACCCTCAGTCTG; 3′ primer, anti-sense GGTGCCTTGGCCCCAGTAG) with DNA from tails. Tg-positive (Tg^+) mice were selected by detection of transgene allotype IgM (μ^a)–expressing B cells in the peripheral blood by immunofluorescence analysis (anti-μ^a, RS-3.1; anti-μ^b, AF6-78). Tg^+ animals were then bred to C.B17 mice for several (>four) generations. Three independent lines showed similar profiles of B cell subsets as represented by the ATA1 line used here.
9. We amplified cDNA generated from individual sorted cells in two rounds of PCR using a single consensus V_κ sequence with nested C_κ primers, then sequenced using taq terminator chemistry on an ABI 377 genetic analyzer. The cannonical $V_\kappa21c$-$J_\kappa2$ light chain was detected in 11 out of a total of 14 sequences from PerC $CD5^+Tg\mu^+Endo\mu^-$ cells, whereas it was not detected in 15 sequences from splenic $CD5^-Tg\mu^+Endo\mu^-$ cells.
10. D. M. Page, Y. Tokugawa, J. Silver, C. L. Stewart, *J. Immunol.* **159**, 5285 (1997).
11. RA3-6B2$^+$ B cells were purified from PerC by cell sorter and stimulated with LPS in vitro. Day 4 culture supernatant was tested for ATA activity as described in Fig. 2.
12. J. Lang, *et al.*, *J. Exp. Med.* **184**, 1685 (1996); S. B. Hartley and C. C. Goodnow, *Int. Immunol.* **6**, 1417 (1994).
13. ATA1 newborn mice were injected intraperitoneally with 2×10^6 C.B17 thymocytes within 24 hours after birth, followed by injection with 3×10^6 to 4×10^6 thymocytes on day 3 and day 7. Serum ATA was analyzed at 2.5 and 6 weeks of age. PerC ATA B cell frequency was determined at 6 weeks. For adult mice, 1×10^7 C.B17 thymocytes were injected intraperitoneally Serum ATA level and ATA B cell frequency were determined 2 and 4 days after treatment. The frequency of injected thymocytes in PerC was analyzed in separate experiments by using combinations of B6.Ly-5 congenic mice and anti–Ly-5 alleles in parallel transfers with identical cell doses.
14. M. Murakami *et al.*, *Nature* **357**, 77 (1992).
15. P. Kisielow and H. von Boehmer, *Adv. Immunol.* **58**, 87 (1995).
16. N. R. Klinman, *Immunity* **5**, 189 (1996).
17. L. G. Hannum, D. Ni, A. M. Haberman, M. G. Weigert, M. J. Shlomchik, *J. Exp. Med.* **184**, 1269 (1996).
18. K.-P. Lam, R. Kühn, K. Rajewsky, *Cell* **90**, 1073 (1997).
19. I. C. M. MacLennan and D. Gray, *Immunol. Rev.* **91**, 61 (1986); H. Gu, D. Tarlinton, W. Müller, K. Rajewsky, I. Förster, *J. Exp. Med.* **173**, 1357 (1991).
20. J. I. Healy and C. C. Goodnow, *Annu. Rev. Immunol.* **16**, 645 (1998).
21. J. G. Cyster *et al.*, *Nature* **381**, 325 (1996).
22. G. Dighiero *et al.*, *J. Immunol.* **134**, 765 (1985); J. F. Kearney, *Curr. Top. Microbiol. Immunol.* **182**, 81 (1992).
23. C. A. Pennell *et al.*, *Eur. J. Immunol.* **19**, 1289 (1989); L. W. Arnold, C. A. Pennell, S. K. McCray, S. H. Clarke, *J. Exp. Med.* **179**, 1585 (1994).
24. T. Feizi, *Nature* **314**, 53 (1985).
25. G. Haughton, L. W. Arnold, G. A. Bishop, T. J. Mercolino, *Immunol. Rev.* **93**, 35 (1986); C. A. Pennell, L. W. Arnold, G. Haughton, S. H. Clarke, *J. Immunol.* **141**, 2788 (1988).
26. M. Potter, J. B. Newell, S. Rudikoff, E. Haber, *Mol. Immunol.* **19**, 1619 (1982).
27. Hybridomas were generated from purified $CD5^-$ and $CD5^+Tg\mu^+Endo\mu^-$ cells in spleen and peritoneal cavity, respectively (*7*). The cDNA was reverse transcribed from hybridoma RNA, then V_κ-C_κ message was amplified with a consensus V_κ primer–C_κ PCR. This amplified DNA was then cloned by using TA vector (Invitrogen, Carlsbad, CA), and plasmid clones containing the appropriate size insert were sequenced by using a nested C_κ primer and dye terminator chemistry (Applied Biosystems, Foster City, CA) on an ABI-377 instrument.

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