Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 208–212, January 1997
Immunology

Memory B lymphocytes migrate to bone marrow in humans

EUSTACHE PARAMITHIOTIS AND MAX D. COOPER*

Departments of Medicine, Pediatrics, and Microbiology, Division of Clinical and Developmental Immunology, and the Howard Hughes Medical Institute, University of Alabama, Birmingham, AL 35294-3300

Contributed by Max D. Cooper, October 30, 1996

ABSTRACT IgM-bearing B lymphocytes with mature phenotype (CD10$^-$ CD24^{lo} IgD$^+$) are acquired after birth in the bone marrow of humans. These B cells are defined here as relatively large, nondividing lymphocytes, variable proportions of which express cell surface molecules indicative of relatively recent activation. Analysis of V_H5_2 (heavy chain variable region) gene transcripts indicated point mutations throughout the Ig variable region from the mature IgM$^+$ B population but not from the immature B cells in the bone marrow. The mutations were concentrated in the complementarity determining regions, and amino acid substitutions were favored over silent mutations, findings indicative of antigen selection within germinal centers in peripheral lymphoid tissues. The V_H sequence analysis also revealed the existence of clonal relatives in individual bone marrow samples. These antigen-experienced lymphocytes did not secrete Ig spontaneously but could be induced to do so *in vitro*. The data suggest that a subpopulation of memory B lymphocytes generated during antigen responses recirculates to the bone marrow in humans.

B cells are generated in the bone marrow after a transient phase of production in fetal liver and, to a lesser extent, in other embryonic tissues (1–5). B lymphopoiesis is a lifelong process in the bone marrow in humans (5) although studies in mice suggest a dramatic age-related decay of this differentiation pathway (6). Newly formed B cells in the bone marrow are sensitive to antigen receptor cross-linking, a characteristic that favors elimination of self-reactive B-cell clones (7). B cells that escape this negative selection migrate to the peripheral lymphoid tissues.

B cells are also positively or negatively selected in the periphery when they encounter environmental antigens. Within the germinal centers formed in peripheral lymphoid tissues during an immune response, antigen-presenting dendritic cells and peptide/major histocompatibility complex class II-reactive helper T cells drive the B-cell response (8–11). During this process, B-cell clones proliferate, diversify their Ig variable [V(D)J] gene regions via somatic hypermutation, and are selected for increased affinity for antigen (8–11). Some of the clonal progeny in germinal centers switch their Ig heavy chains from IgM to other Ig isotypes (12). The germinal center interactions thereby culminate in the generation of antigen-selected memory B cells and mature plasma cells that produce isotypically diverse antibodies of relatively high affinity.

Representatives of the earliest stages in the B-cell pathway, including pro-B, pre-B, and immature B cells, prevail in the bone marrow during fetal life whereas mature B cells predominate in the bone marrow of adults (5, 13, 14). The transition is initiated in early childhood when a subpopulation of B cells with the mature phenotype CD10$^-$ CD24^{lo} IgD$^+$ begins to accumulate in the bone marrow to become the predominate B-cell subpopulation in adults (5). These B cells are characterized in the present study as an antigen-selected population of memory B cells that may have resided previously in the peripheral lymphoid tissues.

MATERIALS AND METHODS

Cell Suspensions. Bone marrow cells were prepared (5) from rib and long bone specimens obtained from healthy kidney donors and aborted fetuses in accordance with policies established by an institutional review board for human experimentation.

Antibodies and Immunofluorescence Flow Cytometry. The mouse mAb against human CD95 (μ isotype) was obtained from Upstate Biotechnology (Lake Placid, NY), the anti-CD74 (γ1) and anti-CD75 (μ) mAbs were from Zymed, the anti-CD77 mAb (rat μ) was from Immunotech (Westbrook, ME), the phycoerythrin (PE)-conjugated anti-CD71 (γ1) mAb was from Leinco Technologies (Ballwin, MO), and the fluorescein isothiocyanante-conjugated (FITC) anti-CD10 (γ2a), anti-CD34 (γ1), anti-CD3 (γ1) and PE-conjugated anti-CD5 (γ2a), anti-CD22 (γ2b), anti-CD23 (γ1), anti-CD25 (γ1), anti-CD38 (γ1), anti-CD54 (γ2b), anti-CD69 (γ1), and anti-CD80 (γ1) mAbs were from Becton Dickinson Immunocytometry Systems. Biotin-conjugated peanut agglutinin (PNA) was obtained from Vector Laboratories, and the biotin-conjugated goat antibodies to human IgM and mouse IgG1, FITC-conjugated goat antibodies to rat Ig, and PE-conjugated goat antibodies to human IgD and IgM were from Southern Biotechnology Associates. Viable cells were stained with FITC-conjugated mAbs, with the PE-conjugated mAbs for two-color immunofluorescence analysis, and then with the addition of biotin-conjugated antibodies and streptavidin PE/CY5 (Southern Biotechnology Associates) for three-color staining. Cells were incubated with Hoechst 33342 dye (6 μg/ml; Molecular Probes) and counterstained for cell surface antigens for cell cycle analysis. Flow cytometric analysis of cells with lymphocyte light scatter characteristics was performed with a FACScan instrument (Becton Dickinson). Positive- and negative-cell sorting and cell cycle analyses were performed with a dual laser FACStar^{plus} (Becton Dickinson).

Ig V(D)J Region Cloning and Sequencing. Total RNA was isolated from sorted subpopulations of bone marrow cells by a single-step method with Tri-Reagent (Molecular Research Center, Cincinnati), and cDNA was prepared using oligo(dT) as primer and avian myeloblastosis virus reverse transcriptase. The cDNA-encoding, rearranged V5-51, one of two functional nonpolymorphic members of the V_H5 (heavy chain variable region gene) family (15, 16), was amplified by PCR using primers specific to the leader sequence of the V5-51 (5′-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Copyright © 1997 by THE NATIONAL ACADEMY OF SCIENCES OF THE USA
0027-8424/97/94208-5$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: PE, phycoerythrin; PNA, peanut agglutinin; CDR, complementarity determining regions.

Data deposition: The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U77534–U77567).
*To whom reprint requests should be addressed. e-mail: Max.Cooper@ccc.uab.edu.

AGAATTCGGTCAACCGCCATCCTCG-3′ or 5′-CTCCT-CCTGGCTGTTCTCC-3′) and to the first exon of the C_μ region (5′-GGAGAAAGTGATGGAGTCGGG-3′, 5′-CTG-TGAGGTGGCTGCGTACTT-3′, or 5′-GGTGCTGCTGAT-GTCAGAGTT-3′). Annealing was at 57°C for 30 cycles of PCR. PCR products were visualized by electrophoresis on 1% agarose gels containing 5 μg/ml ethidium bromide. Reactions that failed to generate visible bands were reamplified via a nested or semi-nested PCR primer protocol using 1 μl from the first reaction as template. PCR products of two to four independent reactions were pooled, and ≈600-bp fragments purified by the Wizard PCR purification system (Promega) were ligated into pCRII vector (Invitrogen). Competent bacteria were transformed and selected according to the manufacturer's instructions. Plasmids from positive transfectants were prepared by alkaline lysis, and the presence of appropriately sized insert was confirmed by *Eco*RI restriction enzyme digestion. Plasmid DNA (5 μg) was sequenced in both directions using the Sequenase II kit (United States Biochemical) and the sequencing primers SP6 (5′-ATTTAGGTGACAC-TATA-3′), T7 (5′-TAATACGACTCACTATAGGG-3′), and H14 (5′-ATTCTCACAGGAGACGAG-3′).

Tissue Culture. Negatively sorted subpopulations of bone marrow mononuclear cells were plated at a density of 1×10^4 B cells in 200 μl of RPMI 1640 medium/10% fetal bovine serum. The cultures were supplemented with supernatant (40 μl) from peripheral blood lymphocytes cultured for 3 days with phytohemagglutinin (10 μg/ml).

Ig Measurement. Supernatants from cultures of bone marrow cells were deposited in duplicate onto nitrocellulose filters (Schleicher & Schuell), and adherent Ig was detected as described (17). Exposed film was analyzed using a FUJIX (Tokyo) Bas 1000 phosphoimager. The values were corrected for background and converted to micrograms per milliliter by interpolation onto the standard curve constructed using purified RS3-1 mAb (mouse γ1).

RESULTS

Mature B Cells with Variable Activation Features Accumulate with Age in the Bone Marrow. Analysis of the distribution of the immature ($CD10^+$) and mature ($CD10^-$) subpopulations of IgM^+ B cells (Fig. 1*A*) confirmed an age-related increase in the proportion of mature phenotype B cells in the bone marrow (Fig. 1*B*). Cells of mature B cell phenotype were relatively rare in fetal bone marrow (1.8 ± 0.5% of IgM^+ cells) but increased to represent the majority of IgM^+ B cells in the bone marrow of individuals over 20 years of age (71.5 ± 14.3%). Assessment of relative cell size by light scatter analysis indicated that the $CD10^-$ B cells were larger than the immature $CD10^+$ B cells (Fig. 1*C*). Nevertheless, analysis of their DNA content indicated that 99.8% of the mature subpopulation of bone marrow B cells were in the G_0/G_1 phase of the cell cycle (Fig. 1*D*).

To assess the activation status of the relatively large $CD10^-$ B cells, a diverse array of cell activation markers was used. A variable proportion of the $CD10^-$ B cells in adult bone marrow samples expressed intermediate to late activation markers (Fig. 2; Table 1). The acute activation markers CD69 and CD80 (B7-1) were rarely expressed, but transferrin receptors (CD71), a more sustained feature of activation (18), were frequently expressed. Over 95% of the $CD10^-$ IgM^+ cells in bone marrow expressed the CDw75 antigen, high levels of which are found on activated B cells in the germinal centers (19). The bone marrow $CD10^-$ B cells also expressed CD38 at low levels, similar to those found on germinal center B cells (20, 21), but had lower levels of PNA than typically seen on germinal center B cells (22). The mature subset of bone marrow B cells was negative for CD77, a cell surface molecule expressed on germinal center centroblasts (20) and on cells

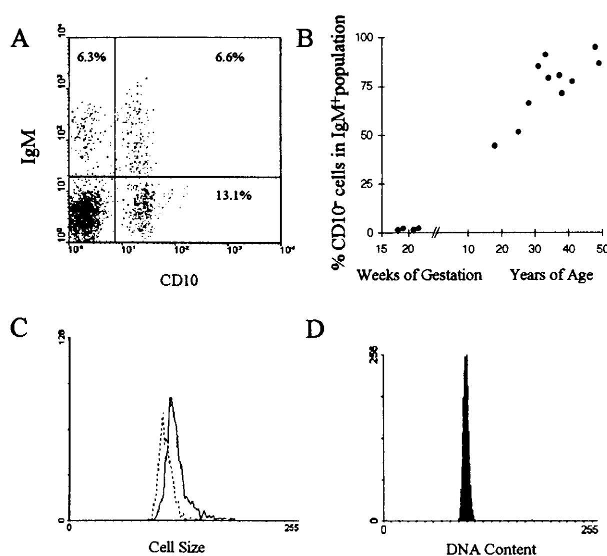

FIG. 1. The $CD10^-$ IgM^+ cells in the bone marrow are relatively large, nondividing cells that accumulate with age. (*A*) Representative dot plot of bone marrow lymphocytes stained with anti-IgM and anti-CD10; 5000 events are displayed. (*B*) Analysis of the frequency of $CD10^-$ IgM^+ cells in bone marrow as a function of age, expressed as proportion of total IgM^+ bone marrow lymphocytes. Each dot represents a separate tissue sample. (*C*) Relative cell size of $CD10^-$ IgM^+ lymphocytes (solid line) and $CD10^+$ IgM^+ lymphocytes (dotted line) as estimated by forward light scatter; 5000 events are displayed. (*D*) Flow cytometric analysis of DNA content of $CD10^-$ IgM^+ lymphocytes in adult bone marrow; 10,000 events are displayed.

entering apoptosis (23). On the other hand, it frequently expressed the CD95/Fas antigen (57 ± 14.2%), indicative of potential susceptibility to Fas ligand-induced apoptosis. The individual variation was greatest in one of the seven bone marrow samples, in which 95% of the mature B cells expressed a transferrin receptor (CD71) and 20% expressed the acute activation marker CD69, suggesting that this exceptional sample contained recently activated cells. Mature B cells in other

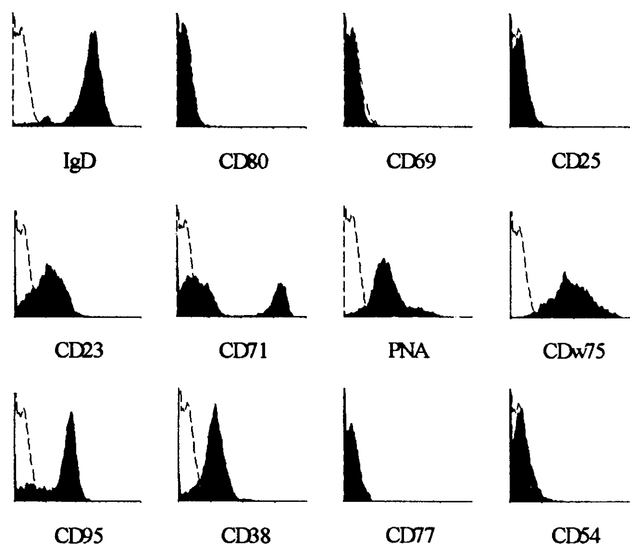

FIG. 2. Phenotypic characterization of the $CD10^-$ IgM^+ bone marrow population. Bone marrow cells were stained with a panel of mAbs to activation and B-lineage markers. Lymphocytes, gated on the basis of forward and side light scatter and $CD10^-$ IgM^+ immunofluorescence characteristics, were analyzed; 5000 events are displayed. Dashed, open histograms represent background fluorescence, and solid histograms represent staining, with the activation and B-lineage markers indicated. The bone marrow donor was 46 years old.

Table 1. Cell surface antigen expression by adult CD10$^-$ IgM$^+$ bone marrow cells

Antigen	Mean ± SE*	Antigen	Mean ± SE*
CD23	49.5 ± 21.2	IgD	83.7 ± 13.3
CD69	4.2 ± 6.5	PNA†	39.0 ± 7.1
CD80	0.7 ± 0.6	CD38†	65.1 ± 15.5
CD71	51.1 ± 24.6	CD95	57 ± 14.2
CDw75	96.7 ± 3.0	CD77	0.1 ± 0.0
CD54	23.3 ± 19.9	CD22	92.1 ± 7.4
CD25	0.5 ± 0.4	CD5	23.0 ± 7.4

*N = 7 for most antigens assayed.
†Detectable as a fluorescence shift, not as a discrete population.

bone marrow samples rarely expressed CD69, and the frequency of CD71 expression ranged from 34 to 74%.

Variable Region [V(D)J$_\mu$] Genes Are Often Hypermutated in Mature Bone Marrow B Cells. The phenotypic characteristics of the mature subpopulation of bone marrow B cells suggested that they could be derivative of a germinal center immune response despite the lack of isotype switching. To test this possibility, RNA from the mature (CD10$^-$ IgM$^+$) and immature (CD10$^+$ IgM$^+$) subpopulations of bone marrow B cells that was converted to cDNA and V(D)J sequences of representative clones amplified by PCR were examined for evidence of somatic mutations. For this analysis, transcripts encoded by the V5-51 gene, one of the two functional nonpolymorphic V_H5 genes (15, 16), were examined. The sequences of randomly selected V_H5 clones derived from the immature subpopulation of B cells did not indicate significant deviation from the germ line V5-51 sequence (Fig. 3). In contrast, the sequences of V5-51 clones obtained from the mature bone marrow subpopulation of IgM$^+$ B cells from five individuals frequently contained point mutations (Fig. 3), most of which were transition-type mutations, with G to A transitions predominating (20.5%), as previously noted for somatic hypermutation of V(D)J genes (20, 24, 25). Analysis of the point mutations (8) indicated preference for those resulting in amino acid replacement mutations over silent mutations (Fig. 3). The point mutations were distributed throughout the framework, complementarity determining regions (CDR), and J regions, but replacement mutations were more frequent in the CDR regions when normalized for length. The replacement/silent mutation ratios for CDR1 and CDR2 of the V5-51 clones were 4.5 and 3.1, respectively, whereas those for frameworks 1, 2, and 3 were 2.2, 2.4, and 5.1, respectively. Comparison of the sequences from individual bone marrow samples indicated the presence of clones sharing the same V(D)J rearrangement while having distinct patterns of point mutations. The clone pairs 1A4 and 1A11, 2A5 and 2A3, 5A25 and 5A16, 48A1 and 48A10, 54A12 and 54A13, and 54A2 and 54A10 isolated from five individual bone marrow samples appeared to be relatives in that they shared the same J regions, identical CDR3 regions, and most, but not all, point mutations (Fig. 4).

Limited *in Vitro* Survival and Ig Production by CD10$^-$ IgM$^+$ Bone Marrow Cells. The mature IgM$^+$ B cells in adult bone marrow exhibit the characteristics of B cells that have participated in an antigen response within germinal centers of peripheral lymphoid tissues and expressed the Fas antigen, so we wished to determine whether they were able to survive and undergo plasma cell differentiation. The CD10$^-$ IgM$^+$ bone marrow cells were isolated for this *in vitro* analysis by a negative selection procedure (which included removal of the CD34$^+$ and CD10$^+$ early B-lineage cells) to avoid altering the activation status of the target B cells. CD3$^+$ T cells, which accounted for 10–15% of bone marrow lymphocytes, were also removed in some experiments. Approximately 60% of these T cells were CD8$^+$, and they frequently expressed the acute activation marker CD69 (50 ± 17%), whereas the CD4$^+$ T cells were rarely CD69$^+$ (15 ± 8%); ≈25% of both T cell CD4$^+$ and CD8$^+$ subpopulations expressed the transferrin receptor CD71.

Mature B cells, thus negatively isolated from bone marrow samples (n = 4), were maintained alone or in the presence of autologous T cells and cytokines produced by activated T cells. Regardless of the culture conditions, >90% of the B cells died within 4 days. The CD10$^-$ IgM$^+$ B cells alone did not produce Ig, irrespective of whether T-cell-derived cytokines were supplied or not. However, when cultured together with autologous bone marrow T cells and T-cell-derived cytokines, IgM was produced in levels ranging from 0.4 to 23.6 μg/ml (12.0 ± 9.6 μg/ml).

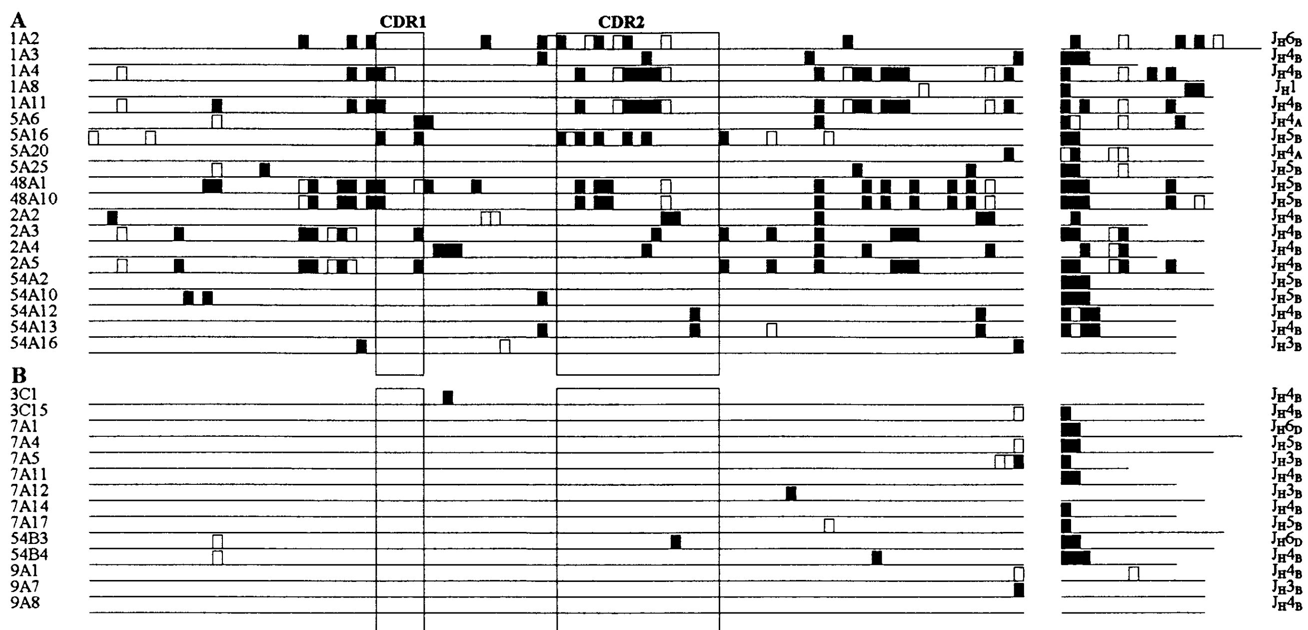

FIG. 3. V_H5_2-containing variable region [V(D)J_H] genes in the mature subpopulation of B cells within the bone marrow are frequently mutated. Diagrammatic representation of cloned sequences from CD10$^-$ IgM$^+$ (*A*) and CD10$^+$ IgM$^+$ (*B*) bone marrow B cells. For each clone, the line represents the deduced germ line V5-51 amino acid sequence. Solid boxes represent replacement amino acid mutations, and open boxes represent silent amino acid mutations. The J region gene of each V region clone is indicated; subscripts A, B, and D designate alleles.

```
       FR1                                                                                        CDR1            FR2
V5-51  GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGCCCGGGGAGTCTCTGAAGATCTCCTGTAAGGGTTCTGGATACAGCTTTACC AGCTACTGGATCGGC TGGGTGCGC
1A4    ---------T------------------------------------------------------------------------C----G-- -A---T--------- ---------
1A11   ---------T------------------------------G-----------------------------------------C----G-- -A------------- ---------
2A5    -----------A---------------C---------------------------------------C--A-------A----T------ -------------C- ---------
2A3    -----------A---------------C---------------------------------------C--A------GC----T------ -------------C- ---------
5A25   -----------------------------------------G------------G----------------------------------- --------------- ---------
5A16   --A-----------------C--------------------------------------------------------------------- T-----------A-- ---------
48A1   ------------------------------------CCAAG---------------------------A-A-------A---A----G-- GA------------T G--------
48A10  --------------------------------------------------------------------A-A-------A---A----G-- GA------------- ---------
54A12  ------------------------------------------------------------------------------------------ --------------- ---------
54A13  ------------------------------------------------------------------------------------------ --------------- ---------
54A10  -------------------------------C-----G---------------------------------------------------- --------------- ---------
54A2   ------------------------------------------------------------------------------------------ --------------- ---------

                                         CDR2                                                FR3
V5-51  CAGATGCCCGGGAAAGGCCTGGAGTGGATGGGG ATCATCTATCCTGGTGACTCTGATACCAGATACAGCCCGTCCTTCCAAGGC CAGGTCACCATCTCAGCCGACAAGTCCATC
1A4    --------------------------------- -------GG-----------CAG--T--A-AGT--T--------------- ------------------------------
1A11   --------------------------------- -------GG-----------CAG--T--A-AGT--T--------------- ------------------------------
2A5    --------------------------------- --------------------------------------------------- -GA-------------T-------------
2A3    --------------------------------- ------------------------------A-------------------- -GA-------------T-------------
5A25   --------------------------------- --------------------------------------------------- ------------------------------
5A16   --------------------------------- -GG--TG-----A--------T------AC--------------------- --C--------------T------------
48A1   ------G-------------------------- ------A-----C--A-------------------T--------------- ------------------------------
48A10  --------------------------------- ------A-----C--A-------------------T--------------- ------------------------------
54A12  --------------------------------- ------------------------------------------C-------- ------------------------------
54A13  --------------------------------- ------------------------------------------C-------- -----------------T------------
54A10  ---------------------------G----- --------------------------------------------------- ------------------------------
54A2   --------------------------------- --------------------------------------------------- ------------------------------

                                                                          N/D/N
V5-51  AGCACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGA ...........................................
1A4    -C---------TA--AC-----C--A-G----------------------------T----T---- GGG AGG GGA TAT TGT AGT GCT GGT AGC TGC TAC
1A11   -C---------TA--AC-----C--A-G----------------------------T----T---- GGG AGG GGA TAT TGT AGT GCT GGT AGC TGC TAC
2A5    -A-----------------------C-G---C---------------------------------- CAA ATA GTG GCA ACT ACC GAT................
2A3    -A-----------------------C-G---C---------------------------------- CAA ATA GTG GCA ACT ACC GAT................
5A25   -------------------------------------------------C---------------- CTC AGC AGT GGC TGG T......................
5A16   -----T------------------------------------------------------------ CTC AGC AGT GGC TGG T......................
48A1   -A---------------C----A--------G----------T-------A-----T--------- CGC TTA TAC TAT G..........................
48A10  -A---------------C----A--------G----------T-------A-----T--------- CGC TTA TAC TAT G..........................
54A12  ---------------------------------------------------A-------------- GGC TAC TAT TAC GAT TTT TGG AGT............
54A13  ---------------------------------------------------A-------------- GGC TAC TAT TAC GAT TTT TGG AGT............
54A10  ------------------------------------------------------------------ GAC AGT TTC GGC GGT AAA AAT ATA A..........
54A2   ------------------------------------------------------------------ GAC AGT TTC GGC GGT AAA AAT ATA A..........

JH4b   TACTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA   JH5b   AAAACTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
1A4    G-T-----------------G-------C----G-----------   5A25   -TC-A---------------A-----------------------------
1A11   G-T----T------------G------------G-----------   5A16   -TC-A---------------------------------------------
2A5    CC-GGG-----------AG-G------------CG----------   48A1   -C-C-CTCC-------------------------T---------------
2A3    CC-GGG-----------AG-G------------------------   48A10  -C-C-CTCC-------------------------T---------A-----
54A12  GGT-A-TCGA--------------------------            54A10  T-GG-C----------------------------------
54A13  GGT-A-TCGA--------------------------            54A2   T-GG-C----------------------------------
```

FIG. 4. Related clones of $CD10^-$ IgM^+ bone marrow cells in individual donors. Sequence comparison of the V5-51, J_H4_B, and J_H5_B germ line sequences with clones isolated from sorted $CD10^-$ IgM^+ bone marrow cells. Each set of related clones was isolated from an individual bone marrow donor.

DISCUSSION

These studies indicate that mature B cells in the bone marrow of adults represent a dynamic population of lymphocyte clones that have undergone prior stimulation, somatic diversification, and antigen selection. These events typically occur within germinal centers, so the data imply prior sojourn in the secondary lymphoid tissues and recirculation to the bone marrow.

This recirculating population of IgM^+ B lymphocytes can be readily distinguished from the immature B cells generated *in situ* by the lack of CD10 (neutral endopeptidase), diminished CD24 (heat-stable antigen), and expression of IgD (5). The present results confirm the postnatal acquisition of B cells with mature phenotype in the bone marrow and indicate that they are relatively large, but nondividing, lymphocytes. A significant proportion of these express cell surface molecules reflective of prior activation, such as the transferrin receptor CD71, CD23, and the CDw75 antigen, high levels of which are expressed by cells in the light zone of germinal centers (19). A variable proportion of the mature B cells in the bone marrow expressed lower levels of PNA relative to the high PNA levels typical of germinal center B cells. The $CD10^-$ IgM^+ bone marrow cells thus resemble germinal center B cells although their phenotypic profile does not match precisely with that of any of the germinal center B-cell subpopulations (20, 26, 27) or that of memory B cells in the circulation (28). The phenotypic profile thus suggests that this bone marrow B-cell population includes a mixture of more or less recent B-cell immigrants of germinal center origin that may have undergone phenotypic modification before or after entry into the bone marrow.

A defining feature of the mature B-cell population characterized here is IgM expression, thereby excluding from our study clonal members that might have undergone an isotype switch in germinal centers. Somatic hypermutation of the Ig VJ_L and $V(D)J_H$ genes is an important feature of germinal center B cells (8–11, 24, 29–31), and our analysis of this possibility focused on B cells expressing one of the two functional V_H5 genes. The results indicate that $CD10^-$ IgM^+ bone marrow cells have extensively mutated V(D)J regions with preference toward replacement amino acid mutations in the CDR, a pattern that typically evolves during antigen selection of germinal center B cells (8, 24, 29, 30). The mutation frequency observed for the Ig variable region bases, $3.3 \pm 2.9\%$, is typically seen late in an immune response (24) and contrasts with unmutated germ line sequences of the V_H5 transcripts observed in the immature $CD10^+$ IgM^+ B cells. Mutational levels similar to these have been reported for IgM transcripts in B cells from human spleen and blood (31–33).

It is interesting to note that clonal relatives with the same V(D)J regions, but having some unique point mutations, were found in each of the five bone marrow samples examined in this way. Together with the variability in activation status of the

mature B cells in bone marrow, the relatively high proportion of clonally related cells in this population at a given point in time suggests that clones of B cells undergoing variable region hypermutation and antigen selection in germinal centers may migrate to the bone marrow in waves. It is also possible that the mutations may have occurred *in situ* because recent evidence indicates that inefficient hypermutation can occur in the absence of germinal centers (34, 35). However, the extensive hypermutation and the lack of cell division noted for the $CD10^{-}$ IgM^{+} cells suggest that affinity maturation did not take place *in situ*. The data also suggest that this mature B-cell population in bone marrow does not represent a pool of memory cells acquired gradually over a lifetime of immune responses but rather represents a dynamic population continually updated by the influx of recently generated memory B cells.

Variable region point mutations begin to accumulate in murine germinal centers $\approx$1 week after primary immunization (24), and, by 3 weeks, IgM transcripts contain as many mutations as the IgG transcripts (36). Murine B lymphoblasts originating in germinal centers of peripheral lymphoid tissues may home to the bone marrow, where they undergo differentiation into antibody-secreting plasma cells (37–39). The germinal center cells that are capable of emigrating to the bone marrow in mice have been characterized by high levels of PNA expression and spontaneous secretion of specific antibody when placed in culture (38), characteristics that were not found for the mature B cells in human bone marrow. If the $CD10^{-}$ IgM^{+} B cells migrating from germinal centers to the bone marrow had received the necessary instructions to differentiate into plasma cells, they would be expected to undergo spontaneous differentiation (40). Instead, the $CD10^{-}$ IgM^{+} B lymphocytes were unable to undergo spontaneous differentiation into antibody-secreting cells although some manifested this differentiative capacity when given appropriate T-cell help. The current data thus indicate that these memory B cells must receive additional environmental signals before they can undergo terminal differentiation within the bone marrow or elsewhere. In conclusion, our data implicate human bone marrow as an important destination in the route of recirculating memory B cells. Further analysis of their homing receptors, duration of bone marrow residence, and ultimate fate is needed for an understanding of clinical disorders, such as multiple myeloma, in which aberrant B lineage cells accumulate in the bone marrow.

We thank Drs. Ted Bertrand and Linda Billips for helpful discussions; Marion Spell, Guo Qiang, and Larry Gartland for fluorescence-activated cell sorting at a moment's notice; and Drs. Garnett Kelsoe, Harry Schroeder, Jr., and Peter Burrows for reading the manuscript. This work was supported by a grant from the National Institute of Allergy and Infectious Diseases (AI34568). E.P. is supported by the Irvington Institute for Immunological Research and the Natural Sciences and Engineering Research Council of Canada. M.D.C. is an Investigator of the Howard Hughes Medical Institute.

1. Owen, J. J., Raff, M. C. & Cooper, M. D. (1976) *Eur. J. Immunol.* **5,** 468–473.
2. Gathings, W. E., Lawton, A. R. & Cooper, M. D. (1977) *Eur. J. Immunol.* **7,** 804–810.
3. Solvason, N. & Kearney, J. F. (1992) *J. Exp. Med.* **175,** 397–404.
4. Osmond, D. G. (1990) *Semin. Immunol.* **2,** 173–180.
5. Nunez, C., Nishimoto, N., Gartland, G. L., Billips, L. G., Burrows, P. D., Kubagawa, H. & Cooper, M. D. (1996) *J. Immunol.* **156,** 866–872.
6. Rolink, A., Haasner, D., Nishikawa, S.-I. & Melchers, F. (1993) *Blood* **81,** 2290–2300.
7. Goodnow, C. C. (1992) *Annu. Rev. Immunol.* **10,** 489–518.
8. Shlomchik, M. J., Marshak-Rothstein, A., Wolfowicz, C. B., Rothstein, T. L. & Weigert, M. G. (1987) *Nature (London)* **328,** 805–811.
9. Berek, C. & Milstein, C. (1987) *Immunol. Rev.* **96,** 23–41.
10. Kocks, C. & Rajewsky, K. (1989) *Annu. Rev. Immunol.* **7,** 537–539.
11. MacLennan, I. C. M. (1994) *Annu. Rev. Immunol.* **12,** 117–139.
12. Harriman, W., Volk, H., Defranoux, N. & Wabl, M. (1993) *Annu. Rev. Immunol.* **11,** 361–384.
13. Duperray, C., Boiron, J.-M., Boucheix, C., Cantaloube, J.-F., Lavabre-Bertrand, T., Attal, M., Brochier, J., Maranichi, E., Bataille, R. & Klein, B. (1990) *J. Immunol.* **145,** 3678–3683.
14. LeBien, T. W., Wörmann, B., Villablanca, J. F., Law, C.-L., Steinberg, L. M., Shah, F. O. & Loken, M. R. (1990) *Leukemia* **4,** 354–358.
15. Matsuda, F., Shin, E. K., Nagaoka, H., Matsumura, R., Haino, M., Fukita, Y., Taka-ishi, S., Imai, T., Riley, J. H., Anand, R., Soeda, E. & Honjo, T. (1993) *Nat. Genet.* **3,** 88–94.
16. Sanz, I., Kelly, P., Williams, C., Scholl, S., Tucker, P. & Capra J. D. (1989) *EMBO J.* **8,** 3741–3748.
17. McGinnes, K., Keystone, E., Bogoch., E., Hastings, D., Messner, H. A., Jamal, N. & Paige, C. J. (1990) *Blood* **76,** 896–905.
18. Kehrl, J. H., Muraguchi, A. & Fauci, A. S. (1984) *J. Immunol.* **132,** 2857–2861.
19. Bast, B. J. E. G., Zhou, L-J., Freeman, G. J., Colley, K. J., Ernst, T. J., Munro, J. M. & Tedder, T. F. (1992) *J. Cell Biol.* **116,** 423–435.
20. Pascual, V., Liu, Y-J., Magalski, A., de Bouteiller, O., Banchereau, J. & Capra, D. (1994) *J. Exp. Med.* **180,** 329–339.
21. Liu, Y-J., Berthélémy, C., de Bouteiller, O., Arpin, C., Durand, I. & Banchereau, J. (1995) *Immunity* **2,** 239–248.
22. Weinberg, D. S., Ault, K. A., Gurley, M. & Pinkus, G. S. (1986) *J. Immunol.* **137,** 1486–1494.
23. Mangeney, M., Richard, Y., Couland, D., Turz, T. & Wiels, J. (1991) *Eur. J. Immunol.* **21,** 1131–1140.
24. Jacob, J., Pryzela, J., Miller, C. & Kelsoe, G. (1993) *J. Exp. Med.* **178,** 1293–1307.
25. Maizels, N. (1995) *Cell* **83,** 9–12.
26. Lagresle, C., Bella, C. & Defrance, T. (1993) *Int. Immunol.* **5,** 1259–1268.
27. Hardie, D. L., Johnson, G. D., Khan, M. & Maclennan, I. C. M. (1993) *Eur. J. Immunol.* **23,** 997-1004.
28. Irsch, J., Irlenbusch, S., Radl, J., Burrows, P. D., Cooper, M. D. & Radbruch, A. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1323–1327.
29. Jacob, J., Kelsoe, G., Rajewsky, K. & Weiss, U. (1991) *Nature (London)* **354,** 389–392.
30. Berek, C., Berger, A. & Apel, M. (1991) *Cell* **67,** 1121–1129.
31. van Es, J. H., Gmelig Meyling, F. H. J. & Logtenberg, T. (1992) *Eur. J. Immunol.* **22,** 2761–2764.
32. Insel, R. A., Varade, W. S. & Marin, E. (1994) *Mol. Immunol.* **31,** 383–392.
33. Varade, W. S. & Insel, R. A. (1993) *J. Clin. Invest.* **91,** 1838–1842.
34. Matsumoto, M., Lo, S. F., Carruthers, C. J. L., Min, J., Mariathasan, S., Huang, G., Plas, D. R., Martin, S. M., Geha, R. S., Nahm, M. H. & Chaplin, D. D. (1996) *Nature (London)* **382,** 462–466.
35. Chu, Y. W., Marin, E., Fuleihan, R., Ramesh, N., Rosen, F. S., Geha, R. S. & Insel, R. A. (1995) *J. Clin. Invest.* **95,** 1389–1393.
36. Tao, W., Hardardrottir, F. & Bothwell, A. L. M. (1993) *Mol. Immunol.* **30,** 593–602.
37. Koch, G., Osmond, D. G., Julius, M. H. & Benner, R. (1981) *J. Immunol.* **126,** 1447–1451.
38. Dilosa, R. M., Maeda, K., Masuda, A., Szakal, A. K. & Tew, J. G. (1991) *J. Immunol.* **146,** 4071–4077.
39. Bachmann, M. F., Kündig, T. M., Odermatt, B., Hengartner, H. & Zinkernagel, R. M. (1994) *J. Immunol.* **153,** 3386- 3397.
40. Hibi, T. & Dosch, H-M. (1986) *Eur. J. Immunol.* **16,** 139–145.