Antibody Repertoires of Four- and Five-Feature Translocus Mice Carrying Human Immunoglobulin Heavy Chain and κ and λ Light Chain Yeast Artificial Chromosomes

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We have produced mice that carry the human Ig heavy (IgH) and both κ and λ light chain transloci in a background in which the endogenous IgH and κ loci have been inactivated. The B lymphocyte population in these translocus mice is restored to about one-third of normal levels, with preferential (3:1) expression of human λ over human κ. Human IgM is found in the serum at levels between 50 and 400 μg/ml and is elevated following immunization. This primary human Ab repertoire is sufficient to yield diverse Ag-specific responses as judged by analysis of mAbs. The use of Dκ and J segments is similar to that seen in human B cells, with an analogous pattern of N nucleotide insertion. Maturation of the response is accompanied by somatic hypermutation, which is particularly effective in the light chain transloci. These mice therefore allow the production of Ag-specific repertoires of both IgM,κ and IgM,λ Abs and should prove useful for the production of human mAbs for clinical use. The Journal of Immunology, 1999, 163: 6898–6906.

The effective clinical application of mAb in the treatment of disease is limited by the development of an anti-Ig response in the patient. The response to foreign mAbs can be minimized by the engineering of chimeric molecules that replace the structural domains with equivalent regions from human Abs and retain only the Ag binding domains of the rodent Ab (1, 2). The disadvantage of this “humanization” process is that it must be repeated for each new Ab, placing practical limitations on the variety of target molecules that can be developed for therapy. Synthetic repertoires of human Ab fragments have been developed using combinatorial phage libraries (3); however, Ag-specific isolates from these may require further manipulation before they can provide the functional activity of mAbs from conventional hybridomas.

The alternative to these artificial libraries is to create transgenic animals carrying human Ig loci in germline configuration in the presence of defective endogenous Ig loci. The aim is that the introduced human loci function instead of the mouse loci and are rearranged and expressed to produce a fully human Ab repertoire that can be exploited to yield human mAbs of desired specificities. Early efforts using plasmid-based mini loci have revealed the feasibility of the approach, although the structural diversity of the human Ab repertoires produced is restricted owing to the limited number of germline V gene segments included in these miniloci (4–7). More recent experiments using yeast artificial chromosome (YAC) technology have demonstrated that large regions of human DNA can be introduced into the mouse germline, allowing the production of human Ab repertoires that derive from increased number of germline V gene segments (8–13). To date, such repertoires of wholly human Abs are only composed of human IgHk Abs. However, in humans (as opposed to mice) a large component of the functional Ab repertoire is provided by the Igλ locus, with Igλ-containing Abs accounting for almost half the serum Ig (14). Here we describe the production of mice that carry YAC-based human IgH, human Igκ, and human Igλ transloci in a background in which endogenous mouse IgH and Igκ chain expression has been inactivated. The introduced human transloci function to yield a fully human Ab repertoire that can be exploited to produce human mAbs of desired specificities.

Materials and Methods

Production of human Ig transgenic mice

The production of transgenic mice containing either the HuIgκ YAC or the HuIgλ YAC has been described previously (9, 14). The HuIgκ YAC (12, 13) was modified so as to incorporate two copies of a neomycin resistance cassette in the acentric YAC arm using homologous recombination in yeast as previously described (15). The modified YAC was transferred into HM-1 ES cells (16) by protoplast fusion (15), and a clone carrying a complete copy of the Igκ YAC, determined by PCR and Southern blot (data not

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shown), was used to derive mice following injection into BALB/c blastocysts and implantation into foster animals (8, 17). Mice with their endogenous H chain or κ L chain loci rendered nonfunctional have been described previously. The μMT-/- modification (18) insert s a stop codon and neo cassette into the membrane exon of the IgM C region, preventing the surface Ig expression during B cell development, while the MoGκ-/- modification (19) disrupts Igκ expression by the insertion of a neo cassette in the κ C region. Transgenic animals were crossed with μMT-/- and MoGκ-/- animals to produce mice expressing human IgM,κ Ab (referred to as five-feature mice). The transgenic status of the offspring was confirmed by Southern hybridization of genomic DNA with probes for the following: human IgM C region exons 1, 2, and 3; the human κ C region; and the human λ3 C region.

Analysis of expression of human Ab proteins

Flow cytometric analysis of translocus-derived Ig expression on the surface of spleen cells was performed using standard techniques. B lymphocytes were identified using B220-APC (PharMingen, San Diego, CA). Human IgM was detected using anti-human Igμ-PE (PharMingen). Human Igκ was detected using biotinylated anti-Igκ (Zymed, South San Francisco, CA) followed by streptavidin-PerCP (Becton Dickinson, Mountain View, CA), and human Igλ was detected using anti-Igλ-FITC (Sigma-Aldrich, Poole, U.K.). Mouse Igκ was detected using anti-mouse Igκ-FITC (PharMingen). Stained cells were analyzed with a FACS Calibur flow cytometer (Becton Dickinson). Data were collected on 100,000 events and were analyzed using CellQuest software (Becton Dickinson). For sorting, Peyer’s patch cells were isolated, and germinal center B cells were stained with B220-APC (PharMingen). Stained cells were analyzed with a FACSCalibur flow cytometer (Becton Dickinson). Data were collected on 100,000 events and were analyzed using CellQuest software (Becton Dickinson).

RT-PCR and sequencing of Ig rearrangements

Total cytoplasmic RNA was isolated from hybridoma cells or sorted Pey er’s patch cells using TriPure reagent (Boehringer Mannheim U.K., Lewes, U.K.), following the manufacturer’s instructions, and reverse transcribed using oligo(dT)₁₂, and Superscript II Reverse Transcriptase (Life Technologies, Paisley, U.K.). The primers used for PCR amplification of rearranged Ig genes are listed in Table I. Rearranged human Ig H chains were amplified using the family-specific leader primers and the IgM Constant primer (20), with separate reactions set up for each leader primer. Rearranged Igκ genes were amplified using the Vκ primer and the κ C primer (21). Rearranged λ genes were amplified using the VA2 and VA3 in separate reactions with the AC primer (14). The amplified rearrangements were purified using the QIAquick Prep System and cloned into the pGem-T vector (Promega U.K., Southampton, U.K.). Recombinant colonies were screened for appropriate rearrangements by PCR using primers to the respective framework 1 and framework 4 regions (Table I). Plasmid was isolated and sequenced using standard M13 forward or reverse sequencing primers. Sequence data were aligned to the germline V, D, and J region sequences known to be on the YAC constructs using MacVector software (Oxford Molecular, Oxford, U.K.), and the sequences used by the rearrangement and any point mutations present were identified. The nomenclature for the various gene segments follows a family- and position-based scheme, as described in the IMGT database (22).

Results

Production of human Ig transgenic mice

The three different transloci are illustrated in Fig. 1. For the human heavy chain translocus, we used a 240-kb YAC (HulHg) that contains the core region of the human IgH locus comprising five VХ regions, and the complete DХ and JХ loci linked to Cμ-δ in correct germline configuration; the isolation and characterization of this YAC have been previously described (13). The HulHg YAC was modified by insertion of a neomycin resistance gene into the acentric YAC arm and introduced into embryonic stem cells using the protoplast fusion technique as described in Materials and Methods. Mice carrying the HulHg and Hulλ3 transloci have been previously described (9, 14). The 1.3-Mb Hulλ3 YAC (9) contains a complement of 103 Vκ region gene segments comprising 20

### Table I. Primers for amplification of human Ig gene rearrangements from translocus mice

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Primers</th>
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</thead>
<tbody>
<tr>
<td>5'-ATG GAC TGG ACC TGG AG-3'</td>
<td>VХ leader</td>
</tr>
<tr>
<td>5'-ATG GAC ATA CT TGT TCC ACC C-3'</td>
<td>VХ leader.1</td>
</tr>
<tr>
<td>5'-ATG GAC ACA TTT TGC TCC ACC C-3'</td>
<td>VХ leader.2</td>
</tr>
<tr>
<td>5'-ATG AAA CAC CTG TGG TTC TTC-3'</td>
<td>VХ leader.3</td>
</tr>
<tr>
<td>5'-ATG TCT GTC TTC TTC ATC ATC-3'</td>
<td>VХ leader.4</td>
</tr>
<tr>
<td>5'-CGT ATC CGA CGG GGA ATT CTC ACA-3'</td>
<td>VХ leader.5</td>
</tr>
<tr>
<td>5'-GAG GTG (AC)(AG) CTG CAG (CG)AG ATC(TAT) GGA-3'</td>
<td>VХ leader.6</td>
</tr>
<tr>
<td>5'-AGT GAC CAG CAG ACC TGG GCC CCA G-3'</td>
<td>VХ leader.7</td>
</tr>
<tr>
<td>5'-GA(AC) A(CT) (CT) GAG TTC ACC CAG CTT CCA-3'</td>
<td>VХ leader.8</td>
</tr>
<tr>
<td>5'-CGG GAA GAT GAC AGA TGG TGC-3'</td>
<td>VХ leader.9</td>
</tr>
<tr>
<td>5'-G TTT GAT CTC CAG CTT GGG CCC-3'</td>
<td>VХ leader.10</td>
</tr>
<tr>
<td>5'-CAG TCT GCC CTG ACT CAG CCT-3'</td>
<td>VХ leader.11</td>
</tr>
<tr>
<td>5'-TCC TAT GAG CTT AC(AT) CAG-3'</td>
<td>VХ leader.12</td>
</tr>
<tr>
<td>5'-CG TGT GGC CTT GTG GCC T-3'</td>
<td>VХ leader.13</td>
</tr>
<tr>
<td>5'-TAG GAC GGT (CG)(AG) CTT GGG CCC-3'</td>
<td>VХ leader.14</td>
</tr>
</tbody>
</table>
repeats of five V\kappa regions obtained from the cosmid cos106 (23), attached to the core of the germline locus, including three V\kappa regions, the complete J\kappa cluster, C\kappa, and the \kappa deleting element. One of the repeated V\kappa region segments (V\kappa2D-10) and one from the core region (V\kappa7-3) are pseudogenes, making a complement of 82 functional V\kappa segments in the locus. The 410-kb HuIg\kappa YAC (24) contains 28 VA region gene segments (with 16 functional segments) and the seven paired JA and CA segments (four of which are functional), in the correct germline configuration.

Four-feature human Ig-expressing mouse strains were derived by crossing of strains carrying the HuIg\kappa YAC and the \muMT^{-/-} knockout with mice carrying the HuIg\kappa YAC and the Mox^{-/-} knockout (9). The remaining HuIg\kappa translocus was introduced by crossing the four-feature mice with mice carrying the HuIg\kappa YAC and the Mox^{-/-} knockout (14), with care to retain the \muMT^{-/-} knockout. The strains were bred to carry two alleles of each of the transloci. Test breeding showed that the three transloci and two knockouts were not linked, indicating that the integration of the YACs was random and independent.

Cell surface and serum expression of human Igs

Flow cytometric analysis of spleen populations was conducted to test whether the human transloci were capable of rescuing B cell development in the \muMT^{-/-}, Mox^{-/-} background. The percentage of B lymphocytes (B220-positive) in the spleens of immunized four-feature and five-feature mice maintained in barrier conditions ranged from 5–25%; representative animals are shown in Fig. 2. This is compared with spleens from normal BALB/c mice, which contain ~40–45% B220-positive cells, and \muMT^{-/-} mice, which contain <2% B220-positive cells (Fig. 2A). This level of B cell reconstitution is equal to or greater than what has been reported for other human Ig mice (6, 11).

Human Ig\kappa L chain was expressed on the surface of 45–65% of B cells from spleens of four-feature animals, with 16–25% of B cells expressing mouse Ig\kappa, a \kappa:\lambda ratio of ~3:1 (Fig. 2B). In five-feature animals, 10–20% of B cells expressed human \kappa L chain, while 45–60% expressed human \lambda, a \kappa:\lambda ratio of 1:3 (Fig. 2B). The level of mouse Ig\kappa L cells in the five-feature animals was <5%. A small number of L chain double-positive spleen cells were detected, which probably means that (as described in normal mice (Ref. 14 and references therein)) there is a low degree of leakage in isotype exclusion at the light chain loci. Human peripheral B lymphocytes typically have a \kappa:\lambda ratio of 3:2, while in normal mice the ratio is typically 19:1. The high contribution of the HuIg\kappa YAC to the Ig repertoire was also seen in mice containing the HuIg\kappa YAC in the presence of a functional mouse Igk locus (up to 40% of the splenic B cells expressing human \kappa) and is in contrast to the low expression of the HuIg\kappa locus to the Ig repertoire when a functional mouse Ig\kappa locus is present (with up to 15% of B cells expressing human \kappa L chain on the cell surface) (9).

In the four- and five-feature mice, human IgM was present at between 50 and 400 \mug/ml (Fig. 3). The level varied between individual animals and tended to be higher in the five-feature animals, but was not correlated to the number of B cells present in the spleen in these animals. The increased serum IgM levels in five-feature mice may result from higher secretion by cells expressing human IgM, Ab due to the stronger transcriptional activity of the Ig\kappa 3' enhancer (25). Normal BALB/c mice maintained under pathogen-free conditions have serum IgM levels of ~500 \mug/ml and total IgG levels of ~400 \mug/ml (26). The level of human IgM in the translocus mice is similar to what was found in mice made using plasmid-based minioloci (6, 27), although these smaller loci contain fewer germline segments to form a diverse repertoire. The distribution of human L chain in the serum of five-feature mice paralleled what was found on the surface of spleen B cells, with Ig\kappa levels 3- to 4-fold lower than human Ig\kappa levels (data not shown).

Immunizations and hybridoma production

Following immunization and boosts, the total serum IgM concentration was elevated, and specific Ab could be detected (Fig. 3). The titer of specific IgM produced was lower than that seen during parallel immunization of BALB/c mice (data not shown). Hybridomas secreting Ag-specific human IgM, \kappa Ab were obtained from four-feature mice following immunization with the Ags listed in Table II. In culture, the hybridomas secreted ~2 \mug of human IgM/ml of supernatant, compared with levels of 10 \mug/ml or more from mouse IgM hybridomas grown in parallel.
The distribution of L chain expression by clones obtained following immunization also paralleled the distribution on the surface of spleen cells. In one fusion from a four-feature animal, human Igκ was expressed by 14 of 16 hybridoma clones, with the remaining clones expressing mouse Igλ. In seven fusions from five-feature animals, more human Igλ-expressing clones were obtained on four occasions, with from 4- to 8-fold more human Igλ-expressing clones isolated. In the remaining fusions, the ratio of human Igκ to human Igλ-expressing clones was almost 1:1. Combined L chain expression from different loci, i.e., a combination of human κ with human λ or mouse λ L chains, was not observed.

Generation of diversity of Ig rearrangements

The H chain and L chain rearrangements from the specific hybridomas described above were sequenced to determine the diversity of the immune response. The H chains showed a very restricted use of the V region segments, dominated by VH 1-2, accompanied by a highly diverse CDR3 rearrangement (Table II and Fig. 4). The use of the L chain V segments was not so restricted, and this was coupled with the use of different J regions. The potential diversity of the response to a single Ag was shown by the several hybridomas that was in the correct reading frame to produce a functional Ab protein.

Further analysis confirmed this restriction in H chain variability. Of 41 H chain rearrangements, 36 used the VH 1-2 V gene, with VH 6-1 and VH 4-4 being found rarely. The other genes, VH 1-3 and VH 2-5, were not found in the sequences analyzed, and rearrangements using the VH 2-5 segment could not be detected by RT-PCR from splenic B cells (data not shown). For the H chain rearrangements, the source of repertoire variability is the CDR3 region formed by the selection of D and J segments and by the V-D and D-J junctions. The length of the CDR3 region ranged from 7–21 aa (Fig. 5), with a distribution similar to H chain rearrangements from human B lymphocytes (28). The use of H chain J segments is also similar to what is seen in human rearrangements, with the JH 4 (18 of 41) and JH 6 (19 of 41) segments being used most often. Nongermline-encoded N nucleotides were found in all rearrangements, with 26 of the 39 sequences containing D segments having insertions at both the V-D (average, 5.2 ± 2.9 bases) and D-J (average, 2.3 ± 2.7 bases) junctions. The number of N nucleotides added in the H

FIGURE 2. Flow cytometric analysis of B lymphocytes from spleens of four- and five-feature mice. A, Percentage of B cells in spleens of wild-type, μMT−/− and representative four- and five-feature mice. Essentially no B cells are present in the μMT−/− mice, and the introduction of the human Ig YACs restores the B220+ cell population in four- and five-feature mice to 25–45% of wild-type levels. B, Percentages of B220+ cells (gated as in A and comparing the numbers of B220+ cells in 100,000 spleen cells) expressing surface human Igκ and mouse Igλ (four feature, upper panels) or surface human Igκ and human Igλ (five feature, lower panels). In the four-feature mice the κ translocus is dominant over the endogenous λ locus, while in the five-feature animals the λ translocus is dominant over the κ translocus.
The Igκ rearrangements used the Vk4–1 and Vk1D-12 segments most frequently (7 of 19 and 9 of 19, respectively), with Vk3D-11 and Vk1D-13 being used in only a few rearrangements. The other functional Vk genes in the construct, Vk5–2 and Vk1D-9, were not found in the sequences examined. The six Igλ rearrangements were obtained from a single animal and show a limited use of the V gene segments, with VA3–19 used in five sequences. Given the high contribution to the B cell repertoire seen in FACS and serum analysis, it is likely that the rearrangement of the locus in the five-feature mice is similar to what is seen in mice in which HuIgλ YAC is in the presence of a functional mouse Igκ locus (14). Junctional diversity is less obvious for the λ L chain rearrangements and was provided mainly through the use of different J segments. The CDR3 length was restricted, with 18 of the 19 Vk-Jκ sequences encoding a 9-aa CDR3, and the remaining sequence encoding an 8-aa CDR3. The same restriction has been reported for mouse Vk-Jκ sequences, where 39 of 41 productive Vk-Jκ rearrangements encoded a 9-aa CDR3, with the remaining sequence encoding CDR3 longer than 9 aa (33). The increased variation in human L chain sequences is due to the insertion of N nucleotides at the V-J junction, which is not seen in L chain rearrangements from mice (32, 33). Little or no N insertion is found in the translocus-derived L chains either in four- or five-feature mice or in mice with the HuIgκ λ YAC in the presence of a functional mouse H chain locus (14, 21). This would suggest that the L chain translocus rearranges at the same developmental stage as the endogenous L chains, at which time terminal deoxynucleotide transferase activity is reduced.

**Somatic hypermutation of human Ig rearrangements**

The Ig rearrangements were examined for evidence of somatic hypermutation, which would indicate whether the B lymphocytes expressing human IgM were able to participate normally in immune responses. The majority of L chain rearrangements (13 of 19 Vk-Jκ and five of six Vλ-Jλ) contained two or more differences from the germline sequence, with up to 15 point mutations/sequence, while in contrast only four of 41 H chain rearrangements contained two or more mutations (Fig. 7). The frequency of mutations in the κ L chains was similar to what was observed in mice carrying the HuIgκ X λ YAC in the presence of a functional mouse H chain locus (21). In the paired sequences from 19 hybridomas, six clones had no mutations in either the H chain or L chain, 10 clones had an unmutated H chain with a mutated L chain, and three clones had mutations in both chains. The most mutated H chain sequence came from the same hybridoma as the most mutated κ L chain sequence (hybridoma 1431/ABS).

The distribution of the mutations in the κ L chain rearrangements shows that of the 28 changes in the CDRs, 25 lead to amino acid replacement, with a replacement:silent ratio near 8:1, while of

**FIGURE 3.** Human IgM expression in serum of four- and five-feature mice. A, Levels in serum of representative four-feature mice (light panels) and five-feature mice (dark panels) kept under barrier conditions, in which the IgM concentration was usually greater in serum from five-feature animals. The four-feature mice 6130 and 6133 and five-feature mice 5079 and 5086 are the animals analyzed for Fig. 2 above. The human IgM background level in normal mice is \(<0.1 \mu g/ml (27). B, Elevation of human IgM in serum of a four-feature mouse following immunization, performed as described in Materials and Methods. C, Specific anti-progesterone-BSA response following immunization of a four-feature mouse. The Ag-specific response was determined by coating the ELISA plate with 3 μg/ml progesterone-11-BSA in PBS. Ag-specific hybridomas 1451-A5 and 1451-B9 (Table II and Fig. 4) were cloned following fusion of the spleen of this mouse.

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Clone Name</th>
<th>Vκ-Dκ-JH Segments</th>
<th>Vk-Jκ Segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAP</td>
<td>1414/20</td>
<td>Vκ1D-2-Dκ2.2-Jκ6</td>
<td>Vκ1D-Jκ4</td>
</tr>
<tr>
<td>Human IgE</td>
<td>1431/ABS</td>
<td>Vκ1D-2-Dκ3.16-Jκ3</td>
<td>Vκ1D1-Jκ1</td>
</tr>
<tr>
<td>Prog-BSA</td>
<td>1451/A5</td>
<td>Vκ1D-2-Dκ1.7-Jκ4</td>
<td>Vκ1-Jκ2b</td>
</tr>
<tr>
<td>Prog-BSA</td>
<td>1451/B9</td>
<td>Vκ1D-Dκ1.7-Jκ6</td>
<td>Vκ1D-Jκ4</td>
</tr>
<tr>
<td>Prog-BSA</td>
<td>1477/13</td>
<td>Vκ1D-Dκ1.7/11-Jκ4</td>
<td>Vκ1-Jκ5</td>
</tr>
<tr>
<td>Prog-BSA</td>
<td>1477/14</td>
<td>Vκ1D-Dκ1.7-Jκ6</td>
<td>Vκ1D-Jκ4</td>
</tr>
<tr>
<td>IGF-1</td>
<td>1559/3</td>
<td>Vκ1D-Dκ1.16-Jκ4</td>
<td>Vκ3D-Jκ1</td>
</tr>
</tbody>
</table>

The Igκ rearrangements of specific hybridomas from four-feature human Ig mice
A. Heavy Chain Rearrangements

50 point mutations in the framework regions, 27 were replacement mutations in the encoded Ab, with a replacement:silent ratio near 1:1. The bias in favor of replacement mutations in the CDR regions is associated with selection-improved Ag binding (34). No such bias was observed for the mutations in the H chain rearrangements. These results suggest that the B cells expressing human surface Ig are capable of participating in an immune response and undergoing affinity maturation, but that the HuIgHeavy YAC is a poor target for the introduction of mutations.

B. Kappa Light Chain Rearrangements

Discussion

The four- and five-feature human Ig mice we have produced are capable of forming large Ab repertoires and can produce a diverse response following immunization. As such, they may be useful for the production of human mAbs for clinical use. In addition, the characteristics of the B cell repertoires that are developed suggest that these mice may be useful in studying the role of Ig loci in B cell development.

Reconstitution of B cell population by Ig transloci

The human Ig transloci are introduced into a background in which the endogenous mouse H chain and κ L chain loci are nonfunctional, with only the mouse λ locus unaltered. While it is clear that there is significant reconstitution of the B cell repertoire in these mice, the number of B cells present in the mice is reduced compared with that in mice with functional endogenous loci (Fig. 2A). The comparison of several strains carrying different transgene and knockout combinations suggests that the low B cell pool results in a smaller repertoire.
from poor function of the HuIgHeavy YAC in replacing the mouse H chain locus.

The HuIgκ and HuIgλ YACs are each capable of substituting for the mouse Igκ locus during B cell development, leading to the complete restoration of the B cell pool. The knockout of the κ locus (19), with a functional mouse H chain locus present, reduces the B cell population from around 40% of spleen cells to about 15%, with these cells expressing mouse λ L chains. The introduction of either the HuIgκ YAC or the HuIgλ YAC to this background restores the B cell population to near normal levels (9, 14). In contrast, the introduction of the HuIgH YAC only increases the B cell population to 5–25% of that of spleen cells, from the <2% present with the μMT−/− modification (Fig. 2A). The failure of the introduced locus to restore the B cell repertoire is not due to competition with endogenous H chain loci, which are still able to rearrange in the μMT−/− background before the functional defect is manifest. The restoration of the B cell repertoire is also not complete if a germline configuration H chain translocus is introduced into a knockout background in which the entire J segment locus is removed, and no rearrangement of the endogenous alleles is possible (11, 35). Similarly, the introduction of a larger germline configuration H chain YAC containing the majority of functional V regions does not restore the B cell population to a greater extent than a locus containing five V regions (10, 11).

Analysis of various L chain transgenes indicates that elements in the locus downstream of the C regions may be necessary during the development of the B cell repertoire (21). No H chain translocus has been described that includes these regions in germline configuration, and those transloci that do include downstream elements, such as other C region genes or downstream enhancers (6, 10), place these elements in close proximity to the IgM and IgD domains and do not allow for the presence of any essential regulatory elements in the regions between the C domains.

Expression of translocus κ and λ light chains

The distribution of surface L chain in the five-feature animals further indicates that the structure of the translocus can affect the contribution to the B cell repertoire. The reconstitution of the B cell population was similar in both four-feature and five-feature mice (Fig. 2A), yet the total serum IgM levels tended to be higher in the five-feature mice (Fig. 3). Although the individual transloci can both substitute for the mouse Igκ locus, the contribution of the HuIgλ locus appears to be dominant when in direct competition with the HuIgκ locus, with a μ:λ ratio of 1:3. In human B cells, Igκ is expressed slightly more frequently, with a typical μ:λ ratio of 60:40. In the human genome, both κ and λ V regions are gathered

![FIGURE 6. Occurrence of individual D segments in H chain rearrangements from four- and five-feature mice (a total of 39 sequences) and from human lymphocytes (data from Ref. 30). D segments are numbered according to their family and position in the locus (30), with segment D1-1 being furthest from, and D7-27 being nearest to the J regions. D4-4 and D4-11 have identical coding sequences, and the appearance of this sequence in rearrangements has been divided equally between the two segments.](image)

![FIGURE 7. Somatic hypermutation of translocus-derived rearrangements from four- and five-feature animals. The proportion of H chain and κ and λ L chain sequences with zero, one, two, etc., mutations are shown using pie charts. The number of sequences analyzed for each locus is indicated by the number in the center of the chart. The CDR3 region was excluded from the mutation analysis, as this may be affected by the junctional diversity during rearrangement.](image)
in clusters along their respective loci (36, 37), and the majority of L chain rearrangements (κ or λ) use V segments from the proximal cluster, nearer the core of the locus (38, 39). The dominant HuIgYAC is arranged in the correct germline configuration (24), whereas the HuIgX YAC was produced by attaching a multimer of five V regions to the core of the human κ locus of three V regions, the J and C segments, and the enhancers (9). Both transloci contain the 3’ enhancers in their correct location, but the format of the Igκ YAC may remove other regulatory elements from upstream of the core of the locus. The absence of these elements may affect the rearrangement of the κ translocus and therefore lower its contribution to the Ig repertoire in the five-feature animals. As the mouse λ locus rearranges so poorly, even when the mouse κ locus is nonfunctional (9), this deficiency in HuIg YAC is not evident in the absence of HuIg YAC, either in the four-feature animals or when HuIgX YAC is present with the functional mouse H chain locus.

Diversity of the translocus-encoded repertoire

The characteristics of the human Ig rearrangements indicate that the formation of the Ab repertoire from the translocus elements follows the same rules as that for the equivalent mouse loci. The similar use of D and J segments suggests that the recognition of recombination signal sequences is all but identical in mouse and man. The insertion of N nucleotides in the H chains, but not the L chains, indicates that the transloci rearrange at the same time as their endogenous counterparts. The major limitation on the potential variability of the repertoire arises from the limited number of V region gene segments present in the HuIgHeavy YAC, apparent by the overexpression of the V_{H1}-1-2 segment. The Ig rearrangement process is affected by variations both in the promoter of the V region (40) and in the recombination signal sequences (41). Given the small number of V regions in the locus, even a relatively small advantage in rearrangement frequency during B cell development may manifest as a large bias in the utilization of the region in the Ig repertoire (42). The sequencing of the human H chain locus indicates that the segments in the HuH YAC differ in both the promoter region and the recombination signals (43), which may account for the observed utilization of V_{H1},1-2 in preference to the other segments. The inclusion of more V region segments in the translocus should overcome this limitation.

Somatic hypermutation of translocus-encoded Ig rearrangements

The presence of point mutations in rearranged Ig genes indicates that they are from a B cell that has undergone affinity maturation following exposure to Ag. Although it has been reported that mutations can be present in the H or L chain but not in the partner sequence, the most common state is that both chains contain mutations (44, 45). Here we found frequent mutations in L chain rearrangements, but almost no mutation in the H chains. The contrast was particularly evident in the paired sequences isolated from hybridoma clones. The presence of mutations in the L chain rearrangements confirms that the B cells expressing human Ab are able to participate in germinal center reactions, and the biased distribution of replacement mutations indicates that these mutations were subject to antigenic selection (34). The consistent absence of mutations in the H chain rearrangements, with only four of 41 rearrangements containing two or more point mutations, would indicate that the H chain translocus provides a poor target for the introduction of somatic hypermutation mechanism. Efficient hypermutation of the V_{H} segments has been described in the case of several, but not all, human IgH transloci (6, 10, 13, 46). This variation could indicate a sensitivity to integration position effects, particularly since transloci are unlikely to contain the full complement of IgH locus cis-acting regulatory elements.

Concluding remarks

We have produced mice carrying germline H chain and κ and λ L chain transloci in a background where the mouse H chain and κ L chain are nonfunctional. The transloci can substitute for their mouse counterparts, leading to the formation of diverse repertoires of fully human Ab. Sequence analysis indicates that the transloci are rearranged at the same stage as their mouse equivalents, and that the segments are used as they would be in human cells. The mice we have developed will be useful in the study of Ig gene rearrangement during B cell development. They can be used to produce fully human mAb for therapy, avoiding the adverse reactions that can be induced by rodent Ab.

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References


