Heterogeneous allele expression of pulmonary SP-D gene in rat large intestine and other tissues

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Lin, Zhenwu, and Joanna Floros. Heterogeneous allele expression of pulmonary SP-D gene in rat large intestine and other tissues. Physiol Genomics 11: 235–243, 2002; 10.1152/physiolgenomics.00061.2002.—Random allele expression has recently been observed for several genes including interleukins and genes of the lymphoid system. We studied the hypothesis that the surfactant protein D (SP-D) gene, an innate host defense molecule, exhibits random allele expression in a tissue-specific manner. SP-D gene expression is tissue specific in the 14 tissues studied. Study of SP-D allelic expression in several tissues revealed a balanced biallelic (BB) in lung, and, in several extrapulmonary tissues, a heterogeneous pattern: BB, unbalanced biallelic (IB), and monoallelic (MO). The results from 103 heterozygous rats showed an expression profile in large intestine of BB (22%), IB (58%), and MO (20%). Among eight families, the percent of BB in siblings varied from 0 to 41%, MO from 0 to 33%, and IB from 49 to 83%. The parent-of-origin does not play a role in SP-D allele-specific expression. However, acquired epigenetic factors, family background, or other factors may contribute to the overall pattern of expression.

innate immunity; monoallelic expression; imbalanced biallelic expression; surfactant protein

Recently, random monoallelic or heterogeneous allele expression has been described for several groups of genes (27). These include the family of odorant receptor genes, the first ones to be described (5), and genes of the lymphoid system such as the Ly49 gene family and genes encoding different interleukins. The Ly49 family of genes code for inhibitory receptors on the surface of natural killer (NK) cells, and help distinguish self from nonself (23). Most of the NK cells express one Ly49 allele, and a small subpopulation of NK cells express both alleles (33). The cytokine gene cluster that includes IL-4, IL-13, and IL-5 (18) is under allele-specific regulation. Both monoallelic and biallelic expression were observed in IL-4 expressing CD4+ T cell clones. Approximately 80% of the clones expressed IL-13 and IL-5 from the same (IL-4-expressing) allele, indicating a coordinate regulation of this gene cluster regarding allelic expression patterns. Expression of RT6 (a marker of T cell differentiation) shows monoallelic expression in a subpopulation of T cells (29). Pax5 is the first transcription factor gene described with a predominant monoallelic expression. In early B-lymphoid progenitors and mature B cells, a single Pax5 allele is expressed, but both alleles are expressed in immature B and pre-B cells (25).

The surfactant protein D (SP-D) gene was initially found to be expressed in the epithelial cells of the distal air spaces in the lung. Subsequently, SP-D expression has been observed in mucosal surfaces of a variety of tissues (3, 24, 32, 35). SP-D, a member of the collectin family, plays an important role in the innate host defense of the lung, and alterations in the levels of SP-D in lungs may promote susceptibility to infection (6). For example, SP-D enhances phagocytosis of bacteria by alveolar macrophages through its ability to agglutinate and/or opsonize microorganisms and enhances killing of the ingested microorganisms, through its ability to enhance nitric oxide production by macrophages (26). SP-D also plays a role in viral host defense by both reducing viral load and enhancing the response of neutrophils against the virally produced inactivation of the respiratory burst activity in the phagocyte (12). Variants of Klebsiella strains encouasing with SP-D increase cytokine production by macrophages and peripheral blood monocytes (17). Ablation of SP-D in mice results in type II cell morphological abnormalities and alterations in surfactant homeostasis (2, 16, 19). SP-D knockout mice exhibit decreased...
ability for viral clearance and in response to viral infection show increased proinflammatory cytokine (TNF-α, IL-1, IL-6, and MIP-2) production (20). Moreover, recent findings indicate that SP-D may provide a link between innate and adaptive immunity (30) through its ability to bind to immature but not mature dendritic cells and enhance antigen presentation by these cells (4).

Therefore, given the role of SP-D in innate immunity and the observation that random monallelic and/or heterogeneous allele expression occurs, mostly, in genes involved in immunity, we hypothesized that SP-D exhibits monallelic or heterogeneous allele expression in a tissue-specific manner. To investigate this hypothesis, we examined SP-D expression in a variety of tissues in the rat as well as in three-generation rat pedigrees. The focus for the latter was on SP-D expression in colon. The results revealed a biallelic expression in lung with a random heterogeneous allele expression in tissues such as colon and vagina. Acquired epigenetic, family background, or other factors that do not depend on parent-of-origin may contribute to this heterogeneous allele expression.

MATERIALS AND METHODS

Rat Tissues

We performed all animal protocols according to Institutional Care and Use committee of The Pennsylvania State University College of Medicine. A piece of tail was taken for DNA and RNA Isolation.

DNA and RNA Isolation

Genomic DNA was isolated from rat tissues by QIAamp DNA Kit (Qiagen, Valencia, CA), and total RNA was isolated by RNAzol (Tel-Test, Friendswood, TX) method (22).

Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Gene</th>
<th>Position</th>
<th>Sequences 5′—3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>971</td>
<td>SP-D</td>
<td>4–26</td>
<td>F: GCCACGTTGCGCACTTCTGGA</td>
</tr>
<tr>
<td>972</td>
<td>SP-B</td>
<td>1426–1448</td>
<td>R: TACAGGGGTTGCTGCTGCTG</td>
</tr>
<tr>
<td>973</td>
<td>SP-B</td>
<td>602–624</td>
<td>F: GCCACGGTTGCTGCTGCTG</td>
</tr>
<tr>
<td>974</td>
<td>SP-B</td>
<td>636–657</td>
<td>R: CAGACACCTTGGGAATACCA</td>
</tr>
<tr>
<td>986</td>
<td>SP-B</td>
<td>1194–1214</td>
<td>F: ACCCTTGGCGAGGCTGTAG</td>
</tr>
<tr>
<td>987</td>
<td>SP-B</td>
<td>1297–1319</td>
<td>R: AGCCCCCTCTGAGAGCAGT</td>
</tr>
<tr>
<td>959</td>
<td>SP-D</td>
<td>8–28</td>
<td>F: ACCAGGAAAGGCTCTCCAG</td>
</tr>
<tr>
<td>959A</td>
<td>SP-D</td>
<td>8–28</td>
<td>R: TACAGGGGTTGCTGCTGCTG</td>
</tr>
<tr>
<td>1044</td>
<td>T7</td>
<td>1191–1212</td>
<td>F: GCCACGTTGCGCACTTCTGGA</td>
</tr>
<tr>
<td>961</td>
<td>SP-D</td>
<td>228–247</td>
<td>R: CAGACACCTTGGGAATACCA</td>
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<td>968</td>
<td>SP-D</td>
<td>1046–1066</td>
<td>F: ACCCTTGGCGAGGCTGTAG</td>
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<td>966A</td>
<td>SP-D</td>
<td>1046–1066</td>
<td>R: AGCCCCCTCTGAGAGCAGT</td>
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<td>1045</td>
<td>SP6</td>
<td>1011–1212</td>
<td>F: GCCACGTTGCGCACTTCTGGA</td>
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<tr>
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<td>Actin</td>
<td>1–19</td>
<td>R: TACAGGGGTTGCTGCTGCTG</td>
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<td>1039</td>
<td>Actin</td>
<td>1940–1960</td>
<td>F: ACCCTTGGCGAGGCTGTAG</td>
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<td>1064</td>
<td>Actin</td>
<td>343–363</td>
<td>R: TACAGGGGTTGCTGCTGCTG</td>
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</table>

References for sequences are SP-B7, SP-D31, and actin36. Lowercase letters denote mismatched nucleotides. Italic letters denote non-gene-specific sequences. Underlined sequence is T7 or SP6 promoter. F, forward; R, reverse.

Northern Analysis

Poly(A)+ mRNA (3.5 µg) was separated on 1% agarose gel containing formaldehyde (0.22 M) and transferred to GeneScreen Plus membrane (NEN Life Science, Boston, MA) and fixed under UV Stratalinker 2400 (Stratagene, La Jolla, CA). The blot was hybridized with [32P]UTP-labeled SP-D antisense RNA probe. The probe was prepared with Strip-EZ RNA Kit (Ambion, Austin, TX) (22). The template for antisense RNA synthesis was rat SP-D PCR fragment that was amplified by a first round of PCR with primers 959A and 966A, then nested with primers 1044 and 1045 to increase specificity and sensitivity. To enable the PCR fragment to direct in vitro transcription, the T7 promoter sequence was attached to the SP-B gene specific sequence at the 5′ end of primer 959A for sense strand transcription, and for antisense strand transcription the SP6 promoter sequence was attached to the SP-B gene specific sequence at the 5′ end of primer 966A. Nested primers 1044 and 1045 contained T7 and SP6 promoter sequences at the 3′ end, respectively, and a sequence of 11 random nucleotides were added at each 5′ end of these primers (Table 1). After decay, the blot was probed with 32P-tailed oligos 1039 and 1064 for Actin. The hybridization was performed in UltraHyb solution (Ambion). The hybridization and washing were done according to the manufacturer’s instruction.

Reverse Transcription and Polymerase Chain Reaction

Total RNA (1 µg) or poly(A)+ mRNA (0.1 µg) was used for RT in vitro by MMLV reverse transcriptase (Invitrogen, Carlsbad, CA) with poly(T)+ as primer. The RT product from poly(A)+ mRNA was used for genotype analysis of different rat tissues. One microliter of RT was used as template for PCR amplification (22). Nearly full-length cDNA sequences for SP-B (1,445 bp) (7), SP-D (1,219 bp) (31), and Actin (1,370 bp) (36) were amplified with primer pairs of 971 and 972, 959 and 961, and 1012 and 1038, respectively (Table 1).
Characterization of SNPs in SP-B and SP-D

We searched for single nucleotide polymorphisms (SNPs) in SP-B and SP-D by direct sequencing of RT-PCR product from 10 randomly selected rats. A cDNA sequence of SP-B cDNA (1,445 bp) was amplified with primers 971 and 972, and the PCR products were used as template to amplify two fragments with primers 971 and 974 (654 bp), and 973 and 972 (847 bp) (Table 1), respectively. A cDNA sequence of SP-D sequence (1,219 bp) was amplified with primers 1012 and 961, and the PCR products were used as template for PCR to amplify two fragments with primer pairs 959 and 966 (1,059 bp), and 965 and 961 (985 bp), respectively (Table 1). The PCR products were purified and directly used for sequencing with T7 Sequenase PCR products Kit (Amersham Pharmacia Biotech) according to the manufacturer’s instruction. The PCR products were used as template in converted PCR with primer pairs 986/H9262 and 971/H11032 restriction fragment length polymorphism (RFLP) by the use of the modified primer 969 (C allele sequence provides a recognition site for BfiI and T does not). The SP-B C/T was a natural MboII RFLP (T allele sequence provides a recognition site and C allele does not). One microliter of cDNA from RT or 100 ng DNA was used as template for PCR amplification. The PCR was carried out in a volume of 30 μl containing 0.1 mM of each dNTP, 60 ng of each primer, 1x mix of buffers 1 and 2 and 1 U Taq polymerase. All the reagents for PCR were from Roche Boehringer Mannheim Diagnostics (Indianapolis, IN). The regular PCR profile was a denaturation step at 95°C for 2 min, 35 cycles at 95°C for 30 s, 55°C for 1 min, and a final extension of 72°C for 1 min per kilobase PCR fragment to be amplified. In converted PCR (see below), one of the primers contained a mismatch, and the PCR profile was 5 cycles at 95°C for 30 s, 50°C for 1 min and 72°C for 1 min, followed by 25 cycles at 95°C for 30 s, 55°C for 1 min and 72°C for 1 min.

PCR Amplification

One microliter of cDNA from RT or 100 ng DNA was used as template for PCR amplification. The PCR was carried out in a volume of 30 μl containing 0.1 mM of each dNTP, 60 ng of each primer, 1x mix of buffers 1 and 2 and 1 U Taq polymerase. All the reagents for PCR were from Roche Boehringer Mannheim Diagnostics (Indianapolis, IN). The regular PCR profile was a denaturation step at 95°C for 2 min, 35 cycles at 95°C for 30 s, 55°C for 1 min, and a final extension of 72°C for 1 min per kilobase PCR fragment to be amplified. In converted PCR (see below), one of the primers contained a mismatch, and the PCR profile was 5 cycles at 95°C for 30 s, 50°C for 1 min and 72°C for 1 min, followed by 25 cycles at 95°C for 30 s, 55°C for 1 min and 72°C for 1 min.

PCR-Based RFLP/RFLP for Genotype Analysis

The SNPs used were C/T at nucleotide 1296 of the SP-B gene and C/T at nucleotide 100 of the SP-D gene. The SP-D C/T was converted to BfiI restriction fragment length polymorphism (RFLP) by the use of the modified primer 969 (C allele sequence provides a recognition site for BfiI and T does not). The SP-B C/T was a natural MboII RFLP (T allele sequence provides a recognition site and C allele does not). One microliter of RT was used as template for PCR amplification with primer pairs 971 and 972 (SP-B), and 1012 and 961 (SP-D), respectively. Then, 1 μl of the PCR product was used as template in converted PCR with primer pairs 986 and 987 (SP-B), and 988 and 969 (SP-D). The final natural or converted PCR products were subjected to RFLP analysis. These were digested with MboII (for SP-B) or BfiI (for SP-D) (NEB, Beverly, MA), separated on 8% PAGE, and allelic PCR products were discriminated based on size (21).

Quantification of Allelic Products and Classification of the Allele-Specific Expression Pattern

Nonradioactive method. The gel photograph was taken under UV light with Polaroid 55 film. The intensity of the band on the negative film was quantified by densitometry (Molecular Dynamics model 100A scanner, Amersham Pharmacia Biotech; and Quantity One software, The Discovery Series, Bio-Rad, Hercules, CA).

The ratio of the PCR product of allele T to allele C was used to classify the allele-specific expression pattern in each individual. If the size of the PCR T and C products was the same, then the classification would have been as follows. If amounts of the two allele products differed less than 30%, then these would have been classified as balanced biallelic (BB) expression, if more than 90% as monoallelic (MO) expression, and between 30–90% as imbalanced biallelic (IB) expression. However, for easy experimental detection, the size of the PCR product of allele T was designed to be 96 bp, and of allele C 76 bp. Therefore, in theory, with equal number of T and C molecules, the intensity of allele C band should be lower than that of allele T by ~26%. Based on the initial assumption, i.e., the amount of T and C allele is identical, then a T/C ratio between 0.7 and 1.3 would have been arbitrarily used to denote BB expression. Therefore, to adjust for the theoretical differences in intensity between T and C alleles (since the PCR size of T and C is not the same), we multiplied by a correction of 1.26. Thus a T/C ratio between 0.9 and 1.6 was used to denote BB expression. MO was noted if the expression of one allele was at least 10-fold higher than the other. Thus MO expression of the C allele (MO-C) was noted if the T/C < 0.1 × 1.26 (correction factor), and MO expression of the T allele (MO-T), if the T/C > 11 × 1.26 (correction factor) or T/C > 14. Alleles T and C in MO-C and MO-T, respectively, are minimally or not expressed.

In summary, the ratio of allele T to allele C was used for classification of BB, MO, or IB expression as follows: BB = 0.9–1.6 (not including 0.9 and 1.6); MO < 0.1 (MO-C) or >14 (MO-T); and IB = 0.1–0.9 (IB-C) or 1.6–14 (IB-T). In the IB expression, IB-C or IB-T denotes that in the given individual, allele C or allele T was expressed in higher amounts than allele T or allele C, respectively.

Radioactive method. To eliminate possible interference of heteroduplexes generated by PCR, we also used “hot-stop” PCR (34), which is a simple and a general assay for linear quantitation of allele ratios. The PCR reaction system and profile were the same as those described for the regular converted PCR, except that the reaction volume was 20 μl instead of 30 μl and the number of cycles 25. At the last PCR cycle the PCR reactions were held at 80°C while 1 μl of 32P-labeled primer 988 was added to each reaction (final concentration 2 × 104 cpm/μl). Following this addition, the reactions were carried out through the last PCR cycle with a 5-min extension step at 72°C. The PCR products were digested with BfiI and separated on 8% PAGE containing 3% glycerol. The gel was dried and exposed to X-Omat AR film (Kodak, Rochester, NY) for 2 h at ~80°C. The bands on the films were quantified by densitometry as described above. The ratio of allele T to allele C was used for classification of BB, MO, or IB expression as follows: BB = 0.7–1.3 (not including 0.7 and 1.3); MO < 0.1 (MO-C) or >10 (MO-T); and IB = 0.1–0.7 (IB-C) or 1.3–10 (IB-T).

Comparison of nonradioactive and radioactive methods. The overall conclusion from the results of the 103 heterozygous rats was similar between the two methods. With the nonradioactive method, the results were 22% of BB, 60% IB, and 18% MO, and with the radioactive method, 22% BB, 58% IB, and 20% MO. Although the overall pattern seemed to be the same, the specific pattern of allelic expression changed slightly, probably due to the higher sensitivity and accuracy of the hot-stop PCR. With the nonradioactive method there must have been heteroduplexes formed after 30 cycles of PCR; these in turn must have resulted in an overestimate of the T allele (the uncut allele) and in an underestimate of the C allele (the cut allele). Among the 60% of the IB expression obtained by the nonradioactive method, 30% were IB-T and 30% IB-C, whereas with the radioactive method among the 58% IB, 22% were IB-T and 36% IB-C. A similar change was found in MO. Among the 18% MO (nonradioactive method), 16% were MO-T and 2% MO-C, and among the 20% of MO
(radioactive method), 8% were MO-T and 12% MO-C. Figure 1 shows an example of the gel pattern of SP-D allele expression in seven colon tissues. The nonradioactive method clearly overestimates T allele expression (lanes 2, 5, 6, and 7). Therefore, for the rat pedigrees, we show only the results obtained with the radioactive method.

RESULTS

SP-D Gene Expression in Various Tissues

First, to assess SP-D gene expression in our system, we examined the expression of SP-D by Northern and RT-PCR analysis in 14 tissues that included brain, ear, eye, tongue, lung, heart, liver, kidney, spleen, stomach, small and large intestine, vagina, and penis. Actin was used as reference because it is expressed in all 14 tissues although with different levels of expression at different tissues and with differences in expression levels of the two isoforms (13) (Fig. 2).

The results (Figs. 2 and 3) indicate that SP-D expression is tissue specific. SP-D is expressed in lung, small and large intestine, stomach, vagina, ear, liver, and penis, but it is not expressed in brain, heart, tongue, kidney, and spleen, as assessed by either Northern analysis or RT-PCR. SP-D expression was at high level in lung. For lung and large intestine, SP-D mRNA signal detection required only 1.5 h of film exposure at −80°C, whereas SP-D signal detection by Northern in stomach, eye, and vagina required 24 h of film exposure at −80°C. In small intestine, ear, liver, and penis, signal detection was achieved only by RT-PCR. In Northern analysis, an mRNA of larger size than SP-D was detected in ear, eye, and tongue (Fig. 2). This hybridized to the SP-D collagen domain but not to the noncollagen domain (data not shown). In RT-PCR, a PCR product (smaller than SP-D) was detected from small intestine, kidney, and penis (Fig. 3). It is unclear whether this is an SP-D homolog or an artifact.

All the tissues (except liver) shown to express SP-D are constantly exposed to a foreign environment replete with potentially harmful substances and microorganisms. We speculate that SP-D in these tissues, as in the lung, plays a role in innate immunity (3, 24, 32, 35).

SP-D Allele Expression in Various Tissues

To study allele-specific expression of SP-D in rat, we first characterized genetic markers that would identify
each allele. These are C/T at nucleotide 100 or amino acid 25 (third nucleotide) of SP-D, and C/T at nucleotide 1296 in the 3’-UTR of SP-B, which is used as control. We genotyped DNA and cDNA of 14 tissues from a single male rat and vaginal tissue from his female sibling. Both rats were heterozygous for SP-D, and for results shown in Fig. 4, genomic DNA and cDNA from each tissue were genotyped. To assess experimental variation, PCR preferential amplification, or other errors, we performed three independent PCR reactions for each of the 14 tissues. Two of these reactions for the eight tissues shown to express SP-D are shown in Fig. 4B. We also performed duplicate RT and duplicate PCR for each RT repeat in the six vaginal tissues (Fig. 5B). Among these repeat controls, no major difference in allele-specific expression pattern was observed. Also when the surfactant protein gene, SP-B, was used as a reference, the results from 24 lung tissues indicated that SP-B expression is exclusively balanced biallelic (Fig. 5C). The SP-B findings provide additional support that the differential allele expression observed for SP-D is biological and not due to experimental error.

The results (Fig. 4B) indicated that both SP-D alleles are expressed in lung, ear, eye, stomach, small and large intestine, and penis. However, the level of expression of each allele differs as evidenced by band intensity. In lung and eye, both alleles are expressed at similar levels. We refer to this as balanced biallelic (BB) expression. In stomach and penis, the expression of allele C is lower than that of allele T; in large intestine the expression of allele C is higher than that of allele T, and in small intestine and ear, the expression of allele C appears to be higher than that of allele T. We refer to this as imbalanced biallelic (IB) expression. In vagina, allele T was barely detectable. We refer to this as monoallelic (MO) expression.

**Fig. 4.** SP-D allele-specific expression among rat tissues. A: genotype of tissue genomic DNA. B: genotype of tissue cDNA. The PCR products were digested with BfaI, and the digested PCR products were separated on 8% PAGE and stained with ethidium bromide. Two of the three PCR repeats are shown as lanes 1 and 2. Uncut, PCR products were not incubated with BfaI; dH2O, dH2O served as template in RT, and the RT served as template for PCR. Marker, 1-kb DNA ladder (Invitrogen). Arrows indicate the SP-D allelic products.

**Fig. 5.** SP-D allele-specific expression among individual rats. A: SP-D genotype analysis of DNA and cDNA from lung and large intestine. B: SP-D genotype analysis of cDNA from vaginal tissues. “RT1” and “RT2” are duplicate RT reactions, and lanes 1 and 2 are duplicate PCR amplifications for each RT. C: SP-B genotype analysis of DNA and cDNA from lung tissues. The PCR products were digested with MboI for SP-B and BfaI for SP-D. The digested PCR products were separated on 8% PAGE and stained with ethidium bromide. Notations M2, M3, . . . S2, and 1, 2, . . . 24 denote different individual rats. Uncut, PCR products were not incubated with the appropriate restriction enzymes. dH2O, dH2O served as template in RT and the RT served as PCR template. Marker, 1-kb DNA ladder (Invitrogen). The allelic PCR products are indicated with arrows. Experiments in A and B, and C were repeated three and two times, respectively.

**SP-D Allele Expression in Different Individual Rats**

To study differences in allele-specific expression among individual rats, we compared SP-D allele expression in lung, large intestine, and vagina from tissues of several individual rats. The individual SP-D genotype was determined by tail genomic DNA. The allelic expression in lung was BB in the eight tissues shown but heterogeneous in large intestine: two BB (332 and G5), three IB (m3, 380, and G1), and three MO (383, G4, and J2) (Fig. 5A). A BB expression in lung was also observed in the ~100 heterogeneous tissues studied. These were obtained from members of several families described in Fig. 6 for SP-D expression in colon. In the six vaginal tissues shown, SP-D allelic
expression was also heterogeneous: one BB (J2), three IB (M4, J1, and S2), and two MO (M2 and J4) (Fig. 5B). Out of a total of 28 vaginal tissues studied, the percent of BB, IB, and MO expression observed was 47%, 21%, and 32%, respectively, and the percent of allele-specific expression in IB and MO in this study group was IB/H18528T (14%), IB/H18528C (7%), MO/H18528T (11%), and MO/H18528C (21%).

Pedigree Study of SP-D Allele Expression in Large Intestine

To determine whether inheritable factors play a role in SP-D differential allele expression, we studied SP-D allele expression in several rat pedigrees using the radioactive method. The allelic PCR products were quantified by densitometry of DNA bands, and the ratio of alleles T/C was used for classification, as described in MATERIALS AND METHODS: BB = 0.7–1.3 (not including 0.7 and 1.3); MO < 0.1 (MO·C) or >10 (MO·T); and IB = 0.1–0.7 (IB·C) or 1.3–10 (IB·T). In MO·C or MO·T, allele C or T is predominantly expressed and the expression of allele T or C is either absent or minimal, respectively. In IB·C or IB·T, allele C or T is expressed at higher level than allele T or C, respectively.

Using the radioactive method along with densitometric quantification, we analyzed SP-D allele expression in the large intestine from 103 heterozygous individual rats of 8 families (Fig. 6). The results revealed an overall expression profile for BB = 22%, IB = 58%, and MO = 20%, with BB expression varying from 0 (S1) to 41% (S8), and MO from 0 (S2 and S5) to 33% (S4). The overall profile of IB and MO expression for each allele in this study group (n = 103) was IB·T (22%), IB·C (36%), MO·T (8%) and MO·C (12%).

For further interpretation of data, we focused on results from 4 families (S3, S6, S7, and S8) for which there were more than 10 heterozygous siblings available for study. The data showed that in a given family if the BB frequency was high (>0.25), the MO frequency was low (<0.25) (S8), and conversely, if the frequency of BB was low (<0.25), the MO frequency was high (>0.25) (S3, S6, S7). We examined whether the parent-of-origin was involved in SP-D allele-specific expression in these pedigrees. The parents of S6...
were from families S3 and S4, the father from S3 and the mother from S4. The parents of S7 were from the same families S3 and S4, but the father was from the S4 and the mother from S3. Among these four rat parents, two were heterozygous (IB·C) from S3, and the other two were homozygous (C/C) from S4. The results indicated that the parent-of-origin does not appear to play a role in this regard, because there was no significant difference detected between S6 and S7. However, interactions between genetic background and cellular microenvironment may favor expression of one allele over another.

The overall impact of the family background on the transmission of BB or MO expression was investigated in three generations of pedigrees 3, 4, and 5 (Fig. 6). Two rats, a male and a female from S4 with a family background of low BB (17%) and high MO (33%), were each crossed with a rat from S3 with low BB (13%) and high MO (31%). Their offspring showed a low BB (20% and 15.4%) and a high MO (26% and 30.8%), respectively. A third rat from S4 was crossed with a rat from family S5 with high BB (33%) and low MO (0%) family background, and their offspring showed high BB (41%) and low MO (10%). This observed overall pattern of high or low BB and of high or low MO expression does not appear to depend on parent-of-origin since comparable observations were made for the two homozygous C/C rats (one male and one female) from S4 when each of these was crossed with a member of S3. The number of heterozygous siblings in the first two families (S6, S7) was 28, and in the third one was 29 (S8). These results indicate that factor(s) involved in SP-D allelic expression may not depend on parent-of-origin, but other unknown and/or inheritable factors either alone or in combination may contribute to this random heterogeneous allele expression.

**DISCUSSION**

Random monoallelic and/or heterogeneous allele expression has been recently observed in signaling (18), regulation (25), and receptor genes (23, 33) of the immune system and the odorant receptor gene family (5). Because of the role of SP-D in innate host defense in lung and perhaps in other tissues, in this report, we investigated the hypothesis that SP-D is subject to random monoallelic and/or heterogeneous regulation in a tissue-specific manner. We first studied SP-D gene and allele-specific expression in 14 and 7 tissues, respectively, and differences of SP-D allele expression among individual rats in lung, large intestine, and vagina. Finally, to determine whether inheritable factors contribute to the heterogeneous SP-D allele expression, we studied SP-D expression in colon in eight rat pedigrees. The results showed that under normal conditions, SP-D expression in lung is BB but in other tissues may be heterogeneous and may vary from BB to IB to MO. Differences among individual rats and among family pedigrees were observed, and the SP-D MO expression did not depend on parent-of-origin. The heterogeneous allele expression pattern might be associated with family background and/or other unknown factors.

The SP-D SNP, C/T, used to identify the two SP-D alleles in the present study is located in the third position of the codon for amino acid 25, which is a leucine. This SNP does not change the encoded amino acid. Although, the possibility of functional and/or structural allele differences due to this SNP can be excluded, it cannot be excluded at present that other linked variations contribute to functional and/or structural differences between the two alleles. Therefore, it is unknown whether the SP-D heterogeneous allele expression results in quantitative or qualitative differences in the phenotype of each allele, i.e., whether it reflects an “expression level” phenotype or a “functional” phenotype due to allele protein product differences.

It is of interest that coordinate allele-specific expression has been observed for linked interleukin genes (18), indicating that at least in some cases the characteristics of the mechanism in question allow for simultaneous regulation of several linked alleles. The SP-D gene is linked to surfactant protein A and the mannose binding protein loci (15). Because these molecules also play a role in host defense and/or regulation of inflammatory cytokine production (6, 28), it would be of interest to determine whether these genes also exhibit heterogeneous allele expression and whether this is a coordinate regulation as observed for the interleukin gene cluster (18).

The significance of the monoallelic and/or the heterogeneous allele expression of SP-D under normal or adverse conditions is unknown and subject to speculation. However, in other systems, this random heterogeneous (primarily monoallelic) gene expression of the Ly49 family of genes is thought to be important in the generation of a diverse repertoire of NK cell Ly49 receptors (14). In a similar manner, the heterogeneous expression of SP-D alleles may help diversify the repertoire of this host defense molecule to combat a larger variety of foreign agents (i.e., microbes, viruses, other) and assure its evolutionary survival. Alternatively, aberrant monoallelic expression may cause haploinsufficiency, a rare genetic phenomenon in mammals (9), where expression of a single allele cannot fulfill the function of the gene. The expression of both alleles is required for normal function. Haploinsufficiency has been described for several Pax5 genes and has been associated with several disease states. Of interest, several of Pax5 genes, transcribed during B cell development, have been identified with allele-specific expression (25).

The mechanisms involved in the heterogeneous allele-specific expression are currently poorly understood. Methylation, shown to play an important role in the regulation of expression of imprinted genes (1, 11), has also been implicated in the random allele-specific expression of the RT6 gene and its tissue-specific expression (29). Mechanisms of asynchronous allele replication and cis-element control have been implicated in the regulation of monoallelic expression of the odorant receptor gene family (5). It is proposed that assyn-
chronous replication inactivates in one of the chromosomes the allelic array of the linked odorant receptor genes and that cis control is involved in the active allelic array to promote expression of a single gene.

Regardless of the mechanisms involved, allele expression differences point to yet another level of genetic variability where deranged allele expression may contribute to disease susceptibility through phenotypic differences in the “expression level” and/or the loss of the biallelic expression advantage (25). In this regard, genotypic association with disease may also require pattern association of allele-specific expression. Moreover, the observations made point to novel ways of thinking and open up new areas of investigation for SP-D and perhaps for the other collectins under both normal and compromised conditions.

Further experimentation is warranted on this topic. For example, it would be important to develop methodologies and reagents to study whether in BB or IB allele expression, both alleles are expressed in a given cell at different levels or whether some cells express one and others the other allele, and/or whether a subpopulation of cells expresses both. It would also be important to study the importance of a putative mosaic cell expression pattern and its potential role in disease pathogenesis should aberrations in this complex expression pattern do occur.

In summary, SP-D expression in a variety of extrapulmonary tissues appears to exhibit heterogeneous allele-specific expression that includes BB, IB, and MO. This type of expression does not depend on parent-of-origin, but it may be dependent on other yet unknown family background or acquired epigenetic factors.

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