Human Immunoglobulin (IgG) Induced Deletion of IgM Rheumatoid Factor B Cells in Transgenic Mice

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Summary

The singular ability of immunoglobulin genes to hypermutate their variable regions, while permitting the generation of high-affinity antibodies against foreign antigens, poses a problem in terms of maintenance of immunological self-tolerance. Immunoglobulin gene hypermutation driven by a foreign antigen has the potential to generate antibodies that cross-react with self-components. Consequently, there must exist a mechanism in the periphery for inactivation of mature autoreactive B cell clones. The classical experimental system used to address this problem is the induction of tolerance to soluble, deaggregated human IgG. We have analyzed the mechanism of induction of tolerance to human IgG using transgenic mice that express a human IgM rheumatoid factor (IgM RF) on a large proportion of their B cells. Injection of deaggregated human IgG caused a specific deletion of those B cells that express an intact IgM RF on their cell surface. The degree of RF B cell deletion was proportional to the reduction in the proliferative response of splenocytes to antigen (aggregated human IgG), or to $F(ab')_2$ fragments of anti-human IgM antibodies. Control experiments showed that IgG administration had little effect on the numbers of mouse Ig-bearing cells or their ability to proliferate to a nonspecific mitogen. Thus, the effects of IgG on the human IgM RF B cell are antigen specific and are not due to nonspecific toxic effects of the human IgG preparation. These experiments demonstrate that peripheral exposure to IgG induces deletion of reactive B cells, without any evidence for anergy, and differ from data obtained by other investigators studying tolerance to soluble protein antigens. The results imply that human Igs have distinct properties as soluble antigens, and that peripheral nonresponsiveness to IgG may be due to lymphocyte deletion.

The induction of immunologic nonresponsiveness to soluble, deaggregated human IgG is one of the classical experimental systems for analysis of peripheral tolerance in both B and T cells (1). Although data have shown that the number of antigen-binding B cells is reduced in the periphery, the low frequency of these cells makes it difficult to determine whether this reduction is due either to downregulation or modulation of surface immunoglobulin, or to lymphocyte deletion. Experiments carried out more recently using transgenic mice which express immunoglobulins specific for hen egg lysozyme (HEL)[1] (2–5) and for H-2 antigens (6, 7) have confirmed that the predominant mechanisms responsible for B lymphocyte nonresponsiveness to self antigens are physical deletion of self-reactive cells, termed clonal deletion, and functional silencing of self-reactive cells, termed anergy. It has been suggested that the degree of immunoglobulin receptor cross-linking determines the fate of an autoreactive B cell (4). According to this hypothesis, recognition of monomeric or soluble antigen induces anergy and recognition of highly multivalent membrane bound antigen results in deletion. Consequently, the injection of soluble, heterologous immunoglobulins would be expected to lead to B cell inactivation by induction of anergy.

We have previously shown that B cells from transgenic mice which express a human IgM rheumatoid factor (RF) share many properties with IgM RF–bearing B cells from normal individuals (8). They comprise a significant proportion of the adult B cell population, but secrete only low levels of RF into the serum. B cells expressing the RF transgene show characteristic localization to primary follicles and the mantle zone regions of secondary follicles in the spleen. Although serum IgM RF levels are low in these mice, they can be substantially increased by breeding the transgenic mice onto the autoimmune prone MRL/lpr background.

[1] *Abbreviation used in this paper:* HEL, hen egg lysozyme.

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In this paper, we present data showing that deaggregated human IgG induces deletion of IgM RF B cells in transgenic mice. These results lead us to believe either that the properties of IgG interacting at high concentrations with B cells expressing this IgM RF may distinguish it from other soluble antigens that induce anergy in reactive B cells, or that division of antigens into those that induce deletion and those that induce anergy may not be as absolute as previously considered.

Materials and Methods

Mice. The initial AB29 IgM RF transgenic line was described previously (8). It derived from B6 × SJL F_2 injections and has since been maintained by crossbreeding with C57Bl/6 mice (The Jackson Laboratory, Bar Harbor, ME). Those mice used in the described experiments were aged 6 wk or older, and were derived from sixth generation, or later, backcrosses to C57Bl/6.

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, 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 Chemical & Scientific Corp., Westbury, NY), streptavidin-peroxidase (Kirkegaard & Perry Laboratories, Inc., 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.

Tolerance Induction To Human IgG. Mice were injected intraperitoneally with 2 mg deaggregated human IgG unless stated otherwise. Initial experiments used pooled human IgG obtained from a commercial source (Cappel). However, the majority of the experiments described here used human IgG1 which was purified from the serum of a myeloma patient. Briefly, serum was filtered and then purified on a protein A–agarose column (Sigma Chemical Co., St. Louis, MO). After extensive dialysis against PBS and testing for levels of endotoxin, the human IgG was considered suitable for use. Human IgG was deaggregated by centrifugation at 100,000 *g* for 150 min at 4°C. The top 25% of the gradient was carefully removed, the Ig concentration was checked and adjusted with pyrogen-free 0.15 M NaCl to 4 mg/ml. 2-mg aliquots were then immediately injected intraperitoneally into mice.

Cell Preparation for Fluorescence Activated Cell Sorter Analysis. Spleens were removed from AB29 transgenic mice or nontransgenic littermates and gently teased apart in RP10 medium (RPMI 1640 [Irvine Scientific, Santa Ana, CA] supplemented with penicillin and streptomycin, glutamine, 2-ME and 10% heat-inactivated FCS [Gemini Biolabs, Calabasas, CA]). Peripheral blood was collected from the mice into heparinized tubes. Before analysis by FACS® (Becton Dickinson & Co., San José, CA), viable white cells were isolated using cell separation gradients (Lympholyte M, Accurate, Westbury, NY). Cells obtained from the interface of these gradients were washed with FACS® medium (PBS/3%FCS/0.1% sodium azide) before use.

FACS® Analysis of Spleen or Peripheral Blood. Cell staining was carried out as previously described (8), using the following reagents: goat anti-human IgM-FITC (Jackson Immuno Research Laboratories, West Grove, PA); anti-B220-PE (PharMingen, San Diego, CA); anti-mouse κ-PE (Fisher Scientific Co., Pittsburgh, PA); goat anti-human kappa-biotin (Sigma Chemical Co.); and streptavidin-PE (Molecular Probes, Inc., Eugene, OR). Cells were either analyzed on a FACScan® (Becton Dickinson & Co.) immediately after staining or fixed with 1.5% paraformaldehyde for 45 min on ice. Fixed samples were analyzed at a later time.

B Cell Proliferation Assays. Spleens were removed from transgenic mice or nontransgenic littermates. Cells were teased apart, washed and resuspended at 2 × 10^6/ml in RP10 medium. 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, goat anti-human IgM (Jackson Immuno Research Laboratories); aggregated human IgG (Cappel); or LPS from *Salmonella minnesota* (Sigma Chemical Co.). 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 and proliferation was measured at day 3–4 by the addition of 1 μCi of [^{3}H]TdR per well. The assay was terminated 18 h after addition of the [^{3}H]TdR by harvesting plates using a cell automated harvester (Cambridge Technology, Inc., Watertown, MA).

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 or affinity-purified goat anti-mouse IgM-biotin (Jackson Immuno Research Laboratories) and streptavidin-linked alkaline phosphatase (KPL). Briefly, 100 μl of medium or an optimal dilution of the biotinylated reagents was added to each slide and incubated for 30 min at room temperature. Slides were then washed with pH 8.0 borate buffered saline (BBS), before adding 100 μl of streptavidin-linked alkaline phosphatase for a further 30 min. After washing the slides with BBS, the bound alkaline phosphatase was detected using a Histomark Red staining kit (KPL) in which specific staining is blue and the tissue background is red.

Results

Time Course of Deletion of IgM RF B Cells. AB29 mice were injected intraperitoneally with 2 mg of purified deaggregated human IgG1 myeloma protein. The mice were bled periodically and the phenotype of their PBL analyzed over time. Data shown in Table 1 express the results in terms of the percentage of B cells ($B220^+$) and human IgM positive cells (hIgM, μ specific).

It is apparent that injection of human IgG results in the removal of peripheral B cells that express human IgM on their cell surface. Interestingly, the time after injection of human IgG, at which levels of IgM positive B cells are no longer significantly different ($p > 0.05$) from saline-treated controls i.e., ~7 wk, is the time at which B cell responsiveness to human IgG returns in classical HGG (human IgG) tolerance as a result of tolerogen catabolism (9). This implies that nonresponsiveness in this latter system may indeed be due to deletion.

Effect of Human IgG on the Phenotype and Functional Capacity of the RF B Cell. The effect of human IgG on splenic lymphocytes in terms of their phenotype and functional capacity was then analyzed more fully. In the experiments shown in Table 2, we analyzed mice 3 wk after injection of deaggregated human IgG.

Deletion of human IgM-expressing B cells is accompanied

Table 1. *Time Course of the Effect of Deaggregated Human IgG on the Peripheral Blood Phenotype*

Time	B220		hIgM	
	hIgG inj.	Saline inj.	hIgG inj.	Saline inj.
Prebleed	34.5 ± 3.8	42.4 ± 11.1	18.3 ± 1.5	20.8 ± 1.7
Week 1	12.9 ± 2.0	32.3 ± 2.7	1.5 ± 0.3	16.8 ± 0.9
Week 3	15.0 ± 1.9	32.4 ± 2.0	6.2 ± 1.7	18.3 ± 1.6
Week 5	17.4 ± 1.5	32.1 ± 1.0	8.5 ± 1.0	16.0 ± 0.5
Week 7	27.1 ± 1.5*	32.1 ± 2.5	15.8 ± 2.3‡	19.3 ± 1.1

Mice were bled at various times relative to intraperitoneal injection of either deaggregated human IgG (hIgG inj.) or saline (sal. inj.) as control. PBL were then stained for surface expression of either B220 (total B cells) or human IgM and analyzed on the cytofluorograph. Data are expressed as percentage of positive cells ± SE for groups of three or four mice. Levels of both B cells (B220+) and hIgM cells are significantly reduced in the hIgG-injected group compared with the saline control from weeks 1–5 ($p < 0.002$). Values at week 7:
* $p = 0.18$ for B220;
‡ $p = 0.20$ for hIgM.

by a reduction in the proliferative response of splenocytes to both antigen (aggregated human IgG) and to $F(ab')_2$ fragments of anti-human IgM antibodies (Table 2). It is obvious that the effect of the deaggregated human IgG is specific for the human IgM RF-expressing B cells and is not due to nonspecific toxicity of the preparation. Cells expressing mouse immunoglobulin are unchanged in number. A similar lack of significant effect on the levels of mouse kappa bearing cells was obtained in six out of seven experiments. IgG administration does not alter the proliferation of splenocytes in response to LPS.

The antigen specificity of the deletion is confirmed when splenocytes are costained for both human IgM (μ chain specific) and human kappa (Table 3). Double staining B cells are pref-

Table 2. *Effect of Deaggregated Human IgG on Phenotype and Functional Properties of Spleen Cells*

Transgenic	Tolerization	Phenotype			Proliferation in response to:		
		B220	hIgM	mkappa	Anti-hIgM	Agg. hIgG	LPS
			%				
+	Saline	51.7 ± 5.1	22.6 ± 1.5	21.8 ± 4.3	31,954 ± 2,753	15,577 ± 894	18,047 ± 2,216
+	hIgG	39.4 ± 3.9	6.7* ± 2.2	23.2‡ ± 1.3	7,014§ ± 1,965	1,146‖ ± 557	17,235¶ ± 1,444
−	Saline	64.2 ± 3.8	0.5 ± 0.1	57.6 ± 2.8	843 ± 47	0	40,974 ± 934
−	hIgG	63.4 ± 3.5	0.3 ± 0.1	57.9 ± 2.6	574 ± 306	0	47,837 ± 3,749

AB29 transgenic mice (+) or nontransgenic littermates (−) were killed 3 wk after intraperitoneal injection of either deaggregated human IgG (hIgG) or saline as control. Data are expressed as the mean ± SE of groups of three mice. Spleen cells were prepared and then stained for phenotypic analysis on the cytofluorograph (left three columns) or for their proliferative response to a variety of stimuli (right three columns). The phenotypes are expressed as the percentages of cells within a lymphoid scatter distribution (defined by forward versus side scatter) that stain for either surface B220 (B cells), human IgM (μ chain specific, hIgM), or mouse kappa (mkappa). The proliferation data are expressed as counts per minute measured on day 3–4 after stimulation of 2×10^5 splenocytes with $F(ab')_2$ fragments of goat anti-human IgM antibodies at 10 μg/ml; heat aggregated human IgG (agg. hIgG) at 200 ng/ml; or LPS at 10 μg/ml. Background proliferation in the presence of medium alone has been substracted from all values.
* $p = 0.0004$.
§ $p = 0.002$.
‡ $p = 0.77$.
‖ $p = 0.0002$.
¶ $p = 0.77$.

Table 3. *Preferential Deletion of B Cells Expressing both Human IgM and Human Kappa*

Tolerization	Phenotype (total lymphocytes)				Phenotype (total hIg$^+$ lymphocytes)		
	B220	hκ	hκ plus hIgM	hIgM	hκ	hκ plus hIgM	hIgM
				%			
Saline	29.1 ± 1.5	0.6 ± 0.2	14.1 ± 1.1	1.8 ± 0.5	4	86	11
Human IgG	12.8 ± 1.2	0.8 ± 0.5	1.2* ± 0.2	1.0 ± 0.1	27	40	33

Transgenic mice were bled 3 wk after intraperitoneal injection of either human IgG (n = 9) or saline (n = 7) as control. Peripheral blood cells were then stained for phenotypic analysis on the cytofluorograph. Data in the left four columns are expressed as the percentage of cells within a lymphoid scatter distribution (defined by forward versus side scatter) that stain for either surface B220 (B cells), human kappa light chain (hκ) alone, human kappa plus human IgM (μ chain specific, hIgM), or human IgM alone. Data in the right three columns show these values as a percentage of the total cells that express human Ig (either kappa or μ). Background staining of the anti-human Ig reagents with nontransgenic lymphocytes has been deducted from the percentages shown.
* p = 0.0001.

erentially deleted, and residual B cells which express either human kappa or human IgM, presumably in association with mouse immunoglobulin, are relatively spared. Such data are also shown in Fig. 1 as a double-color FACS® profile. It should be noted that in ~15% of cases (6/38 mice in seven separate experiments), deaggregated human IgG fails to induce a substantial reduction (>50%) in the levels of human IgM-expressing cells. It has previously been shown that the presence of LPS prevents induction of tolerance to human IgG (10). It is possible that the few mice which did not delete were suffering from an acute bacterial infection at the time of human IgG injection which would have prevented the induction of tolerance. Data from such mice are not included in the results shown, although their inclusion would still have resulted in a significant reduction (p <0.05) in the number of B cells expressing both surface human IgM and κ.

The reduction in total B cell levels made it unlikely that human IgG was merely downregulating human IgM RF from the B cell surface. Immunohistologic analysis of treated mice confirms this conclusion (Fig. 2). Spleens were removed from mice 3 wk after injection of either saline (*A* and *B*) or human IgG (*C* and *D*), sectioned, and stained for the presence of mouse IgM (μ chain specific, *A* and *C*), or human IgM (μ chain specific, *B* and *D*). IgM RF B cells in the AB29 IgM RF transgenic mice characteristically localize to primary follicles and the mantle zones of secondary follicles in the white pulp (8). This is the area in which small resting B cells are normally located, and is similar to the localization pattern of RF B cells in normal humans (11). In addition, scattered positive cells are located in the red pulp, a region through which all blood cells percolate. Presumably, some of these latter cells include the plasma cells that secrete the low levels of IgM RF detected in the serum. Treatment of AB29 mice results in preferential loss of cells from the primary follicles and mantle zones of secondary follicles and relative sparing of cells located within the red pulp. This is not unexpected as the existing plasma cells lack surface immunoglobulin and consequently cannot be signaled in either a positive or negative manner by antigen. Staining for mouse IgM confirms that treatment does not destroy the follicles or otherwise disrupt the normal architecture of the spleen. These results confirm that IgM RF-bearing B cells are deleted, and that

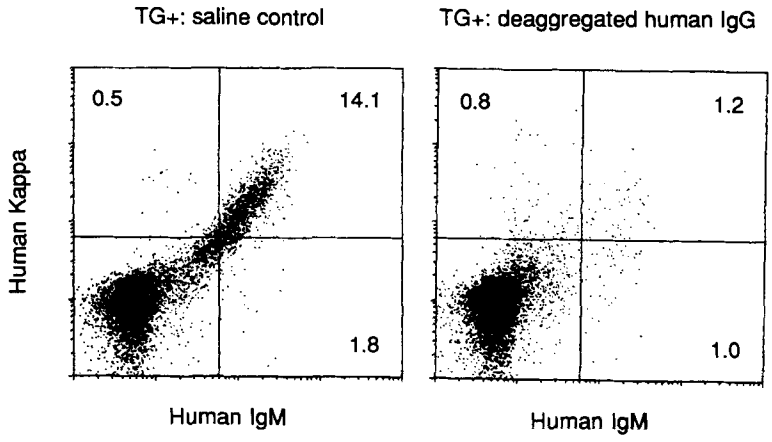

Figure 1. FACS® analysis of PBL from transgenic (TG$^+$) mice. TG$^+$ mice were bled 3 wk after intraperitoneal injection of either saline as control (*left*) or deaggregated human IgG (*right*). PBL were then stained for surface human kappa (*y-axis*) or surface human IgM (μ, *x-axis*).

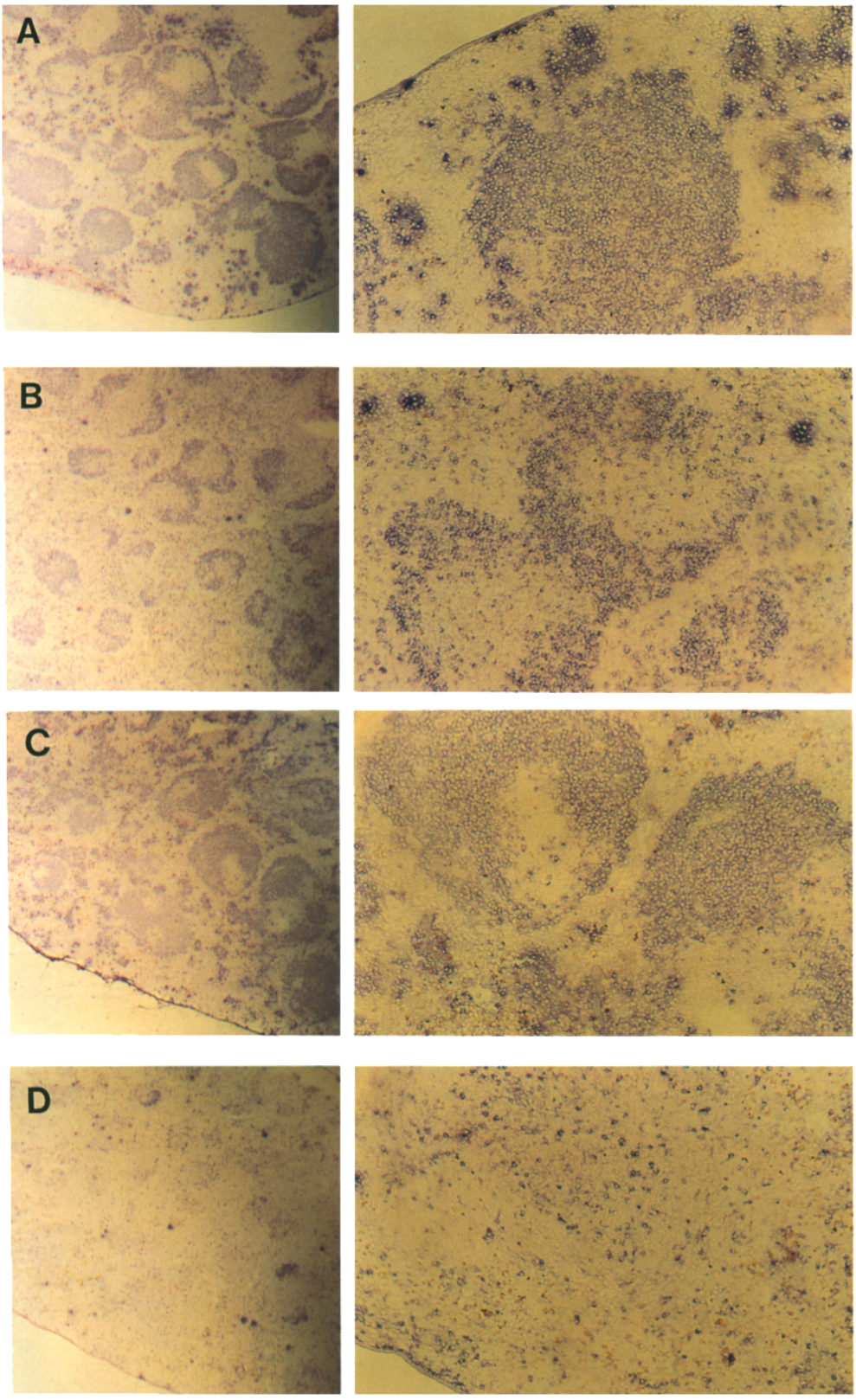

Figure 2. Immunohistochemical analysis of spleen sections from AB29 mice (TG^+) injected with either saline (control) or deaggregated human IgG. TG^+ spleens were harvested 3 wk after intraperitoneal injection of saline (*A* and *B*) or 2 mg deaggregated human IgG (*C* and *D*). Sections were stained for the presence of mouse IgM (μ specific, *A* and *C*) or human IgM (μ specific, *B* and *D*). Each panel is divided to show both low power views (×100) and high power views (×400) of the same section.

surface immunoglobulin is not merely modulated or downregulated. In addition, they suggest that soluble human IgG does not cause B cell loss through maturation to the plasma cell level, because IgM RF-expressing cells do not appear to increase in the red pulp after the host is treated with soluble IgG.

Discussion

Somatic hypermutation of Ig genes makes possible the selection and expansion of high affinity antibodies to foreign antigens. However, a consequence of this hypermutation is that is that it imposes a necessity for the immune system to not only maintain central tolerance to self-components but also to induce peripheral tolerance in mature B cells that have left the bone marrow and, because of diversification, may have acquired reactivity to self. Such a mechanism of peripheral tolerance may be essential for the prevention of autoimmune diseases.

Immunologic unresponsiveness in the adult mouse after injection of soluble, deaggregated human IgG is the most thoroughly studied system of acquired peripheral tolerance (1, 9, 12–14). The doses of antigen and kinetics of induction in both the T and B cell populations have been fully documented. After a single dose of tolerogen, nonresponsiveness is long-lasting in the T cell compartment but of shorter duration in the B cell compartment. However, the exact mechanism of B cell inactivation is not known because of the low frequency of human IgG-specific B cells under normal circumstances. To overcome this barrier, we have studied AB29 transgenic mice that express a human IgM antibody with specificity for human IgG (a human IgM RF). The AB29 transgenic mice express the Les human IgM RF on a large proportion (mean = 70%, range = 46–90%) of their mature B cells, but similarly to normal humans, show characteristically low levels of IgM RF in the serum (mean level in AB29 mice = 12.9 μg/ml; 8). The transgenic heavy and light chain Ig genes were isolated from the DNA of a chronic lymphocytic leukemia patient who had associated systemic vasculitis (15–17). The Vk derives from Vk328, a conserved Vk3 subgroup gene that often encodes light chains of human autoantibodies. The *Vh* gene most likely derives from Vh4.21 (17–19). A number of point mutations result in the production of a higher affinity RF than would normally be produced by the germline genes themselves.

Injection of soluble, deaggregated human IgG results in deletion of IgM RF-bearing B cells from the periphery of AB29 transgenic mice. Lack of IgM RF B cells was not due to downregulation or internalization of surface Ig because there was a similar reduction in the total number of B cells in both the spleen and blood. Neither could it be explained as a loss of B220-expressing cells due to maturation of IgM RF B cells to a plasma cell stage, as staining of treated spleen sections showed a significant reduction of IgM RF B cells in the primary follicles and mantle zones of secondary follicles, and there was no indication of a greater number of plasma cells in the red pulp. The antigen specificity of the deletion was confirmed by a preferential reduction in B cells which expressed both human IgM (μ) and kappa and so had the potential for binding human IgG, and a relative sparing of those B cells that expressed either human IgM (μ) or kappa alone, presumably in association with mouse immunoglobulin. Nonspecific toxicity of the human IgG preparation was also ruled out by a generally insignificant effect on the numbers of mouse κ-bearing B cells. Moreover, the IgG administration did not reduce responsiveness to the nonspecific B cell mitogen LPS.

The effects of deaggregated human IgG in transgenic mice show a number of striking similarities to previously published studies of human IgG (HGG) tolerance (1, 9, 12–14). In both systems, induction of tolerance is dose dependent. Thus, 0.1, 0.5, and 2.5 mg of deaggregated human IgG induced 9, 56, and 70% B cell unresponsiveness, respectively, in normal mice (9), whereas in the AB29 mice, injection of 0.25 and 2.5 mg resulted in deletion of 39 and 90% of human IgM (μ) positive B cells, respectively (data not shown). In normal mice, B cell tolerance was lost 49 d after injection of tolerogen. In AB29 mice, levels of human IgM (μ)-bearing B cells were restored to normal by 49 d. In both cases it is likely that the circulating concentrations of human IgG drop with time (20), eventually enabling B cells which have newly emerged from the bone marrow to survive.

Our finding of B cell deletion in mice exposed to deaggregated human IgG contrasts with earlier data obtained in transgenic models of peripheral tolerance in which soluble antigens induced anergy, whereas multimeric or cell surface antigens induced deletion (2–4, 6, 7, 21, 22). However, human IgG has distinct immunological properties that distinguish it from other antigens such as an ability to bind to Fc receptors and fix complement, besides interacting with IgM RF. Crosslinking of surface Ig by either antigen or $F(ab')_2$ fragments of anti-Ig antibodies usually results in B cell activation. These activation signals are downregulated if surface Ig is cocrosslinked with FcγII receptors (23–31). Although the high affinity binding site for the Les RF is in the Fc portion of human IgG (Carson, D. A., unpublished results), the antigen–antibody complex is very poorly soluble. Thus, it is possible that a portion of the deaggregated human IgG which is injected into the mice aggregates at the B cell surface, aided either by the low levels of human IgM RF present in the circulation or by low affinity, polyspecific mouse IgM of a type similar to that naturally found in humans. The presence at the B cell surface of small aggregates of human IgG/IgM RF could potentially lead to negative signaling through simultaneous binding to surface Ig and Fc receptors. It is interesting in this regard that classically the most effective antigens for induction of tolerance have been gamma globulins (32). In addition, it was recently reported (33) that anergic B cells have a reduced life span in the periphery, indicating that a distinction between B cell anergy and deletion may not be absolute. Instead, the effects of antigen on lymphocyte numbers may depend on the kinetics of cell turnover after antigen exposure, compared with the rate of cell generation.

Results from other studies (34) analyzing the effect of IgG on central tolerance of mouse IgM RF-expressing B cells show

that the RF B cells were neither deleted nor anergized, and may even have been expanded by antigen. Whereas it is not altogether clear why these results differ from our finding of peripheral tolerance by deletion, it is likely that the amount of antigen present in these mice (~50 μg/ml of mouse IgG2a) is probably less than the peak achieved in AB29 mice injected intraperitoneally with 2 mg of deaggregated human IgG. In addition, this level of IgG is also less than the concentration of IgG present in the serum of normal individuals.

Lymphocytes bearing cell surface IgM RF constitute a significant proportion of adult B cells in humans (35). Obviously then, some IgM RF B cells which react with human IgG remain in normal individuals in spite of the presence of antigen. This is potentially due to differences in affinity of the IgM RFs. Germline encoded IgM RFs are generally of low affinity and frequently polyspecific (10^{-4} to 10^{-5} M; 36). The Les IgM RF is specific for human IgG and of higher avidity because of a number of point mutations (14–16, and our unpublished results). Secretion of this autoantibody led to immune complex formation and vasculitis in the patient from which these genes were derived.

Higher affinity RFs may arise naturally as a consequence of somatic hypermutation and diversification of immunoglobulin genes during normal antigen-driven immune responses. We and others (8, 37) have previously shown that one of the physiological functions of normal IgM RF B cells may be to present antigen, in the form of immune complexes with human IgG, to antigen-specific T cells. Therefore, while it is unlikely that IgG-specific T cells would escape central tolerization, T cells reactive to foreign antigens also have the potential for stimulating the RF B cell. Recent data with anti-HEL transgenic mice indicate that anergic B cells maintain their nonresponsive state in the presence of helper T cells and soluble antigen. However, use of HEL in a more potent membrane-bound form in the presence of T cell help (38), or transfer of anergic B cells together with T helper cells and HEL emulsified in adjuvant to irradiated third party recipients (39) leads to antibody production by the previously anergic B cells. RF-expressing B cells must be exposed intermittently to highly cross-linked human IgG in the form of an immune complex, together with T cells reactive to the antigen component of the immune complex. Under these circumstances, deletion may be essential, otherwise higher affinity RF B cells retained in an anergic state might reactive and trigger autoimmunity.

We postulate that RF B cells which express low affinity IgM RFs fail to be inactivated or stimulated by soluble IgG. They may however, serve as excellent APCs for any antigen present in the form of an immune complex with IgG, resulting in an expansion of responsive T cells early in a secondary immune response. This would account for the increase in RF precursor B cells which normally accompanies a secondary immune response (40). IgM RFs in humans have been considered unusual in their apparent lack of somatic mutation and class switching. We would suggest that IgM RFs may indeed have the ability to undergo affinity maturation. In addition, the normal process of somatic mutation in other antibody genes may generate antibodies that cross-react with IgG. The presence of immune complexes and antigen-specific T cells would then lead to the activation of both low and high affinity RFs. However, after clearance of antigen, B cells expressing higher affinity RFs would be deleted by high levels of circulating soluble IgG in the absence of T cell help. Under circumstances where such peripheral deletion is defective, T cells reactive with any antigen capable of forming an immune complex with human IgG, would be able to stimulate and maintain production of high affinity RFs. This may be the situation that we observe in diseases such as rheumatoid arthritis and systemic lupus erythematosus.

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