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Human Memory B Cells Transferred by Allogenic Bone Marrow Transplantation Contribute Significantly to the Antibody Repertoire of the Recipient

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The bone marrow is an important source of Abs involved in long-term protection from recurrence of infections. Allogenic bone marrow transplantation (BMT) fails to restore this working memory. Attempts to overcome this immunodeficiency by immunization of the donor have not been very successful. More needs to be known about transfer of B cell memory by BMT. We tracked memory B cells from the donor to the recipient during BMT of a girl with leukocyte adhesion deficiency. Vaccination of her HLA-identical sibling donor 7 days before harvest induced Haemophilus influenzae type b (Hib) capsular polysaccharide (HibCP)-specific B cells readily detectable in marrow and blood. BMT did not lead to spontaneous production of HibCP Abs, but the recipient responded well to booster immunizations 9 and 11 mo after BMT. HibCP-specific B cells were obtained 7 days after the vaccinations, and their V\(_\gamma\) genes were sequenced and analyzed for rearrangements and unique patterns of somatic hypermutations identifying clonally related cells. Ninety (74%) of 121 sequences were derived from only 16 precursors. Twelve clones were identified in the donor, and representatives from all of them were detected in the recipient where they constituted 61 and 68% of the responding B cells after the first and second vaccinations, respectively. No evidence for re-entry of memory clones into the process of somatic hypermutation was seen in the recipient. Thus, memory B cells were transferred from the donor, persisted for at least 9 mo in the recipient, and constituted the major part of the HibCP-specific repertoire. The Journal of Immunology, 2004, 172: 3305–3318.

The recovery of the immune system after allogenic bone marrow transplantation (BMT)\(^1\) requires at least 12 mo to be completed. Both cellular and humoral immune functions are affected, rendering the patients susceptible to serious infections (1, 2). B cells recover slowly in numbers and function and may be of host or donor origin (3, 4). In cases of complete B cell chimerism, it is currently unknown to what extent the cells are recruited de novo from the emerging marrow or by expansion of donor-derived mature or even memory B cells.

BMT recipients generally become seronegative to previous immunizations during follow-up (5–9), and long-term immunity defined as persistent Ab is not maintained regardless of the immune status of the donor (8, 10). Therefore, reimmunization of long-term survivors after BMT is necessary (1, 8, 11). Nevertheless, several observations indicate that the effect of such immunizations may be influenced by the immune status of the donor, and it has been proposed that immunization of the donor before transplantation combined with immunization of the recipient may be beneficial for Ab protection throughout the posttransplantation period (12).

Evidence for adoptive transfer of immunity from donor to recipient by BMT has so far been sparse and indirect. Sharing of plasma Ab spectrotypes and Ids between donor and recipient has indicated transfer of immune B cell progeny to the recipient (13–16), but it is not clear whether these cells were transferred as plasma cells or memory B cells. Also, the demonstration of secondary-like Ab responses to the first immunization of the recipient after BMT points to transfer of immunological memory (13, 17–19). However, these studies yield limited information as to which cell types are responsible for this transfer (memory B cells, Th cells, Ag-loaded APCs). To settle these questions, studies are needed using cellular markers coidentifying the level of cellular differentiation and clonal relatedness.

In this study, we used clonal tracking based on unique patterns of somatic hypermutations to study the transfer of vaccine-specific memory B cells during BMT of a girl with leukocyte adhesion deficiency (LAD) of type I. We demonstrate that vaccination of her HLA-identical bone marrow donor 7 days before harvest induced Haemophilus influenzae type b (Hib) capsular polysaccharide (HibCP)-specific hypermutated memory B cells, which were transferred to the recipient by BMT. These cells survived in the recipient for at least 9 mo, constituted a major part of the HibCP-specific B cell repertoire at this time point, and responded to repeated immunizations of the recipient. Re-entry into the process of somatic hypermutation could not be demonstrated.

**Materials and Methods**

**Donor-recipient pair**

The patient was diagnosed as LAD type I at 7 mo of age. LAD is a rare inherited immunodeficiency characterized by defective adherence and migration of leukocytes caused by mutations in the β\(_2\) integrin (CD18)-encoding...
gene (20). The diagnosis was based on the following clinical picture: leukocytosis with marked neutrophilia and recurrent bacterial infections (e.g., omphalitis) combined with complete absence of CD18 on blood mononuclear cells (MNC) as judged by flow cytometry (data not shown). Lymphocyte subpopulations were normal including normal numbers of B cells. There were normal proliferative responses to mitogens and allogeneic cells. Plasma Ig levels were normal for the age with easily detectable Abs to tetanus toxoid (TT) and HibCP.

The patient was conditioned by etoposide, busulphan, and cyclophosphamide, and received at 10 mo of age an unseparated bone marrow graft (2.33 × 10^8 cells/kg) from her 3-year-old healthy HLA-identical sister. The patient developed signs of veno-occlusive disease at day 19 after BMT but recovered completely without signs of graft-vs-host disease at any time after BMT. IgG substitution was given at days 7 and 31 post-BMT.

The study was approved by the regional ethics committee, and written consent was obtained from the parents.

**Vaccination schedule**

The donor was immunized 7 days before marrow harvest with HibCP-TT consisting of 10 µg of HibCP covalently coupled to 24 µg of TT (Act-Hib; Pasteur Mérieux Sérums et Vaccins, Lyon, France) given s.c. in the supracrural region. She had previously received three routine immunizations (referred to as "Do" in the following) and from the recipient after the two experimental vaccinations (referred to as "Rec1" and "Rec2", respectively) were thawed, washed, and pelleted. RNA was extracted by the guanidinium thiocyanate method (23), dissolved in sterile water, and stored at −80°C until use. The RNA was used for H chain cDNA synthesis with the Gene Amp RT-PCR kit (PerkinElmer/Cetus, Emeryville, CA) for each vaccination occasion (Do, Rec1°, Rec2°). Three 10-µl isotype-specific first-strand reaction mixes were set up with final concentrations of 1× PEII PCR buffer (PerkinElmer/Cetus), 5 mM MgCl₂ (Life Technologies, Rockville, MD), 1 mM dNTP (Pharmacia, Peapack, NJ), 1 U/ml of RNase inhibitor (PerkinElmer/Cetus), and 2.5 U/µl of Moloney murine leukemia virus reverse transcriptase (PerkinElmer/Cetus), and incubated at 42°C for 30 min with 1 pmol of the relevant C°/C region primer: IgM133rc, IgA269rc, or IgG264rc (Table I). In addition, a new set of primers corresponding to regions situated further downstream in C°/C was designed for cDNA synthesis from mRNA obtained from the second post-BMT vaccination of the recipient (Rec2°). IgM140rc, IgA277rc, and IgG274rc (Table I). These primers were used to formally exclude amplification of contaminant DNA derived from the donor or the recipient after the first post-BMT vaccination.

Four microelits of the isotype-specific first-strand reaction mixes from each vaccination occasion (Do, Rec1°, Rec2°) were used as template in each of six independent 5-µl PCR reactions with final concentrations of 0.2 mM dNTP, 1.5 mM MgCl₂, 1× PEII PCR buffer, and 1.25 U of AmpliTaq and anti-Taq (PerkinElmer/Cetus), and incubated at 94°C for 3 min with 1 pmol of the relevant C°/C region primer: IgM133rc, IgA269rc, or IgG264rc (Table I). The resulting amplicons were sequenced corresponding to the entire variable domain (codons 1–113) and a part of the first constant domain allowing identification of the V°/V family available in the GenBank database and in the directory of Kabat (24). The PCRs were hot-started and after an initial denaturation for 2 min at 94°C, 40 PCR cycles were performed, consisting of 30 s at 94°C, 1 min at 55°C, and 1 min at 72°C, and a final 5-min step at 72°C.

The PCR products were cloned into the pCR2.1 vector by use of the Original TA Cloning kit (Invitrogen, San Diego, CA) as instructed by the manufacturer. Insert length was analyzed by running a control PCR using the flanking primers M13bio and M133Rbio (Table I). Plasmid DNA was purified using the Quantum Prep Plasmid Miniprep kit (Bio-Rad, Hercules, CA); the plasmids were extracted with phenol/chloroform/isooamyl alcohol (25:24:1; pH 6.7/8.0; Amresco, Solon, OH) before use as template. Clones were sequenced corresponding to the entire variable domain (codons 1–113) and a part of the first constant domain allowing identification of isotypes and subclasses using the Ready Reaction kit (PerkinElmer/Cetus) and an ABI 373 automatic sequencer (PerkinElmer/Cetus) according to the instructions of the manufacturer.

Candidate germline V° genes were assigned to each sequence based on maximum homology (codons 1–94) with sequences of the GenBank database. Similarly, candidate germline J°/J genes were assigned based on maximum homology from codons 101–113 with the published germline sequences (25). D°/D segments were assigned to a germline D° gene if they matched at seven continuous bases or more than eight continuous bases

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Table I. Primers used in the study

<table>
<thead>
<tr>
<th>Name</th>
<th>Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-23c89rc</td>
<td>5'-TCTTTTGACAGATCTTAT-3'</td>
</tr>
<tr>
<td>3-23c1al</td>
<td>5'-TGAGGTGGCAAGCCTGTTG-3'</td>
</tr>
<tr>
<td>3-23c9mut-TE</td>
<td>5'-GATTGCTAGCTGATTTTCTTG-3'</td>
</tr>
<tr>
<td>3-23c12-G</td>
<td>5'-ATTGCCAAGGTGAGTGC-3'</td>
</tr>
<tr>
<td>IgA18s5c</td>
<td>5'-GCTGTGCTGCTGGTGGAT-3'</td>
</tr>
<tr>
<td>IgA269rc</td>
<td>5'-TGCACTGTAGGATCTTCCT-3'</td>
</tr>
<tr>
<td>IgA277rc</td>
<td>5'-AGG0CATCCCCAGGCCC-3'</td>
</tr>
<tr>
<td>IgG183rc</td>
<td>5'-GCTGTGCTGCTGATTTTCTTG-3'</td>
</tr>
<tr>
<td>IgG264rc</td>
<td>5'-G0TCTGCTGCTGATTTTCTTG-3'</td>
</tr>
<tr>
<td>IgG274rc</td>
<td>5'-CTGCAGCTGCTGCTGCTG-3'</td>
</tr>
<tr>
<td>IgG140rc</td>
<td>5'-TGACAGCATCAGGCTCAC-3'</td>
</tr>
<tr>
<td>IgM126rc</td>
<td>5'-CGAGAGCATCAGGCTGCTG-3'</td>
</tr>
<tr>
<td>IgM133rc</td>
<td>5'-GCTGGTGGCTGCTGCTGCTG-3'</td>
</tr>
<tr>
<td>IgM140rc</td>
<td>5'-GTTCCATCTGCGCTGCTGCTG-3'</td>
</tr>
<tr>
<td>M13bio</td>
<td>5'-TTTCTCCAATGCTCAAGGCTGTTGAAACCCGGCCACAG-3'</td>
</tr>
<tr>
<td>M133Rbio</td>
<td>5'-AGGGATACAAATTTACTACAGGACGAAAAAGATGTATGAC-3'</td>
</tr>
<tr>
<td>V°/3-sig-deg</td>
<td>5'-G(T/C)T(T/G)GCT(A/C)T(A/T)TA(A/G)(A/G)AGGTGCTCA-3'</td>
</tr>
</tbody>
</table>
The frequency of RT PCR mutations was estimated from the sequences encoded by the germline gene using 5 pmol of gene-specific primers placed in framework region (FR1) (3-23cn1) and FR3 (3-23cn9rc), respectively (Table I). PCR was performed as described above but with 30 cycles instead of 40.

Amplification of germline gene sequences

Genomic DNA was isolated by a salting-out procedure (28) from 5 × 10⁶ MNC obtained before vaccination of the donor. Five hundred nanograms of DNA were used for PCR amplification of the 3-23 germline gene using 5 pmol of gene-specific primers placed in framework region (FR) (3-23cn1) and FR3 (3-23cn9rc), respectively (Table I). PCR was performed as described above but with 30 cycles instead of 40.

Measures to exclude cross-contamination

Great care was taken to avoid mixing of sequences derived from the three experimental vaccinations. Besides ordinary PCR precautions such as handling of cells and pre-PCR RNA and DNA samples in facilities other than those used for post-PCR material, handling of samples from the three vaccinations were separated in time too. Thus, PCR, cloning, and sequencing of material from the three vaccinations were done with weeks to months between; first from Do, then from Rec1°, and finally from Rec2°. In addition, different primers amplifying different lengths and isotypes of the PCR products were used to allow for detection of colonies not containing the proper PCR product. No indication of cross-contamination was seen throughout the study. To formally exclude contamination of Rec2° samples with Rec1° or Do samples, specially designed primers (see above) were used recognizing a template in the C region gene not present in any PCR product from the donor or first post-BMT vaccination of the recipient.

Clonal analysis

The software program TREECON for Windows (version 1.3b) was used to generate a cluster analysis dendrogram based on the neighbor joining method (29). All mutated sequences (codons 1–94) derived from the canonical Vπ3-23 and 3-49RB germline genes were subjected to analysis. The presence of shared and unshared somatically acquired mutations was used to calculate evolutionary distances and an evolutionary tree minimizing these was formed using the respective germline sequences as roots.

Analysis of CDR3 lengths

For CDR3 length analysis, mRNA was extracted from thawed MNC using the Dynabeads mRNA DIRECT kit (Dynal). The mRNA was used for cDNA synthesis in an 80-μl reaction mix with a final concentration of 1.25 μM oligo(dT)₆₄ primer (DNA Technology, Aarhus, Denmark), and reaction conditions were otherwise as described above. The cDNA was stored at −20°C until use. The cDNA was used for IgG Vπ3-23-specific PCR as follows. Four microliters of cDNA was used in a 20-μl PCR with a final concentration of 1 × PCR buffer, 1.5 mM MgCl₂, 0.2 μM PlatinumTaq polymerase (Life Technologies), 0.2 mM dNTP, and 0.1 μM isotype-specific Cµ region primer (IgG183rc or IgM133rc) combined with a Vπ3-23 signal peptide primer (0.1 μM; 3-23cn12-G; Table I). PCR was hot-started and after an initial denaturation for 2 min at 94°C, 22 PCR cycles were performed, consisting of 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, and a final 10-min step at 72°C. One microliter of these PCR products was thereafter used as template in a 50-μl nested PCR with IgG140rc primer or IgM133rc primer combined with a fluorochrome-coupled (TET) Vπ3-23 signal peptide primer 3-23cn9mut-TET (Table I); both primers were in a final concentration of 0.1 μM. The reaction mix was otherwise as described above. PCR was hot-started, and after an initial denaturation for 2 min at 94°C, 20 PCR cycles were performed, consisting of 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, and a final 10-min step at 72°C. The lengths of the PCR products were analyzed in a fluorochrome-coupled fragment analysis using PE Applied Biosystems (Foster City, CA) ABI Prism 310 genetic analyzer.

Statistics

The hypergeometric distribution was used to evaluate the distribution of sequences with different joints among the clusters defined by the neighbor joining method. The probability that any of the different joints would appear by chance as often (or more often) as the one found to be most numerous in each cluster was calculated. Subgroups were compared by

Table II. Numbers of IgSC, and TT and HibCP AbSC 7 days after immunization of the donor and the recipient with HibCP-TT (Act-Hib)°

<table>
<thead>
<tr>
<th></th>
<th>IgSC and AbSC per Gram of Blood and Bone Marrow</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before purification</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Isotype distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgM (%)  IgG (%)  IgA (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor blood</td>
<td>23,736</td>
<td>950</td>
<td>11,480</td>
<td>16</td>
<td>42</td>
<td>42</td>
<td>48</td>
</tr>
<tr>
<td>Donor marrow</td>
<td>24,975</td>
<td>4,538</td>
<td>8,313</td>
<td>21</td>
<td>44</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Recipient 1st</td>
<td>17,679</td>
<td>143</td>
<td>1,527</td>
<td>22</td>
<td>29</td>
<td>49</td>
<td>9</td>
</tr>
<tr>
<td>immunization</td>
<td>14,692</td>
<td>62</td>
<td>338</td>
<td>59</td>
<td>ND</td>
<td>41</td>
<td>2</td>
</tr>
</tbody>
</table>

° Isotype distribution is indicated for HibCP AbSC. Recovery after purification of HibCP-specific cells are given, and the purity of the latter is given in percentage of total IgSC (right column). Confidence limits (95%) are given in parentheses (Poisson distribution).

° IgG-secreting cells were not measured.
Clinical course

After transplantation at 10 mo of age, the patient recovered completely from her immunodeficiency and rapidly developed complete amnion regeneration. She had received a single immunization with HibCP-TT 5 mo after BMT, and the numbers of B and T lymphocytes were normal after 6 and 12 mo, respectively. Cell-mediated immunity, as determined by the delayed-type hypersensitivity skin test, was normal after 6 mo and the recipient remains healthy with a normal childhood development.

**Table III. Numbers of sequences using specific gene combinations and CDR3 sequences (joints) among 121 canonical and noncanonical VDJ sequences from the donor (Do) and the first (Rec1°) and second (Rec2°) immunizations of the recipient**

<table>
<thead>
<tr>
<th>Joints (No.)</th>
<th>V&lt;sub&gt;H&lt;/sub&gt; Gene</th>
<th>D&lt;sub&gt;H&lt;/sub&gt; Gene</th>
<th>J&lt;sub&gt;H&lt;/sub&gt; Gene</th>
<th>Sequence of CDR3 (Codons 95–102)</th>
<th>Number of Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do</td>
<td>Rec1°</td>
<td>Rec2°</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canonical rearrangements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3-23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>JH6B1</td>
<td>GGC TAC GGT ATG GAC GTC</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>3-23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>JH6B1</td>
<td>GGC TAC GGT ATG GAC GTC</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>3-23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>JH6B1</td>
<td>GGC TAC GGT ATG GAC GTC</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td>3-23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>JH6B1</td>
<td>GGC TAC GGT ATG GAC GTC</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>DS-18&lt;sup&gt;f&lt;/sup&gt;</td>
<td>JH6B1</td>
<td>GGC TAT TAT TTC GAC TGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncanonical rearrangements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3-49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>D1-1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>JH6B1</td>
<td>GAT TCT AAG GTC GAC GGG AAA CTG GAT GCT TTT GAT ATT</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3-49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>D3-10</td>
<td>JH6B1</td>
<td>GAG CGG TTA TAC GAT TTT TGG AGT GGA ACC CTT GGG TAC TAC TTT GAC TAC</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3-49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>D3-10</td>
<td>JH6B1</td>
<td>GAG CGG TTA TAC GAT TTT TGG AGT GGA ACC CTT GGG TAC TAC TTT GAC TAC</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3-21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>D3-10</td>
<td>JH6B1</td>
<td>GAG CGG TTA TAC GAT TTT TGG AGT GGA ACC CTT GGG TAC TAC TTT GAC TAC</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>3-21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>D3-10</td>
<td>JH6B1</td>
<td>GAG CGG TTA TAC GAT TTT TGG AGT GGA ACC CTT GGG TAC TAC TTT GAC TAC</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>3-30</td>
<td>D6-19</td>
<td>JH6B1</td>
<td>GAT GAG AGG GCG GGA GTA ACT GSG GAC AAC TAC TAC TAC TAC GGT ATG GAC GTC</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>3-30</td>
<td>D6-19</td>
<td>JH6B1</td>
<td>GAT GAG AGG GCG GGA GTA ACT GSG GAC AAC TAC TAC TAC TAC GGT ATG GAC GTC</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>3-30</td>
<td>D6-19</td>
<td>JH6B1</td>
<td>GAT GAG AGG GCG GGA GTA ACT GSG GAC AAC TAC TAC TAC TAC GGT ATG GAC GTC</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>3-30</td>
<td>D6-19</td>
<td>JH6B1</td>
<td>GAT GAG AGG GCG GGA GTA ACT GSG GAC AAC TAC TAC TAC TAC GGT ATG GAC GTC</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Where present, nucleotides from DH genes are underlined. All sequences have been submitted to the European Molecular Biology Laboratory Data Library under accession nos. AJ519219–AJ519339. Joint nos. 6–9 are identified as ANBPM229, ANBPM233, ANBPM230, and ANBPM234, respectively.

<sup>b</sup> V<sub>H</sub> genes have been previously described in HBcCP responses (31, 34, 35).

<sup>c</sup> Could also be DK5-5/DK4.

<sup>d</sup> Could also be D1-7 or D1-20.

<sup>e</sup> Fisher's exact test, the x<sup>2</sup>-test, and the Kruskal-Wallis test. In all analyses, two-sided p values of <0.05 were considered statistically significant.
Somatic hypermutations

All sequences were compared with the germline VH gene with highest homology in the GenBank database, and differences were considered to represent somatically acquired hypermutations. By these criteria, 119 of 121 sequences were mutated with a median prevalence of mutations of 5.1% (range, 0.7–7.8%) (codons 1–94). For comparison, the estimated prevalence of RT PCR errors was 0.1% (see Materials and Methods). For the predominant VH43, identity between the published sequence and the donor germline sequence was confirmed by sequencing of 14 cloned germline PCR products generated by use of Vp23-specific primers recognizing areas of FR1 and FR3 shared by the published germline gene (data not shown). Thus, substitutions of the 103 canonical sequences had to be somatically acquired and therefore potentially applicable as clonal markers.

Definition of clonality

Two DNA sequences were considered clonally related if they derived from two B cells descending from the same precursor cell in which a functional H chain gene rearrangement had occurred. The first criterion for clonal relationship was the requirement of identical VDJ rearrangements. The second criterion was sharing of a number of mutations not explainable by chance or selection by Ag. To establish safe limits for the numbers of shared mutations defining a clone, a sample of published HibCP-specific, canonical DNA sequences (31, 32, 34, 35) and five sequences from this study representing five different canonical joints were analyzed for mutations shared by chance. Among the 17 unrelated sequences, <5% of randomly selected pairs shared five or more identical mutations in the VH gene, and no pair shared more than six mutations (Fig. 2). A provisory requirement of at least eight shared mutations was used to define a clone in this report. In Fig. 2, the sequences of the five canonical joints were analyzed by pairwise comparisons of shared somatic mutations. It is evident that several sequences within each type of joint shared many more mutations than explainable by chance and thus had to be clonally related.

Clonal analysis

Fig. 3 shows a cluster analysis for the 103 canonical sequences based on the neighbor-joining method. Fifteen clusters encompassing 80 (78%) of the sequences are indicated, in which all sequences fulfill the second criterion of clonality, i.e., share at least eight mutations with all the other sequences in the cluster. Because the genealogical tree was exclusively based on the VH gene sequences (codons 1–94), codons possibly shaped by the rearrangement process (i.e., codon 95) and the JH minigene-encoded codons 96–113 could be used to test the reliability of the clustering. For 12 of 15 clusters, only sequences with identical codon 95 and JH gene had been allocated to each cluster. For the six major clusters where statistical analysis was meaningful (n > 4), all differed significantly from expectations if sequences were allocated randomly (Fig. 3; p < 0.03). In cluster C, however, one sequence (LIBPM261) used another JH gene (joint 1.1 rather than 2) indicating erroneously allocation to this cluster by chance. Alternatively, this sequence could have arisen by a PCR or cloning crossing-over artifact. This sequence was not considered truly clonally related to the other sequences of that cluster, and the sequence is omitted in Table IV. The four sequences of cluster A using joint 1.3 rather than 1.1 (ANBPM234, AN2PG497, ANBP2G58, and AN2PG444), however, are readily explained by a single mutation event in codon 95 in a precursor B cell in the genealogical tree and therefore likely clonal members of the cluster despite the difference in codon 95 (Figs. 3 and 4). Similarly, a single mutation event may explain the presence of a sequence with joint 1.1 (ANBP2G289) in clone F. In all, these data strongly validate the identification by this algorithm of 15 clonal progenies comprising 79 canonical sequences.

FIGURE 2. Shared somatic mutations. Shared somatic mutations among canonical HibCP-specific VH3–23 sequences (codons 1–94) analyzed by comparison of all possible pairs of sequences and enumeration of the number of pairs that shared a defined number of mutations. Sequences from donor and recipient were pooled and divided according to the five joints representing different glycine codons in position 95 and JH gene (Table III). Shared mutations among 17 clonally unrelated HibCP-specific canonical VH3–22 sequences (codons 1–94) are shown as control for random sharing of mutations. The presence within each joint group of several sequences sharing many more mutations than explained by chance indicates clonal relationships among the cells from which the sequences were derived.
FIGURE 3. Legend continues
Table IV. Isotype of 90 (of 121) mRNA sequences that could safely be allocated to definitive clones (A–P) and their origin from donor (Do) or from the first (Rec1°) or second (Rec2°) vaccination of the recipient

<table>
<thead>
<tr>
<th>Clone</th>
<th>Joint</th>
<th>Donor (Do)</th>
<th>Rec1°</th>
<th>Rec2°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canonical clones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.1: GCC-JH6B1</td>
<td>M, M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2: GCC-JH4B1</td>
<td>M, A1, A1, A1, A2</td>
<td>A2</td>
<td>G2</td>
</tr>
<tr>
<td>D</td>
<td>1.2: GGT-JH6B1</td>
<td>M</td>
<td>M, M</td>
<td>G2, G2</td>
</tr>
<tr>
<td>E</td>
<td>1.1: GCC-JH6B1</td>
<td>M</td>
<td>A1</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1.1: GCC-JH6B1</td>
<td>M, M</td>
<td>M, M</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>1.4: GGA-JH6B1</td>
<td>M, M, A1, A1</td>
<td>G1, G1</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.1: GCC-JH6B1</td>
<td></td>
<td>G2, G2, G2, G2</td>
<td>G1, G2</td>
</tr>
<tr>
<td>J</td>
<td>1.4: GGA-JH6B1</td>
<td></td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>K</td>
<td>1.1: GCC-JH6B1</td>
<td>A2</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>1.1: GCC-JH6B1</td>
<td>M, G1</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.1: GCC-JH6B1</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.3: GGG-JH6B1</td>
<td>M</td>
<td>A2</td>
<td>A1</td>
</tr>
<tr>
<td>O</td>
<td>1.1: GCC-JH6B1</td>
<td>M, M</td>
<td>M, G1</td>
<td></td>
</tr>
<tr>
<td>Noncanonical clone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>3:3-49-JH3B1</td>
<td>A2</td>
<td>M, M, M, M, G1, G1, G1, A1, A1</td>
<td></td>
</tr>
</tbody>
</table>

* Isotypes and subclasses: M, IgM; G1, IgG1; G2, IgG2; A1, IgA1; A2, IgA2.

Table IV shows the isotypes and origins of the 79 canonical sequences allocated to the 15 identified clones. The clones consisted of from 2 to 20 DNA sequences each (median, 4). The median number of mutations shared by all members of a single clone was 9 (range, 8–14), but some sequences shared up to 23 mutations. Although most mutations tended to be concentrated in certain areas of the gene (hot spots) (see Fig. 4), the clones clearly differed with respect to mutational patterns (clonal markers).

A similar analysis could be performed for the noncanonical sequences. Fig. 5 shows a genealogy tree for the 14 3-49-derived sequences. One cluster of 11 sequences (clone P) fulfills the second criterion of clonality. Unlike the remaining three sequences, the 11 sequences share the same CDR3 sequence and the JH gene: JH6B1. Furthermore, they share a median of 16 (range, 13–16) mutations with each other. Thus, these 11 sequences are definitively clonal. The remaining four noncanonical sequences using Vγ genes other than 3-49 (ANBP2M229, ANBP2M223, ANBP2M230, and ANBP2PM454) had different joints (joint nos. 6–9 (Table III), respectively) and were therefore clonally unrelated.

In all, 90 sequences of the 121 DNA sequences (74%) could be assigned to 16 clonal progenies (Table IV).

Transfer of memory clones by BMT

After definition of the clones, the contributions from the two individuals could be analyzed. The amplified part of the donor’s response was largely limited to 12 clones representing at the most the progeny of 12 precursor B cells (Table IV). Interestingly, representatives from all of these clones could be retrieved in the recipient (Table IV) constituting 27 sequences (61%) from the first experimental vaccination of the recipient (Rec1°) and 25 sequences (68%) from the second (Rec2°). Within most clones, memory cells of the IgM isotype were also transferred as evident from the finding of IgM transcripts in the recipient sharing clonal markers with transcripts from the donor. In addition, four clones were obtained from the recipient without representatives in the donor, and three new clones were obtained after the first and one after the second experimental immunizations. Nevertheless, >64% of the Vγ3 family gene sequences used by the recipient for the anti-HibCP responses were clearly derived from mutated (i.e., memory) B cells transferred from the donor.

Clonal diversification before and after transfer

The numbers of mutations were higher for canonical IgA and IgG sequences (medians of 16 (5.5%) and 17 (5.7%) mutations in codons 1–113 (345 bases), respectively) compared with IgM sequences (median, 11.5 (3.8%); p < 0.002). Because the isotype distribution varied with the vaccination situation, switched and unswitched cells were studied separately in relation to the three experimental vaccinations. Interestingly, no significant differences were seen in the number of somatic hypermutations after the three experimental immunizations, neither for unswitched sequences (medians of 12, 11, and 17 for Do, Rec1°, and Rec2°, respectively; p = 0.60; n = 49; Kruskal-Wallis test), nor for switched sequences (medians of 15, 16, and 18; p = 0.17; n = 72). This was also the case when only clonally related sequences were studied (medians of 13, 15, and 17 for unswitched; p = 0.10; n = 31; and 17, 16, and 20; p = 0.12; n = 59) and also when the analysis was restricted to clones with representatives in both donor and recipient

FIGURE 3. Cluster analysis of canonical sequences. Dendrogram demonstrating a cluster analysis based on the neighbor joining method for the 103 vaccine-induced HibCP-specific canonical sequences (pooled data from all three vaccination situations). The germline Vγ3-23 sequence (codon 1–94) was used as root. Clusters in which all sequences shared eight or more mutations with all other members of the cluster are indicated by gray background and presumed to contain clonally related sequences only and are designated clones A–O. The five different joints are indicated by the following symbols: ♦, joint 1.1 (GGG-Jp6B1); ♦, joint 1.2 (GGT-Jp6B1); ○, joint 1.3 (GGG-Jp6B1); □, joint 1.4 (GGA-Jp6B1); ○, joint 2 (GGC-Jp6B1). The validity of the clone assignment was assured by the preferential allocation of sequences with the same joint to each clone and tested statistically against random allocation (see Materials and Methods). *, This sequence was not selected to be a member of clone J, because it shared eight mutations with some but not all of the sequences in the clone.
FIGURE 4. (Legend continues)
were any signiﬁcant sequences had identical combined with the paucity of unique mutations. In fact, 5 of the silent mutations was noted either (acid-replacing mutations, because no difference in the prevalence 0.17). These results were not due to negative selection of amino.

First post-BMT immunization. However, this apparent repertoire 11 sequences seen after the last immunization and none after the indication of sequential acquirement of mutations from vaccinia.

Sequences from clone A. Entire variable domain (codons 1–113) of 20 clonally related vaccine-induced HibCP-speciﬁc cDNA sequences from clone A (joints 1.1 and 1.3). Dots mean identity with the donor, strongly indicating that most if not all mutations in clone P had occurred in the donor. Similar observations were made for clones C, F, G, K, N, and O (Fig. 6), which all contain donor-derived sequences with high numbers of mutations similar to or even outnumbering the mutations in recipient-derived sequences. In the case of clone F, the two most heavily mutated sequences were of donor and recipient origin, respectively, but shared all mutations, proving that the B cell providing the sequence ANBPG284 did not mutate in the recipient.

Inﬂuence on the general Ab repertoire of the recipient

The finding of >33% of the IgSC being of HibCP speciﬁcity in the transferred bone marrow raised the question as to whether the general Ab repertoire of the recipient was skewed after the transplantation. Because almost all of the HibCP-speciﬁc cells used the canonical V3-23 gene, transcripts of this gene were ampliﬁed using H chain-speciﬁc downstream primers followed by ﬂuorochrome-coupled fragment analysis to reveal the CDR3 length spectrum of the molecules. Fig. 7 reveals that the expressed IgG V3-23 repertoire was almost as diverse after transplantation as before (a–e), and that canonical size transcripts found among HibCP-speciﬁc cells from the donor and recipient (g and h) constituted only minor fractions (if any) of the repertoire at these time points despite the two restimulations with Ag. Similar results were obtained for IgM and IgA (data not shown) with the exception that the IgM repertoires were bell-shaped polyclonal at all time points (example given in Fig. 7f). In conclusion, although the transferred cells had a dramatic effect on the HibCP-speciﬁc Ab repertoire, little if any effect was detected on the general Ab repertoire.

Discussion

The normal bone marrow plays a central role in the maintenance of humoral immunity. Not only is it the principal site of B cell lymphopoiesis after birth (36); it is also a source of high-afﬁnity Abs protecting the individual from reinfection for years. After infections like polio, yellow fever, smallpox, and measles, humans may be protected by Abs for most of their lives (37–41). How the immune system manages this remarkable task is not understood. Until recently, it was generally assumed that plasma cells were short lived with life spans of a few days or perhaps weeks (42). However, new studies in mice indicate that individual plasma cells of the bone marrow may live and produce Ab for at least a year after immunization, and do so independently of the persistence of Ag (37, 43, 44). Although these plasma cells may be transferred to recipient mice by BMT, the generated Ab levels are, however, low, suggesting that long-lived plasma cells either fail to home to the proper compartment or need signals for their activity only provided by the immune host. Other murine studies fail to detect long-lived plasma cells (45). In these studies, continued production of Ab depends on the persistence of Ag (and T cell help), and involves continued recruitment of new plasma cells by activation and differentiation of memory B cells. The basis for these discrepancies is not clear, but it may be related to differences in the Ags used (replicating vs nonreplicating Ags).

The lifetime of a human bone marrow plasma cell is yet unknown. Unlike the mouse, humans depend on the production of Abs for decades, and it is possible that recruitment of new plasma cells from the memory compartment or even de novo may be needed to obtain lifelong protection by Abs. After birth, an increasing number of Ag-experienced, somatically hypermutated

(i.e., exclusion of clones I, J, L, and M; p = 0.19 and 0.14). Neither were any signiﬁcant differences found when members of the largest clones were studied separately (Fig. 6, clones A and F; p > 0.17). These results were not due to negative selection of amino acid-replacing mutations, because no difference in the prevalence of silent mutations was noted either (p = 0.67 and 0.65 for switched and unswitched canonical sequences; data not shown).

Also, the shape of the genealogical trees (Fig. 6) are compatible with little if any somatic diversiﬁcation (and selection) between the immunizations. Thus, sequences from the three vaccinations appear more or less randomly distributed in the trees with no clear indication of sequential acquisition of mutations from vaccination to vaccination. Of the 121 studied sequences, only four pairs of sequences with identical VDJ sequences were found (clone D, AN2PG481 (G2) and AN2PG443 (G2); clone F, LIBPG316 (G2) and ANBP284 (G2); clone H, LIBPA232 (A1) and LIBPA214 (A1); and clone I, AN2PG568 (G1) and AN2PG489 (G2)). In clones J and P, 3 and 5 sequences with identical VDJ sequences were found, respectively. Clone P is somewhat atypical, with 10 of 11 sequences seen after the last immunization and none after the ﬁrst post-BMT immunization. However, this apparent repertoire shift is likely to be a sudden ampliﬁcation of a pre-existing, mutated clone rather than a sign of continued intraclonal diversiﬁcation. This is judged by the high number of shared mutations (14) combined with the paucity of unique mutations. In fact, 5 of the sequences had identical VDJ sequences. Moreover, 13 of the 14 shared mutations were also found in the sequence LIBPA231, which was isolated from the donor, strongly indicating that most if not all mutations in clone P had occurred in the donor. Similar observations were made for clones C, F, G, K, N, and O (Fig. 6),

FIGURE 4. Sequences from clone A. Entire variable domain (codons 1–113) of 20 clonally related vaccine-induced HibCP-speciﬁc cDNA sequences from clone A (joints 1.1 and 1.3). Dots mean identity with the V3-23 germline sequence given above. Capital and lowercase letters indicate amino acid replacing and silent substitutions, respectively. N indicates nucleotides probably added by N addition. Sequence name, isotype, and origin from the donor (Do) or from the recipient after the ﬁrst (Rec1°) or second vaccination (Rec2°) are indicated on the left.

FIGURE 5. Cluster analysis of noncanonical 3-49RB sequences. Denrogram demonstrating a clonal relationship between 11 of the 14 vaccine-induced HibCP-speciﬁc 3-49RB sequences in clone P (shaded area). The germline 3-49RB sequence (codon 1–94) was used as root. The three different joints among the sequences are indicated as follows: ○, joint 3 (3-49RB; D1-1; J3; G1); □, joint 4 (3-49RB; D3-10; J4; G1); ○, joint 5 (3-49RB; D3-3; J4; G1). The validity of the clone assignment was assured by the preferential allocation of sequences with the same joint to the clone and tested statistically against random allocation (see Materials and Methods).
FIGURE 6. Legend continues
Marrow, but in lower numbers. It is possible that memory cells

FIGURE 6. Dendrograms of 16 identified clonal progenies (clones A–P) showing the clonal evolution, isotypes, and relation to vaccination episodes. The number of mutations in each sequence (codons 1–113) is indicated at the bottom of the figure. Sequences from the donor are named LIBP (○, □); from the first immunization of the recipient, Rec1*, are named ANBP (□, □); and from Rec2* are named AN2P (●, □). The next letter indicates the isotype: M, IgM; G, IgG; and A, IgA. In addition, every sequence is given a particular number. ○, □, ●, IgM sequences. □, □, ■, IgA and IgG sequences.

FIGURE 7. CDR3 length spectra before and after BMT. The applied

IgM + IgD + CD10 + memory B cells are found in the human bone

marrow (46). Isotype-switched memory B cells are also found in

the marrow, but in lower numbers. It is possible that memory cells

constitute a source of plasma cells, and it has been proposed that

they divide in a self-renewing (stem cell-like) way, allowing con-

tinued production of plasma cells without exhausting the memory

pool (47). Recent data from Bernasconi et al. (48) support this

hypothesis by showing that human memory B cells respond to

polyclonal stimuli by differentiating into plasma cells in vitro. This

sets focus on the memory B cells present in the bone marrow as an

indirect source of long-term Ab production in humans.

Memory B cells are resting but Ag-experienced B cells (49)

characterized by the expression of CD27 and the presence of

somatic hypermutations in the rearranged genes (VDJ and VJ)

coder the H and the L chains of their Ag receptors, respectively.

They constitute 40% of circulating B cells in adults and may be

divided into three populations by expression of surface Ig:

IgM + IgD + (15%), IgM + IgD − (10%), and isotype-switched

IgM + IgD − (15%) (50). Mutations are abundant and acquired by

largely stochastic processes (51–53). The primary site of somatic

hypermutation is in the germinal center in which memory cells are

generated in response to thymus-dependent Ags (54). Other sites

of mutation probably exist, because memory B cells may also be

generated in knockout mice lacking germinal centers (55). Humans

lacking germinal centers due to CD40 ligand deficiency also pro-

duce somatically mutated B cells (56). Mutations are inherited by

the cellular offspring and therefore may be used as clonal markers

suitable for tracking of memory cells and their progeny in a BMT

context. However, clonal tracking is only technically feasible if the

B cell response is oligo- or monoclonal.

In this study, we exploit the well-characterized and genetically

restricted Ab response to the capsular polysaccharide from the

important childhood pathogen Hib (31, 57, 58) to perform clonal

tracking of memory B cells. We clearly demonstrate that after

recent immunization of the donor—at a time point where numer-

ous vaccine-specific plasmablasts are present in the marrow—the

graft contains Ag-experienced and somatically hypermutated B

cells (i.e., memory B cells) capable of persisting in the recipient for

a prolonged time (at least 9 mo). This persistence was not accom-

panied by significant production of Ab in the recipient, because Ab

levels were low at 6 mo after BMT. Nevertheless, memory B cells

clearly resided in the body in positions and numbers allowing them
to constitute a major part of the responding population at subse-

quent immunizations. In fact, 61% of V H 3 family transcripts
cloned after the first post-MT immunization of the recipient rep-

resented B cell clones present in the transplant. For the remaining

39%, it is unknown whether they originated from donor memory

cells, but some of them may have done so, because minor clones

for statistical reasons may have been overlooked among the donor-
derived sequences. These proportions are probably representative

for the overall response, because virtually all Abs to HibCP use

V H 3 genes in normal individuals (31, 34, 57, 59). However, it

cannot be excluded that HibCP-specific B cells induced after BMT

might differ by including B cells using V H 3 genes from families

other than V H 3. Whereas about one-half of the general population

of B cells in normal adults use V H genes from the V H 3 family (51,

60), this family is less represented shortly after BMT (61, 62).

However, the use of V H 3 genes catches up and reaches the normal

level in the first year after BMT (61, 63). It is therefore unlikely

that the recipient lacked sufficient naive cells using V H 3 genes at

the time of vaccination (9 and 11 mo post-BMT), and indeed Fig.
7 documents polyclonal involvement of B cells using the canonical V_{H}3-23 gene at all time points before and after BMT.

Interestingly, the donor-derived cells retained or reacquired the memory phenotype after the first post-BMT immunization of the recipient, because 66% of the mRNA cloned after the second post-BMT vaccination could be tracked to the donor. It is noteworthy that even the apparent repertoire shift represented by the appearance of 3-49RB-derived clones after the second posttransplant immunization (Table IV) could be tracked to the donor and must represent a selective expansion of the progeny of memory B cells present in the donor before transplantation. The clonal relationship between these sequences was unequivocally determined by the sharing of 12 nucleotide substitutions in the between these sequences was unequivocally determined by the sharing of 12 nucleotide substitutions in the V_{H} region and a unique 17-bp N addition between the V_{H} and the D_{H} gene. It is interesting that the donor-derived sequence was isotype switched to the most downstream C_{H} gene (Ca2), from which no further isotype switching is possible, whereas the recipient-derived sequences comprised unswitched sequences and sequences switched to upstream C_{H} genes (C_{H}1 and C_{H}4). This clearly shows that at least some of the transferred memory B cells must have been unswitched, and that some of them refrained from isotype switching even after two rounds of antigenic stimulation. The same pattern was seen in five of the canonical clones (clones A, D, G, K, M, and P; Table IV). This is compatible with the idea of a stem cell-like phenotype of memory B cells allowing them to stay undifferentiated (and unswitched) and yet generate isotype-switched offspring (47). The IgM-IgD^−CD10^- memory B cells could be candidates for such a function, because they are preponderant in the human bone marrow (46) and retain the capacity to switch isotype unlike IgM-only and isotype-switched memory B cells, as demonstrated in vitro (64).

Another question is whether hypermutated B cell progeny may re-enter the process of somatic hypermutation when rechallenged with Ag. The fact that B cells are sometimes found with mutation prevalences exceeding by far the normal level of ~5% mutated nucleotides in the VDJ region could point to such a possibility (65), but so far, no direct evidence for this has been given. The finding in mice that the number of mutations (and affinity) sometimes increases after reimmunization (66) is not found in other experimental systems (67) and could alternatively be explained by selection of rare variants with many mutations generated during the primary immune response. In adoptive transfer experiments in mice, continued mutation of memory B cells was not seen (68, 69), and the present study finds the same in this donor-recipient pair. Thus, mutation prevalences were ~5% among cloned transcripts from Ag-purified isotype-switched HibCP-specific cells in the donor, which is close to the general average of transcripts from the peripheral blood (51), and these values did not increase significantly after the first or even the second immunization of the recipient (Fig. 6). Also, when the genealogical tree structures for the individual clones were examined, they were found to be compatible with expansion without mutation in the recipient, and in some clones, individual donor-derived sequences contained as many mutations as the most mutated sequences derived from the recipient. This indicates that memory B cells transferred by BMT are unable to increase their affinity or to participate in other immune responses where further somatic hypermutation would be required. This could contribute to the humoral immunodeficiency following BMT. However, it should be noted that the experimental design may have overlooked a low level of somatic hypermutation in the recipient for statistical reasons. Moreover, RT-PCR favors detection of circulating cells rich in mRNA like plasmablasts and Ab-forming cells, whereas resting memory cells may have been overlooked either because of less mRNA content or because they were not circulating on postvaccination day 7 where blood samples were taken. However, memory cells mutated after the first immunization of the recipient should reveal themselves by seeding hypermutated progenies into the circulation as Ab-forming cells after the second immunization which was apparently not the case. RT and Taq errors were calculated to one mutation per 1200 bp, which corresponds to 0.2 per VDJ sequence (~4% of all mutations). Because these errors affect donor and recipient sequences equally, this has no consequences for the conclusions concerning the reinduction of mutations in the recipient. Because these errors for statistical reasons are unique for each affected sequence, they will not affect the definition of clones, which is based on shared mutations irrespective of unshared ones. For the same reason, the RT-PCR errors will only reveal themselves terminally in the tree where the terminal branch will increase by one mutation in approximately one in five branches. This has no influence on the conclusions in this study.

However, failure to mutate after BMT does not prove that memory B cells under normal conditions are unable to re-enter the process of somatic hypermutation. The failure could relate to the BMT procedure itself. Thus, it is possible that the ability to re-enter somatic hypermutation is confined to a subpopulation of cells not present in the graft or restricted to compartments that adaptively transferred cells do not reach. Another possibility is that the BMT procedure induces an intrinsic deficiency of somatic hypermutation in B cells in general as suggested by Suzuki et al. (63) and recently by in vitro studies by Glas et al. (70). Finally, the failure to mutate in the present study could be related to the lack of CD18, which may not be fully reconstituted by BMT in all compartments of the immune system in this recipient. However, we find this unlikely, because somatic hypermutation was demonstrated in the recipient before BMT showing that CD18 is not obligatory for this process (data not shown).

This study adds evidence to that provided by other groups (12, 13, 18, 19) indicating that BMT recipients may benefit from immunization of their donors, but optimal immunization schedules concerning both the donor and the recipient must be studied in the context of the relevant vaccine. It is unknown whether immunization before marrow harvest is mandatory for transfer of memory cells. The close time relationship between the immunization of the donor and the BMT in our study could have facilitated the efficient transfer of HibCP-specific memory B cells, because one-third of the IgSc in the bone marrow were HibCP specific at that time point. This might raise the concern that recent immunization of the donor could skew the repertoire of the recipient to a similar extent. However, this was not the case in this study, in which we find broadly distributed CDR3 length spectra of which the canonical (HibCP-specific) rearrangement constituted only a few percent—at most—of the V_{H}3-23 repertoire.

It is concluded that, after recent immunization of the donor, memory B cells may be transferred to the recipient, stay there for at least 9 mo, and constitute the major source of cells responding to vaccination of the recipient without perturbing the general repertoire of the recipient. At least some of the transferred cells are not isotype switched (but Ag experienced and hypermutated) and may stay unswitched despite antigenic stimulation in the recipient. Transferred memory acquired little if any somatic hypermutations in this setting.

References


