Recurrent Identical Rearrangement and Repeated Expression of Identical Heavy and Light Chains in Single Anti-phosphatidylcholine B Cells[a]

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V(D)J recombination confers a vast potential for expressing a great number of different immunoglobulin (Ig) molecules (10^{18} possible combinations[1]) and hence for generating a population of B cells producing a highly diverse set of antibodies. Limited heavy-chain (IgH) and light-chain (IgL) gene usage, however, is common in immune responses to certain antigens, such as $\alpha(1\text{->}3)$ dextran, phenyloxazolone, phosphorylcholine, and phosphatidylcholine (PtC), the antigen studied here. Anti-PtC antibodies, produced exclusively by cells of the B-1 lineage,[2] lyse mouse erythrocytes treated with bromelain to expose PtC. Previous studies have shown that the IgH and IgL of anti-PtC antibodies produced by independently isolated hybridomas and neoplasms tend to be encoded by V_H11-Vk9 or V_H12-Vk4.

Collectively, these earlier studies raised the question of whether identical anti-PtC IgH rearrangements are derived from a common progenitor or from multiple progenitors that develop into a limited PtC-repertoire. Single PtC-binding cells, detectable by staining with PtC-liposomes, were sorted from four C57BL/6J mice (C57), two separate pools of peritoneal cells from BALB/c mice, and two C.B-17 mice (FIGURE 1). The PtC-liposome$^+$ IgM$^+$ phenotype of the sorted cells was recorded for each cell.

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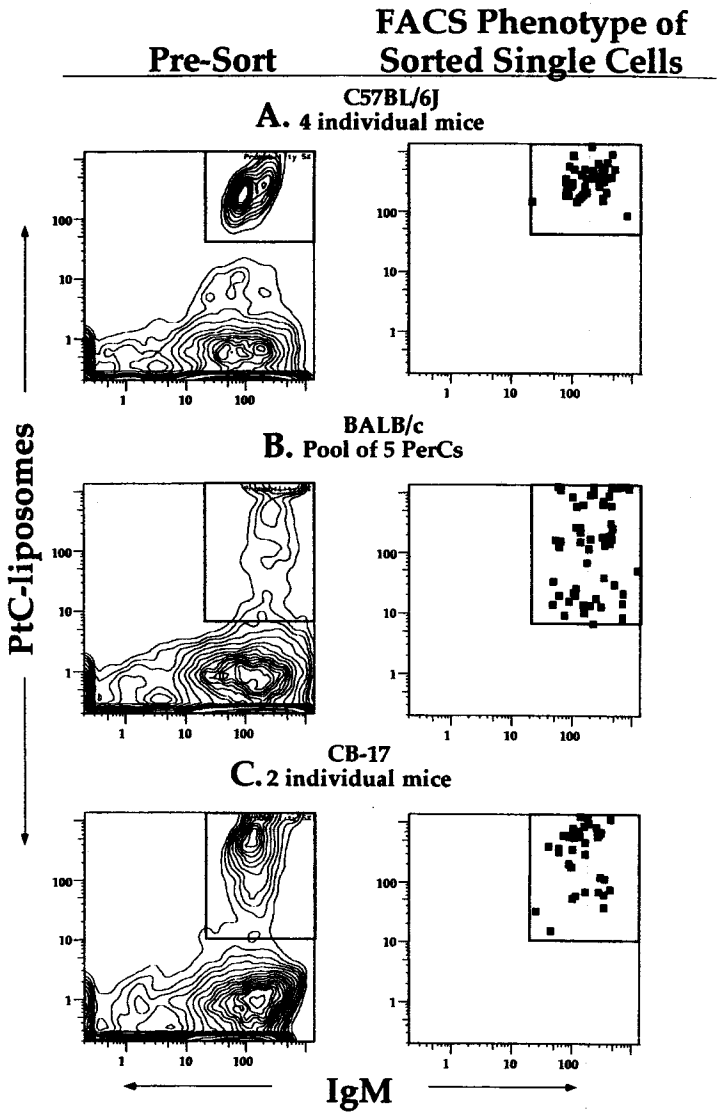

FIGURE 1. Single cell sorting of PtC-binding B-1 cells. PtC-binding cells were identified by FACS using fluorescein encapsulated in PtC-liposomes and surface IgM staining. PtC-binding cells were sorted from four C57 mice (A), a pool of peritoneal cells from nine BALB/c mice (Expt 1), a pool of peritoneal cells from five BALB/c mice (B) (Expt 2), and two C.B-17 mice (C). Gates used for sorting are indicated. The typical FACS profile for a mouse from each strain is shown. PtC-liposome-binding cells were first bulk sorted, based on size, viability, and expression of PtC-liposomes and IgM. Single cells were sorted into lysis solution and snap-frozen on dry ice for future analysis. Each square represents a single sorted cell.

We sequenced the expressed Ig from individual B cells using an unbiased FACS-based PCR method.[3,4]

Among the 47 C57 individual cells analyzed, 33 out of 38 V_H11 rearrangements were identical (87%; V_H11 type 1, see TABLE 1). PtC-binding cells from BALB/c mice

TABLE 1. Recurrent Heavy-Chain Rearrangements and Their Light-Chain Pairings[a]

Strain	Heavy-Chain Rearrangement Type[b]	Mouse (Ms) #/ Expt #	Percent of Total[c]	Light Chain	Number of Cells[d]
C57BL/6J	V_H11 type 1	Ms 1	60 (9/15)	Vκ9 Jκ2	2
				Vκ9 Jκ4	2
				Vκ21E Jκ2	1
		Ms 2	75 (9/12)	Vκ9 Jκ2	5
				Vκ9 Jκ4	3
		Ms 3	90 (9/10)	Vκ9 Jκ2	3
				Vκ9 Jκ4	3
		Ms 4	60 (6/10)	Vκ9 Jκ2	3
				Vκ9 Jκ4	3
BALB/c	V_H11 type 1	Expt 1	5 (2/43)	Vκ9 Jκ4	1
		Expt 2	9 (5/58)	Vκ9 Jκ2	1
				Vκ9 Jκ4	4
C57BL/6J	V_H11 type 3	Ms 4	10 (1/10)	Vκ9 Jκ1	1
BALB/c		Expt 1	5 (2/43)	Vκ9 Jκ1/Vκ2 Jκ1	2
C.B-17	V_H11 type 4	Ms 1	13 (2/15)	Vκ9 Jκ2	1
				Vκ9 Jκ4	1
C57BL/6J	V_H12 type 1	Ms 1	13 (2/15)	NA[e]	2
BALB/c		Expt 2	3 (2/58)	Vκ4/5 Jκ2	2
BALB/c	Q52 type 1	Expt 2	3 (2/58)	Vκ4/5 Jκ5[f]	1
				Vκ20 Jκ4	1
C57BL/6J	V_H11 type 2	Ms 4	20 (2/10)	Vκ9 Jκ4	2
C.B-17	V_H11 type 5	Ms 1	11 (2/18)	Vκ9 Jκ1	2
BALB/c	V_H12 type 2	Expt 2	3 (2/58)	Vκ4/5 Jκ5/NA	2
BALB/c	Q52 type 2	Expt 1	5 (2/43)	NA	2
C.B-17	Q52 type 3	Ms 2	11 (2/18)	Vκ4/5 Jκ2[f]/NA	2

[a]A more complete data set can be found in reference 18.

[b]Rearrangement type is defined by identity of V, D, J segments and N/P additions within a heavy-chain family.

[c]Numbers in parentheses are the number of cells expressing that rearrangement type/total cells analyzed in that mouse or pool of mice.

[d]Light-chain analysis is not shown for all V_H11 type 1–expressing cells.

[e]NA = not available.

[f]Different germline gene than other Vκ4/5 rearrangements.

also expressed this rearrangement, albeit much more rarely (7/101). Furthermore, although we failed to isolate V_H11 type 1 from C.B-17, others have found it within unseparated populations of B cells from C.B-17[5] and other mouse strains.[6–10]

IgL sequences were obtained for the V_H11 type 1–expressing cells isolated from the four C57 mice and the two pools of cells from BALB/c mice (see TABLE 1). Many of the cells analyzed expressed the same IgH/IgL pair. Nevertheless, unique instances of IgH/IgL pairing in each of the individual mice show that at least 2 to 3 of the V_H11 type 1–expressing cells either have arisen from different B cell progenitors prior to IgH rearrangement or have arisen from pre-B cells that have expanded after IgH rearrangement, but prior to IgL rearrangement.[11] However, there is some question whether B-1 pre-B cell populations expand like conventional pre-B cells given the differences in MHC class II expression between B-1 and conventional pre-B cells.[12,13] In total, at least 12 IgH-IgL pairs (39%) of the 31 V_H11 type 1 cells studied for IgL expression in our data set are not due to clonal expansion of mature B cells by the described criteria (TABLE 1).

In addition to V_H11 type 1, we isolated V_H11, V_H12, and V_HQ52 IgH rearrangements, which each occurred at least twice in our data set. Consideration of the animal origin and IgL expression of cells with these additional recurrent rearrangements brings the total number of identical rearrangement events observed in this study not due to mature B cell clonal expansion to 21.

Many of the V_H11 and V_H12 IgH described here exhibit sequence identity at the coding junctions; V_H11 type 1 shows homology at both coding joints. If sequence homology at the coding joints reflects a constraint of rearrangement outcome,[14–16] this double homology could explain the high proportion of V_H11 type 1 rearrangements. Such conserved sequences could also reflect antigen selection.

In summary, cells expressing recurrent IgH rearrangements with identical variable-region sequences isolated from separate animals must have rearranged independently. Cells expressing identical IgH rearrangements, but different IgL rearrangements, are derived from different pro-B or pre-B progenitors; that is, they cannot be explained solely by clonal expansion of mature B cells. These findings expand on initial findings of dominant idiotype expression.[17] Thus, by single cell analysis, we show evidence for a mechanism that generates recurrent identical IgH and IgL rearrangements.

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