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SCIENCE DIRECT
Molecular Immunology 43 (2006) 1827–1835
Molecular Immunology
www.elsevier.com/locate/molimm

Rearrangement of only one human IGHV gene is sufficient to generate a wide repertoire of antigen specific antibody responses in transgenic mice

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Received 19 July 2005; received in revised form 28 October 2005; accepted 30 October 2005
Available online 15 December 2005

Abstract

In recent years, mice carrying human IG transgenes are being generated for the production of human monoclonal antibodies as an alternative approach to the conventional use of mouse or chimeric-humanized antibodies. Theoretically, the size of the repertoire of human antibodies that these mice could produce would be critically dependent on the number of human V genes introduced in the transgene. This could be the case for BABκ and BABκ,λ transgenic mice, which carry several genes from the human IGK (BABκ), and IGK and IGL (BABκ,λ) loci, but only five human IGHV genes and the entire IGHD-IGHJ cluster linked to two human IGHC (IGHM-IGHD) genes. We analyzed the expressed human IG genes in 30 IgM-secreting hybridomas generated from transgenic mice immunized either with soluble proteins (human IgM coupled to KLH) or with cells (human PBMC, tumour cell lines or rat cells transfected with human CD69). The results show that all hybridoma cells analyzed rearranged exclusively the IGHV1-2 gene, in contrast with naive spleen B cells that used three out of the five IGHV genes present in the transgene. The configuration of the rearranged CDR3 region revealed a much higher heterogeneity in the heavy chains. A variety of IGHJ and IGHD genes were used in hybridomas, and somatic mutations were also seen in some hybrids. Regarding the rearranged light chains genes, it was a much higher variety in the use of V and J genes in both, κ and λ chains, than in the heavy chain, and also in the level of mutation. The results indicate that only one IGHV gene is sufficient to generate a wide repertoire of antigen specific antibody responses. Thus, efforts aimed at the generation of new transgenic mice should focus more on the integrity of the D/J region and on the DNA regions regulating somatic hypermutation, rather than on the number of V genes present in the transgene.

Keywords: Somatic hypermutation; Human IG transgenes; Immunoglobulin; Hybridomas; Monoclonal antibodies

1. Introduction

Recent advances in transgenic technology permit large amounts of foreign DNA to be introduced into the mouse genome, including several V, D and J genes from the human heavy and kappa loci (Brüggemann and Neuberger, 1996; Jakobovits, 1995; Zou et al., 1996). Transgenic mice carrying human IG genes are being used for the generation of human monoclonal antibodies (mAbs) with potential therapeutic uses (Green, 1999). Our group has generated several human mAbs directed against human antigens: surface molecules on leucocytes (Magadán et al., 2002), CD69 antigen (Molina et al., 2003) and tumour idiotype IgM/κ from lymphomas (Suárez et al., 2004), using the BABκ and BABκ,λ transgenic mice. Both mouse strains have non-functional endogenous mouse IGH and IGK loci (Kitamura et al., 1991; Zou et al., 1995), and they carry a human transgene region containing five IGHV (IGHV1-2, IGHV1-3, IGHV2-5, IGHV4-4 and IGHV6-1) genes, and the complete human D-J cluster linked to two IGHC (IGHM and IGHD) genes in correct genomic configuration (Nicholson et al., 1999; Wagner et al., 1996; Lefranc, 2001a). Mice also

Abbreviations: CDR-IMGT, complementarity determining region; FR-IMGT, framework region; IG, immunoglobulin; PBMC, peripheral blood mononuclear cells

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doi:10.1016/j.molimm.2005.10.015

carry a human IGK translocus containing 20 repeats of five IGKV (IGKV1D-9, IGKV2D-10, IGKV3D-11, IGKV1D-12 and IGKV1D-13) genes (Nicholson et al., 1999; Pech et al., 1985) attached to the core of the germline locus which includes three IGKV (IGKV7-3, IGKV5-2 and IGKV4-1) genes, the complete IGKJ cluster and the IGKC gene. (Zou et al., 1996; Lefranc, 2001b). BABκ,λ mice carry an additional human IGL transgene with 28 IGLV genes and the seven-paired IGLJ-IGLC genes, in correct genomic configuration (Popov et al., 1996; Lefranc, 2001c; Lefranc and Lefranc, 2001). Other strains of transgenic mice that have been used to obtain human mAbs contain different numbers of IGHV genes (Davies et al., 1993; Green et al., 1994; Lonberg et al., 1994). Theoretically, the size of the repertoire of human antibodies that these mice could produce would be critically dependent on the number of human V genes introduced in the transgene. However, how many human V genes are required in a transgenic mouse in order to generate an adequate immune response against different antigens, is still to be resolved.

Here, the human IG genes in splenocytes from unimmunized BABκ and BABκ,λ mice, and those in hybridomas derived from the fusion of splenocytes from immunized mice and myeloma cells have been analyzed and compared. The results show that although splenocytes from unimmunized transgenic mice can rearrange different IGHV genes, all hybridomas analyzed used the same IGHV gene, the IGHV1-2, irrespective of the type of antigen used in the immunization (either human cells or human IgM-KLH soluble proteins). The use of different IGHD-IGHJ, IGKV-IGKJ and IGLV-IGLJ genes, and in some cases, the introduction of punctual mutations along the genes, lead to increase the repertoire of antigen recognition. That only one IGHV gene in the repertoire of human IG is sufficient to generate a specific immune response to several antigens, is a significant finding that has important implications for the future development of transgenic mice carrying human IG transgenes.

2. Experimental

2.1. Materials and methods

2.1.1. Translocus mouse strains

The BABκ and BABκ,λ mice strains containing YAC-based human IG transloci were kindly provided by Drs. M. Brüggemann and M. Neuberger. Both strains were developed from mice carrying five human IGHV genes and the entire IGHD-IGHJ cluster linked to two human IGHC (IGHM-IGHD) genes (Wagner et al., 1996), and a human IGK translocus (Zou et al., 1996). The BABκ,λ strain carries an additional IGL translocus (Popov et al., 1996). BABκ and BABκ,λ mice had been crossed to homozygosity with mice in which the endogenous mouse IGHM and IGK loci were rendered non-functional by gene targeting (Kitamura et al., 1991; Zou et al., 1995). The mice were housed in a pathogen free environment, and used according to the guidelines of Spanish regulations (Royal Decree 223/1988) regarding the use of animals in scientific research.

2.1.2. Preparation of hybridomas

Several hybridomas were obtained from immunized BABκ and BABκ,λ mice against human leucocytes, human CD69 or against soluble tumour IgM/κ proteins. Briefly, a group of transgenic mice was intraperitoneally immunized with (1–2) $\times 10^6$ of either human peripheral blood leukocytes (PBMC), HMy-2 (Edwards et al., 1982), or human CD69-transfected rat cells (Sancho et al., 2000), emulsified with complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, MI, USA). Two to three weeks later, animals were immunized a second time with the same doses of respective cells emulsified in incomplete Freund's adjuvant (IFA) (Difco). A second group of transgenic mice was immunized three times with 100 μg of human monoclonal IgM/κ proteins conjugated to keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA, USA). The purpose of these immunizations was to generate hybridomas directed against tumour idiotypes obtained as previously described (Barrios et al., 2002) from patients with non-Hodgkin's lymphomas. Either CFA/IFA, alum or cholera toxin were used as adjuvants in the immunizations (Magadán et al., 2002). Three days after a final intravenous boost immunization, with either 5×10^5 of respective human cells or with 100 μg IgM-KLH in saline solution, the animals were sacrificed and their spleens removed for the preparation of splenocytes. The splenocytes were washed and used for fusion with NSO or Sp/2.0 mouse myeloma cells according to standard protocol as detailed previously (Galfre and Milstein, 1981). Fused cells were suspended in DMEM supplemented with 20% fetal calf serum (FCS) (PAA Laboratories, Linz, Austria) plus hypoxanthine–aminopterin–thymidine (HAT) (Sigma–Aldrich, St. Louis, MO, USA). Hybridoma supernatants were screened for specific human IgM secretion using ELISA. Hybridomas secreting antibodies directed against surface molecules on human leukocytes, tumour cell lines or human CD69-transfected rat cells were selected by flow cytometry. Each monoclonal antibody showed a specific pattern of recognition of tumour cell lines and of human leukocytes (Magadán et al., 2002; Molina et al., 2003). In the case of hybridomas secreting antibodies against human lymphoma IgM/κ proteins or to the carrier molecule KLH, only those secreting human IgM/λ monoclonal antibodies were selected by ELISA in order to be able to distinguish between the immunizing protein (human IgM/κ) and the monoclonal antibody (human IgM/λ) (Suárez et al., 2004). In total, 30 different hybridomas secreting antigen specific human IgM antibodies were selected in this study and cloned twice by limited dilution in 96-well plates (Costar, Cambridge, MA, USA).

2.1.3. RNA Isolation and cDNA synthesis

A suspension of splenocytes was prepared from two unimmunized BABκ,λ mice and red cells were lysed by osmotic shock with distilled water. RNA was isolated from 5×10^6 nucleated splenocytes or from 2.5×10^6 hybridoma cells using SV total RNA-isolation system (Promega, Madison, WI, USA) according to the manufacturer's instructions. First-strand cDNA was obtained by incubating an aliquot of RNA with 5 units of AMV reverse transcriptase (RT) (Promega), 40 units of RNasin RNase inhibitor (Promega), dNTPs 5 mM and buffer with 1 mM of