Production of Immunoglobulin Isotypes by Ly-1$^+$ B Cells in Viable Motheaten and Normal Mice

CHARLES L. SIDMAN, LEONARD D. SHULTZ, RICHARD R. HARDY, KYOKO HAYAKAWA, LEE A. HERZENBERG

Almost all B cells in autoimmune mice with the viable motheaten (*me*^{v}) mutation express the Ly-1 cell surface antigen, which marks a minor population of B cells constituting a separate lineage in normal mice. Immunoglobulins primarily of the M and G3 classes, which in both normal and *me*^{v} mice contain high levels of lambda light chain, are produced in excess in *me*^{v} mice. These and other observations suggest that the development of B cells that express Ly-1 is regulated independently from the development of B cells that do not express Ly-1. B cells bearing the Ly-1 surface antigen may play specialized roles in the normal immune system and in autoimmunity by regulating other B cells via lymphokines, by producing antibodies to self and certain foreign antigens, and by preferentially secreting immunoglobulin M and immunoglobulin G3.

MICE HOMOZYGOUS FOR EITHER of the autosomal recessive mutations motheaten (*me*) or viable motheaten (*me*^{v}) have the most severe autoimmune syndromes known in mice, living on the average only 22 or 61 days, respectively (*1–3*). Sera from these mice contain 10 to 20 times the normal concentrations of serum immunoglobulin (Ig) and include many species of autoantibodies that react with various tissues (*3, 4*). B cell hyperactivity in *me* and *me*^{v} mice is accompanied by, and may be caused by, overproduction of two B cell maturation–promoting lymphokines, at least one of which is secreted by B cells themselves (*4*). To examine the surface phenotype of the lymphokine- and autoantibody-secreting *me*^{v} B cells, we isolated cells from various lymphoid organs of C57BL/6J and C57BL/6J-*me*^{v} mice, doubly stained them with antibodies to immunoglobulin M (IgM) and with antibodies to IgD, to Ly-1, or to Ly-2, and examined them on a fluorescence-activated cell sorter (FACS). As previously described (*5–7*), the levels of membrane-bound IgM, IgD, and Ly-1 distinguish several populations of B cells in normal mice. Staining with antibody to Ly-2 (anti–Ly-2), which has not been detected on B cells, serves as a negative control for Ly-1 staining, since the latter is much weaker on B than on T cells.

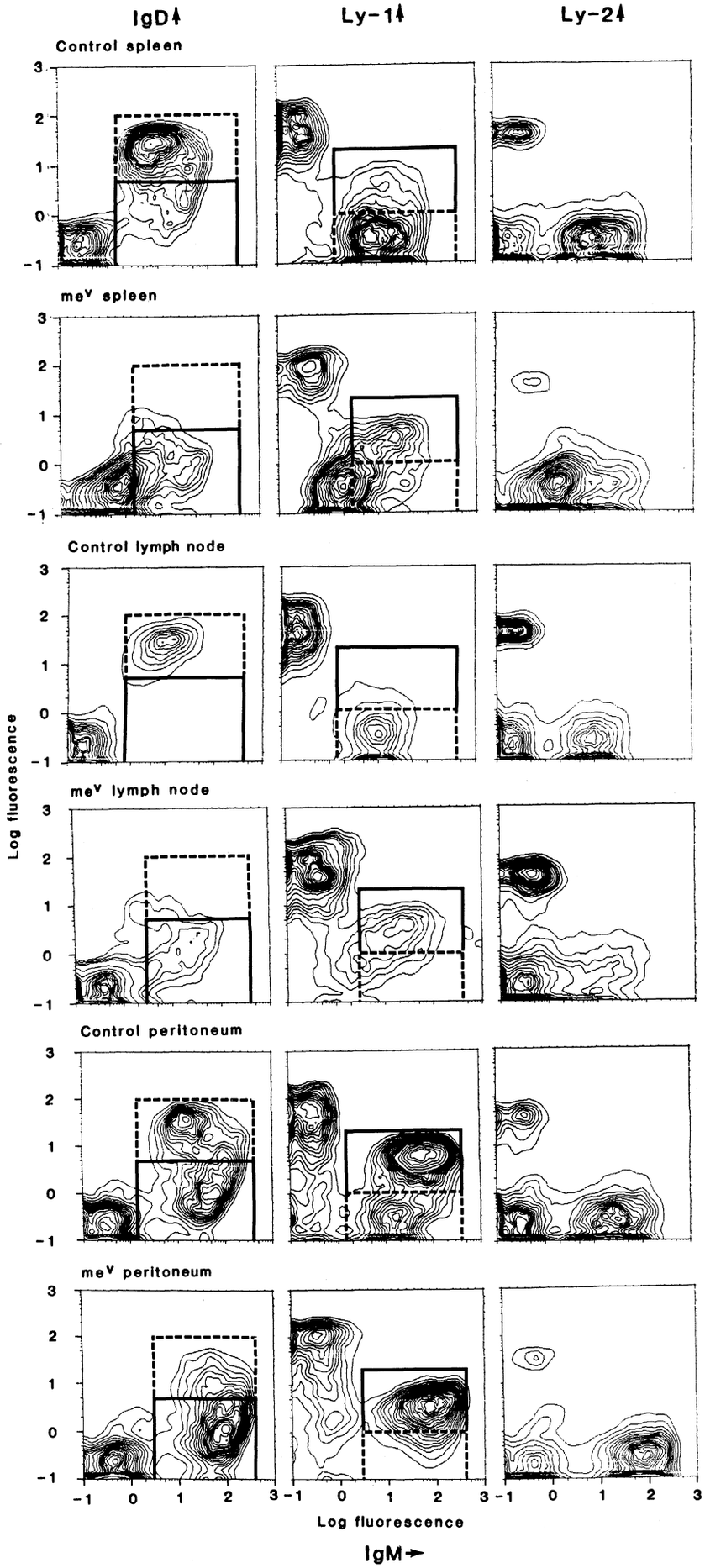
Figure 1 shows the results of such staining, presented as contour plots of the distribution of cells with different amounts of various combinations of markers. In young adult control mice, two populations of B cells (those with high IgM, low IgD, and detectable Ly-1, and those with low IgM, high IgD, and no detectable Ly-1) were seen in the peritoneum in approximately equal numbers. Control spleens had relatively few, and control lymph nodes almost none, of the B cell population marked by high IgM, low IgD, and detectable Ly-1. In contrast, nearly all B cells of age-matched *me*^{v} mice, from all three organs, had the high IgM, low IgD, detectable Ly-1 surface phenotype. The regions of the staining patterns that represent the two B cell populations are shown in Fig. 1 by hatched and solid lines. Most of the *me*^{v} B cells are judged to express Ly-1 because, although the distribution of Ly-1 staining of *me*^{v} B cells partially overlaps the range of control Ly-2 staining, most *me*^{v} B cells show Ly-1 staining clearly above the level of Ly-2 staining, and few, if any, show the absence of staining that leads to an accumulation of cells represented near the *x*-axis, as is seen with non-*me*^{v} tissues. Since *me*^{v} organs contain approximately the same total number of B cells as their control counterparts (*8*), *me*^{v} mice show significant increases in the absolute number of B cells bearing the Ly-1 antigen (Ly-1$^+$) and significant decreases in the absolute number of B cells without this antigen (Ly-1$^-$) in all organs, as compared to their numbers in age-matched control mice.

The immunoglobulins from normal and *me*^{v} mice were quantitated with enzyme-linked immunosorbent assays (ELISA) with antibodies to heavy (H) chains as the first layer and enzyme-conjugated antibodies to light (L) chains as the second and developing layer. These antibodies were produced and rendered specific by immunization of goats with purified myeloma and hybridoma products, passage over columns containing immunoglobulins of other isotypes, and positive adsorptions to two columns of immunoglobulins different from the immunizing antigens but of the desired isotype. All reagents showed less than 1 percent

C. L. Sidman and L. D. Shultz, The Jackson Laboratory, Bar Harbor, ME 04609.
R. R. Hardy, K. Hayakawa, L. A. Herzenberg, Department of Genetics, Stanford University Medical School, Stanford, CA 94305.

Table 1. Serum immunoglobulin levels. Values represent geometric means (standard error of the mean) for 6 to 15 animals per group. %λ = λ/(κ + λ) × 100.

Age of mice (months)	Serum immunoglobulin (μg/ml)							
	L chain	IgM	IgG3	IgG1	IgG2b	IgG2a	IgA	Total
				me^v *mice*				
2	κ	3570 (1.4)	3325 (1.4)	425 (1.5)	476 (1.3)	187 (1.2)	1428 (1.1)	9411
	λ	1674 (1.4)	1018 (1.3)	132 (1.3)	51 (1.3)	50 (1.3)	680 (1.3)	3905
	%λ	32	23	24	10	21	32	29
				Control mice				
2	κ	68 (1.2)	185 (1.1)	352 (1.2)	195 (1.1)	49 (1.0)	203 (1.1)	1052
	λ	33 (1.4)	62 (1.2)	51 (1.2)	12 (1.1)	5 (1.2)	13 (1.3)	176
	%λ	33	25	13	6	9	6	14
11	κ	438 (1.0)	284 (1.1)	237 (1.2)	528 (1.3)	72 (1.1)	269 (1.1)	1828
	λ	101 (1.1)	58 (1.1)	33 (1.1)	20 (1.1)	4 (1.2)	31 (1.5)	247
	%λ	19	17	12	4	5	10	12
30	κ	549 (1.2)	240 (1.2)	261 (1.2)	390 (1.4)	65 (1.1)	492 (1.4)	1997
	λ	64 (1.2)	43 (1.2)	32 (1.2)	13 (1.2)	4 (1.2)	39 (1.7)	195
	%λ	10	15	11	3	6	7	9



cross-reactivity to nontarget isotypes. The antibody to lambda L chain reacted with both lambda-1 and lambda-2. Concentrations of IgM and IgG3 in serum from *me*^{v} mice were 50 and 20 times greater, respectively, than those in serum from control age-matched (littermate) mice, but concentrations of IgG1, IgG2b, IgG2a, and IgA were only 1.4 to 10 times greater than those in controls (Table 1). All classes of immunoglobulins from *me*^{v} mice, and IgM and IgG3 from age-matched control animals, contained two to five times as much lambda relative to total L chains as did the other H chain isotypes (IgG1, IgG2b, IgG2a, and IgA) of normal C57BL/6J mice. The particularly high level of lambda L chains in the IgM and IgG3 classes of normal mice disappeared as the animals aged.

On the basis of cell transfer experiments, Ly-1$^+$ and Ly-1$^-$ B cells are thought to represent distinct cell lineages; allotype-marked peritoneal cells injected into irradiated allotype-congenic mice develop exclusively into Ly-1$^+$ B cells, whereas similarly injected adult bone marrow gives rise only to Ly-1$^-$ B cells (*6*). Our data indicate that severely autoimmune *me*^{v} mice have primarily one of these two major B cell lineages. Even though the mechanism whereby *me*^{v} mice express mainly the Ly-1$^+$ B cell subset is not yet understood, the finding suggests the independent and essentially exclusive development of the Ly-1$^+$ B cell subset in the autoimmune *me*^{v} mutant mouse strain. An alternative possibility, that a large number of Ly-1$^-$ B cells were present at some stage of *me*^{v} life history but have been eliminated, cannot be formally ruled out at this time but is considered unlikely because of the lack of clearly detectable *me*^{v} Ly-1$^-$ B cells in any organ examined, and the striking increase in the absolute number of *me*^{v} Ly-1$^+$ B cells even in organs such as lymph nodes that do not normally contain appreciable numbers of such cells.

Fig. 1. Two-color FACS analysis of cell surface phenotypes from a pair of 6-week-old littermate C57BL/6J-+/− and C57BL/6J-*me*^{v}/*me*^{v} mice. Data are presented as computer-generated probability contours that show the likelihood of finding cells with given intensities of staining for various cell surface antigens. [See (*6*) for details of reagent preparation and staining procedures.] Because of the somewhat greater background staining of *me*^{v} cells with anti-IgM (due, for example, to nonspecific stickiness, or to cytophilic or specifically bound antibody), IgM staining was considered significant when it was greater than 1.5 (linear) units for control cell populations and greater than 3.0 (linear) units for *me*^{v} cells. Ly-1 staining of B cells was considered significant when it was above the level of Ly-2 staining. In all cell populations, two main B cell subsets are indicated as (——) high IgM, low IgD, and Ly-1$^+$ and (– – –) low IgM, high IgD, and Ly-1$^-$.

These experiments identify two parameters that distinguish IgM and IgG3 from the other immunoglobulin isotypes: IgM and IgG3 are found in the highest concentrations in *me*^{v} mice, and in both *me*^{v} and age-matched control mice, these immunoglobulins show unexpectedly high levels of lambda L chain expression. (The pattern of initially higher but later decreasing levels of lambda L chain expression in IgM and IgG3, as compared with other isotypes of serum immunoglobulin, has been investigated and confirmed in the CBA/CaJ strain, in addition to C57BL/6J.) We hypothesize that the IgM and IgG3 classes, and lambda L chains, may preferentially arise from the Ly-1^{+} B cell subset. This suggestion, however, applies only to the immunoglobulins secreted from B cells and not to cell surface immunoglobulins. The representation of different H (*9*) and L (*10*) chains in serum does not necessarily correlate with their expression on the B cell surface. Increased expression of lambda L chains by immature B and pre-B cells has been reported (*11, 12*) but not confirmed (*13–16*). One reason why previous workers (*10, 16*) have not reported higher levels of lambda-containing immunoglobulins may be that they examined total serum immunoglobulin rather than specific isotypes. A second reason may be that the decline in the proportion of lambda in IgM and IgG3 and the increase in the levels of predominantly kappa-containing IgG1, IgG2b, IgG2a, and IgA, both of which occur gradually over several years in our mouse colony, may depend on the level of antigenic stimulation and may occur more rapidly or to a greater extent in other colonies.

Mice of the CBA/N strain, carrying the *xid* gene (*17*), show a pattern opposite to that demonstrated here for *me*^{v} mice. CBA/N mice have essentially no Ly-1^{+} B cells (*5*) and are markedly deficient in background and induced IgM, IgG3, and lambda-containing antibodies (*10, 17–19*).

Ly-1^{+} B cells may have several distinctive roles in the immune system. We have described two lymphokines from *me*^{v} mice that potentiate the maturation of normal and certain tumor B cells to active immunoglobulin secretion (*4, 20*); at least one of these lymphokines is produced by the *me*^{v} Ly-1^{+} B cells themselves (*4*). Others have reported the production of lymphokines (*21*), and the regulation of specific antibody responses (*22, 23*), by Ly-1^{+} B cells. In NZB mice, most of the autoantibody-producing cells are Ly-1^{+} (*24*), whereas in normal mice, some antigens (polysaccharides, for example) induce mainly IgM and IgG3 responses (*19, 25*). These IgM and IgG3 antibody responses are greatly reduced in CBA/N mice (*10, 18, 26–28*) but are among the few responses detectable in *me*^{v} mice (*8*). Background antibody-secreting cells in the lymph nodes of normal mice are almost totally eliminated by antigen deprivation, whereas those in the spleen are essentially unaffected (*29, 30*). It has been suggested that B cells with lambda L chains and those with kappa L chains differ in their triggering requirements for antibody formation (*10, 31–33*) and in their representation in certain classes of tumors (*34–36*). Underlying all of these observations may be the distinctive properties of Ly-1^{+} as opposed to Ly-1^{-} B cells, including a preference for IgM, IgG3, and lambda L chain production, differential triggering requirements, and the release of immunoregulatory lymphokines.

REFERENCES AND NOTES

1. M. C. Green and L. D. Shultz, *J. Hered.* **66**, 250 (1975).
2. L. D. Shultz and M. C. Green, *J. Immunol.* **116**, 936 (1976).
3. L. D. Shultz, D. R. Coman, C. L. Bailey, W. G. Beamer, C. L. Sidman, *Am. J. Pathol.* **116**, 179 (1984).
4. C. L. Sidman, J. D. Marshall, N. C. Masiello, J. B. Roths, L. D. Shultz, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7199 (1984).
5. K. Hayakawa, R. R. Hardy, D. R. Parks, L. A. Herzenberg, *J. Exp. Med.* **157**, 202 (1983).
6. K. Hayakawa, R. R. Hardy, L. A. Herzenberg, L. A. Herzenberg, *ibid.* **161**, 1554 (1985).
7. R. R. Hardy, K. Hayakawa, J. Haaijman, L. A. Herzenberg, *Nature (London)* **297**, 589 (1982).
8. C. L. Sidman *et al.*, in preparation.
9. E. S. Vitetta and J. W. Uhr, *J. Exp. Med.* **134**, 242 (1971).
10. L. C. Burkly, R. Zaugg, H. N. Eisen, H. H. Wortis, *Eur. J. Immunol.* **12**, 1033 (1982).
11. G. Haughton, L. L. Lanier, G. F. Babcock, *Nature (London)* **275**, 154 (1978).
12. S.-I. Nishikawa, T. Kina, J.-I. Gyotoku, Y. J. Katsura, *J. Exp. Med.* **159**, 617 (1984).
13. K. L. McGuire and E. S. Vitetta, *J. Immunol.* **127**, 1670 (1981).
14. S. Kessler, K. J. Kim, I. Scher, *ibid.*, p. 1674.
15. J. M. LeJeune, D. E. Briles, A. R. Lawton, J. F. Kearney, *ibid.* **129**, 673 (1982).
16. T. Takemori and K. Rajewsky, *Eur. J. Immunol.* **11**, 618 (1981).
17. D. F. Amsbaugh *et al.*, *J. Exp. Med.* **136**, 931 (1972).
18. R. M. Perlmutter *et al.*, *ibid.* **149**, 993 (1979).
19. J. Slack, G. P. Der-Balian, M. Nahm, J. M. Davie, *ibid.* **151**, 853 (1980).
20. C. L. Sidman, L. D. Shultz, R. Evans, *J. Immunol.* **135**, 870 (1985).
21. K. H. Brooks, J. W. Uhr, E. S. Vitetta, *ibid.* **133**, 3133 (1984).
22. K. Okomura, K. Hayakawa, T. Tada, *J. Exp. Med.* **156**, 443 (1982).
23. D. H. Sherr and M. E. Dorf, *ibid.* **159**, 1189 (1984).
24. K. Hayakawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2494 (1984).
25. R. M. Perlmutter, D. Hansburg, D. E. Briles, R. A. Nicolotti, J. M. Davie, *J. Immunol.* **121**, 566 (1978).
26. P. L. Cohen, I. Scher, D. E. Mosier, *ibid.* **116**, 301 (1976).
27. J. J. Mond, R. Lieberman, J. K. Inman, D. E. Mosier, W. E. Paul, *J. Exp. Med.* **146**, 1138 (1977).
28. C. Fernandez and G. Moller, *ibid.*, p. 1663.
29. R. Benner, A.-M. Rijnbeek, R. R. Bernabe, C. Martinez-Alonso, A. Coutinho, *Immunobiology* **158**, 225 (1981).
30. R. Benner *et al.*, *Int. Arch. Allergy Appl. Immunol.* **66**, 404 (1981).
31. R. S. Jack, T. Imanishi-Kari, R. Rajewsky, *Eur. J. Immunol.* **8**, 559 (1977).
32. L. C. Burkly, J. Nikolic, H. H. Wortis, *J. Immunol.* **135**, 1573 (1985).
33. L. C. Burkly and H. H. Wortis, *ibid.*, p. 1577.
34. H. Mellstedt, D. Pettersson, G. Holm, *Acta Med. Scand.* **204**, 485 (1978).
35. H. Stein, J. Gerdes, D. Y. Mason, *Clin. Haematol.* **11**, 531 (1982).
36. S. H. Swerdlow *et al.*, *Am. J. Pathol.* **113**, 181 (1983).

37. Supported by NIH grants CA-35845, AI-20232, CA-20408, GM-17367, HD-01287, and CA-04681. We thank W. Murphy for expert technical assistance in these studies. D. Harrison for providing the aged normal mice, and G. Carlson, B. Sanford, P. Laler, and A. Stall for critical reading and comments on the manuscript.

6 December 1985; accepted 28 April 1986