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Determination of neutrophil Fcγ receptor IIIb antigens (HNA-1a, HNA-1b and HNA-1c) by fluorescence-primed allele-specific polymerase chain reaction

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Summary We evaluate a technique for genotyping HNA-1a, -1b and -1c antigens, resorting to fluorescence-primed allele-specific polymerase chain reaction (FPAS-PCR), and determine the frequency of the different genotypes in a normal Portuguese population. Our results indicate that the FPAS-PCR system is a reliable and simple tool for genotyping the neutrophil Fcγ receptor IIIB antigens. The HNA-1a, -1b and -1c gene frequencies of 42.98, 84.21 and 6.14%, respectively, found in this study are similar to those reported for other white populations.

Keywords FcγRIIIB antigens, neutrophil, polymerase chain reaction

Introduction

The Fcγ receptor III (FcγRIII) or CD16 is one of the three types of receptor for the Fc region of immunoglobulin G (IgG) antibodies (Hessner *et al.*, 1996; Bux *et al.*, 1997, 1999). This receptor is coded by two highly homologous genes, FcγRIIIA and FcγRIIIB, located on chromosome 1 (Hessner *et al.*, 1996; Bux *et al.*, 1997). The former, a non-polymorphic gene, is expressed in macrophages and natural killer cells, while the latter, which has two major allelic forms [HNA-1a (NA1) and HNA-1b (NA2)], is expressed in neutrophils (Hessner *et al.*, 1996; Bux *et al.*, 1997, 1999; Kissel *et al.*, 2000). These two distinct alleles of the FcγRIIIB gene differ in five bases within exon 3 (nt 141, 147, 227, 277 and 349), four of which result in amino acid substitutions (Hessner *et al.*, 1996; Bux *et al.*, 1999). More recently, two additional alleles have been found in the FcγRIIIB gene. That described by Bux *et al.* (1997), designated HNA-1c (SH), has an identical sequence to HNA-1b except that nucleotide 266 is a cytosine instead of an adenine. Similarly, NA2M, described by Watanabe *et al.* (2000), differs from HNA-1b by a single guanine to adenine change at nucleotide 193. The frequency of this last allele was estimated to be 0.008% in the Japanese population (Watanabe *et al.*, 2000).

Genotyping the FcγRIIIB antigens is of clinical relevance, because those antigens are associated with allo- and autoimmune neutropenias as well as transfusion-related acute lung injury (Hessner *et al.*, 1996; Bux *et al.*, 1997, 1999; Bux, 2002). The HNA-1a and -1b antigens have also been studied in the context of other autoimmune disorders, such as systemic lupus erythematosus and Guillain-Barré syndrome (Pol *et al.*, 2000; Bux, 2002). Several DNA-based techniques have been developed for reliable, rapid and inexpensive genotyping of FcγRIIIB antigens, namely polymerase chain reaction-single-strand conformational analysis (PCR-SSCP) (Satoh *et al.*, 1994), PCR – preferential homoduplex formation assay (Fujiwara *et al.*, 1999) and allele-specific PCR (AS-PCR) (Hessner *et al.*, 1996). In this study, we evaluate a modification of the AS-PCR technique, employing fluorochrome-labeled sequence-specific primers [fluorescence-primed allele-specific polymerase chain reaction (FPAS-PCR)], for the HNA -1a, -1b and -1c antigens, and determine the frequency of these genotypes in a normal Portuguese population.

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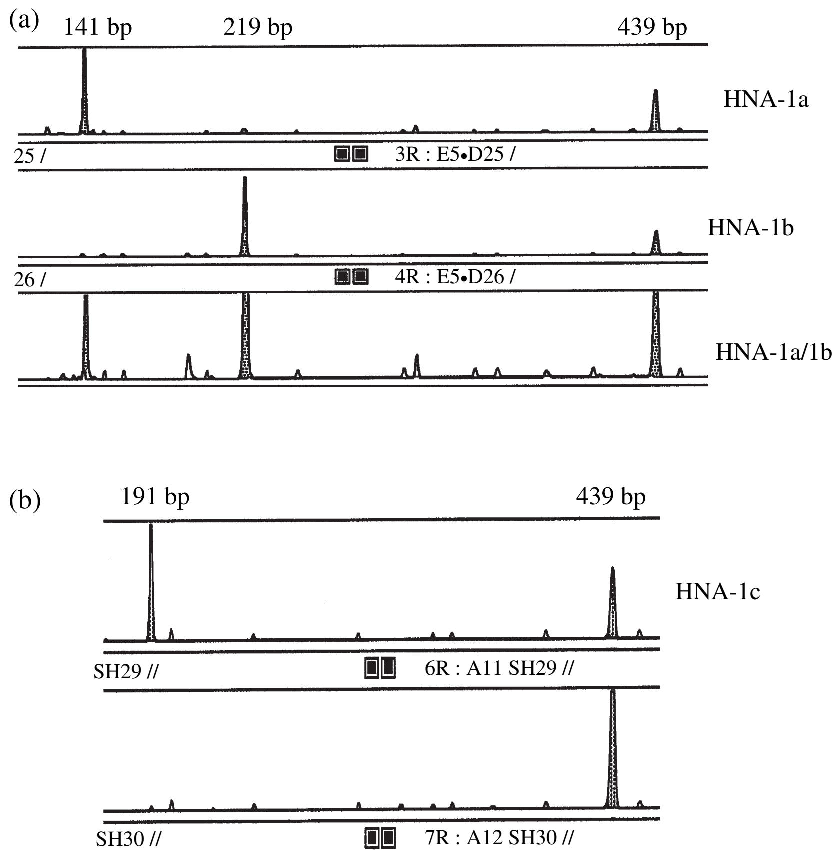

Figure 1. FPAS-PCR analysis. (a) Detection of the HNA-1a and -1b alleles, with DNA fragments of 141 bp (HNA-1a), 219 bp (HNA-1b) and 439 bp (internal control). (b) Detection of DNA fragment of 191 bp corresponding to the HNA-1c allele. The presence of a 439-bp DNA fragment alone demonstrates an HNA-1c negative result.

Materials and methods

Fluorescence-primed allele-specific-polymerase chain reaction was performed on 114 blood donor samples. HNA-1a, -1b and -1c alleles as well as an internal positive PCR control [a 439-bp fragment of the human growth hormone gene (HGH)] were amplified using previously described primers (Kissel *et al.*, 2000), with the introduction of a flourochrome (NED) in the reverse primer of each pair. Two PCR reaction mixtures were set up, one with the HNA-1a, -1b and HGH primers and the other with the HNA-1c and HGH primer pairs. The resulting amplicons were separated by automated capillary electrophoresis and analysed with the ABI GeneScan program (Applied Biosystems, Foster City, CA, USA).

In 20 of the samples, PCR-SSCP typing and sequencing was also performed, in order to validate the results obtained by FPAS-PCR. Here the two FcγRIII genes were co-amplified using a single primer set (5′-AAG GCT GTG GTG TTC CTG GA-3′ and 5′-ATG GAC TTC TAG CTG CAC CG-3′) under the conditions described by Satoh *et al.* (1994), with minor modifications. The bands obtained upon PCR-SSCP analysis were excised from the gel, purified and re-amplified for subsequent sequencing in both directions, using the same forward and reverse primers in independent sequencing reactions.

Results

HNA-1a only and HNA-1b only alleles were found in 18 and 59 DNA samples, respectively. HNA-1a and -1b alleles were found together in 30 samples. HNA-1c was found in seven samples, associated with HNA-1b in six cases and with both HNA-1a and -1b alleles in a single case (Figure 1). Antigen frequencies of HNA-1a, -1b and -1c may be extrapolated to be 42.98, 84.21 and 6.14%, respectively. Full concordance was observed between the three different genotyping assays (FPAS-PCR, PCR-SSCP and sequencing).

Discussion

DNA-based techniques for genotyping FcγRIIIB antigens have been the method of choice when compared with serologic typing methods, such as granulocyte agglutination or immunofluorescence tests. The first is less labour-intensive, does not require fresh samples and can be performed on a small amount of material. The second

method is cumbersome, time-consuming and requires a well-characterized typing serum (Satoh *et al.*, 1994; Fujiwara *et al.*, 1999).

Polymerase chain reaction with sequence-specific primers was previously described as a powerful technique to discriminate alleles arising from single or multiple base substitutions, like those related to the FcγRIIIB gene. Introduction of a flourochrome in the reverse primers enabled us to detect the amplified products in an automated system, thereby increasing the speed and sensitivity of the test.

Worldwide distribution of FcγRIIIB antigens is not homogeneous. In Chinese, Japanese and Native American populations HNA1a is more frequent than HNA-1b, whereas in Caucasians, African Black and Indians the opposite holds true. While HNA-1c antigen has not been found in Chinese and Japanese populations, it is reported to be expressed on neutrophils of 23–38% of Africans and American Blacks and 5–7% of Caucasians (Bux, 2002). In our study FcγRIIIB antigen frequencies were found to be similar to those previously described in other caucasian populations.

In summary, these results indicate that the FPAS-PCR system is a very useful tool for genotyping the neutrophil FcγRIIIB antigen. The HNA-1a, HNA-1b and HNA-1c gene frequencies estimated for the studied population are similar to those reported for other white populations.

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