Function of B Cells Expressing a Human Immunoglobulin M Rheumatoid Factor Autoantibody in Transgenic Mice

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Summary

We have generated transgenic mice that express the immunoglobulin (Ig)M heavy chain and κ light chain genes coding for a human IgM rheumatoid factor (RF), Les. Transgenic B cells expressing human IgM RF show striking similarities to their counterparts in normal humans. They comprise a significant proportion of the adult B cell population, but secrete only low levels of RF into the serum. The RF transgene–expressing B cells localize to primary B cell follicles and the mantle zone regions of secondary follicles in the spleen. Using these mice we have been able to show that one of the central functions of normal RF-expressing B cells may be to act as highly efficient antigen-presenting cells for low concentrations of immune-complexed antigen. High levels of secretion of IgM RF can not be induced under normal circumstances, although RF-expressing B cells proliferate well in vitro to both aggregated human IgG and anti-human IgM antibodies. However, these mice are not intrinsically secretion deficient. By crossing the RF transgenic mice with the autoimmune MRL/*lpr* background, we find a dramatic increase, >200-fold, in levels of serum RF. The results strongly suggest that a major function of normal resting RF B cells is unrelated to antibody secretion. Rather, the RF B cells in the follicles may play a role in antigen presentation and regulation of immune responses to antibody-bound nonself-, and possibly self-antigens. This physiologic role of RF B cells may be disrupted in RF-associated autoimmune disease.

Rheumatoid arthritis (RA)[1] is a chronic inflammatory autoimmune disease that primarily affects the joints. It is characterized by the presence of high titers of anti-IgG antibodies (rheumatoid factors [RFs]). However, IgM RFs also can occur in patients with lymphoproliferative disease, and in normal individuals. These "natural" autoantibodies derive from a small set of conserved heavy and light chain variable region genes, that can encode RF in the absence of somatic mutation and are selectively rearranged early in fetal development (1–5).

Igs serve two major physiologic functions. The secreted antibodies of plasma cells play a critical role in the effector phase of the immune response. Surface-bound antibodies on B cells focus and internalize antigen and therefore play a role in the afferent arm of the immune response. Whether or not these functions are always fulfilled by the same set of B cells is not clear.

It is thought that IgM RFs amplify the early, low affinity, polyclonal immune response to bacterial antigens by increasing the avidity of antigen-bound IgG, and the fixation of complement. However, recent observations suggest that a major physiologic function of IgM RF antibodies is in the cell-bound as opposed to the soluble form. Lymphocytes bearing cell surface RF constitute a significant proportion of adult B lymphocytes (3), although under normal circumstances there are only low levels of circulating autoantibody. RF precursors are particularly abundant in the mantle zones of lymph nodes and tonsils of normal individuals who lack this autoantibody in their sera (6). A number of investigators have shown B cells to be very effective APCs, using their Ig receptor to capture small amounts of their specific antigen. In this way they are able to present antigen at 1,000-fold lower concentrations than monocytes and macrophages, suggesting that

[1] *Abbreviations used in this paper:* CLL, chronic lymphocytic leukemia; RA, rheumatoid arthritis; RF, rheumatoid factor; TT, tetanus toxoid.

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B cells may play a role in environments where the concentration of antigen is low (7–12). Recent experiments (13) using an RF-expressing EBV-transformed B lymphoblastoid cell line showed that these cells were highly efficient at presenting low concentrations of specific antigen to helper T cells provided that the antigen was present in the form of an immune complex with human IgG. Presumably, the activated RF-expressing B cells bound the IgG-antigen complexes and internalized them for processing and presentation. However, EBV-transformed lines do not necessarily reproduce the characteristics of normal RF-expressing B cells in vivo. The EBV-transformed cells divide rapidly and constitutively express high levels of the costimulatory molecules that promote T cell activation. Compared with stimulated B lymphoblasts, resting B cells have been reported to inefficiently present antigen to T cells (14–17) and may induce T cell anergy (18).

To analyze the normal immune function of B cells expressing IgM RF requires large and uniform populations of RF-positive B cells that have developed in the presence or absence of IgG. However, cells expressing mouse IgM RF normally exist in the presence of their target autoantigen. In addition, a murine counterpart of the human, low affinity IgM RFs that show regulated expression and tissue distribution has not been isolated. The use of a human IgM RF transgene in mice avoids these problems. Low affinity, germline-encoded autoantibodies are characteristically polyspecific, reacting with a variety of autoantigens and potentially crossreacting with mouse IgG. The Les IgM RF derives from a patient with atypical chronic lymphocytic leukemia (CLL; 19–21). The Les RF genes show a number of point mutation differences from germline and encode a protein that does not crossreact detectably with mouse IgG.

Here, we have analyzed the localization and function of transgenic B cells bearing the Les human IgM RF. We find that the transgenic B cells accumulate in primary B cell follicles and the mantle zones of secondary follicles, and display the antigen-presenting functions that have been postulated for RF B cells in normal humans. The results provide further support for the concept that RF B cells may regulate the afferent arm of the immune response to antibody-complexed self- and nonself-antigens.

Materials and Methods

DNA Constructs. The rearranged Ig heavy and light chain genes were isolated from the DNA of CLL cells that expressed the Les RF (19–21). After initial cloning into λ Dash (Stratagene, La Jolla, CA), a 13-kb SalI fragment containing the VkIIIa (Vk328) gene and Ck C region, 3.1 kb of 5′ sequences, and 5.0 kb of 3′ sequences was subcloned into pUC18. A 15-kb BamHI fragment containing the VhIV gene and Cμ C region together with 0.6 kb of 5′ sequences and 0.4 kb of 3′ sequences was subcloned into pSVG-gpt. HindIII linkers were added to a 0.8-kb EcoRI fragment containing the murine Ig heavy chain enhancer, and this fragment was cloned into the HindIII site on the heavy chain plasmid between the V and C regions 3′ of the human enhancer.

Creation of Transgenic Mice. Heavy and light chain genes coding for the human IgM RF were excised from vector sequences by SalI 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. For microinjection, the concentration of the fragment was adjusted to 2 μg/ml total DNA containing equimolar concentrations of the heavy and light chain constructs. Ova used for microinjection were derived from matings of (C57Bl/6 × SJL/J)F_1 mice. Founder lines were then maintained by backcross mating with C57Bl/6 or MRL/lpr strains (The Jackson Laboratory, Bar Harbor, ME).

ELISA. Positive progeny of transgenic matings were identified by measuring the level of human IgM RF in the serum of 4–5-wk-old mice. Plates were coated with human IgG (Cappel Laboratories, Durham, NC) at 10 μg/ml, and binding of serial dilutions of mouse serum was determined by sequential addition of anti-human IgM biotin (Accurate Scientific, Co., Westbury, NY), streptavidin-peroxidase (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and peroxidase substrate (KPL). Absorbance was measured at 450 nm. Levels of expression were determined by comparison with a standard curve of binding by purified Les IgM RF. Levels of IgM RF in supernatants from LPS-stimulated cultures were determined in a similar manner. Levels of mouse Ig in serum and culture supernatants were determined by coating plates with affinity-purified goat anti-mouse Ig (KPL) and detected with affinity-purified goat anti-mouse Ig-biotin (Jackson Immunoresearch, West Grove, PA).

Fluorescence Analysis. Spleen cells for fluorescence analysis were separated on Lympholyte M gradients (Accurate Scientific Co.). Cell phenotype was determined using the following reagents: goat anti-human IgM-FITC (Jackson Immunoresearch); anti-B220-PE (Pharmingen, San Diego, CA); anti-mouse IgD-PE (Pharmingen); anti-mouse κ-PE (Fisher Scientific Co., Pittsburgh, PA); anti-mouse CD5-PE (Boehringer Mannheim Biochemicals, Indianapolis, IN); goat anti-human κ-biotin (Sigma Chemical Co., St. Louis, MO); goat anti-mouse IgM-biotin (Jackson Immunoresearch); goat anti-mouse IgG-biotin (Kirkegaard & Perry Laboratories); rat anti-mouse CD4-biotin (YTA3.1); rat anti-mouse CD8-biotin; streptavidin-PE (Molecular Probes, Eugene, OR); and streptavidin-FITC (Molecular Probes), and analyzed on a FACScan® flow cytometer (Becton Dickinson & Co., San Jose, CA).

Immunohistochemistry. Tissues were snap frozen in optimal cutting temperature medium (Miles Laboratories, Inc., Naperville, IL). 4-μm sections were prepared from the tissue blocks and stained with affinity-purified goat anti-human IgM-biotin (Accurate Scientific Co.), affinity-purified goat anti-human κ-biotin (Sigma Chemical Co.), avidin-D HRP (Vector Laboratories, Burlingame, CA), and affinity-purified goat anti-mouse Ig-peroxidase (Boehringer Mannheim). Briefly, 100 μl of medium or an optimal dilution of the biotinylated reagents was added to each slide and incubated for 60 min at room temperature. Slides were then washed with PBS (pH 7.2) before adding 100 μl of either anti-mouse Ig coupled to peroxidase or avidin D coupled to horseradish peroxidase for a further 60 min. After washing the slides with PBS, the bound peroxidase was detected by incubation with 3-amino-9-ethylcarbazole (Sigma Chemical Co.) at 0.4 mg/ml in 0.015% hydrogen peroxide in 0.1 M sodium acetate (pH 5.2) for ~20 min. Washed slides were then stained with Mayer's hematoxylin for 2 min before mounting.

Generation of RF-secreting Hybridomas. Spleens were removed from AB29 transgenic mice and then teased apart in serum-free IMDM (Irvine Scientific, Santa Ana, CA). 10^8 spleen cells were combined with 6×10^7 NSO cells (kind gift of Dr. H. Waldmann, University of Cambridge, UK), washed in serum-free medium, and then pelleted. 0.8 ml of polyethylene glycol 1500 (Boehringer Mannheim) was added slowly to the pellet while stir-

ring. 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 + 10% FCS. After 24 and 48 h, the wells were fed with medium supplemented with HAT (hypoxanthine, thymidine, aminopterin) (Sigma Chemical Co.). Culture supernatants were tested at days 14–21 after fusion for the presence of human IgM RF. Positive wells were then cloned and positive hybridomas selected.

T Cell Proliferation Assay. C57/Bl6 mice were immunized at the base of the tail with 100 μg tetanus toxoid in CFA (Sigma Chemical Co.). 10 d later draining lymph nodes were removed, teased apart, and used as a source of T helper cells. After washing, the cells were resuspended at 6×10^6/ml in complete Clicks (Clicks/Eagle's Hanks amino acids [Irvine Scientific] with the addition of antibiotics, 10 mM Hepes, 50 μM 2-ME, and 10% heat-inactivated FCS). Spleen cells were removed from transgenic mice, nontransgenic littermates, or C57/Bl6 control mice, teased apart, and resuspended at 6×10^6/ml. After receiving 1,000 rad, these cells were used as a source of APC. 3×10^5 lymph node cells and 3×10^5 APC were added to each well in a total volume of 100 μl. Wells contained either tetanus toxoid alone or preformed immune complexes of tetanus toxoid and human IgG anti-tetanus toxoid (Hypertet, Cutter Biological, West Haven, CT) in a total volume of 100 μl. Immune complexes were preformed by incubation for 2 h at 37°C. All tests were performed in triplicate. After 3 d, 1 μCi of [^{3}H]thymidine (Tdr) was added per well and the assay harvested after a further 18 h using a cell harvester (PHD; Cambridge Technology Inc., Watertown, MA). [^{3}H]Tdr incorporation was measured using a β scintillation counter.

B Cell Proliferation Assays. Spleens were removed from transgenic mice, nontransgenic littermates, or control C57/Bl6 mice and teased apart. Cells were washed and resuspended at 2×10^6/ml in RPMI 1640 (Irvine Scientific) with the addition of antibiotics, glutamine, 50 μM 2-ME, and 10% heat-inactivated FCS. 2×10^5 cells were added to each well of a round-bottomed 96-well plate in a total volume of 100 μl. A further 100 μl of medium was then added containing one of a number of antibodies: F(ab')$_2$ fragments of affinity-purified, species-specific goat anti-human IgM, goat anti-mouse Ig, or goat anti-mouse IgM (Jackson Immunoresearch); soluble or aggregated human IgG (Cappel, Durham, NC). Other experiments utilized LPS from *Salmonella minnesota* (Sigma Chemical Co.). Soluble human IgG was prepared by pelleting a 10–20 mg/ml solution of human IgG at 160,000 *g* for 1–2 h; the upper 25% was then removed and used in the assays. Aggregated human IgG was prepared by heating a 10–20 mg/ml solution at 63°C for 1 h followed by cooling on ice for 2 h. All tests were performed in triplicate. Proliferation was measured on day 3 by the addition of 1 μCi of [^{3}H]Tdr per well and the assay terminated 18 h later.

Results

Establishment of Human RF Transgenic Mice. Transgenic mice were created that express the heavy and light chain genes coding for the Les human IgM RF. These genes had been isolated from RF-expressing B-CLL cells (19–21). The Vκ gene is derived from Vk328 (19–20), a conserved Vk3 subgroup gene that often encodes light chains of human autoantibodies. The Vh gene is most likely derived from Vh4.21 (21, 22). The products of these genes are readily detectable in the mouse background by use of human Ig-specific reagents; in addition, the κ light chain is recognized by the murine antiidiotypic antibody 6B6.6 (20, 23), whereas the heavy chain is detected by the monoclonal antiidiotype 9G4 (24). The constructs used for microinjection are shown in Fig. 1. The heavy chain gene was modified by the insertion of the mouse heavy chain enhancer at the HindIII site just 5′ of the switch region. Of seven founder lines that had human IgM RF in their serum, two (AB8 and AB29) were found to simultaneously express human IgM and human κ on the B cell surface. These latter lines were selected for further experimentation.

B Cell-specific Expression of Human IgM RF. Approximately 15% (range, 13–16%) of AB8 splenic B cells and 70% (range, 46–90%) of AB29 splenic B cells express human RF on the cell surface. Fig. 2 shows a representative dual-color FACS® analysis of spleen cells from AB29 mice. The two transgenes were coordinately expressed on the surface of B cells. There was no coexpression with T cell markers such as CD4 (or CD8; data not shown). There appears to be complete allelic exclusion between the transgenic protein and mouse IgG and IgD in B cells. In most experiments, human IgM RF-positive cells expressed neither mouse IgM nor κ (as shown in Fig. 2). However, occasionally in some experiments, it appeared that there may be a small proportion (<5%) of cells that express very low levels of murine IgM and κ in conjunction with human IgM RF. Table 1 shows the phenotype of AB29 spleen cells. Results are expressed as mean percentage and range of the total splenic mononuclear cell population that expresses each antigen. Seven mice are included in each

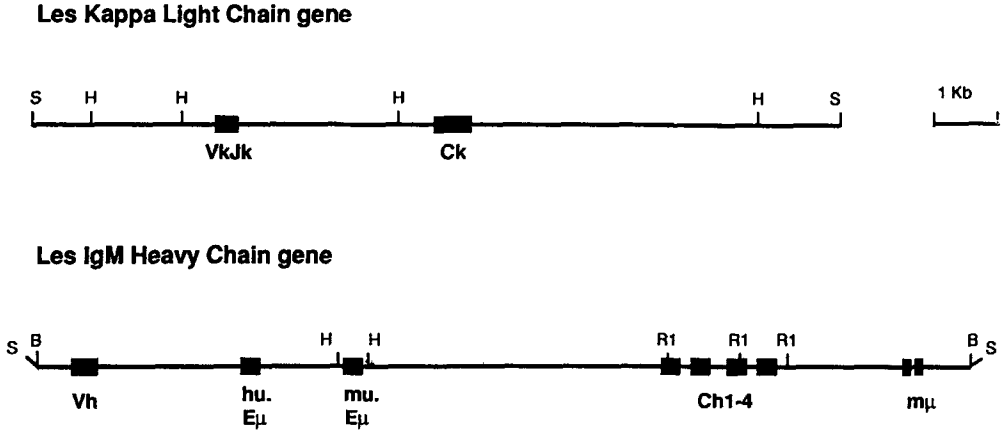

Figure 1. Human IgM heavy and κ light chain DNA coding for the Les human IgM RF.

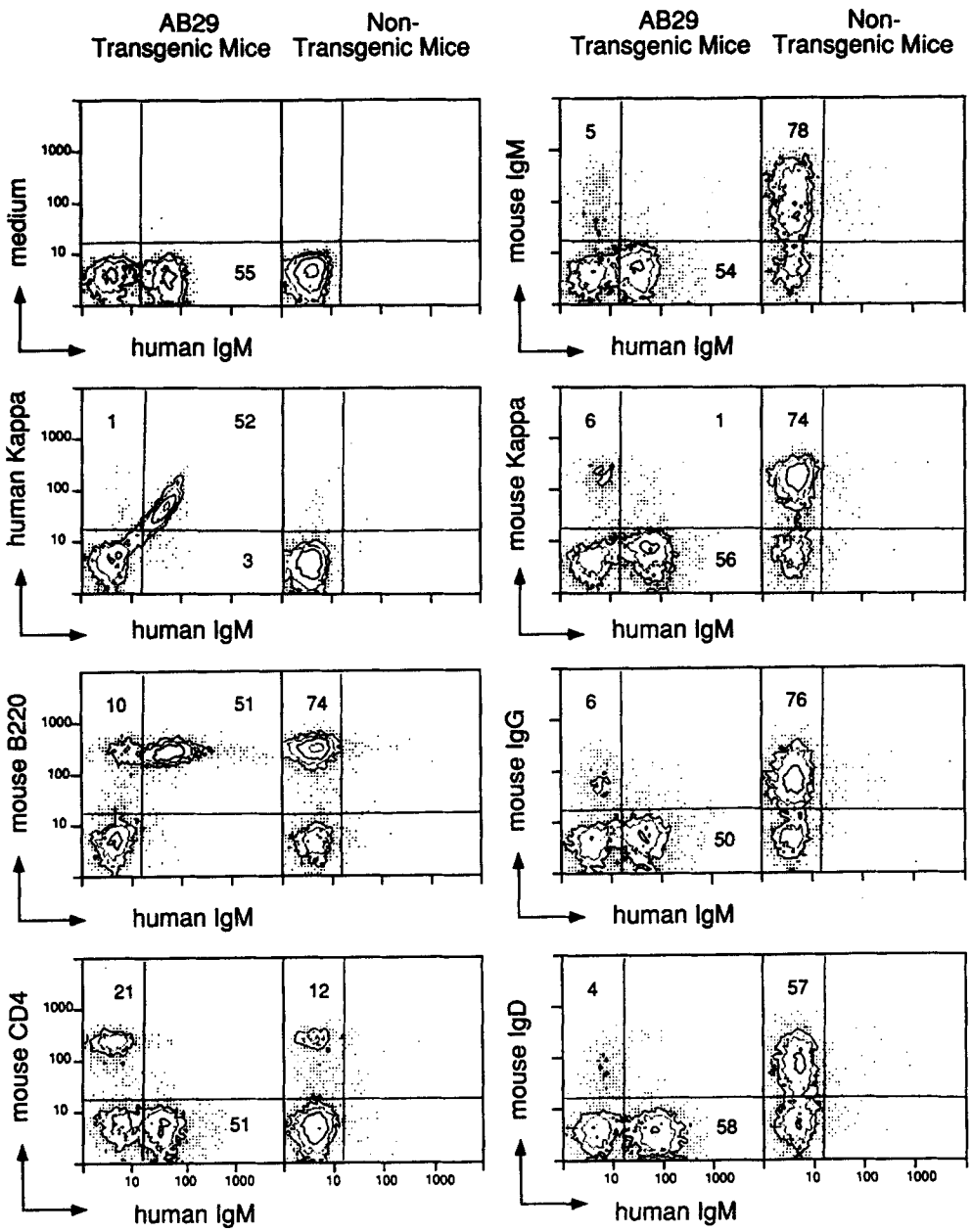

Figure 2. FACS® analysis of AB29 spleen cells. Note the anti-mouse IgG reagent crossreacts weakly with other murine Igs. Consequently, the percentages shown represent more than just mouse surface IgG-positive cells.

group unless stated otherwise. AB29 transgenic mice have 80% of normal B cell levels; consequently there is a compensatory increase in the percentage of total cells that express T cell markers. Cells expressing mouse Ig are reduced to 15% of the levels of control littermates, due to allelic exclusion by human IgM RF.

Fig. 3 shows staining of transgenic spleen for human and mouse Igs. RF-positive cells show characteristic staining in the primary B cell follicles of the white pulp (*A*) and in the mantle zones of secondary follicles (*B*). These are areas where small B lymphocytes are characteristically located. There are few RF-positive cells within the germinal centers of secondary follicles, which are usually the sites of antigen-induced B cell expansion. The localization of B cells expressing the transgene is consistent with a lack of antigen-induced expansion of human IgM RF in the mice. In transgenic spleen the number of mouse Ig-bearing cells is reduced compared with normal spleen, as shown in Fig. 3, *C* and *D*. The remaining mouse Ig-positive cells are located within the follicles and the marginal zone of the white pulp.

High Levels of Surface IgM RF Expression Are Not Accompanied by High Levels of Secretion. AB8 and AB29 mice secreted an average of 2 μg/ml (n = 21) and 12.9 μg/ml (n = 36) human RF at 4–5 wk of age corresponding to ~1 and 6% of total serum IgM. Thus, although 70% of the B cells from AB29 mice express human IgM on their cell surface, this human Ig comprises a small percentage of total serum Ig levels. This is not surprising, as these mice are not exposed to human IgG and so the RF B cells lack antigenic selection. However, injection of the mice with 50 μg/ml aggregated human IgG

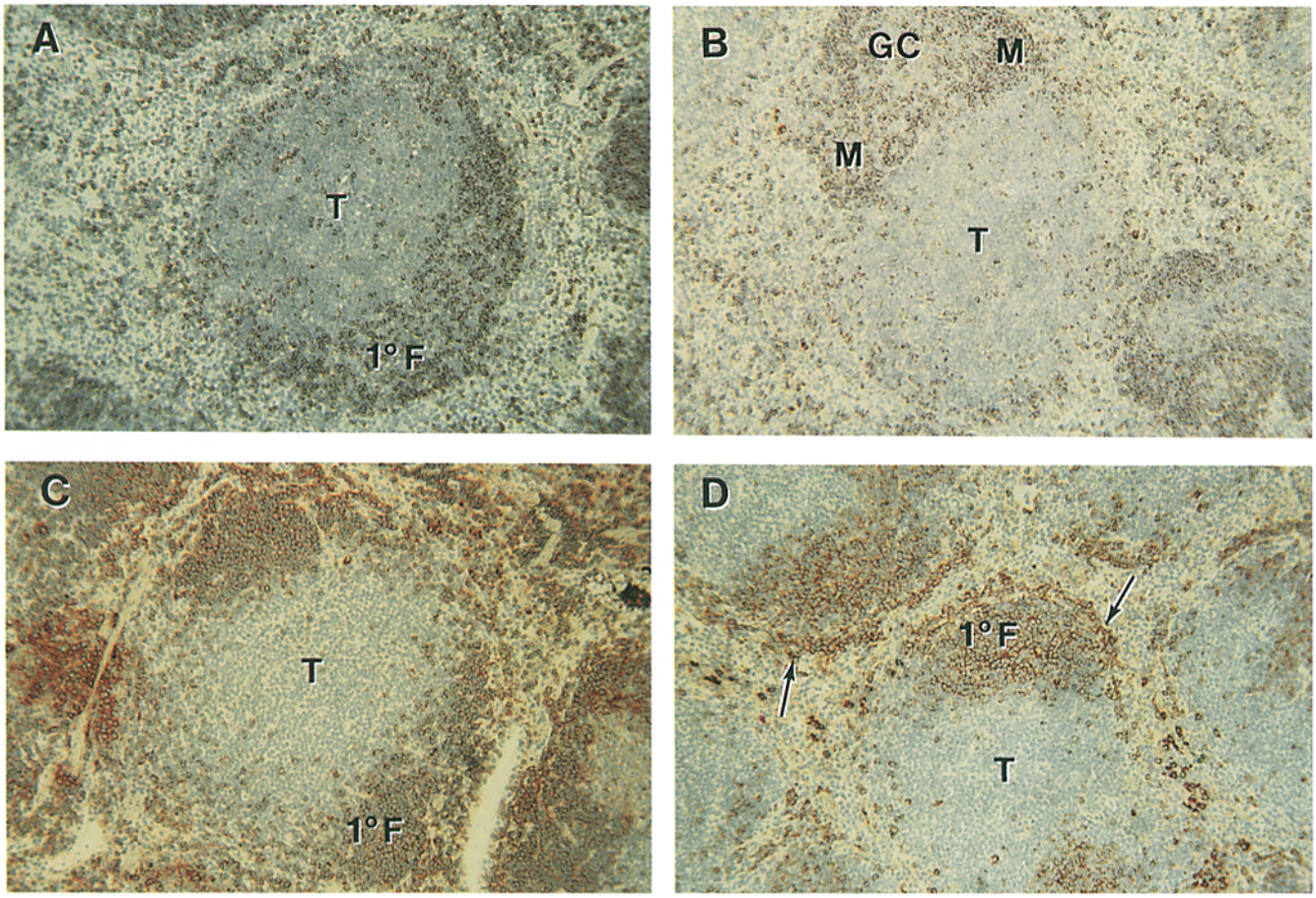

Figure 3. Immunohistochemical analysis of spleen sections from AB29 mice and control littermates. (*A* and *B*) High-power view (×400) of AB29 spleen stained for human IgM. (*C*) High-power view of control spleen stained for mouse Ig. (*D*) High-power view of AB29 spleen stained for mouse Ig. *1°F*, a primary B cell follicle; *T*, the periarteriolar lymphatic sheath (T cell area). *M*, the mantle zone of the secondary follicle; *GC*, the germinal center. The marginal zone of the white pulp is indicated by arrows.

Table 1. *Phenotype of AB29 Spleen cells*

Cell surface marker	Percentage of total cells	
	AB29 mice (n = 7)	Control littermates (n = 7)
B cells	55.4 (43–67)	69.6 (57–78)
Human IgM	39.1 (28–55)	0.0
Human κ	40.6 (26–53)	0.0
Mouse IgM	10.7 (5–24)	60.6 (46–78)
Mouse κ	10.3 (6–22)*	64.7 (52–74)
Mouse IgD	4.8 (3–10)*	52.2 (39–64)*
T cells (CD4 + CD8)	35.0 (24–43)‡	20.5 (20–29)‡

The results show the mean percentages of total splenic mononuclear cells that express each antigen; the ranges are shown in parentheses.
* n = 6.
‡ n = 5.

emulsified in CFA followed by boosting 2 wk later with aggregated human IgG in IFA did not increase serum levels of human IgM although a mouse anti-human IgG response was induced (data not shown). We concluded that, although unlikely, the presence of low levels of soluble IgM RF in the circulation may interfere with human IgG binding to the RF B cell surface. Consequently, to avoid the problem of competing soluble antibody, we stimulated freshly obtained spleen cells in vitro with bacterial LPS and then analyzed the culture supernatants 6 d later for their content of human and mouse Ig. LPS (20 μg/ml) induced much higher levels of secretion of mouse Ig (mean of AB8, 207 μg/ml; mean of AB29, 356 μg/ml; mean of nontransgenic littermate, 366 μg/ml) than of human IgM (mean of AB8, 0.2 μg/ml; mean of AB29, 0.4 μg/ml), despite, in the case of AB29 mice, a higher percentage of cells expressing human Ig. In addition, stimulation of the transgenic splenocytes in vitro with aggregated human IgG in the presence of T cell replacement factors derived from Con A–stimulated mouse lymphocytes did not augment human IgM secretion (data not shown).

These data would suggest that the RF-positive B cells that have differentiated in the transgenic environment normally release only small amounts of IgM RF and that high levels of secretion can not be readily induced.

Splenocytes from Transgenic Mice Are Immunocompetent. In AB29 mice, 70% of splenic B cells express human IgM. However, the mice do not appear to be immunocompromised by the reduction in mouse Ig–expressing B cells, as demonstrated by apparently normal levels of mouse Ig compared with their nontransgenic littermates (data not shown). In addition, the transgenic mice generated high titers of mouse antibodies to either keyhole limpet hemocyanin, or to other human IgM paraproteins. Thus, AB8 and AB29 mice would appear to be a useful system in which to examine the function of RF-expressing cells in the relative absence of secreted antibody or antigen, as a substantial proportion of cells express the human Ig in a B cell–specific manner and with a normal anatomical distribution, but this does not, at least superficially, interfere with endogenous immune competence.

Transgenic B Cells Can Be Signaled through Their Human IgM Surface Receptor. The human IgM RF on the cell surface of transgenic spleen cells can act as a receptor in much the same was as endogenous mouse Ig, as shown by the ability of anti–human IgM to stimulate the cells to proliferate (Table 2). Fig. 4 shows the results of a representative experiment in which AB29 splenocytes were stimulated in vitro with human IgG as an antigen. Pulsing spleen cells for 1 h on ice with 20 μg/ml soluble human IgG, followed by washing away excess, induced little proliferation of either transgenic or nontransgenic spleen cells. However, pulsing with aggregated human IgG induced proliferation of transgenic, but not control spleen cells. The results show that the aggregation of the antigen was able to achieve a sufficient degree of crosslinking to stimulate proliferation of transgenic B cells.

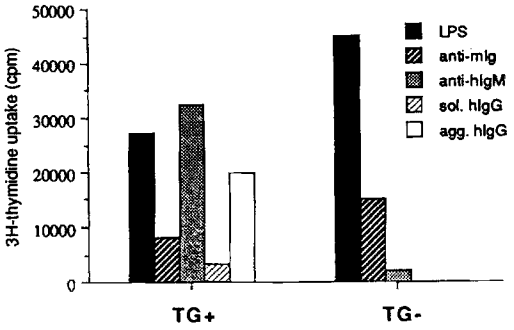

Figure 4. Proliferation of AB29 transgenic spleen cells in response to stimulation by human IgG. 2 × 10^5 spleen cells were cultured in the presence of LPS (*S. minnesota*) at 10 μg/ml, affinity-purified $F(ab')_2$ goat anti–human IgM (species specific) at 10 μg/ml, affinity-purified $F(ab')_2$ goat anti–mouse Ig (species specific) at 10 μg/ml, or were incubated with either soluble or heat-aggregated human IgG at 20 μg/ml for 1–2 h at 4°C, washed, and then cultured in medium. Results are expressed as means of triplicates. Background proliferation in the presence of medium alone has been subtracted from all values. [^{3}H]Thymidine incorporation was measured after 3 d. *TG+*, AB29 mouse; *TG−*, control littermate.

Table 2. *Proliferation of Spleen Cells in response to Anti-Ig Stimulation*

Spleen cell donor	Mouse	[^{3}H]Thymidine incorporation in response to: Anti–human IgM	Anti–mouse Ig	LPS
		cpm		
AB8	1	68,494	78,055	94,089
AB8	2	67,936	90,154	110,456
AB29	1	78,046	4,441	126,476
AB29	2	114,010	4,717	138,056
Non-Tg	1	2,004	115,754	101,699
Non-Tg	2	664	117,893	132,145

2 × 10^5 spleen cells from pairs of AB8, AB29 mice, and nontransgenic littermates, age 11–17 wk, were cultured in the presence of 20 μg/ml of $F(ab)_2$ fragments of goat anti–human IgM or goat anti–mouse Ig (affinity absorbed for crossreactivity with other species) or LPS (*Escherichia coli*). Results are expressed as means of triplicates. Background cpm in the presence of medium alone have been subtracted from all values. [^{3}H]Thymidine incorporation was measured after 3 d. The results shown here are from a representative experiment.

Transgenic RF B Cells Are Highly Efficient APC for Immune Complexes. Lymph node cells from C57/Bl6 mice immunized 10 d previously with 100 μg tetanus toxoid (TT) in CFA were used as a source of TT-specific Th cells. Spleen cells from transgenic mice or nontransgenic littermates were used as APCs. These cells received 1,000 rad to inhibit their proliferation, while preserving their ability to process and present antigen (25). Immune complexes were preformed in the culture wells by incubating TT with human IgG anti-TT in varying proportions for 2 h at 37°C. Lymph node Th and APC were then added and proliferation of Th measured at days 3–4. As shown in Fig. 5 *A*, AB29 spleen and control spleen were equally able to present soluble TT. However, when increasing amounts of human IgG anti-TT were titrated against a constant amount (10 ng/ml) of TT in order to form immune complexes, the ability of AB29 spleen to present antigen was substantially enhanced, whereas that of control spleen was unchanged (Fig. 5 *B*). The spleen cell population containing RF-expressing B cells was able to process and present 500–1,000-fold lower concentrations of antigen when that antigen was present in the form of an immune complex, compared with soluble antigen alone. As little as 10 ng/ml anti-TT antibody, in the presence of antigen, induced vigorous T cell proliferation, as determined by [^{3}H]Tdr uptake. As spleen cells from nontransgenic littermates showed no such enhanced ability to present small amounts of immune complexed antigen to T cells, we conclude that the enhanced antigen presentation capacity of the AB29 spleen was due to the specificity of its Ig receptor.

High Levels of Secretion of IgM RF Can Be Induced. The lack of secretion of human IgM RF by B cells in people could be the result of continuous exposure to high concentrations of monomeric IgG. However, the inefficient secretion of IgM

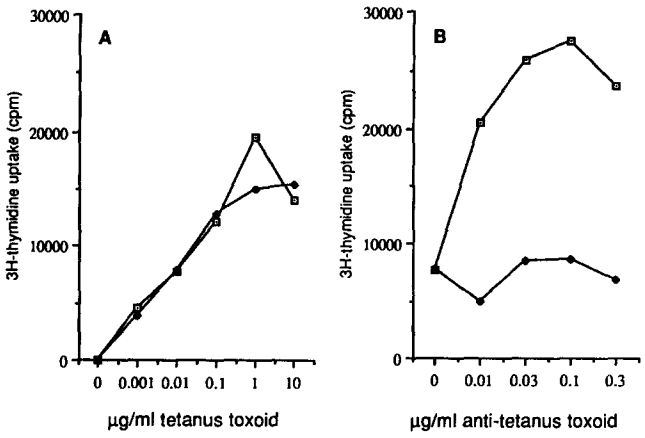

Figure 5. Comparison of the ability of spleen cells from AB29 mice and control littermates to present TT-human IgG murine complexes to TT-specific T cells. (*A*) Proliferation of TT-specific T cells to increasing concentration of TT presented by spleen cells from AB29 mice (*open symbol*) and control littermates (*filled symbol*). (*B*) Proliferation of TT-specific T cells to preformed immune complexes composed of 10 ng/ml TT and increasing concentrations of human IgG anti-TT presented by spleen cells from AB29 mice (*open symbol*) and control littermates (*filled symbol*).

Table 3. *Serum Levels of Human IgM RF and Mouse Ig in AB8 Mice Backcrossed with MRL/lpr Mice for Two Generations*

Mice	Human IgM RF	Mouse Ig
	µg/ml	*mg/ml*
TG$^+$ *lpr/lpr* (n = 7)	364 ± 240	23.3 ± 2.6
	(0.2–1,768)	(15.4–34.1)
TG$^+$ *lpr/–* (n = 6)	1.6 ± 0.5	3.7 ± 1.0
	(0.6–3.8)	(1.1 ± 8.4)
TG$^-$ *lpr/lpr* (n = 6)	0.0	34.4 ± 13.0
	(0.0)	(8.3–88.7)
TG$^-$ *lpr/–* (n = 10)	0.0	4.4 ± 0.5
	(0.0)	(1.8–6.7)

Serum Ig levels were measured at week 16 and are expressed as mean ± SE. The range for each group is in parentheses. TG, IgM RF transgene.

RF by the transgenic B cells cannot be attributed to tolerance caused by a weak crossreactivity with murine IgG. The IgM RF, Les, did not show any greater binding to an ELISA plate coated with aggregated mouse IgG than other antigens such as TT or even an uncoated plate. This was in spite of being tested at concentrations as high as 40 µg/ml, this level being approximately three times higher than that usually seen in AB29 mouse serum. To determine if the low secretion of IgM RF represented an irreversible state, the spleen cells from the AB29 transgenic mice were fused with NSO myeloma cells. The mean human IgM level secreted in vitro by the resultant hybrids was 40 µg/ml (n = 4), a level indistinguishable from that typically secreted by mouse Ig–producing hybridomas. These results indicate that IgM RF could be secreted at high levels if appropriate cofactors were provided.

To examine whether breeding the transgene onto strains of mice prone to autoimmunity could result in increased IgM RF production in vivo, the AB8 mice were bred onto the MRL/*lpr* background for two generations. The *lpr/lpr* (homozygous) mice were identified by the development of lymphoid enlargement by week 16. Table 3 shows that by 16 wk, secretion of human IgM RF increased >200-fold in the serum of AB8 mice that are homozygous for the *lpr* gene, reaching a mean serum level of 364 µg/ml. In contrast, heterozygous animals maintain low levels of secretion. This suggests that recessive genetic factors can lead to high-level secretion of the human IgM RF autoantibody transgenes.

Discussion

In this report we describe two lines of transgenic mice, AB8 and AB29, that produce a human IgM RF that is specific for human IgG. The transgene-expressing B cells from these mice share many properties with RF-expressing B cells in normal humans. The B cells expressing the IgM RF in the Les transgenic mice make up a substantial proportion of the total B cell pool. In AB8 mice 13–16% of splenic B cells express human IgM and κ, and in AB29 mice between 46 and 90% of splenic B cells express transgenic Igs. Such high percentages of RF-expressing B cells exist in these mice in the apparent absence of antigenic selection. Human IgM RF does not bind to mouse IgG (as detectable by ELISA) and the high degree of allelic exclusion seen between human and mouse Igs means that we cannot ascribe expansion to coexpression and stimulation of mouse Ig.

Analysis of immunohistological sections of the transgenic mouse spleens showed that RF-expressing B cells home to primary B cell follicles and the mantle zones of secondary follicles and apparently survive in the absence of self-antigen. It seems either that these cells do not require continued antigen stimulation for long-term survival, or that they are continuously being replenished by new precursors from the bone marrow.

Expression of the human IgM RF apparently does not substantially interfere with endogenous immune function as determined by assays that measure mouse Ig serum levels or antibody responses to foreign antigens. Furthermore, although a large percentage of B cells express surface human RF, the proportions of human-to-mouse Ig in such animals are low, comprising ~1 or 6% of serum IgM in AB8 and AB29, respectively. The discordance in the cellular ratios of RF transgene–expressing cells and serum IgM RF has striking parallels with that observed in human physiologic situations (5). In the transgenic mice, this is not surprising, as the transgene RF B cells exist in the absence of their target antigen and, therefore, presumably are not activated. This hypothesis is supported by the relative paucity of RF transgene–expressing B cells in germinal centers of secondary lymphoid follicles arguing against their recruitment into secondary immune responses. In addition, IgM RF–expressing B cells are indis-

tinguishable in size from normal mouse splenic B cells, as determined by light scatter (data not shown). However, it is surprising, that after exposure in vitro to a polyclonal B cell mitogen such as LPS, the B cells expressing mouse Ig synthesize 20 times more Ig in AB8 mice, or 100–1,000 times more Ig in AB29 mice, than cells expressing the RF transgenes. The RF transgene–expressing B cells, however, can be stimulated to proliferate in vitro by aggregated human IgG, as well as by bivalent anti–human IgM antibody. Thus, such RF transgene–expressing B cells can be signaled through their human IgM receptor, but are not readily induced to differentiate into antibody-secreting plasma cells by polyclonal B cell activators.

A number of investigators have shown B cells to be highly effective APC, using their Ig receptor to capture very small amounts of their specific antigen (8–12). IgM RF–expressing B cells may likewise have a role as a universal APC for immune-complexed antigen. For such a function it would be advantageous to the organism to have large numbers of cells expressing cell surface human IgM RF, but with little secreted RF, insofar as secreted antibody would compete for immune complex binding and presentation. Our experiments show that transgenic RF B cells function as an excellent source of APC for antigens such as TT present in the form of immune complexes with human IgG. The RF B cells are fully able to process and present antigen and deliver the necessary second signals to normal TT-primed Th cells. It has previously been shown that RF-expressing EBV-transformed B cell lines can act similarly in vitro (13), but this is the first demonstration of antigen presentation by a population of RF-expressing B cells that are untransformed, presumably resting, and consequently more closely represent the physiological state of the RF-expressing B cells present in the mantle zones of lymphoid tissues.

Lymphoid tissues contain several classes of APC, including dendritic cells, macrophages, and B lymphocytes. Immune-complexed antigens internalized by the FcγIII (CD16) receptor on macrophages are efficiently presented (26). In contrast, antigens targeted to the FCγII (CDw32) receptor on B lymphocytes are not presented, and may inhibit B cell activation (27–31). However, surface RF also may be considered an Fc-binding molecule that can facilitate antigen presentation of immune complexes. Upon secondary immunization, antigen arrives in the sinuses of draining lymph nodes in the form of immune complexes (32). At very low antigen concentrations, uptake by conventional APCs may be suboptimal, and the presence of antigen-IgG immune complexes may lead to feedback inhibition of presentation by antigen-specific B cells (29, 30). Under these conditions, RF-expressing B cells may play a major role in antigen presentation and activation of the antigen-reactive T cells required for specific B cell expansion.

Normally, serum levels of IgM RF are low; however, under certain circumstances, secretion of RF can be dramatically increased. Fusion of RF-positive B cells with myeloma cells to produce hybridomas rescues Ig secretion in both humans (5) and, as shown here, in the RF transgenic mice. Hybridomas derived from AB29 spleen cells secrete human IgM at approximately the same levels as mouse Ig–producing hybridomas. Patients with RA, Sjögren's syndrome, and mixed cryoglobulinemia also secrete high levels of IgM RFs in the absence of known antigenic stimulation (3). Similarly, we found that breeding transgenic mice onto the autoimmune MRL/*lpr* background led to very high levels of human IgM secretion: a >200-fold increase compared with normal levels. We interpret this result to show that the RF-expressing transgenic B cells can differentiate into plasma cells that are fully competent for antibody secretion. Mice that are homozygous for the *lpr* mutation have defective expression of the Fas surface antigen, which mediates apoptosis in immature T cells (33). The expression of the autoimmune phenotype in the *lpr* homozygous mice requires B as well as T cells (34). In the RF transgenic mice, high levels of autoantibody secretion may be secondary to the effects of the MRL/*lpr* background on B cell survival or differentiation.

The RF transgene–expressing B cells analyzed in this report show striking similarities to their normal human counterparts, in spite of development in a milieu where they have no apparent ligand. Use of transgenic mice expressing rearranged antigen receptor genes has contributed significantly to our understanding of the fate of autoreactive cells in the presence of antigen. It appears that these cells may either be anergized, be deleted, or persist unchanged in the circulation (35–43). The factors that decide these different outcomes have not been fully elucidated, but seem to be a function of antibody binding affinity, whether the antigen is soluble or bound to a cell surface, and antigen concentration. Experiments are in progress to determine the effect upon RF B cell function of development in an environment containing high levels of human IgG.

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