Lewis and Secretor Gene Dosages Affect CA19-9 and DU-PAN-2 Serum Levels in Normal Individuals and Colorectal Cancer Patients

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ABSTRACT

The effect of doses of the secretor (Se) and Lewis (Le) genes on the serum levels of CA19-9 and DU-PAN-2 was investigated in four hundred normal individuals. It was clearly demonstrated that the Se gene dosage negatively affected both the CA19-9 and DU-PAN-2 values, whereas the Le gene dosage positively affected the CA19-9 value and negatively affected the DU-PAN-2 value.

The 400 normal individuals were separated into nine groups by their Le and Se genotypes, as follows: group 1, LeLe and se/se; group 2, LeLe and se/se; group 3, LeLe and SeSe; group 4, LeLe and SeSe; group 5, LeLe and SeSe; group 6, LeLe and SeSe; group 7, LeLe and SeSe; group 8, LeLe and SeSe; and group 9, leLe and SeSe. The group 1 individuals, having homozygous inactive Se alleles (se/se) and homozygous active Le alleles (Le/Le), exhibited the highest mean CA19-9 value. The CA19-9 value clearly ranged from a high in group 1 to a low in group 9. All of the Le-negative individuals who had the leLe genotype (groups 7, 8, and 9) had completely negative CA19-9 values, i.e., under 1.0 unit/ml, irrespective of the Se genotype. Group 7 individuals (leLe and se/se) showed a higher mean DU-PAN-2 value than did individuals in other groups. The Le-negative individuals in groups 8 and 9 also showed a higher mean DU-PAN-2 value than did the Le-positive individuals in groups 1–6.

We recommend that the revised Le and Se genotype-dependent positive/negative cutoff values for CA19-9 and DU-PAN-2, determined in this study, be applied for more accurate cancer diagnoses.

The Le and Se genotypes of 168 patients with colorectal cancer were also examined, and the CA19-9 and DU-PAN-2 values were measured before surgical resection. All 15 Le-negative patients (lele) with colorectal cancer again showed undetectable CA19-9 values, i.e., under 1.0 unit/ml, but many of them exhibited highly positive DU-PAN-2 values. In contrast, many of the Le-positive patients (LeLe or LeSe) had positive CA19-9 values, whereas very few of them exhibited positive DU-PAN-2 values. CA19-9 measurement is more useful than is DU-PAN-2 measurement for Le-positive patients, but it is not useful for Le-negative ones. DU-PAN-2 measurement should be performed in Le-negative patients for cancer diagnosis.

INTRODUCTION

The biosynthetic pathways of the Le histo-blood group system and the CA19-9 and DU-PAN-2 carbohydrate epitopes that are involved in ABH antigen synthesis, shown in Fig. 1, are considered to function in the epithelial cells of digestive organs. Both the CA19-9 and DU-PAN-2 epitopes are well-known tumor markers that are useful for cancer diagnosis of the colon, pancreas, and other organs (1, 2). The carbohydrate antigens are detected by the CA19-9 and DU-PAN-2 antibodies that are defined as the sLeα (NeuAcα2,3Galβ1,3(Fucα1,4)GlcNAcβ1-R) and sLeβ (NeuAcα2,3Galβ1,3GlcNAcβ1-R) carbohydrate structures, respectively (3, 4). sLeα, lacking α-1,4-fucosylation, is the precursor structure of sLeβ. Both antigens are known to be carried on mucins that are secreted into the plasma by cancer cells.

A CA19-9 value of 37 units/ml has been used as the positive/negative cutoff value for cancer diagnosis. However, it has been observed that some individuals often have a CA19-9 value higher than 60 units/ml but are not cancer patients.

Recent progress in the molecular analysis of Le antigen expression has clearly revealed the following points. First, the Le blood group antigens, Leα and Leβ, are the products of two kinds of fucosyltransferases, Le and Se enzymes. Leα antigen expression on erythrocytes definitely requires both the Le and Se enzymes, whereas Leβ antigen expression requires only the Le enzyme. Individuals who genetically lack the Le enzyme show the Le-negative erythrocyte phenotype [Le (a− b−)], irrespective of the Se enzyme activity (5–16). Individuals who genetically lack the Se enzyme but possess the Le enzyme show a Leα-positive and Leβ-negative erythrocyte phenotype [Le (a+ b−)] (5–16). Individuals who genetically possess both the Le and Se enzymes show a Leα- and Leβ-positive erythrocyte phenotype [Le (a+ b+)]. Unequivocally, the Le blood group phenotypes are genetically determined according to the Mendelian rules. In a series of previous studies, we found that the Le (a− b−) and Le (a+ b−) individuals are homozygotes with inactive Le alleles (lele) and inactive Se alleles (se/se), respectively (6, 9, 13). Point mutations inactivating the Se and Le genes have been found in various ethnic groups and seem to be ethnic group-specific because the mutations found in Caucasians are different from those found in the Japanese population (5–16).

In addition, Koda et al. (15) recently reported some other inactive Se alleles in the Japanese population that are different from those we have found. We finally determined all predominant Le and Se alleles distributed in the Japanese population. As summarized in Fig. 2, Le alleles could be divided into four kinds, one active allele, Le, and three inactive alleles, le1, le2, and le3. The Se alleles were divided into six kinds, two active alleles, Se1 and Se2, and four inactive alleles, se1, se3, se4, and se5 (13, 15). To date, we have examined the Le and Se genotypes of more than 600 Japanese individuals. From the results of a random sampling study on a large number of individuals, we have determined the frequency of each Le or Se allele in the Japanese population, as summarized in Fig. 2.

The le1 and le2 alleles are completely inactivated by the single missense mutations G508A and T1067A, respectively, in the catalytic domain of the enzymes (6, 9). In addition to the catalytic domain mutation, they possess and share a single missense mutation in the transmembrane domain of the enzymes that severely affects the Golgi retention of the enzyme. The impaired Golgi retention of the le3 enzyme profoundly reduces the ability of fucosylation of glycocon-
jugates. The Se2 allele possesses only one silent mutation, C357T.

In fact, it was demonstrated that all Japanese nonsecretors examined were homozygotes for the inactive sej allele, whereas all secretors possessed at least one of the active Se alleles, i.e., the Sel and Se2 alleles, homozygously or heterozygously (13).

We have established two different methods, a PCR-RFLP method (9, 13) and an ASO hybridization method (16), to determine the Le and Se genotypes through detection of the above mutations in the mutant alleles. These methods were described in detail previously by us and others (6-16). By determining the Le and Se genotypes, we have proven that the expression of type 1 Le antigens, such as Lea, are markedly reduced activity, i.e., only 3% of the activity of the wild-type enzymes, Se1 and Se2 (13). In our previous study, we demonstrated that all Japanese nonsecretors examined were homozygous for the inactive sej allele, whereas all secretors possessed at least one of the active Se alleles, i.e., the Sel and Se2 alleles, homozygously or heterozygously (13).

Considering the biosynthetic pathways for type 1 Le antigens, shown in Fig. 1, three enzymes, Le, Sel, and ST3Gal(s) (18), might compete for a common acceptor substrate, the type 1 chain. Le and ST3Gal are more dominant than the others, the pathway would flow to H type 1 and Le synthesis. If ST3Gal is more dominant than the others, it would flow to sLea and sLeb synthesis. Although there has been no proof of competition among the three enzymes, the Le blood group phenotypes have been considered to be somehow correlated to the CA19-9 value. In fact, it has been reported that many Le (a— b—) patients with pancreatic and colorectal cancers tend to show negative CA19-9 values, but there were some exceptional Le (a— b—) patients who showed positive CA19-9 values (19). However, Le blood group phenotyping is often ambiguous because of the small amounts of the antigens compared with those of other blood group antigens. In particular, individuals with some specific biological conditions, such as pregnancy and cancer, often show weakened phenotypes because of inhibition of antigen adsorption to erythrocytes and are sometimes mistyped, in a false-negative manner, as Le (a— b—) individuals (20). Recently, a paper describing the effect of the Le gene dosage on the CA19-9 value in colorectal cancer patients was published (11). The authors proposed that different cutoff values should be applied to patients, depending on the Le gene zygosity. However, they did not divide the samples according to the Se gene zygosity or refer to the DU-PAN-2 value. Here, we examined larger numbers of normal individuals and colorectal cancer patients, 400 normal individuals and 168 patients, respectively, than they examined and determined both of the Le and Se genotypes to determine to what extent the Le and Se gene dosages affect the CA19-9 and DU-PAN-2 values.

The results clearly indicate that the cutoff values for CA19-9 and DU-PAN-2 are profoundly influenced by the genotypes of both genes, i.e., the Le and Se genes. Therefore, cutoff values based on the Le and Se zygosity should be applied to patients depending on their Le and Se genotypes. In particular, the DU-PAN-2 value should be applied to cancer diagnosis for genuine Le-negative individuals, whatever the CA19-9 value is.

MATERIALS AND METHODS

Samples from 400 Normal Individuals and 168 Patients with Colorectal Cancer. Peripheral blood samples were randomly donated by 400 normal volunteers. Peripheral blood samples from 168 patients with colorectal cancer were also obtained before surgical resection. A total of 5 ml of peripheral blood were obtained with heparin. Leukocytes were separated and subjected to extraction of genomic DNA, which was used for genotyping of the Le and Se genes. A total of 2 ml of serum were subjected to measurement of the CA19-9 and DU-PAN-2 values.

Determination of CA19-9 and DU-PAN-2 Values. Both the CA19-9 and DU-PAN-2 values were determined by routine methods using commercially available kits.

Genotyping of Le and Se Genes. The methods for determining the genotypes of the Le and Se genes of Japanese individuals were described in detail previously (9, 13). Here, we used two different methods, the PCR-RFLP and ASO methods. We first carried out genotyping of the Le and Se genes by the ASO method, with which a large number of samples can be examined. Then, we subjected those samples that gave ambiguous results with the ASO method to the PCR-RFLP method, which requires much labor but gives more accurate results than does the ASO method.

ASO and PCR-RFLP Methods for Le and Se Genotyping. The ASO method was carried out as follows. The Le and Se genes encode intron-less ORFs. The primer sets, h1 and h2 or h3 and h4, for PCR amplification of the ORFs of Le and Se genes, respectively, which encode all point mutations that were examined in this study, are listed in Table 1. The sequences of the oligonucleotide probes used for hybridization to detect each point mutation, hybridization conditions, and washing conditions for the probes are also listed in Table 1. For example, we used primer set h1 and h2 for amplification of the full-length ORF of the Le gene. The amplified fragment was denatured with alkali and neutralized and then blotted onto a filter using a 96-well microplate blotting apparatus (model SRC96D; Schleicher & Schuell, Dassel, Germany). Thus, 96 individual blotted samples were duplicated on two filters. One filter was subjected to hybridization with a probe, hIT59, which has the same sequence as the active Le allele, to detect the Le allele having T59, and the other filter was hybridized with a primer, hIT59, which has the same sequence as hIT59, except for having G at position 59. All of the oligonucleotide probes used for hybridization were labeled with biotin. After hybridization, the filters were washed under the conditions listed in Table 1, and the color was developed with an avidin-biotin kit (Southern-Light, Tropix, MA), followed by

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divided into Sel, Se2, sej, se3, se4, and se5. The point alleles found in the Japanese population. The Le alleles are presented. The enzyme activity directed by each allele, mutations and cross-overs in each allele are schematically presented. The enzyme activity directed by each allele, which was determined by the transient transfection experiments on COS-1 cells (7, 10, 14, 16), is also presented. Right, frequencies of each allele occurring in the Japanese population examined in the previous studies (10, 14, 16) and those in this study. • missense mutation; ■ silent mutation; and ▲ nonsense mutation. * 551 alleles were found among the 800 alleles of the 400 normal individuals. The Le and Se genotypes of 400 randomly sampled normal individuals were determined. The Le and Se genotypes of individuals were determined. Identification of the se5 Allele. Koda et al. (15) reported the finding of three inactive Se alleles, se3, se4, and se5, in the Japanese population, which we did not find in the previous study on 205 randomly chosen Japanese samples (13). Here, we used the ASO method to detect the point mutations of C571T and C628T in the se3 and se4 alleles, respectively, as described by Koda et al. (15). Again, we did not find the se3 and se4 alleles in the samples in this study, indicating that they might be very rare in the Japanese population. As for the se5 allele, we now understand why we could not find it in the previous study. The se5 allele, a null allele, consists of the 5' region of the pseudogene SecI (12) and the 3' region of the functional Sel allele, probably arising from a cross-over between the two genes. In the previous study, we first amplified the full-length ORF of the Se gene using the primer set of the Se gene sequence, and then we subjected the amplified full-length Se gene to the second PCR of PCR-RFLP method to detect the point mutations. Therefore, the se5 allele having a different sequence in the 5' region from that of the original Se gene could not be amplified with our previous method. To detect the se5 allele, we used the primer 5'-TTGGCAAGGGGTGTTGAAAGAAGACTCTCTG-3', which has the 5' region sequence of the se5 allele, as a forward primer and a primer encoding the 3' region of the Sel allele as a reverse primer. With this primer set, the se5 allele was easily amplified and identified in this study.

RESULTS

Genotyping of 400 Randomly Sampled Normal Individuals. The Le and Se genotypes of 400 randomly sampled normal individuals were determined as described in “Materials and Methods.” Their CA19-9 and DU-PAN-2 values were also measured. The results are summarized in Table 2 and Fig. 3. The individuals were divided into nine groups by their Le and Se genotypes, as follows: group 1, Le/Le and se5/Se; group 2, Le/le and se5/Se; group 3, Le/Le and Se/se; group 4, Le/le and Se/se; group 5, Le/Le and Se/Se; group 6, Le/le and Se/Se; group 7, le/le and se/se; group 8, le/le and Se/Se; and group 9, le/le and Se/Se. The numbers of individuals in each group were 34, 34, 97, 97, 59, 39, 6, 17, and 17 for groups 1–9, respectively. The frequencies of the Le alleles, Le, le1, le2, and le3, and Se alleles, Se1, Se2, Se3, Se4, and se5, in the 400 normal individuals examined in this study were 68.9, 24.8, 5.8, and 0.5% and 13.9, 40.8, 40.6, and 4.7%, respectively, as summarized in Fig. 2. The frequency of each allele was almost consistent with that determined in the previous studies, except that of the se5 allele (9, 13). We did not find the se3 and se4 alleles in either the previous study or in this one, probably because their frequencies are very low in the Japanese population. The reason why we did not find the se5 allele in the previous study was discussed in “Materials and Methods.” Here, we found 38 se5 alleles among the 800 alleles of 400 individuals, which showed the frequency of the se5 allele was 4.7% in the Japanese population. The 40 individuals in groups 7, 8, and 9, who were determined to be homozygous for the inactive Le gene, showed the Le (a− b−) erythrocyte phenotype. The 68 individuals in groups 1 and 2, who were determined to be homozygous for the inactive Se gene, showed the Le (a+ b−) erythrocyte phenotype. With the more sensitive hemagglutination assay, many of the se5 homozygotes showed the Le (a+ b+) phenotype, due to the residual activity of the sej enzyme (14). The Le (a+ b+) phenotype was observed in the se/se individuals who had at least one sej allele (data not shown). The rest, 292 individuals who possessed at least one active allele of the Le and Se genes, showed the Le (a− b+) erythrocyte phenotype. Seventy-four individuals, representing 18.5% of the 400 individuals, who belonged to groups 1, 2, and 7 were nonsecretors, meaning they cannot secrete ABH antigens into their saliva.

CA19-9 Values in 400 Normal Individuals. The CA19-9 values in the 400 normal individuals are plotted in Fig. 3, separately for each group. The mean values, SDs, and positive/negative cutoff values, which were calculated as (2 × SD) + mean, are presented in Fig. 3 and listed in Table 2. A CA19-9 value of around 37 units/ml has been summarized in Table 2 and Fig. 3. The individuals were divided into nine groups by their Le and Se genotypes, as follows: group 1, Le/Le and se5/Se; group 2, Le/le and se5/Se; group 3, Le/Le and Se/se; group 4, Le/le and Se/se; group 5, Le/Le and Se/Se; group 6, Le/le and Se/Se; group 7, le/le and se/se; group 8, le/le and Se/Se; and group 9, le/le and Se/Se. The numbers of individuals in each group were 34, 34, 97, 97, 59, 39, 6, 17, and 17 for groups 1–9, respectively. The frequencies of the Le alleles, Le, le1, le2, and le3, and Se alleles, Se1, Se2, Se3, Se4, and se5, in the 400 normal individuals examined in this study were 68.9, 24.8, 5.8, and 0.5% and 13.9, 40.8, 40.6, and 4.7%, respectively, as summarized in Fig. 2. The frequency of each allele was almost consistent with that determined in the previous studies, except that of the se5 allele (9, 13). We did not find the se3 and se4 alleles in either the previous study or in this one, probably because their frequencies are very low in the Japanese population. The reason why we did not find the se5 allele in the previous study was discussed in “Materials and Methods.” Here, we found 38 se5 alleles among the 800 alleles of 400 individuals, which showed the frequency of the se5 allele was 4.7% in the Japanese population. The 40 individuals in groups 7, 8, and 9, who were determined to be homozygous for the inactive Le gene, showed the Le (a− b−) erythrocyte phenotype. The 68 individuals in groups 1 and 2, who were determined to be homozygous for the inactive Se gene, showed the Le (a+ b−) erythrocyte phenotype. With the more sensitive hemagglutination assay, many of the se/se homozygotes showed the Le (a+ b+) phenotype, due to the residual activity of the sej enzyme (14). The Le (a+ b+) phenotype was observed in the se/se individuals who had at least one sej allele (data not shown). The rest, 292 individuals who possessed at least one active allele of the Le and Se genes, showed the Le (a− b+) erythrocyte phenotype. Seventy-four individuals, representing 18.5% of the 400 individuals, who belonged to groups 1, 2, and 7 were nonsecretors, meaning they cannot secrete ABH antigens into their saliva.
Table 1 Primer sets used for amplification of the Le and Se genes and oligonucleotide probes used for hybridization with the ASO method

<table>
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<th>Primer name</th>
<th>Primer sequence</th>
<th>Annealing temperature (°C)</th>
<th>Product size (bp)</th>
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<td>1082</td>
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<tr>
<td>hi2</td>
<td>5'-CAGCGGGCAGCCTTCTGAGGATCCAGG-3'</td>
<td>64</td>
<td>445</td>
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Probe name | Probe sequence | Hybridization temperature (°C) | Washing temperature (°C) |
<table>
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<td>5'-CAGCGGGCAGCCTTCTGAGGATCCAGG-3'</td>
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<td>hiG59</td>
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<td>32</td>
<td>45</td>
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<td>45</td>
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<td>45</td>
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</table>

Washing conditions: 2× SSC, 10 min, twice recommended as the positive/negative cutoff value for cancer diagnosis. As can be seen in Fig. 3, 10 individuals exhibited CA19-9 values that were much higher than 37 units/ml. Seven of them, interestingly, belonged to group 1, and the others belonged to groups 2, 3, and 4. The highest mean value, 26.0 units/ml, for CA19-9 was obtained in group 1, as compared to in the other groups. The second highest CA19-9 mean value, 20.2 units/ml, for CA19-9 was observed in group 2. Groups 3 and 4 showed almost the same CA19-9 mean value, i.e., 10.5 and 9.0 units/ml, respectively, and groups 5 and 6 also showed the same CA19-9 mean value, 7.2 units/ml for both, which was a little lower than those of groups 3 and 4. The mean values for CA19-9 in group 7, 8, and 9 were 0.18, 0.4, and 0.18 units/ml, respectively.

The above facts demonstrate the following points. The Se gene dosage clearly affects the CA19-9 value. The se/se individuals (non-secretors) in groups 1 and 2 appeared to show higher CA19-9 values than did the secretors. A difference in the Le gene dosage effect was also observed in the secretors between those who were heterozygous for the Le allele and those who were homozygous for the Le allele, i.e., between the Se/se individuals (groups 3 and 4) and the Se/Se individuals (groups 5 and 6). A difference in the Le gene dosage effect in the nonsecretors, the se/se individuals, was clearly observed between groups 1 (Le/Le) and 2 (Le/le). However, a difference in the Le gene dosage effect in the secretors (the Se/se and Se/Se individuals) was not observed between groups 3 (Le/Le) and 4 (Le/le), or groups 5 (Le/Le) and 6 (Le/le). The completely negative CA19-9 values in all of the le/le individuals (groups 7, 8, and 9), irrespective of the Se genotype, again confirmed that the Le enzyme is definitely required for synthesis of the sLe<sup>a</sup> epitope, which is the CA19-9 epitope, as we found previously (17).

DU-PAN-2 Values in 400 Normal Individuals. The DU-PAN-2 epitope, which lacks α-1,4-fucosylation, is the precursor structure of the CA19-9 epitope. The mean values, SDs, and cutoff values for DU-PAN-2 for each of the nine groups are summarized in Table 2. As expected, the le/le individuals in groups 7, 8, and 9, who are Le-negative, lacking the Le enzyme, showed much higher mean values than did the Le-positive individuals (groups 1–6). In addition, a Se gene dosage effect on the DU-PAN-2 value was observed in the Le-negative groups, i.e., the mean DU-PAN-2 decreased from group 7 to group 9 (33.7, 15.7, and 13.2 units/ml, respectively). In particular, six individuals in group 7, whose genotypes were le/le and se/se, showed a very high mean value of DU-PAN-2, i.e., 37.3 units/ml. However, the Se gene dosage effect was not observed in the groups of Le-positive individuals (groups 1–6). All of the Le-positive individuals in groups 1–6 showed almost the same mean DU-PAN-2 value of around 6.0–8.9 units/ml. The three groups of Le-negative individuals (groups 7–9) showed significantly higher cutoff values for DU-PAN-2.

Table 2 Le and Se gene dosage effects on the CA19-9 and DU-PAN-2 values in normal individuals

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>Le/Le</td>
<td>Le/Le</td>
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<td>se/se</td>
<td>se/se</td>
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<td>se/se</td>
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<tr>
<td>No. of individuals</td>
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<td>34</td>
<td>97</td>
<td>34</td>
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<td>97</td>
<td>34</td>
<td>34</td>
<td>97</td>
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<tr>
<td>Mean</td>
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<td>20.2</td>
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<td>9.0</td>
<td>7.2</td>
<td>7.2</td>
<td>0.06</td>
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<tr>
<td>SD</td>
<td>17.8</td>
<td>10.6</td>
<td>7.3</td>
<td>6.6</td>
<td>4.7</td>
<td>5.4</td>
<td>0.05</td>
<td>0.2</td>
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<td>41.3</td>
<td>25.2</td>
<td>22.3</td>
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<sup>a</sup> Cutoff value was calculated as: mean + 2 (SD).
(87.6, 44.0, and 37.9 units/ml, respectively) than did the groups of Le-positive individuals, whose cutoff values were less than 30.2 units/ml (group 5).

Le-negative Patients with Colorectal Cancer Never Show a Positive CA19-9 Value but Frequently Show a Positive DU-PAN-2 Value. The Le and Se genotypes of the 168 patients with colorectal cancer, who were randomly sampled, were determined, and their CA19-9 and DU-PAN-2 values in serum before surgical resection were also measured. Fifteen le/le patients were found in the total cancer patients. The 15 le/le patients did not gather in the low clinical stage, i.e., they were distributed into the four Dukes’ stages: two le/le patients in Dukes’ A, six le/le patients in Dukes’ B, five le/le patients in Dukes’ C, and two le/le patients in Dukes D. Because the patients were randomly sampled, the clinical stages of cancer progression varied. Therefore, the Le and Se gene dosage effects on the two tumor marker values, as obtained in the normal individuals, were not clearly observed (data not shown). We applied the cutoff values for CA19-9 and DU-PAN-2, determined in the normal individual experiment in the previous section, to each patient, depending on their Le and Se genotypes.

As can be seen in Fig. 4, it was clearly demonstrated that all 15 Le-negative patients having the le/le genotype, among the 168 patients, never showed a positive CA19-9 value and, again, exhibited an undetectable amount of CA19-9 (<1.0 unit/ml). However, 7 of these 15 Le-negative patients (46.7%) showed apparent positive DU-PAN-2 values, which were much higher than those of the Le-positive patients, who showed positive DU-PAN-2 values (Fig. 5). In particular, four Le-negative patients showed very high DU-PAN-2 values (>4000 units/ml), although they showed undetectable CA19-9 values. The rest, 153 patients, were determined to be Le-positive individuals having at least one active Le allele. Fifty-three of the 153 Le-positive patients (34.6%) showed a positive CA19-9 value, to which the cutoff value determined in this study was applied (Fig. 4). Only eight of the 153 Le-positive patients (5.2%) showed a positive DU-PAN-2 value (Fig. 5). The positive DU-PAN-2 values in the Le-positive patients were much lower than those in the Le-negative patients, who showed positive DU-PAN-2 values. Among the eight Le-positive patients who showed positive DU-PAN-2 value, five showed both positive CA19-9 and DU-PAN-2 values, and the rest (three patients) showed only a positive DU-PAN-2 value; the values were very low but still a little higher than the cutoff values (Fig. 4).

DISCUSSION

The Lea and Leb antigens are blood group antigens found on RBCs. There have been some reports that pancreatic and colorectal cancer patients with the Le blood group-negative phenotype of RBCs tend to show negative CA19-9 values in serum (19, 21). Despite this tendency, there have been some exceptional patients who showed the Le-negative blood group phenotype [Le(a- b-)] but apparently had positive CA19-9 values (20, 22). Lea- and Leb-glycolipid antigens are known to be synthesized in some intestinal tissues and released into the plasma and then adsorbed to RBCs (23). Previously, we demonstrated that the Le blood group phenotype of cancer patients’ RBCs is often mistyped, in a false-negative manner, because the adsorption of Le-active glycolipids to RBCs is inhibited in such patients (20).
was considered to be the reason why some Le (a− b−) patients with cancer, who are mistyped, in a false-negative manner, through a hemagglutination test and, thus, are not genuine Le-negative individuals, showed high CA19-9 values. The genuine Le-negative patients, who genetically lack the Le enzyme, were found to be the homozygotes with null Le alleles (le/le) and never showed the expression of the sLea antigen in colorectal cancer tissue (17) or a positive CA19-9 value in serum (11). We demonstrated that Le genotyping is the most accurate method for distinguishing genuine Le-negative patients from false-negative patients (9, 13). We also demonstrated that the Le enzyme is solely responsible for the expression of sLea antigens in colon cancer tissues and concluded that the sLea epitope recognized by the CA19-9 antibody is a product of the Le enzyme (17).

On examination of the samples from the randomly chosen 400 normal individuals, we observed apparent Le and Se gene dosage effects on CA19-9 and DU-PAN-2 values. As is clearly shown in Fig. 3 and Table 2, the Se gene dosage reduced the CA19-9 value and the DU-PAN-2 value, whereas the Le gene dosage augmented the CA19-9 value. The former's effect on the CA19-9 value was more clearly observed than was the latter's. These facts strongly indicated that the Se enzyme competes for the acceptor substrate, i.e., the type 1 chain with ST3Gal(s), which are responsible for the synthesis of the sLea epitope (DU-PAN-2 epitope). The Le enzyme may also compete with the ST3Gal(s) for the type 1 chain, but it is also required for sLea synthesis from the sLea precursor. The Le enzyme might be involved in both the competition with ST3Gal(s) and the synthesis of sLea, as would be expected from the synthetic pathways in Fig. 1. Thus, the Le gene dosage effect on the CA19-9 value was not as dramatic as the Se gene dosage effect.

Genuine Le-negative individuals (le/le) never exhibited a positive CA19-9 value, and all of them, whether they were cancer patients or normal individuals, showed CA19-9 values of under 1.0 unit/ml. This confirmed that the Le enzyme is merely responsible for the synthesis of sLea (the CA19-9 epitope), as reported previously by us and others (11, 17). The normal genuine Le-negative individuals showed higher DU-PAN-2 values than did the Le-positive ones. This fact clearly demonstrated that the sLea epitope (the precursor structure of sLea) accumulates in the sera of Le-negative individuals because of the lack of α-1,4-fucosylation. In addition, a difference in the Se gene dosage effect on the DU-PAN-2 value was observed between in the Le-negative nonsecretors (le/le, se/se) and the Le-negative secretors (le/le, Se/Se). This fact again indicated that the Se enzyme competes with ST3Gal(s). There are two possible modes of enzyme competition for the acceptor substrate. First, there should be competition between the enzymes that share the acceptor substrate if they are colocalized in the Golgi compartment, and the amount of each product should be determined by the relative activity of each enzyme. Second, if one enzyme is localized in a subcompartment prior to that of another enzyme in the Golgi apparatus, the former enzyme works more efficiently in synthesis, although its amount is very small. It is a very difficult task to determine the Golgi compartmentation of each enzyme. It remains to be elucidated which of the above two modes explains the competition between the enzymes Le, Se, and ST3Gal(s), which are involved in the synthesis of the sLea and sLea epitopes.

Recently, we quantitatively measured the amounts of transcripts for the Le, Se, and ST3Gal(s) genes expressed in normal and cancerous colorectal tissues.5 The Le gene was found to be ubiquitously and abundantly expressed, regardless of whether the tissue was normal or cancerous, from which we concluded that the amount of the Le enzyme expressed in a tissue does not determine the amount of sLea antigens in the tissue or the CA19-9 value in serum. If the precursor structure for the sLea (CA19-9) epitope [the sLea (DU-PAN-2) epitope] exists in the tissues of Le-positive individuals, the Le enzyme seems to be abundant enough to convert almost all of the precursor into the sLea epitope. This would be the reason why Le-positive patients with colorectal cancer showed a high frequency of positive CA19-9 values but a very low frequency of positive DU-PAN-2 values. The Le enzyme is unequivocally the only enzyme responsible for α-1,4-fucosylation of the sLea epitope; therefore, the Le-negative patients, who genetically lack the Le enzyme, never synthesized the sLea (CA19-9) epitope, although the precursor structure, sLea(DU-PAN-2), accumulates at a very high level in the tissues of Le-negative patients. On the other hand, the amount of transcripts for the Se gene expressed in colorectal tissue was not very high, as compared to those for the Le gene. This fact would explain why the observed Se gene dosage effect is more dramatic than the observed Le gene dosage effect.

It is not still possible to routinely determine the Le and Se genotypes in an ordinary hospital laboratory. Here, we raise some important proposals regarding the clinical diagnosis of colorectal cancer, using CA19-9 and DU-PAN-2 values as tumor markers.

First, the Le blood phenotype should be determined by hemagglutination tests, in addition to CA19-9 measurement. In the case of individuals showing the Le-positive phenotype, the CA19-9 value is useful for colorectal cancer diagnosis. The individuals showing the Le-negative phenotype consist of nongenuine and genuine Le-negative individuals. We can distinguish the genuine Le-negative individuals from the nongenuine ones by the CA19-9 value. The genuine Le-negative individuals never show detectable CA19-9 values (usually <1.0 unit/ml), whereas nongenuine Le-negative individuals usually exhibit detectable CA19-9 values (i.e., >5 units/ml), as demonstrated here. Measurement of the CA19-9 value should be applied to the nongenuine Le-negative individuals, whereas measurement of the DU-PAN-2 value should be applied to the genuine ones. Measurement of the CA19-9 value is of no use for genuine Le-negative patients. The frequency of genuine Le-negative individuals is approximately 10–11% in the Japanese population, as found here and previously.

Alternatively, both tumor markers, CA19-9 and DU-PAN-2, should be measured in all individuals for the first-time diagnosis. The patients showing positive CA19-9 values should then undergo CA19-9 measurement to examine the recurrence of cancer. On the other hand, the patients who showed positive DU-PAN-2 values and undetectable CA19-9 values (usually <1.0 units/ml) must be genuine Le-negative individuals (le/le). If the CA19-9 value is <1.0 units/ml and the DU-PAN-2 value is negative, the patient is probably a genuine Le-negative individual. To monitor the cancer recurrence in such genuine Le-negative patients, the DU-PAN-2 value should be measured, whatever the CA19-9 value is.

Second, the Le (a− b−) phenotype of the genuine Le-negative patients never became Le-positive, even after surgical resection of cancer tissues, whereas the nongenuine Le-negative patients, who showed the Le (a− b−) phenotype when they had cancer tissue, became Le-positive after surgical resection. Therefore, the phenotype of the Le blood group should be again determined after recovery of the patients, and then the CA19-9 value or the DU-PAN-2 value should be applied to the Le-positive ones or the genuine Le-negative ones, respectively, for monitoring cancer recurrence.

Third, the patients with the Le (a+b−) or Le (a+b+) phenotype are nonsecretors; therefore, the cutoff values for CA19-9, as determined for the nonsecretors in groups 1 and 2 here, which are much higher than 37 units/ml, should be applied to cancer diagnosis.

In the future, a simpler method for determining the Se and Le
genotypes, which is available as a routine test, is expected to be developed. Such a simpler method will be useful for more accurate cancer diagnosis, by measuring the two tumor markers CAI9-9 and DU-PAN-2.

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Lewis and Secretor Gene Dosages Affect CA19-9 and DU-PAN-2 Serum Levels in Normal Individuals and Colorectal Cancer Patients

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