Differential Expression of V_H Gene Families in Peripheral B Cell Repertoires of Newborn or Adult Immunoglobulin H Chain Congenic Mice

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Summary

The pattern of V_H gene family expression in the primary B cell repertoire of the mouse is strain dependent. In C57Bl/6 mice, the V_H J558 family is expressed by more than 45% of the cells, while the expression of V_H 7183, V_H Q52, and V_H 36–60 families together does not exceed 20%. In BALB/c mice, relative expression of V_H J558 is lower than 35%, while the sum of the other three families reaches 25%. To assess which genetic loci control strain-specific V_H gene family expression, we studied V_H gene family usage in splenic B cell repertoires of different congenic strains of mice. Changes in major histocompatibility complex or immunoglobulin (Ig) K light chain genes did not modify V_H gene family expression in adult mice. Differences at the IgH locus, however, modified V_H gene family usage. In 1-d-old mice, the strain-specific V_H gene family expression pattern is determined by the IgH haplotype. In adult mice, the V_H gene family expression pattern of resting B cells is independent of the IgH locus and follows the genetic background of the congenic strain, while it is determined by the IgH haplotype among Ig-secreting spleen cells. In F_1(B6 × BALB/c) mice, each of the two spleen B cell populations, sorted on the basis of μ heavy chain allotype expression, shows an independent V_H gene family expression pattern, determined by the IgH locus. The implications of these results in the control of V_H gene family expression, and in the selection of peripheral B cell repertoires are discussed.

Variable regions of Ig are generated by recombination of multiple V (D) and J genetic segments (1). Combinatorial association of these elements together with junctional diversity originate a potential B cell repertoire with more than 10^9 different Ig combining sites (2). In mice, H chain variable gene segments (V_H)[1] are clustered in subgroups or families of genes sharing more than 80% of nucleotide sequence homology (3). Twelve V_H gene families have been described with a chromosomic organization within the IgH locus which is conserved across species (4). The mouse strains C57Bl/6 and BALB/c carry different IgH haplotypes (5) of roughly the same genetic complexity (3–6), although the actual number of different active genes per V_H gene family, in each mouse strain, remains to be established (3, 7, 8).

V_H gene family usage does not follow genetic complexity, but varies with the mouse strain (9–11), and is influenced by genetic factors unlinked to the IgH locus (12). Within the same inbred strain, V_H gene family expression is genetically controlled during ontogeny (13–17) and lymphopoiesis (18, 19), varies with cellular environment (17), and is influenced by T-B cell interactions (20). Nevertheless, the strain-specific pattern of V_H gene family expression is stable for age-matched male or female individuals.

In a normal (nonimmunized) mouse, peripheral B cell repertoires are continuously renewed and selected (21). In fact, the number of newly formed B cells produced every day is estimated to be 2–5 × 10^7 cells (22, 23). A part of these cells will integrate the peripheral B cell pool (available repertoire), which consists of $<10^8$ different clones of resting immunocompetent B cells (24). Moreover, only a fraction (1% or less) of all peripheral B cells are naturally Ig-secreting cells (actual repertoire) (25). Hence, the stable expression of V_H gene families in the context of this high turnover of B cells must result from the continuous selection of peripheral B cells leading to the strain-specific V_H gene family usage.

In this study, we investigated which genetic loci could control the strain-specific V_H gene family expression. We quantified V_H gene family expression, by in situ hybridization, both in neonatal and in adult peripheral B cell repertoires of different strains of mice congenic for the MHC, IgK L- and H-chain loci. We observed that changes in MHC or IgK L chain loci did not modify V_H gene family expression of peripheral mature B cells. In IgH congenic mice, however, the V_H gene family usage among splenic resting B cells (available repertoire) changes from 1-d-old newborn, where it is determined by the IgH locus, to adult individuals, where it follows the genetic background of the congenic strain. We also found that V_H gene family expression among splenic Ig-

[1] *Abbreviations used in this paper:* V_H, H chain variable gene segments.

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secreting cells (actual repertoire) of adult IgH congenic mice is determined by the IgH locus, and thus differs from the adult available repertoire. Finally, studies in F_1(C57Bl/6 × BALB/c) mice, sorting adult splenocytes on the basis of their IgM allotype expression, showed that the V_H gene family usage by each allotype sorted population is determined by the IgH locus.

Materials and Methods

Animals. Inbred strains of mice, raised in our colony at the Pasteur Institute, were used at either 1 d after birth or at 2–3 mo of age. The following strains of mice were studied: C57Bl/6 (IgH^b, H-2^b), BALB/c (IgH^a, H-2^d), F_1(C57Bl/6 × BALB/c), B6Igh^a (IgH^a, H-2^b), CB20 (IgH^b, H-2^d), BALB.B10 (IgH^a, H-2^b), BALB.C3H (IgH^a, H-2^k), and AKR (H-2^k). The IgK L chain congenic strains used were: C.AKR and C.C58, bearing the K L-chain locus of AKR and C.58, respectively, and in the BALB/c genetic background (26). For each experiment, pools of 2–3 age-matched animals were used.

Cells. Spleen cell suspensions from adult or newborn mice were prepared as described (18) and used for in situ hybridization, either ex vivo, to assay for Ig-secreting spleen cells, or after in vitro stimulation with LPS from *Salmonella typhimurium* at 25 μg/ml) at 10^6/ml. It has been previously shown that LPS activation does not bias V_H gene family expression (17). LPS-activated cells were harvested on day 3 of culture, cytospun, and fixed onto slides as described in detail elsewhere (17). Sorted F_1 spleen cells, when stimulated with LPS, were cultured for 4–5 d at 0.2 × 10^6/ml with 3 × 10^6/ml irradiated (100 rad) rat thymocytes. The V_H gene family expression did not vary with the different protocols used for cell culture (not shown). Limiting dilution analysis of LPS-reactive cells was performed as described previously (27). The frequency of LPS-reactive B cells bearing each μ allotype was determined in sorted F_1 B cells for quantitating the degree of purity of the sorted cell populations.

Flow Cytometry and Cell Sorting. Cells were stained with FITC-RS3.1 (anti-μ^a, reference 28) and biotin-labeled MB86 (anti-μ^b, reference 29) mAbs, followed by streptavidin-PE from Becton Dickinson & Co. (Grenoble, France). Dead cells were excluded from analysis by light-scatter gatings. All analyses were performed on a FACStar Plus® cell sorter (Becton Dickinson & Co., Sunnyvale, CA) interfaced to a Hewlett-Packard (Palo Alto, CA) computer. Cells were positively sorted for both IgM allotypes. The purity of each sample was controlled by limiting dilution analysis, and contamination never exceeded 4%.

Probes. The Cμ and V_H gene probes were kind gifts of Drs. F. Alt, S. Riley, J. Kearney, U. Krawinkel, L. A. Reininger, M. Sims, and R. Kofler. The V_H gene family probes used (with the family name in parenthesis) were 81-X (V_H 7183), 300-19 (V_H Q52), S107 (V_H S107), X24 (V_H X24), P6.3RI (V_H 36–60), VNPB4 (V_H J558), PBV14 (V_H J606), MS1 (VGAM3.8), V31 (V31/V_H 36-09), CP12 (V_H 11), and have been previously described (17). The VMS1 (VGAM3.8) probe is a 830-bp HaeIII fragment 98% homologous to VGAM3.8 (30), and the V_H10 (V_H 10) probe is a 483-bp PstI fragment (31).

In Situ Hybridization. In situ hybridization was performed using a modification of the method described by Haase et al. (32), as detailed by Freitas et al. (17). cDNA probes were labeled by the random priming method (33) with ^{35}S-dCTP (500 Ci/mmol, Amersham International, Amersham, Bucks, UK) and specific hybridization of probes with intracytoplasmic mRNA was revealed by autoradiography in a Kodak emulsion NTB2 (Eastman Kodak, Rochester, NY). Positive cells were covered by silver grains and analyzed by light microscopy.

Specificity of the V_H Gene Family Probes. The family specificity of the V_H gene family probes and experimental conditions were established by hybridization to cell lines expressing known V_H of the different families (17). It must be noted that although cross-hybridization cannot be excluded between at least some members of the V_H X24 and 7183 families, or the V_H J558 and VGAM3.8 families, the lack of correlation in the expression of these families shows that the respective probes identify distinct cell populations (17, 20, 34). The absolute numbers of positive cells with all V_H gene family probes correlated well with the numbers of cells positive with the Cμ probe, representing between 70% (in LPS-activated cells) and 100–120% (in non-LPS–stimulated spleen cells) of the latter, but showing that cells expressing isotypes other than μ are detected. Hence, V_H gene family usage in the different cell populations studies was expressed as a relative distribution, calculated by the percentage of positive cells for each V_H gene family out of the total number of positive cells detected with all V_H gene family probes.

Detection Limits of in situ Hybridization. All Cμ and V_H positive cells had the morphology of blasts or plasma cells, and the number of Cμ positive cells correlated well (r = 0.83–0.92) with the number of IgM-secreting cells, as determined by the protein A-plaque forming cell assay (17). It follows that our conditions of in situ hybridization exclusively score activated cells, with levels of mRNA severalfold higher than those of resting B cells. We could, therefore, discriminate V_H gene family usage in the actual repertoire (by directly assaying Ig-secreting cells ex vivo) versus the available repertoire (by prior in vitro activation of resting B cells with a polyclonal mitogen).

Evaluation of the Number of Positive Cells. The number of V_H positive cells were scored by counting between 30 and 100 microscopic fields (a total of 2,000–30,000 cells scored), as well as the mean number of total cells per field, from which the frequency of positive cells was calculated. The frequency of positive cells in splenic cell suspensions directly assayed ex vivo was 0.1–0.5%, while after LPS stimulation this frequency increased to 20–30% of total cells. A total of at least 50–200 (ex vivo) or 500–2,000 (after LPS stimulation) V_H positive cells were counted. A cell was scored as positive if it contained 20 or more silver grains in a background with a mean grain number per cell <5. In most instances, positive cells were heavily labeled with an uncountable number of grains.

Statistical Analysis of the Results. Statistical analysis of the differences between cell populations were done using a one way ANOVA computer program (Unité d'Informatique Scientifique, Institut Pasteur, Paris, France).

Results

Peripheral B Cell Repertoires in C57Bl/6 and BALB/c Mice. V_H gene family usage by LPS-activated splenocytes of adult C57Bl/6 and BALB/c mice is shown in Fig. 1. In BALB/c, the relative representation of the V_H J558 family varies between 25 and 35%, and it never exceeds 35%. The V_H 7183 and Q52 families are each expressed by about 10% of the splenocytes, while the V_H 36–60 family is always used by 5% or more of the cells. In C57Bl/6, the V_H J558 family is expressed by 45% or more of the splenic B cells. The V_H 7183 and Q52 families are each used by about 7%, and the V_H 36–60 family by <5% of the cells. A similar strain-

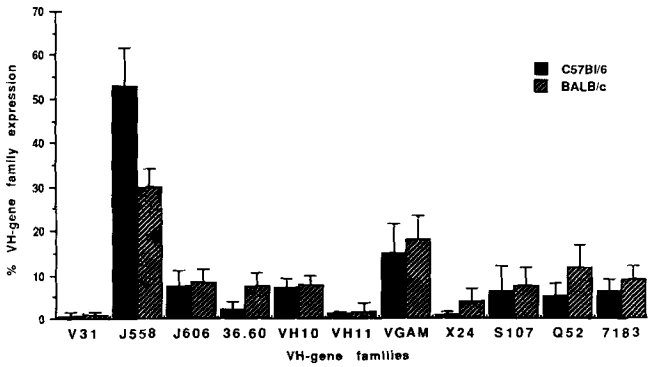

Figure 1. V_H gene family usage among LPS-activated splenocytes (available repertoire) of adult C57Bl/6 and BALB/c mice. Results are expressed as the relative representation of each V_H gene family among 11 families studied and represent the mean of 12 (C57Bl/6) or 26 (BALB/c) experiments. (*Bars*) SD. Ig-secreting spleen cells assayed ex vivo give similar strain-specific V_H patterns (not shown).

Table 1. *Relative Expression of the V_H J558 Family by LPS-activated Splenic B Cells (available) from Newborn and Adult and Among Ig-secreting Spleen Cells (actual) from Adult IgH Congenic Mice.*

	Newborn	Adult	
	Available	Available	Actual
BALB/c	23.7	29.9	31.4
	(4.6)	(2)	(2.3)
B6Igh^a	32.3	47.2*	32.6
	(6)	(2.1)	(1.5)
C57Bl/6	53	52.8	63.8
	(2.6)	(2.5)	(2.8)
CB20	59.7	35.3‡	54.9
	(7.1)	(3.5)	(6.3)

Adult or 1-d-old C57Bl/6, BALB/c, B6Igh^a, and CB20 spleen cells were analyzed by in situ hybridization with 11 V_H gene family probes. The relative representation of the V_H J558 family is shown. Adult spleen cells were either tested ex vivo (actual repertoire) or after LPS stimulation in vitro (available repertoire). Results shown represent the mean (SD) of five (CB20) or nine (B6Igh^a) different experiments and significant differences are given by one factor ANOVA treatment. The relative V_H J558 family expression in the available repertoire of adult B6Igh^a differs significantly from BALB/c, but not from C57Bl/6 while, in the available repertoire of the newborn and in the adult actual repertoire, it is similar to BALB/c, and significantly different from C57Bl/6. Reciprocally, the relative expression of V_H J558 family in the available repertoire of adult CB20 differs significantly from C57Bl/6, but not from BALB/c while, in the available repertoire of the newborn and in the adult actual repertoire, it is similar to C57Bl/6, but significantly different to BALB/c.
* $p < 0.01$.
‡ $p < 0.05$.

specific pattern of V_H gene family expression is kept among the Ig-secreting spleen cells in both strains of mice (17). These findings, in particular the difference in the representation of the V_H J558 family between C57Bl/6 and BALB/c mice, confirm previous reports where V_H gene family expression was studied by either in situ hybridization (10, 17), RNA colony blot assay (9), cDNA phage libraries (11), or Northern blotting (15).

To investigate the genetic factors controlling V_H gene family expression patterns, we analyzed several independent congenic strains of mice. To simplify our presentation, we shall restrict our analysis mainly to the V_H J558 family, and refer to a C57Bl/6 or a BALB/c pattern of V_H gene family usage, respectively, when >45 or <35% of the splenic B cells express the V_H J558 family. In all experiments shown, however, the other ten V_H gene families varied in agreement with the changes observed for the V_H J558 family, after the strain-specific patterns described above.

Available Repertoire of LPS-activated Splenocytes in Adult Congenic Mice. The B6Igh^a and CB20 mouse strains are reciprocal congenics with C57Bl/6 and BALB/c strains for the IgH haplotype. As shown in Table 1, LPS-activated splenocytes of adult B6Igh^a mice show a relative expression of the VH J558 family that differs significantly from BALB/c ($p < 0.01$), but not from C57Bl/6. Reciprocally, LPS-activated splenocytes from CB20 show a relative expression of VH J558 family that is significantly different from C57Bl/6 ($p < 0.05$), but not from BALB/c. The V_H gene family expression pattern in LPS-activated splenocytes (available repertoire) from adult IgH congenic mice segregates, therefore, independently of the IgH locus, and seems determined by the genetic background of the congenic strain.

In an attempt to identify the background genes that could play a role in the regulation of VH gene family expression, we have next investigated MHC and IgK L chain loci. We found that the representation of V_H gene families among LPS-activated splenocytes of MHC congenic BALB.B10 and BALB.C3H strains, as well as in the IgK L chain congenic C.C58 and C.AKR strains, is identical to that observed in the BALB/c strain (Table 2). Similarly, V_H gene family usage by Ig-secreting spleen cells did not differ in these congenic strains of mice. These results show that the strain-specific pattern of V_H gene family expression is kept regardless of MHC and IgK L chain haplotypes.

Available Repertoire of LPS-activated Splenocytes in Newborn IgH Congenic Mice. In contrast to the adult, LPS-activated splenocytes in newborn B6Igh^a and CB20 congenic mice show a pattern of V_H gene family expression identical to BALB/c and C57Bl/6, respectively (Table 1). The usage of the V_H J558 family among LPS-activated splenocytes of newborn B6Igh^a differs significantly from that of the newborn C57Bl/6 ($p < 0.01$), while it is similar to that of the newborn BALB/c. Reciprocally, the V_H J558 family usage in spleen of newborn CB20 is significantly different from that of the newborn BALB/c ($p < 0.01$), but is similar to that of the newborn C578Bl/6. In newborn IgH congenic mice, therefore, the pattern of V_H gene family expression among

Table 2. *Relative Expression of the V_H J558 Family by LPS-activated Splenic B Cells from Adult MHC and IgK L chain Congenic Mice*

BALB/c (H-2d)	C57Bl/6 (H-2b)	AKR (H-2k)	BALB.B10 (H-2b)	BALB.C3H (H-2k)	C.AKR	C.C58
29.9	52.8*	44.7*	33	36.5	31.2	26.8
(0.8)	(2.5)	(4.2)	(4)	(2)	(2.7)	(2)

Spleen cells from adult BALB.B10 or BALB.C3H MHC congenic mice, C.AKR or C.C58 IgK L-chain congenic mice, or BALB/c, AKR, and C57Bl/6 mice were used, either ex vivo or after in vitro LPS stimulation, to determine the relative expression of 11 V_H gene families by in situ hybridization. The relative expression of the V_H J558 family among LPS-activated spleen cells is shown. Results represent the mean (SD) of two (IgK L chain congenic mice) or three (MHC congenic mice) different experiments, and statistically different values are given by one factor ANOVA treatment. The relative representation of the V_H J558 family by LPS-activated splenocytes of the different congenic mouse strains differs significantly from both C57Bl/6 and AKR, but is identical to BALB/c. Similar patterns were observed among Ig-secreting spleen cells (not shown).
* $p < 0.01$.

LPS-activated splenocytes is determined by the IgH locus. The shift in the pattern of V_H gene family usage in the splenic available repertoire from newborn to adult IgH congenic mice suggests that V region selection operates in the establishment of the adult antibody repertoire (14–17).

Actual Repertoire of Adult Splenic Plasmocytes in IgH Congenic Mice. Analysis of spleen cells ex vivo allows assessment of V_H gene family expression among Ig-secreting splenocytes. Plasma cells in adult B6Igh^a and CB20 mouse strains show a pattern of V_H gene family expression determined by the IgH locus. Thus, the expression of the V_H J558 family in B6Igh^a Ig-secreting splenocytes is significantly different from C57Bl/6 ($p < 0.01$), but not from BALB/c plasma cells (Table I). Reciprocally, expression of the V_H J558 among splenic plasma cells of CB20 mice is similar to C57Bl/6, but differs significantly from BALB/c ($p < 0.01$). These results show that the V_H gene family expression pattern among adult Ig-secreting splenocytes is determined by the IgH locus.

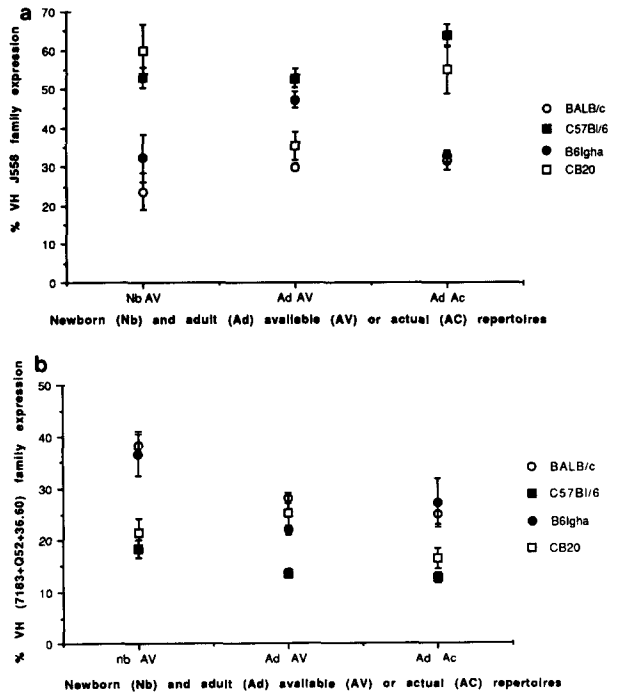

Figure 2. Relative expression of the V_H J558 family (*a*) and the sum of V_H 7183, Q52, and 36–60 families (*b*) by LPS-activated newborn and adult splenic B cells (available) and among adult Ig-secreting spleen cells (actual), from IgH congenic mice. The color of the symbols shows the genetic background of the strain: white for BALB/c, and black for C57Bl/6. The shape of the symbols shows the IgH haplotype: circles for IgH^a, and squares for IgH^b. The B6Igh^a mouse strain (*black circles*) shows a BALB/c pattern for V_H gene family expression in the newborn available and adult actual repertoires and a C57Bl/6 pattern in the adult available repertoire.

The V_H gene family expression patterns in newborn and adult mice of the congenic strains are summarized in Fig. 2. Splenic available repertoires of newborn and adult IgH congenic mice display different patterns of V_H gene family expression. Available and actual repertoires of adult IgH congenics differ, while newborn available and adult actual repertoires have similar patterns of V_H gene family expression.

Available and Actual Repertoires in Newborn or Adult F_1 Spleen Cells. We investigated the relative role of the IgH locus and of the genetic background on the pattern of V_H gene family expression in F_1(C57Bl/6 × BALB/c) mice. In these mice, both C57Bl/6 and BALB/c genetic backgrounds are present, but two subpopulations of B lymphocytes are available, each expressing V_H genes of either the BALB/c or the C57Bl/6 IgH haplotype. We determined the V_H gene family usage, either ex vivo or after LPS stimulation, in FACS®-isolated IgM^a or IgM^b splenic B cells from adult F_1 mice. FACS® profiles of F_1-unseparated spleen cells and allotype-sorted populations are shown in Fig. 3. The relative expression of the V_H J558 family expression by sorted and unseparated B cells from F_1 mice is shown in Table 3. The V_H J558 usage by μ^b cells differs significantly from μ^a ($p < 0.01$) and BALB/c ($p < 0.01$), but not from C57Bl/6 spleen cells. V_H J558 usage among μ^a cells differs significantly from μ^b ($p < 0.01$) and C57Bl/6 ($p < 0.01$), but not from BALB/c splenocytes. Thus, each allotype population of F_1 mice follows an independent pattern of V_H gene family expression, both in resting or Ig-secreting cells. These results show that the B cell repertoires of μ^a and μ^b adult spleen cells in F_1 mice are independent from one another.

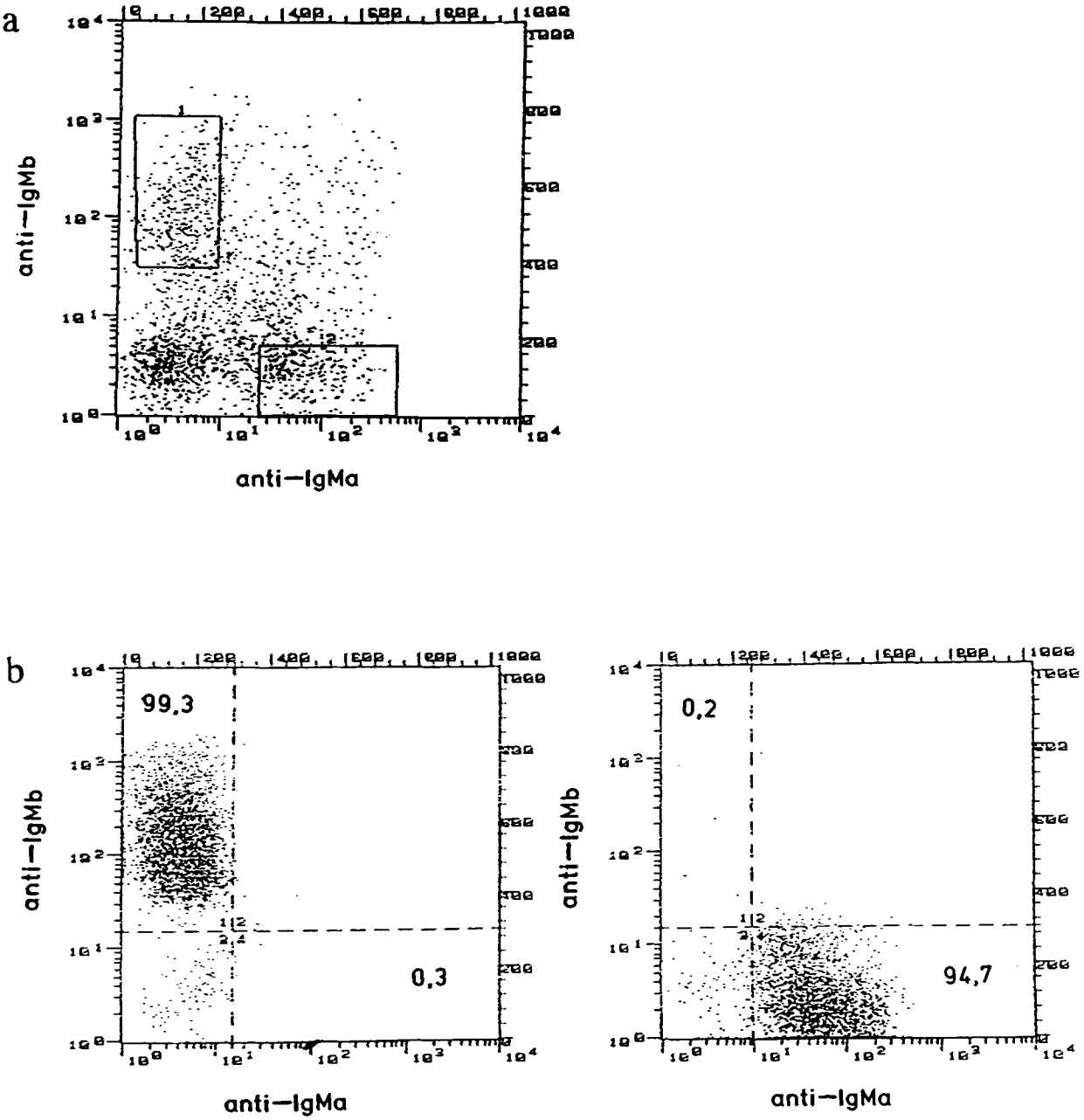

Figure 3. Purification by cytofluorometry of IgM^a and IgM^b cells from adult F_1(C57Bl/6 × BALB/c) splenocytes. Adult spleen cells were labeled with anti-IgM^a and anti-IgM^b antibodies (see Materials and Methods), and sorted by gating each population as shown (*a*). Cytofluorometric reanalysis of the sorted cell populations gave the profiles shown (*b* and *c*). The purity of each sample, given by the percentage of the contaminant allotype, was controlled by limiting dilution analysis. For this experiment, in the IgM^a sorted population, the frequencies of LPS-reactive cells of the a and b allotypes were, respectively, 1:9 and 1:383 i.e., 2.6% contaminating IgM^b cells. In the IgM^b sorted population, the frequencies of LPSr cells of the a and b allotypes were, respectively, 1:875 and 1:10 i.e., 1% contaminating IgM^a cells.

Discussion

The pattern of V_H gene family usage in peripheral B cell repertoires of normal C57Bl/6 and BALB/c mice is strain specific. We have now studied peripheral B cell repertoires of newborn and adult mice from the respective IgH congenic strains, $B6Igh^a$ and CB20. We show here that the pattern of V_H gene family usage in LPS-activated splenocytes (available repertoire) segregates with the IgH locus in newborns, but is determined by the genetic background in adult mice. These results partially confirm a previous report by Wu and Paige (12), studying V_H gene family expression in LPS-induced B cell colonies from spleen cells of adult IgH congenic mouse. In these experiments, V_H gene family usage

Table 3. *Relative Expression of the V_H 558 Family by LPS-activated B Cells (available) from Newborn and Adult and by Ig-secreting Cells (actual) from Adult F_1(C57Bl/6 × BALB/c) Unseparated Spleen Cells or Adult IgM^a and IgM^b Sorted Spleen Cells.*

	Newborn	Adult	
	Available	Available	Actual
BALB/c	23.7	29.9	31.4
	(4.6)	(0.8)	(2.3)
F_1 μ^a sorted		31*	37.5 *
		(2.2)	(0.5)
F_1	48.8	35.1	43.3
	(3.4)	(2.2)	(2)
F_1 μ^b sorted		64.7*	66.6 *
		(3.9)	(0)
C57Bl/6	53	52.8	63.8
	(2.6)	(2.5)	(2.8)

V_H gene family expression by LPS-activated and Ig-secreting cells was determined among either unseparated spleen cells from newborn and adult F_1(C57Bl/6 × BALB/c) mice, or among sorted IgM^a and IgM^b spleen cells from adult F_1 mice. The relative representation of 11 V_H gene families was determined by in situ hybridization, and the relative expression of the V_H J558 family is shown. Values shown represent the mean (SD) of three (sorted cells) or five (unseparated cells) different experiments. The purity of each sorted sample was controlled by FACS® reanalysis and limiting dilution analysis, and never exceeded 4% contamination. The relative V_H J558 family expression by μ^a cells is similar to BALB/c, while significantly different from μ^b and C57Bl/6, both in LPS-activated and Ig-secreting cells. In μ^b cells, the relative V_H J558 family expression, while similar to C57Bl/6, differs significantly from μ^a and BALB/c, both in LPS-activated and Ig-secreting spleen cells.
* $p < 0.01$.

was also determined by genes of the genetic background, and it was proposed that a locus, outside of the V_H region, regulates the strain-specific V_H gene family expression. We have now found that, in newborn IgH congenic mice, the strain-dependent V_H gene family expression is not regulated by a locus outside of the V_H region, but rather determined by the IgH locus itself. We conclude, from the shift of V_H gene family expression pattern in the available repertoire between newborn and adult IgH congenic mice, that the strain-specific V_H gene family usage either is regulated by a locus outside of the IgH locus whose expression is developmentally controlled, or results from cellular selection of the available repertoire during the development and establishment of the adult antibody repertoire. Since V_H gene family usage is not modified in MHC congenic mice, such cellular selection of the adult available repertoire would involve products of the genetic background other than MHC antigens.

The strain-dependent V_H gene family usage could also reflect a different V_H gene family genetic complexity in C57Bl/6 and BALB/c strains, or in the respective IgH congenic strains. Although unlikely (34), the actual number of functional genes could differ among the IgH locus donor and its respective IgH congenic strain. The finding that, within the same IgH congenic strain, splenic B cells expressing the same IgH haplotype display different pattern of V_H gene family expression, depending upon the developmental stage of the mice, demonstrates that the V_H gene family usage does not only rely on genetic complexity.

In adult IgH congenic mice, while the pattern of V_H gene family expression in the available repertoire is determined by the genetic background of the congenic strain, it segregates with the IgH locus among Ig-secreting cells. The different patterns of V_H gene family expression observed between the available and actual repertoires of adult IgH congenic mice suggest that V_H gene family usage among natural splenic plasmocytes of a normal, nonimmunized mouse is not a random representation of the V_H gene families expressed by resting splenic B cells, and results from the activation and terminal differentiation of a selected population of B cells. Selection of the actual antibody repertoire was previously reported in Ig-transgenic mice (35), where it was found that the fraction of B cells expressing endogenous V_H gene families, which did not exceed 5% of the resting splenic B cells, represented over 50% of the Ig-secreting splenocytes.

The present results indicate that, in a nonimmunized mouse, available and actual B cell repertoires are generated through different mechanisms of cellular selection, acting, respectively, on the rate of accumulation and turnover of newly-formed B cells in the peripheral compartments of the immune system, and on the activation and terminal differentiation of immunocompetent B cells. It is interesting to note that V_H gene family usage by both neonatal resting and adult Ig-secreting B cells is determined by the IgH locus. This could suggest that selection for Ig secretion is stably kept from the newborn to the adult mice. We have previously shown, however, that the V_H gene family usage differs in neonatal and adult BALB/c mice, and that these changes are determined by exposure to external antigens, since they are not observed in mice kept under axenic conditions (36). On the other hand, resting nonactivated B lymphocytes have been shown to accumulate during the first 3 wk of age, in the spleen of normal mice (37). Our findings suggest that resting splenocytes would selectively accumulate according to their Ig variable regions through cellular interactions with their environment. The different environmental influences imposed by the C57Bl/6 or BALB/c genetic backgrounds would dictate the differential selection of the available repertoires, in the adult IgH congenic mice, leading to the strain-specific V_H gene family usage. Alternatively, the differences of V_H gene family expression between available and actual repertoires of adult IgH congenic mice could result from different cellular origins of resting and Ig-secreting B cells (38).

We also investigated the relative role of the IgH locus and the genetic background on the pattern of V_H gene family usage in adult F_1(C57Bl/6 × BALB/c) mice. The question

was how, in F_1 mice, the presence of both genetic backgrounds would determine the pattern of V_H gene family usage in each of the two B cell subpopulations expressing C57Bl/6 or BALB/c V_H genes. We found that the pattern of V_H gene family expression in each allotype population differs from unseparated B cells and is determined by the IgH locus, both in resting and Ig-secreting spleen cells. This finding suggests that the strain-specific V_H gene family expression of resting splenocytes, if regulated by genes located outside of the IgH locus, must be under control of a factor acting in *cis*. Such intracellular *cis* regulation of V_H gene family usage could result from differential recombinase activity in C57Bl/6 and BALB/c mice (39). Control of Ig gene rearrangements, however, cannot be the only mechanism involved in regulation of V_H gene family usage, since, in IgH congenic mice, resting B cells and Ig-secreting B cells, although expressing the same IgH haplotype, show different patterns of V_H gene family expression. Alternatively, in adult F_1 mice, each allotype population could be preferentially selected by products of background genes from the parental origin of the IgM allotype expressed. The finding that the V_H gene family usage by μ^a and μ^b cells in the F_1 are independent suggests that V-region interactions of a functional immune network (35) would not operate in the regulation of the V_H gene family expression, unless these interactions distinguish between C57Bl/6 and BALB/c Ig variable regions. Kearney and colleagues (personal communication) have recently found that the frequency of V region interactions among neonatal IgM-secreting hybridomas is higher within the same mouse strain.

We conclude that different selective processes act on resting or naturally activated B cells of normal mice. The selection of the available repertoire in IgH congenic mice is responsible for the strain-specific V_H gene family usage. This selection occurs during ontogeny and involves background genes of the congenic strain, other than MHC. It is not clear, however, how this selection could differentiate between both alleles in F_1 mice.

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