Influence of nucleotide polymorphisms in the CCR2 gene and the CCR5 promoter on the expression of cell surface CCR5 and CXCR4

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Abstract

Polymorphisms in the CCR2 gene (CCR2-64I) and the CCR5 promoter (pCCR5-59029G) have been correlated with slower HIV-1 disease progression. How these polymorphisms influence the rate of AIDS progression has remained unclear. We have therefore investigated whether these nucleotide polymorphisms will reduce the expression levels of surface CCR5 and CXCR4, and thus lead to slower AIDS progression. For this, a cohort of Chinese volunteers in Taiwan was subjected to the determination of CCR2 and pCCR5 genotypes followed by analysis of the surface CCR5 and CXCR4 expression on five cell types derived from peripheral blood mononuclear cells by flow cytometry. Several significant associations were detected between genotypes and expression levels of the proteins. The most important finding was that an increased number of CD4⁺/c9059 cells expressing CCR5 correlated with pCCR5-59029A homozygosity without the interference of both the CCR2-64 and the CCR5∆32 mutations (P = 0.0453), which is consistent with the previous data on the association of the genotype to AIDS progression. Since different genetic polymorphisms co-exist in human beings, the rate of AIDS progression as well as the risk of rheumatoid arthritis may be governed by the interplay of the array of nucleotide changes and their affected proteins.

Introduction

The CC chemokine receptor (CCR) 5, one of many β-chemokine receptors, on CD4⁺ cells is one of the main gates for HIV-1 (predominantly macrophage-tropic or R5 strains) entry into target cells (reviewed in 1). Many correlation studies have suggested that the levels of CCR5 on CD4⁺ cells determine the susceptibility to R5 virus infection as well as govern the rate of HIV-1 disease progression (2–7). For example, a 32 nucleotide deletion in the CCR5 coding region (CCR5Δ32) produces a truncated protein that fails to reach the cell surface (7,8). Consequently, CCR5Δ32 homozygotes resist HIV-1 infection despite repeated sexual contacts (2–4). CCR5Δ32 heterozygotes, however, are susceptible to HIV-1 infection, but with a 2–4 year delay on the development of AIDS, which is likely due to fewer CCR5⁺ cells inside their bodies (1.3,9–11). In vitro experiments have demonstrated that CCR5Δ32 produces a truncated protein with the trans-dominant inhibitory phenotype for CCR5-dependent HIV-1 infection (8). Furthermore, the expression levels and pattern of CCR5 in peripheral blood T cells correlate well with R5 virus infectability in vitro (7,12–14). Thus, the availability of cell surface CCR5 plays an important role in HIV-1 pathogenesis. Another β-chemokine receptor mutant allele, CCR2-64I, also...
correlates with slower HIV-1 disease progression (10,15,16). CCR2 mediates cellular entry of the rare R5 and dual-tropic (R5X4) HIV-1 strains. A change of valine (V) to isoleucine (I) at position 64 in CCR2 has been reported in all races, with one CCR2-64I allele alone conferring 2–4 year slower AIDS progression that is similar to individuals with heterozygous CCR5-32 alleles. However, unlike CCR5, CCR2 is not an essential HIV-1 co-receptor and conservative substitution of V for I in the protein should not cause any serious functional defect—therefore CCR2-64I influences the rate of AIDS progression possibly through an indirect mechanism rather than the mutation itself. An in vitro co-transfection experiment performed by Lee et al. demonstrated that the expression of CCR2-64I intracellularly did not affect surface CCR5 levels (17), suggesting negligible interaction between the proteins. Kostrikis et al. reported, at the same time, that a nucleotide transition from C to T at position 59653 in the CCR5 promoter (pCCR5-59653T) was in complete linkage disequilibrium with the codon 64 missense mutation of CCR2 (15). This observation raises the possibility that the mutation of pCCR5-59653T, instead of the CCR2-64I allele, caused slower HIV-1 disease progression, presumably via down-regulation of CCR5 expression at the transcription level. More recently, evidence has shown that mutant CCR2-64I, but not the wild-type protein, formed heterodimers with CXCR4 that may impair the function of CXCR4 as a co-receptor for the entry of R5 strains (18). Taken together, many potential pathways may be involved in controlling the rate of AIDS progression; however, these have not been confirmed or thoroughly investigated, although a few reports have analyzed the correlation of the CCR2 and pCCR5-59653 alleles with the expression of surface CCR5 or CXCR4 (15,17–19).

Furthermore, a polymorphic change at position 59029 (from G to A) in the CCR5 promoter (pCCR5-59029G) has also been reported to correlate with the rate of HIV-1 disease progression and the expression levels of surface CCR5 (20). The clinical study showed that HIV-1-infected patients with homozygous pCCR5-59029G, but without the CCR5-32 and CCR2-64I alleles, progressed to AIDS on average 3.8 years slower than those with homozygous A. Consistently, in vitro transcription regulatory activity of pCCR5-59029A was approximately twice as high as that of pCCR5-59029G. A strong correlation between the promoter activity in vitro and clinical manifestations thus suggests that the infectability of HIV-1 depends on CCR5 availability. However, how transcriptional activity of the CCR5 promoter with the nucleotide polymorphism affects the levels of surface CCR5 on different blood cell types has not been systematically analyzed. The notion is that there are three possible genotypes derived from the combination of two alleles, G or A, for pCCR5-59029 and each allele contributes partly to total CCR5 expression on the cell surface.

Since the availability of CCR5 or CXCR4 is likely to determine the rate of HIV-1 disease progression, we therefore investigated whether a group of healthy, unrelated Chinese volunteers in Taiwan who harbor these different polymorphic alleles are associated with higher or lower expression of surface CCR5 and/or CXCR4 on their peripheral blood mononuclear cells (PBMC), CD3+ cells, CD4+ cells, CD4+ lymphocytes or CD4+ monocytes. The results may help to explain how genetic variants affect the pathogenesis of HIV-1.

Methods

Subjects and genotyping of β-chemokine receptor alleles
Molecular biology reagents were mainly purchased from Stratagene (La Jolla, CA) unless otherwise specified. All reagents were used according to the recommendations from the manufacturers.

Ninety-three randomly selected, healthy HIV-1-seronegative Chinese volunteers, 21–50 years old (average 28.7 years), in Tainan and Taichung Cities, Taiwan participated in genotype determinations. At the time of collecting blood samples, all volunteers were healthy and did not recall any sickness including common cold in at least 1 month. Genomic DNA of the participants was isolated from 0.5–1.0 ml of whole blood (in EDTA anti-coagulant tubes) with Puregene DNA (Gentry, Research Triangle Park, NC). Then, 1 µg of genomic DNA was used in the routine PCR-RFLP genotyping of the all CCR2-64I, pCCR5-59653T and pCCR5-59029A polymorphisms as per the previous reports (10,20,21), except that the reverse primer for determining the CCR2-64I allele was replaced by 5′-CGTGTGAAATTGGACATTGC-3′ [nucleotide positions from 46436 to 46457 in (22)]. Amplification with the CCR2-64-specific primers yielded a 183 bp PCR product that was subsequently digested with restriction enzyme BsaI (New England Biolab, Beverly, MA). The production of 165 and 18 bp DNA fragments served as a diagnostic determinant for the mutant (64I) allele, whereas the PCR fragment for the wild-type allele resisted such digestion. The PCR products for the genotyping of all alleles have been randomly selected for automated nucleotide sequence analysis (ABI Prism 377 DNA sequencer; Perkin-Elmer, Foster City, CA) to ensure the accuracy of the PCR-RFLP determinations.

Flow cytometry analysis of cell surface CCR5 and CXCR4
Among the participants with genotypes determined, 66 and 57 individuals were selected at random for the analysis of the CCR5 and CXCR4 cell surface expression by flow cytometry. Shortly before FACScan analysis, a small portion of fresh drawn whole blood (10 ml) from each participant was subjected to a complete blood cell count (CBC) performed by medical technicians at the Hematology Section, Department of Pathology, National Cheng Kung University Hospital. The CBC results confirmed that blood cell counts and percentages for the three major cell types (neutrophil, lymphocyte and monocyte) were in normal ranges for Chinese (data not shown), suggesting that no inflammation was present in any of the studied volunteers. The remaining whole blood was fractionated with Ficoll-paque gradient (Pharmacia Biotech, Uppsala, Sweden) individually and the separated PBMC was collected. Approximately 106 cells were stained with one or more mouse anti-human mAb (all purchased from Phar-Mingen, San Diego, CA), which included FITC–anti-CD4 mAb (clone RPA-T4), FITC–anti-CD3 mAb (clone UCHT1), phycoerythrin (PE)–anti-CCR5 mAb (clone 2D7) and PE–anti-CXCR4 mAb (clone 12G5), for 40 min on ice. Approximately 106 cells were separated into three cell populations, including PBMC, CD3+ cells and CD4+ cells. CD4+ cells were further divided into CD4+ lymphocytes and CD4+ CD45+ monocytes by staining with mouse anti-human CD14 mAb (clone M5E2). These cells were analyzed for levels of CCR5 and CXCR4
expression by a FACSCalibur flow cytometer equipped with an argon laser, and calibrated using CaliBRITE beads and FACS COMP software (Becton Dickinson, Mountain View, CA). During the measurements of FITC- and PE-labeled cells, 530DF30 (FL-1 channel) and 575DF26 (FL-2 channel) band-pass filters were used. Data was then acquired and analyzed using CellQuest software (Becton Dickinson).

Results

Genotyping of β-chemokine receptor alleles

PCR-RFLP was used to determine the genotypes of CCR2-64, pCCR5-59653 and 59029 alleles for the 93 individuals in the studied group. Table 1 summarizes the results of the genotyping experiments, showing that CCR2-64 is in complete linkage disequilibrium with pCCR5-59653T in the Chinese so far tested in our study, as was found previously among Caucasians (15). Therefore, CCR2 genotypes of wt/wt, wt/64I and 64I/64I can be represented as genotypes of pCCR5-59029 or pCCR5-59653 genotype were subjected to χ² tests to reveal any significant difference and the respective value of $P = 0.45$ or $P = 0.76$ was found.

### Table 1. CCR2 and CCR5 genotype distributions in the studied Chinese cohort

<table>
<thead>
<tr>
<th>CCR2</th>
<th>C/C</th>
<th>C/T</th>
<th>T/T</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2 wt/wt</td>
<td>58 (62.4)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>58</td>
</tr>
<tr>
<td>CCR2 wt/64I</td>
<td>0 (0.0)</td>
<td>29 (31.2)</td>
<td>0 (0.0)</td>
<td>29</td>
</tr>
<tr>
<td>CCR2 64I/64I</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>6 (6.4)</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>29</td>
<td>6</td>
<td>93</td>
</tr>
<tr>
<td>pCCR5-59029</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>31 (33.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>31 [28]a</td>
</tr>
<tr>
<td>G/A</td>
<td>20 (21.5)</td>
<td>20 (21.5)</td>
<td>0 (0.0)</td>
<td>40 [46]</td>
</tr>
<tr>
<td>A/A</td>
<td>7 (7.5)</td>
<td>9 (9.8)</td>
<td>6 (6.4)</td>
<td>22 [19]</td>
</tr>
</tbody>
</table>

Note: The numbers inside the square brackets represent the expected values by Hardy–Weinberg analysis. Total no. (n) = 93.

Chemotactic activity for monocytes and lymphocytes from CD4+ cells was occasionally used to separate CD4+ lymphocytes and monocytes, as indicated by an oval in the lowest plot of the left panel.

expression for the Chinese population. Interestingly, we observed and expected numbers for each pCCR5-59029 or pCCR5-59653 genotype were subjected to χ² tests to reveal any significant difference and the respective value of $P = 0.45$ or $P = 0.76$ was found.

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<td>0 (0.0)</td>
<td>6 (6.4)</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>29</td>
<td>6</td>
<td>93</td>
</tr>
<tr>
<td>pCCR5-59029</td>
<td></td>
<td></td>
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Expression of CCR5 and CXCR4 on a variety of peripheral blood cell types. A representative flow cytometric analysis of PBMC in the presence or absence of mAb against human CD3, CD4, CCR5 and/or CXCR4 is shown. Three plots at the left panel, from the bottom to the top (y-axis), show cells stained individually with FITC–anti-CD4 antibody, FITC–anti-CD3 antibody or without any antibody staining. Besides treating PBMC under the identical experimental conditions as those plots in the left panel, the plots shown in the center and the right panels (along the x-axis) were also reacted with PE-anti-CCR5 or PE-anti-CXCR4 mAb respectively. Cell percentages in three of four quadrants are indicated in each plot. Since CD4+ cells consist of lymphocytes and monocytes, anti-CD14 mAb was occasionally used to separate CD4+ lymphocytes from CD4+ monocytes, as indicated by an oval in the lowest plot of the left panel.

Genetic variants and CCR5 and CXCR4 expression

Wu et al. have shown that fewer peripheral blood lymphocytes expressed surface CCR5 and there was decreased average protein quantity in healthy individuals heterozygous for CCR5a32, correlating with slower AIDS progression (7). A similar experimental design was applied to study the nucleotide polymorphisms relative to the levels of CCR5 and CXCR4 expression on the cell surface. Flow cytometry analysis was able to determine percentages of five blood cell types, including PBMC, CD3+ cells, CD4+ cells, CD4+ lymphocytes and CD4+ monocytes, that expressed surface CCR5 and CXCR4 (Fig. 1). Flow cytometry also determined mean fluorescence intensities (MFI) for CCR5 and CXCR4 that represented the protein expression levels on cell surface. A typical flow cytometric result in Fig. 1 shows that more CD4+ monocytes than CD4+ lymphocytes express detectable CCR5, whereas the reversed situation is found for these two cell types expressing surface CXCR4 in our study group. Subsequently, the presence of the pCCR5-59653 and 59029 allelic variants could be categorized into 10 haplotypes (P1–P10) have been reported to correlate with AIDS progression (23). Therefore, the strength of the CCR5 promoter is thus likely governed by an array of polymorphic nucleotide changes. Currently, we are determining the frequency of those newly described P1–P10 polymorphisms in our study group so that the relation of the haplotypes with surface CCR5 expression levels can be analyzed.
genotypes in the studied cohort was correlated with the percentages of the different CCR5<sup>+</sup> cell types and CCR5 MFI on cell surface. Furthermore, the CCR2-64I (pCCR5-59653) genotypes were also correlated with CXCR4<sup>+</sup> cell percentages as well as the expression levels of the protein. Statistical analysis for any possible association of the particular genotypes with the levels of surface CCR5 or CXCR4 on all five cell types used the Kruskal–Wallis test for three-group comparisons. When a meaningful result was found, Dunn’s multiple comparison test was then employed to determine which two genotypes exhibited significant difference in expressing the surface proteins.

As in previous reports (15, 17, 19), neither CCR5<sup>+</sup> cell numbers nor the expression levels of surface CCR5 revealed any significant difference in individuals with different pCCR5-59653 genotypes, even though there was a trend for the pCCR5-59653T/T genotype to exhibit the lowest CCR5<sup>+</sup> cell percentages in all cell types examined in this study (data not shown). Since a recent report suggests that CCR2-64I regulates HIV-1 disease progression by interacting with CXCR4 (18), we investigated whether the CCR2-64I (also pCCR5-59653T) allele correlates with less surface CXCR4 expression. Again, no significant difference was found regarding the cell numbers or MFI of the protein, two CXCR4 expression parameters, in all cell types except for PBMC for which the lowest level of surface CXCR4 was detected in individuals with two CCR2-wt alleles (P = 0.0240 by Kruskal–Wallis test), as it is shown in Fig. 2(A). This finding contradicts the earlier report by Lee et al. that lower CXCR4 MFI were found in individuals with CCR2-wt/64I than wt/wt (17).

### pCCR5-59029 polymorphism and expression of surface CCR5

As reported by McDermott et al (20), the pCCR5-59029A mutant allele has been found to produce a higher gene expression in vitro and to associate with a faster AIDS progression. From the results of flow cytometric analysis of our studied cohort, we did not find any significant difference in the two CCR5 expression parameters for any cell types, except for CD3<sup>+</sup> lymphocytes, which expressed a statistically significant (P = 0.0221) higher level of CCR5 in individuals with pCCR5-59029G/G than for the other two genotypes (Fig. 2B). Moreover, the same study showed a 3.8 years faster AIDS progression for 59029A/∆ when detected without the nucleotide substitution of T for C at p59653T allele. For further information, we looked at the correlation of three 59029 genotypes with the CCR5 expression parameters in blood cells derived from homozygous pCCR5-59653C (CCR2-wt) volunteers who were also free of the ∆32 allele. CCR5∆32 does not exist in the Chinese population (21). In this case, we detected significantly more CD4<sup>+</sup> cells expressing CCR5, as shown in Fig. 2(C), with respect to the presence of two 59029A alleles (P = 0.0453). The main subtype of the high CCR5-expressing CD4<sup>+</sup> cells, CD4<sup>+</sup> monocytes, also shows a weak, but not significant, increase in cell population in the identical cohorts (P = 0.0508) as compared to two other genotypes in Fig. 2(C). Thus, without interference of the pCCR5-59653 polymorphism, the influence of pCCR5-59029A on HIV-1 disease progression rate is likely due to the increased numbers of CCR5-expressing CD4<sup>+</sup> cells, which is consistent with the previous report (20).

Since the pCCR5-59653T allele was invariably the pCCR5-59029A allele in our studied group, we investigated the effect of nucleotide polymorphism at the 59653 position on the CCR5 expression in individuals with homozygous 59029A background. We detected less CCR5<sup>+</sup> PBMC, CCR5<sup>+</sup>CD4<sup>+</sup> cells and CCR5<sup>+</sup>CD4<sup>+</sup> monocytes in individuals homozygous for pCCR5-59653T than other genotypes, as shown in Fig. 3. Furthermore, although of marginal significance, a trend of lesser CD4<sup>+</sup> lymphocytes produced detectable surface CCR5 in individuals carrying one or more pCCR5-59653T allele was revealed (data not shown). This result seems consistent with the correlation of slower HIV-1 disease progression to the CCR2-64I mutation (pCCR5-59653T allele) reported previously (10, 15, 16).

### Discussion

This paper describes the determination of the genotype frequencies of three genetic variants of CCR2, pCCR5-59653 and 59029, as well as the investigation of the possibility that the nucleotide polymorphisms affect the expression of CCR5 and CXCR4 on cell surfaces. With the Chinese volunteers, we first performed ~300 genotype determinations. The result confirmed the finding by D. D. Ho’s group that pCCR5-59653T is in complete linkage disequilibrium with CCR2-64I (15), as shown in Table 1. Since mainly Caucasians and a small number of African-Americans were the subjects of the previous investigation, it is likely that the genetic linkage is a general inherited trait in all humans. Interestingly, the genotype distribution of the pCCR5-59653T allele with respect to the pCCR5-59029A allele in our studied group (Table 1) is more similar to that of CCR5∆32 to 59029A than that of pCCR5-59653T to 59029A reported in the group consisting mainly of Caucasians (20). These data suggest that pCCR5-59653T (CCR2-64I) links to 59029A differently in different races, just as CCR5∆32 is not present in all races with an equal frequency (21). Because the number of pCCR5-59653T individuals (n = 6) were far less than the number of 59653C/C (n = 58) as compared to that of pCCR5-59029A/∆ (n = 22) to 59029G/∆ (n = 31) in our studied group (Table 1), we further speculate that the nucleotide substitution of T for C at pCCR5-59653 appeared later than at the 59029 position in Chinese evolution. However, both genotype distributions are in equilibrium as predicted by the Hardy–Weinberg equation and shown in Table 1 (P = 0.76 for pCCR5-59653 and P = 0.45 for pCCR5-59029).

The major purpose of this study was to investigate the hypothesis that the CCR2-64I (pCCR5-59653T) and pCCR5-59029G polymorphisms reduce the expression levels of HIV-1 co-receptor and thus lead to slower AIDS progression. Interestingly, recent studies have showed that CCR5∆32 was absent in patients suffering from rheumatoid arthritis (24, 25), indicating that CCR5 also plays an important role in that disease and the protein may serve as a potential target for therapy. Any genetic influence on the expression of CCR5 certainly affects the risk of developing rheumatoid arthritis. Therefore, it would be interesting to know whether individuals carrying
Genetic variants and CCR5 and CXCR4 expression

Fig. 2. Correlation of genetic polymorphisms with CCR5 and CXCR4 expression. In this study, we determined both percentages of five blood cell types expressing CCR5 and CXCR4 and the expression levels (MFI) of the proteins on cell surface in a Chinese cohort with all pCCR5-59653 and pCCR5-59029 genotypes. Statistical comparisons employed the Kruskal-Wallis test first for three-group analysis. If $P < 0.05$ was found, Dunn’s multiple comparison test was then performed to define if the genotype pairs exhibited significant differences. Among 40 analysis performed, only three comparisons have statistically significant meanings and they are shown. In all plots, each point represents the value obtained for a single individual and the $P$ values derived from Kruskal-Wallis tests are marked at the upper left corners. Whereas the $P$ values written above the inverted square brackets indicate how significant differences are between the particular genotype pairs as determined by Dunn’s multiple comparison test. (A) PBMC isolated from individuals with pCCR5-59653C/C (also CCR2-wt/wt) expressed lower levels (MFI) of surface CXCR4 than 59653C/T heterozygotes. (B) A smaller MFI on CD3+ cell was detected in individuals with pCCR5-59029G/A than 59029G/G. (C) We detected significantly more CD4+ cells expressing 59029G/A alleles (left panel). Furthermore, a greater number of CD4+ monocytes was found to associate weakly, but not significantly, to individuals with pCCR5-59653wt-59029A/A than 59029G/A (right panel).

Before investigating the correlation between genetic variants and surface CCR5 and CXCR4 expression, we first tested the fundamental relationship between the CCR5 mRNA copy number and the expression of surface CCR5 on PBMC isolated from a small number of individuals in the studied cohort. For this, we employed a quantitative RT-PCR system AG-9600 AmpliSensor Minilyzer with AmpliSensor Assay software (Biotronics Technology, Lowell, MA) to determine the levels of cellular CCR5 transcription. We detected that CCR5 MFI was in positive proportion ($r = 0.527$ by Spearman analysis) to the amounts of CCR5 mRNA in PBMC ($P = 0.025$) isolated from 18 randomly selected individuals (data not shown). Since almost two-thirds of the participants examined here carried the pCCR5-59653C/C genotype, we further performed identical statistical analysis with 12 individuals carrying the genotype. Similarly, a linear increase of CCR5 MFI on PBMC ($r = 0.762$) was found to associate with a greater mRNA copy number ($P = 0.004$; data not shown) in vivo. These data strongly indicate that the amounts of CCR5 mRNA synthesized in PBMC are indeed positively proportional to the average numbers of CCR5 expressed on surface of PBMC.

For the CCR2-64I allele, we investigated its effects on both CCR5 and CXCR4 expressions. The reason was 3-fold. First, the mutant allele is tightly linked to the nucleotide substitution at 59653 in the CCR5 promoter (20), which may influence the wild-type protein, has the capability of interacting with CXCR4, suggesting that heterodimer formation between the two proteins abolishes the function of CXCR4 as an HIV-1 co-receptor (18). The report also stated that both CCR2-wt and CCR2-64I could heterodimerize with CCR5. However, whether
Genetic variants and CCR5 and CXCR4 expression

CCR2-64I possesses the transdominant inhibitory phenotype remained to be investigated. Third, only a few investigations have analyzed the relations between the presence of the CCR2-64I allele and the expression of surface CCR5 and CXCR4 (17,19). The experiments performed were with a small number of Caucasian individuals with a single cell type (PBMC) or with one protein expression parameter only (MFI); and the results were contradictory. Therefore, we systematically examined cell percentages of five CCR5- and CXCR4-expressing cell types as well as MFI of the two proteins on the cell surface and correlated the data to the presence of the particular genotypes. Here, we detected a significant reduction of CXCR4 expression level on PBMC only in individuals with 59653C/C or CCR2-wt homozygotes (P = 0.024; Fig. 2A) that contradicted both the hypothesis and the previous result by Lee et al. (17). The significance of this statistical observation is unclear; however, it does indicate that CCR2-64I does not inhibit the transportation of either CCR5 or CXCR4 to the cell surface. Since CXCR4 mediates both the entry of X4 virus and the signals from α-chemokines, it is possible that the cellular regulatory machinery up-regulates protein synthesis to compensate for the loss of functional CXCR4 caused by the heterodimerization with CCR2-64I so that the normal immunological activity can be maintained. However, since only PBMC, and not the four other cell types showed altered expression levels of CXCR4, more analyses with a larger sample size are required to confirm this possibility. On the contrary, the trend was for the pCCR5-59653T/T (CCR2-64I/I) genotype to exhibit the lowest CCR5 cell percentages in all cell types examined in this study (data not shown), which would agree with the correlation of the CCR2-64I mutation to slower AIDS progression (10,15,16), although the analyzed sample size was not sufficient. As illustrated by Wu et al., CCR5 is highly expressed on activated T lymphocytes as well as on a portion of effector/memory T cells in blood and tissue macrophages (7). We cannot rule out that this inconsistent detection of CCR5 expression levels in our studied group with different nucleotide polymorphisms may be due to different levels of lymphocyte activation in these individuals, even though they had been determined to be healthy by the CBC tests at the time of CCR5 expression determination.

With respect to the pCCR5-59029 polymorphism, a higher expression level of CCR5 was detected only on CD3+ cells, but not other, cell types in 59029wt homozygotes (Fig. 2B). The meaning of this statistical data is rather weak. Since 3.8 years faster AIDS progression has been reported to associate with pCCR5-59029A/A homozygotes without other confounding genetic factors like CCR5∆32 and CCR2-64I, we therefore undertook the analysis of the correlation of the three 59029 genotypes with CCR5+ cell percentage and MFI of the protein in individuals with the homozygous pCCR5-59653C (CCR2-wt) allele. In this case, we detected significantly more CD4+ cells expressing CCR5 with respect to the presence of the pCCR5-59029A alleles (P = 0.0453; Fig. 2C). We also detected a weak, hardly significant, increase in the CD4+ monocyte population in identical cohorts (P = 0.0508). Although the previous in vivo experiments used a T (Jurkat) cell line rather than a monocytic cell line as the host cells for transfection (20), we generally regard our data as confirming the earlier

Fig. 3. Correlation of the pCCR5-59653 genotypes with CCR5 expression in individuals carrying the pCCR5-59029A/A genotype. In the studied subjects with the pCCR5-59029A/A genotype, we found that less detectable surface CCR5 on PBMC (A), CD4+ cells (B) and CD4+ monocytes (C) is associated with the presence of the pCCR5-59653T allele (also the CCR2-64I allele). Although not reaching a statistically significant difference, a similar correlation was also detected between the CCR5 expression levels and another subset of CD4+ cells, i.e. CD4+ lymphocytes (data not shown). Therefore, it seems that, in the pCCR5-59029A/A genetic background, the result of this study agrees with the association of CCR2-64I to slower HIV-1 disease progression.
result. Therefore, greater susceptibility of the pCCR5-59029A homozygotes for HIV-1 infection is likely due to more CD4+CCR5+ cells in vivo. In addition to this, an interesting finding was observed that, in the background of pCCR5-59029A/A, a greater percentage of CCR5+ PBMC, CD4+ cells or CD4+ monocytes was associated with the pCCR5-59653T allele (Fig. 3). Since 59029A and 59653T have been associated with the opposite rates of HIV-1 disease progression, our results suggest the effect of 59653T on the expression levels of CCR5 is stronger than the 59029A allele in individuals who harbor these two nucleotide polymorphisms simultaneously.

The results from this and other studies have demonstrated that many polymorphic changes in the co-receptor genes differently influence both the expression levels of surface CCR5 or CXCR4 and the ratios of peripheral blood cells expressing the proteins. All genetic polymorphisms, those already known and those unreported, therefore, should all be accounted for in predicting the expression levels of these two proteins under physiological conditions. Since different genetic polymorphisms may co-exist in human beings, the rate of AIDS progression as well as the risk of rheumatoid arthritis is likely governed by the interplay of an array of nucleotide changes and their affected proteins.

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CBC</td>
<td>complete blood counts</td>
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<tr>
<td>CCR</td>
<td>CC chemokine receptor</td>
</tr>
<tr>
<td>CXCR</td>
<td>CXC chemokine receptor</td>
</tr>
<tr>
<td>MFI</td>
<td>mean fluorescence intensity</td>
</tr>
<tr>
<td>PBL</td>
<td>peripheral blood lymphocytes</td>
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<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
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<tr>
<td>PE</td>
<td>phycoerythrin</td>
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References

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