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Human antibody expression in transgenic rats: Comparison of chimeric IgH loci with human V_H, D and J_H but bearing different rat C-gene regions ☆

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ABSTRACT

Expression of human antibody repertoires in transgenic animals has been accomplished by introducing large human Ig loci into mice and, more recently, a chimeric IgH locus into rats. With human V_H, D and J_H genes linked to the rat C-region antibody expression was significantly increased, similar to wild-type levels not found with fully human constructs. Here we compare four rat-lines containing the same human V_H-region (comprising 22 V_Hs, all Ds and all J_Hs in natural configuration) but linked to different rat C_H-genes and regulatory sequences. The endogenous IgH locus was silenced by zinc-finger nucleases. After breeding, all lines produced exclusively chimeric human H-chain with near normal IgM levels. However, in two lines poor IgG expression and inefficient immune responses were observed, implying that high expression, class-switching and hypermutation are linked to optimal enhancer function provided by the large regulatory region at the 3′ end of the IgH locus. Furthermore, exclusion of Cδ and its downstream interval region may assist recombination. Highly diverse IgG and immune responses similar to normal rats were identified in two strains carrying diverse and differently spaced C-genes.

1. Introduction

Successful strategies to produce human antibodies have involved humanization of rodent monoclonal antibodies (mAbs), selection of antigen-specific human sequences by display technology and the generation of transgenic animals carrying human Ig loci (Green, 1999; Lonberg, 2005, 2008; Brüggemann et al., 2007). All these approaches provided an increasing number of valuable drugs, even though the manipulation of individual antibody H- and L-chains can be quite laborious (with case by case recurrence) while the production of human antibody repertoires in rodents relies on the assembly and integration of large gene constructs (Riechmann et al., 1988; Mendez et al., 1997; Nicholson et al., 1999). In transgenic animals, sizeable germline regions performed better in rearrangement and expression (Xian et al., 1998), nevertheless, rodents with fully human IgH transloci often failed to produce high affinity binders after multiple immunizations (Green and Jakobovits, 1998; Pruzina et al., 2011).

The suboptimal performance of a human IgH locus in transgenic mice, in respect of antibody yield and immune response, was attributed to the imperfect interaction of the human constant region of membrane Ig with the endogenous

Abbreviations: 3′RR, IgH 3′ regulatory region; BAC, bacterial artificial chromosome; YAC, yeast artificial chromosome; ZFN, zinc-finger nuclease.

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rodent cellular signaling machinery (Pruzina et al., 2011). This was supported by work in transgenic rats, carrying human V_H, D and J_H gene segments linked to the rat C-region locus, which displayed IgG immune responses very similar to wild type controls (Osborn et al., 2013). In these transgenic animals a large part of the rat C-region was included in conjunction with ~30 kb downstream of Cα containing the 3′ enhancer regulatory region, termed 3′RR (Vincent-Fabert et al., 2010). It was reasoned that intergenic regions containing cis-acting control sequences might be important in their entirety to secure class-switch recombination and hypermutation.

The size of the complete human IgH locus, ~1.3 Mb with 38–46 V_H, ~23 D and 6 J_H segments (Hofker et al., 1989; Matsuda et al., 1998), provides an immense challenge for engineering of multi-gene constructs and their germline integration by DNA microinjection into fertilized eggs. This can be partly overcome by using a previous finding where co-injection of multiple DNA constructs with homologous overlaps frequently led to co-integration into the genome (Bruggemann et al., 1991; Wagner et al., 1996). The use of large restriction fragments from modified bacterial artificial chromosomes (BACs) with terminal homology sequence enabled a functional Ig locus to be assembled (Osborn et al., 2013). Successful homologous or tandem integration could be verified by transcript analysis, which showed productive rearrangement of diverse V_H-D-J_H-Cγ products brought together from segments accommodated on several BACs.

Here we compare chimeric human IgH expression from four transgenic rat-lines with identical human V_H, D and J_H segments but different rat C-region and 3′ enhancer sequence. We found that there is flexibility in the positioning of the C-genes but that the region downstream of Cα containing multiple transcriptional enhancer elements resulted in optimal immune response, class-switch recombination and somatic hypermutation.

2. Materials and methods

2.1. Construction of chimeric human-rat IgH loci

For the construction of the HC10 translocus, the rat genomic region from BAC clone CH230-408M5 (Invitrogen), including Cμ, Cδ and the region up to the γ2c switch region on a ~49 kb fragment, was extended with a 100 bp homology arm corresponding to the sequence immediately upstream of the rat γ2b switch region using the Red/ET recombination method (Gene Bridges GmbH, Heidelberg, Germany). Briefly, the recombination reaction used the pACYC177 vector DNA amplified via PCR using long primers containing homology arms matching the 5′ and 3′ end of the gene loci of interest followed by NotI restriction sites. The rat genomic region encompassing Cγ2b, Cε, Cα and 3′RR was isolated from BAC clone CH230-162I08 (Invitrogen) as a ~76 kb NruI-fragment using the BAC Subcloning Kit from Gene Bridges. The rat γ2b C_H1 region was replaced by human γ1 C_H1 according to the instructions using the Counter Selection BAC Modification Kit (service provided by Gene Bridges). Finally, HC10 was assembled as a circular YAC/BAC (cYAC/BAC) construct in *Saccharomyces cerevisiae* using 6 overlapping fragments (oligos are listed below): a 6.1 kb fragment 5′ of human V_H6-1 (amplified using oligos 383 and 384, and human genomic DNA as template), a ~78 kb PvuI–PacI fragment containing the human V_H6-1–Ds–J_Hs region cut out from BAC1 (RP11645E6, Invitrogen), a 8.7 kb fragment joining human J_H6 with the rat genomic sequence immediately downstream of the last J_H and containing part of the rat Cμ coding sequence (using oligos 488 and 346, and rat genomic DNA as template), the ~49 kb NotI-fragment covering rat μ up to the γ2c switch region as described above, the ~76 kb NruI-fragment from rat Cγ2b up to the 3′RR as described above, the pBelo-CEN-URA vector with URA3 joined with a homology tail matching the 3′ end of the rat 3′RR, and CEN4 joined with a homology tail matching the 5′ end of human V_H6-1 (using long oligos 385 and 322, and pBelo-CEN-URA as template). Further details, including the purification of the constructs, and the methods for converting a cYAC into a BAC were published previously (Osborn et al., 2013).

For the construction of HC13 a 5.6 kb fragment encompassing the membrane exon 2 as well as 3′ UTR of rat γ2b was amplified from BAC clone CH230-162I08 using primers 547 and 548 with PmlI and AscI sites, respectively. This fragment was cloned into pGEM®-T Easy via TA cloning (Promega). The short 3′ E region, 3′RR hs1,2, located ~17 kb downstream of rat Cα (Pettersson et al., 1990) was amplified from BAC clone CH230-162I08 using primers 549 and 252, and isolated as a 950 bp AscI-SacII fragment. This fragment was cloned downstream of the γ2b 3′ UTR into the multiple cloning sites of pGEM®-T Easy. Finally, the γ2b 3′ region joined together with the 3′RR hs1,2 was isolated as a ~6.6 kb PmlI–SacII fragment. HC13 is an extension of the previously constructed BAC containing humanV_H6-1-Ds-J_Hs followed by the authentic rat μ, δ, and γ2c region on a single ~140 kb NotI fragment (Osborn et al., 2013). The following 5 fragments were used to assemble HC13 as a cYAC/BAC construct: the ~140 kb NotI fragment described above, a ~1.8 kb PCR fragment covering the γ2c 3′ UTR followed by a 65 bp homology tail matching the sequence 3.8 kb upstream of the γ2b switch region (using primer 502 and 503, and rat genomic DNA as template), a ~16 kb SphI-fragment covering the entire modified γ2b locus with rat γ2b C_H1 replaced by human γ1 C_H1 (cut out from the modified BAC clone CH230-162I08 described under 'HC10'), the ~6.6 kb PmlI-SacII γ2b 3′ enhancer fragment described above, and amplified pBelo-CEN-URA vector with homology tail ends (using long oligos 385 and 560, and pBelo-CEN-URA as template).

HC17 is an extension from HC13. The region including humanV_H6-1-Ds-J_Hs followed by the rat μ, δ, γ2c and the modified γ2b region, was cut out from HC13 as a single ~160 kb NotI-AscI fragment. A cYAC/BAC construct was made from 4 fragments: the ~160 kb NotI-AscI region, a ~1.7 kb PCR fragment containing a 58 bp 5′ homology tail matching the sequence ~5 kb downstream of the γ2b membrane exon 2 followed by the sequence located ~3.6 kb upstream of the α switch region (using primers 591 and 592, and rat genomic DNA as template), the ~40 kb FspI-SalI region with Cα and the 3′RR from BAC clone CH230-162I08, and amplified pBelo-CEN-URA vector with homology tail ends (using long oligos 385 and 322, and pBelo-CEN-URA as template). The following oligos have been used:

252 [TGGAACCTGCTTAGGTCAGC];

322 [TATACATCGTCTTAGTATCTGTCTGACCCCACCACCATC TTCCCTGCCTCC GTCCACTCACAGATCTCTGACGCGTCACCGC AGGGTAATAACTG];