



Review

Human Antibody Expression in Transgenic Mice

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Abstract. Human antibody repertoires can be created in transgenic mice following the introduction of human immunoglobulin heavy and light chain genes in their germline configuration. Transgene constructs or transloci have been obtained by plasmid assembly, cloning in yeast artificial chromosomes, and the use of chromosome fragments. Translocus integration and maintenance in transgenic mouse strains has been achieved by pronuclear DNA injection into oocytes and various transfection methods using embryonic stem cells. The human DNA segments rearrange faithfully in the mouse and produce extensive V(D)J combinations. Specific human monoclonal antibodies of high affinity for use in therapeutic applications have been produced from these translocus mice.

Key words: human antibody repertoires; transgenesis; human monoclonal antibodies; embryonic stem cell manipulation.

Introduction

Monoclonal antibodies have become essential for medical intervention of disease, however, those derived from rodents can themselves be seen as antigenic targets, and this created a demand for specific human antibodies. The challenge of producing human monoclonal antibodies has been met through several approaches: immortalization of human lymphocytes, *in vitro* phage or ribosome display libraries, and the derivation of transgenic mice¹⁸. Key concerns for the transgenic approach were whether the human genes would rearrange and whether the murine cellular machinery would allow expression, antigenic selection and clonal expansion. These problems have failed to materialize, as transgenic mouse lines, carrying a combination of human heavy and light chain loci in an endogenous knock-out background, express a comprehensive spectrum of human

immunoglobulin types^{16, 17, 19}. The transgenic approach also has the advantage that immunization procedures and monoclonal antibody production are well established in mice. Table 1 lists the human Ig transloci introduced and their features in respect to origin, size, gene content and antibody titre.

Human Immunoglobulin Minigenes

Minigene constructs have been assembled by placing individual gene segments in artificially close proximity. This means that V_H, D, J_H and C_H gene segments for the heavy chain, and V_L, J_L and C_L for the light chain, were placed immediately adjacent to each other, disregarding any natural intronic spacer regions. Such compact assembly is needed because plasmid-based minilocus constructs can only accommodate

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Table 1. Size, variable region genes and serum titre of mice carrying human Ig transloci

Translocus	Human Ig chains	Size [kb]	Functional V genes	Titre [$\mu\text{g} \cdot \text{ml}^{-1}$]	Reference
Plasmid-based transgenes					
IgH loci					
HuIgH	μ	25	2	20, 300*	1, 28, 29
HuIgH ^{COS}	μ	100	2	50, 350*	3, 28, 29
HC1	$\mu, \gamma 1$	61	1	10*	24
HC2	$\mu, \gamma 1$	80	4	100*	16, 23
IgL loci					
KC1	κ	24	1		24
KCo4	κ	43	4	100*	16
HuIg κ ML	κ	15	5	15, 50*	30
YAC-based transgenes					
IgH loci					
J1.3	μ	85	2	0.2	5
HuIgH ^{P1-2}	μ	210	5	5, 180*	27
HuIgH	μ	240	5	200*	19
yH1	μ	220	5	1, 350*	11
yH2	$\mu, \gamma 2$	1020	~40	700* (IgM); 600* (IgG)	17
IgL loci					
yK1	κ	170	2	16	11
HuIg κ YAC	κ	300	2	50, 800*	8, 30
HucosIg κ YAC	κ	1300	~80	100, 800*	30, 31
KCo5	κ	450	~26		9
yK2	κ	800	~25	800*	17
Ig λ	γ	380	15	400, 1500*	21
Chromosome-fragment transgenes					
IgH loci					
hCF(SC20)	$\mu, \delta, \gamma, \epsilon, \alpha$	> 20 Mb	whole locus	10, 350* (IgM); 3, 300* (IgG)	25, 26
IgL loci					
hCF(2-W23)	κ	5–50 Mb	whole locus	60, 450*	25, 26
hCF[MH(ES) 22-1] ^a	γ	intact chromosome 22	whole locus	20	26

The approximate (usually mean) titre of serum antibody containing human Ig is shown for the different transgenic lines. * – titre in a knock-out background is indicated, ^a – human λ expression was only obtained in chimeric mice, COS – cosmid, H – heavy chain, Hu – human, Ig – immunoglobulin, V – variable region, YAC – yeast artificial chromosome.

a rather limited number of gene segments, due to their small size. The various heavy chain minigene constructs carry up to 4 V genes and can have a region of over 500 kb missing^{2, 16, 23}. One approach to obtain a more authentic heavy chain locus used pronuclei co-injected with two cosmids, permitting the integration of a contiguous 100 kb translocus containing V_H, D, J_H and C_H gene segments. Head-to-tail integration permitted the 5' V_H gene on one cosmid to rearrange to D-J_H on the other cosmid³.

The assembly of κ light chain minigenes was considerably easier because of the lack of diversity segments and multiple constant regions. In one human κ minigene construct, 5 variable region gene segments, all the joining segments, C κ , and all the known regulatory sequences, including enhancers, were assembled artificially close together to form a rather small 15 kb translocus³⁰. This construct, which disregarded the spa-

tial arrangements of the genes, did, however, allow the importance of the natural κ light chain gene segment spacing for rearrangement and expression to be tested. The results showed that DNA rearrangement and expression, although being achieved, operated with substantially reduced efficiency. The 25 kb κ light chain core region, including the B3 V κ gene, all J κ segments, the intron enhancer, C κ and the downstream enhancer, was used in many experiments for the addition of V κ genes^{9, 16, 17, 30}. This allowed rearrangements to form a diverse range of human κ light chain regions with good expression levels.

Despite the small number of V genes included on these heavy and light chain constructs, these miniloci give rise to reasonably large repertoires. It was particularly encouraging to find extensive D usage and non-encoded nucleotide additions at the V-D and D-J junctions^{3, 11, 17, 19, 27}.

Yeast Artificial Chromosome-Based Loci

The use of large genomic regions for the introduction into mouse chromosomes rely on suitable cloning techniques. Libraries made in yeast can produce artificial chromosomes in excess of 1 Mb, but usually yeast artificial chromosome (YACs) carry regions of not more than a few hundred kb. In addition, yeast has the practical advantage that its chromosomes, including the YAC, can be very efficiently manipulated by homologous recombination. This allows site-specific modifications to add or remove sequences within the Ig gene loci and to mark YAC arms, derived from the cloning vector, by the integration of a selectable marker gene^{6, 7}. The largest authentic human Ig core regions on YACs are 340 kb for the heavy chain, including 5 V's, all D's, all J's and C μ , and 320 kb for an Ig κ YAC, including 3 V's, all J's and C κ . A human Ig λ YAC, containing a 380 kb region in the authentic configuration, was created by homologous integration of 3 overlapping cosmids which added J-C segments to a V λ YAC²⁰. Further YAC modification allowed the addition of variable region genes for heavy and light chain and constant region genes for the heavy chain, but these additions did not produce extended YACs in authentic germline configuration. The largest human heavy chain locus created is 1020 kb and contains ~66 V_H genes, with about a third found to be expressed, and an additional constant region proximal to C μ which allows switching to IgG2¹⁷. Human κ light chain YACs have also been extended by YAC and cosmid integration^{17, 30}. Multiple site-specific integration of a cosmid accommodating 5 V κ genes allowed YAC extension to 1.3 Mb which, with about 100 variable region genes, is presently the largest Ig YAC integrated in the mouse genome³⁰.

Chromosome Fragment Transloci

More recently a technology for the transfer of human chromosome fragments into embryonic stem cells allowed the derivation of trans-chromosomal mice^{25, 26}. A >20 Mb region of human chromosome 14, containing the IgH locus in germline configuration and, separately, a 5–50 Mb chromosome fragment for the human κ light chain locus on chromosome 2, were transmitted. These mice produce human antibody repertoires from whole Ig loci, of which the total sizes are, for the heavy chain, 1.5 Mb, the λ light chain 1.1 Mb and the κ light chain over 2 Mb². As pointed out, large chromosome fragments or whole chromosomes may be difficult to maintain and germline transmission was not

achieved from chimaeric trans-chromosomal λ light chain mice. A reason for this could be the presence of a very large part of or, possibly, the entire chromosome 22, which may interfere with meiosis. The trans-chromosomes accommodating either the heavy chain or the κ light chain locus maintain telomeric and centromeric regions with very large deletions in between. Interestingly, the human IgH locus is telomere proximal whilst the human κ light chain locus is close to the centromer. This particular locus position may be important to maintain translocus stability and transmission.

Translocus Introduction

Transgenic mice expressing human antibodies were initially obtained by oocyte pronuclear injection utilising strategies that were developed in the 1980s¹⁰. Improvements in DNA purification over the last 10 years have permitted the use of YACs²² and DNA transfection approaches into embryonic stem cells to derive translocus mice. For both human heavy and κ light chains, regions of several 100 kb have been integrated by microinjection of overlapping clones^{9, 27}. As there are limits to the purification of large DNA fragments, associated with the handling and sheering of large linear molecules, DNA and chromosome transfer techniques without the need to purify or dissociate DNA from cells have been developed. Yeast protoplast fusion, similar to cell-cell fusion used in monoclonal antibody production, is widely used for the transfer of YACs into embryonic stem cells^{7, 8}. For protoplast fusion, a selectable marker gene has to be added to the YAC. In many experiments the neomycin-resistance gene was homologously integrated and vectors which allow simple site-specific additions are readily available⁷. With the protoplast fusion method, single YACs carrying greater than 1 Mb of heavy and light chain regions have been expressed^{17, 30}. The use of unmodified YACs by co-lipofection with a selectable marker gene has also been shown⁵. Here, size fractionated DNA maintained in a gel slice was incubated with a cationic lipid to allow formation of DNA-lipid complexes for DNA transfer into embryonic stem cells.

Much larger regions have been introduced and expressed by the transfer of human chromosome fragments into embryonic stem cells via microcell-mediated chromosome transfer. Preparation for human chromosome transfer involved transfection of human fibroblasts with the neomycin resistance selectable marker gene, identification of marked chromosomes and fusion with mouse cells to obtain hybrids¹⁴. Tagged

human chromosomes are usually of reduced size compared with their intact equivalents. For microcell-mediated chromosome transfer, A9 clones containing the human chromosome of interest were fused with embryonic stem cells in the presence of LPS²⁶. With this technology, 2 germline transmission mouse strains have been obtained expressing an extensive, possibly complete, human heavy and κ light chain repertoire²⁵.

Multi-Feature Mouse Strains

Human heavy and light chain loci can be rearranged and expressed in the mouse. However, competition with endogenous loci, which are usually dominantly expressed, suggested that human Ig levels could be dramatically increased if the transloci lines were crossed into mouse strains in which the endogenous Ig loci had been silenced by gene targeting². Gene targeting and Ig locus silencing has been achieved for mouse heavy chain genes by J_H deletion and, separately, by integration of the neomycin resistance gene into the μ membrane exons^{13, 15}. The mouse κ light chain locus has been silenced by $J\kappa$ and/or $C\kappa$ deletion or $C\kappa$ disruption by marker integration^{15, 19}. The mouse λ light chain locus has so far not been silenced, but is usually only expressed at a low level and, thus, may not interfere with transloci expression.

Several mouse strains with 4 transgenic features and one strain with 5 features have been produced^{4, 17, 19}. A 4-feature mouse refers to an animal carrying a human heavy chain locus and a human κ light chain locus in a background where the endogenous heavy and κ light chain loci are silenced. Five-feature mice refers to animals which carry a human heavy chain locus, a human κ and a human λ light chain locus in an endogenous heavy and κ light chain knock-out background. In the initial experiments, crossing of mice carrying human IgH and Ig κ miniloci gave rise to 4-feature strains with, for example, an ~80 kb heavy chain region accommodating 4 variable region genes and 2 constant region genes, $c\mu$ and $c\gamma 1$ ¹⁶. The κ light chain region was about 40 kb. The heavy chain allowed switching which resulted in IgG1, κ antibodies in the serum. With the use of large YACs, a typical 4-feature mouse strain carries a heavy and light chain locus of at least several hundred kb, and antibody repertoires and expression levels were substantially improved (>0.5 mg/ml of IgM and IgG)^{17, 19}. The 5-feature translocus strain recently produced carries the full complement of human Ig loci¹⁹. The addition of a λ light chain YAC improved antibody expression levels, which are gener-

ally higher in 5-feature mice. Interestingly, the Ig λ translocus is also highly expressed in normal (non-KO) transgenic mice²¹.

A major technological improvement in locus transfer was the generation of a 4-feature trans-chromosomal mouse strain. This mouse strain carries complete human heavy and light chain loci on large Mb chromosome fragments²⁵. Human IgM and IgG serum titres were of a few hundred μ g/ml, with the average proportion of each subclass similar to that observed in humans²⁵. However, it is unclear why in trans-chromosomal mice the regulation of human Ig levels from complete transloci is below that found for YAC translocus mice (see Table 1). Reasons for this could be the pathogen-free conditions for strain maintenance or, alternatively, that trans-chromosome instability may significantly reduce antibody titres.

Human Antibody Repertoires and Monoclonal Antibodies

Human Ig translocus mice with 4- or 5-features are particularly useful for evaluating immune responses and the production of specific human monoclonal antibodies^{12, 15}. Initial sequence analysis of germinal centre or spleen cells by RT-PCR, showed a wide range of V(D)J recombinations and also that hypermutation of variable region genes can be obtained^{19, 23, 27}. The extensive use of the translocus diversity segments is particularly interesting as the human locus contains 27 D's, over twice as many as found in the mouse locus. The addition of non-encoded nucleotides at the V-D and D-J junction of the heavy chain was essentially found in all variable regions. Many rearrangements exhibited extensive CDR3 regions, important for antigen contact, which exceeded the length of mouse V to D and D to J joins^{11, 17, 19}.

Regarding antibody responses, it has been shown that antigens which elicit good responses in normal mice do not only increase human Ig titres in translocus mice, but also allow the production of antigen-specific titres which are increased upon re-immunization¹⁹. An impressive range of human IgM and human IgG antibodies specific for cells, proteins and haptens has been produced; particularly important was the successful production of human monoclonal antibodies against human antigens such as lymphoma cells or cell surface markers⁴. There appears to be an advantage of human IgG antibodies over human IgM in that the affinity of IgG may be superior due to recruitment of hypermutation and selection. Affinity measurements of purified

monoclonal IgG using surface plasmon resonance in BIAcore yielded very good values down to the pM range (1×10^9 – 5×10^{10} /M) for IL-8, EGFR, TNF- α and CD4 specific human antibodies^{9, 16, 17}. Obtaining high affinity human antibodies from translocus mice indicates that the mouse recombination and selection mechanism is fully operative on the introduced human loci, which allows the production of a wide spectrum of human antibodies for diagnostic and therapeutic applications.

Conclusions

In summary, mice carrying large Mb Ig regions have been produced and crossed to obtain strains which express human heavy and light chain loci in endogenous knock-out backgrounds. Immunization with human antigens resulted in specific and high affinity IgM and IgG antibodies. The 4- and 5-feature human Ig mouse strains are available to the scientific community upon request¹⁹ and it is anticipated that they will replace the medical use of rodent antibodies in the future.

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