Linkage of the CCR5Δ32 Mutation with a Functional Polymorphism of CD45RA

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A 32-bp deletion in CCR5 (CCR5Δ32) confers to PBMC resistance to HIV-1 isolates that use CCR5 as a coreceptor. To study this mutation in T cell development, we have screened 571 human thymus tissues for the mutation. We identified 72 thymuses (12.6%) that were heterozygous and 2 (0.35%) that were homozygous for the CCR5Δ32 mutation. We found that thymocyte development was normal in both CCR5Δ32 heterozygous and homozygous thymuses. In 3% of thymuses we identified a functional polymorphism of CD45RA, in which cortical and medullary thymocytes failed to down-regulate the 200- and 220-kDa CD45RA isoforms during T cell development. Moreover, we found an association of this CD45 functional polymorphism in thymuses with the CCR5Δ32 mutation (p = 0.00258). In vitro HIV-1 infection assays with CCR5-using primary isolates demonstrated that thymocytes with the heterozygous CCR5Δ32 mutation produced less p24 than did CCR5 wild-type thymocytes. However, the functional CD45RA polymorphism did not alter the susceptibility of thymocytes to HIV-1 infection. Taken together, these data demonstrate association of the CCR5Δ32 mutation with a polymorphism in an as yet unknown gene that is responsible for the ability to down-regulate the expression of high m.w. CD45RA isoforms. Although the presence of the CCR5Δ32 mutation down-regulates HIV-1 infection of thymocytes, the functional CD45RA polymorphism does not alter the susceptibility of thymocytes to HIV-1 infection in vitro. The Journal of Immunology, 2000, 165: 148–157.

Materials and Methods

Thymus tissue

Human thymuses were obtained from the Department of Pathology at Duke Medical Center as discarded tissues taken in the course of corrective cardiovascular surgery or therapeutic thymectomy for myasthenia gravis using a Duke institutional review board-approved protocol. No tissue was removed that was not clinically indicated by the surgical procedure being performed. Fresh thymus tissue was either teased with forceps and scissors, and thymocyte suspensions were prepared, or a portion (~0.5 × 0.5 cm) was placed in RPMI 1640 medium supplemented with 7.5% DMSO and 15% FCS and snap-frozen in liquid nitrogen.

Monoclonal Abs

Mouse anti-human CD45 mAb FI0-89-4 and CD45RA mAb F8-11-13 were provided by Rosemarie Dalchau (London, U.K.) (17). Anti-CD45 RO mAb UCHL-1 was provided by P. C. L. Beverley (London, U.K.) (18). Anti-CD45RB mAb N-L162 and anti-CD45RC mAb N-L121 were obtained through the Fifth International Workshop on Human Leukocyte Differentiation (19). P3×3 IgG1 paraprotein (P3) was produced by the P3×3 Ag8.652 myeloma cell line (20) and used as a control Ab. PE-conjugated anti-CD4-PE and Cy5-conjugated CD8 were purchased from Pharmingen (San Diego, CA).

Cells and tissue culture conditions

Frozen thymocytes were thawed by incubation at 37°C for 1 h in RPMI 1640 medium containing 10% FCS and 10 μg/ml of DNase I (Sigma, St. Louis, MO). After thawing, thymocytes were passed through a Ficoll-Hypaque gradient by centrifugation at 1500 rpm for 30 min and washed twice with RPMI 1640 containing 10% FCS. Thymocytes were cultured in RPMI 1640 medium supplemented with 10% FCS and 10 ng/ml of IL-2 and were maintained at 37°C in a humidified 5% CO2 incubator. Human PBMCs were prepared from buffy coats of healthy, HIV-1-seronegative individuals obtained through the laboratory services of the American Red Cross, Carolina region (Charlotte, NC). PBMC were isolated by Ficoll-Hypaque gradient centrifugation and were used as positive control cells for in vitro HIV-1 infection assays. PBMC were washed twice in RPMI 1640 medium containing 10% FCS, resuspended at a density of 2.5 × 107 cells/ml in the same medium containing 10% DMSO, and frozen in 1-ml aliquots in liquid nitrogen. PBMC were prescreened for the ability to support the replication of syncytium-inducing (SI) and non-SI (NSI)
primary isolates of HIV-1 to confirm the expression of appropriate coreceptors, including CCR5.

**HIV-1 virus stocks**

Seven different strains of HIV-1 belonging to the genetic clade B subtype were used to infect thymocytes and PBMC in vitro. The IIB strain is an SI, T cell line-adapted strain that uses CXCR4 as its major coreceptor (3, 21, 22). 89.6 is an SI primary isolate that infects macrophages and CD4+ lymphocytes (23) and is capable of using multiple coreceptors (22), although CXCR4 usage dominates (24). We used the uncloned stock of 89.6 that had been passaged minimally in PBMC and was expanded in RPMI 1640 containing heat-inactivated FBS (20%), gentamicin (50 μg/ml), and delectinized IL-2 (5%, v/v; Advanced Biotechnologies, Columbia, MD). The cells were incubated for 4 h before and after activation with PHA were incubated for 30 min at 4°C with various mAbs against the cell surface molecules, including CD4, CD8, CD45RA, CD45RO, CD45RB, and CD45RC or control mAb P3 in PBS containing sodium azide for 30 min at room temperature, washed twice with PBS, and then incubated with saturating amounts of goat anti-mouse IgG-FITC for 30 min at room temperature. These sections were washed three times with PBS, and then viewed under fluorescence microscopy. Thymocytes in suspension (0.5 × 10^7 cells/tube) before and after activation with PHA were incubated for 30 min at 4°C with various mAbs against the cell surface molecules, including CD4, CD8, CD45RA, CD45RO, CD45RB, CD45RC, and CXCR4 and control mAb P3 in μl of PBS containing 0.2% BSA and 0.1% sodium azide. Cells were washed twice with PBS with BSA/sodium azide, incubated with goat anti-mouse IgG-FITC for 30 min at 4°C followed by washing twice with PBS with BSA/sodium azide. In some experiments in which three-color flow cytometric analysis was performed, thymocytes were incubated with PE-conjugated mouse anti-CD4, and Cy5-conjugated mouse anti-CD8 after completion of incubation with the goat anti-mouse IgG-FITC. After staining, thymocytes were washed, fixed in 4% paraformaldehyde in PBS, and protected from light at 4°C until analysis by a flow cytometer. Three-color flow cytometry was performed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Data acquisition and analysis were conducted with CellQuest software. For each cell sample, a total of 10^4 cell events were analyzed. Data were expressed as the mean fluorescence channel (MFC), an indication of the intensity of cells stained with specific mAbs.

**Statistical analysis**

The Fisher exact test was used to analyze association of the heterozygous or the homozygous CCR5Δ32 mutation with the presence of the abnormality of CD45RA expression in thymocytes.

**Results**

**Detection of the CCR5Δ32 mutation in a bank of human thymus tissues**

The Human Thymus Bank at Duke University contains 571 normal or myasthenia gravis thymuses. These thymuses were from patients with congenital heart disease (age range, 1 day to 17 years) and patients with myasthenia gravis (age range, 3–80 years). We screened these 571 thymus tissues for the CCR5Δ32 mutation with the presence of the abnormality of CD45RA expression in thymocytes.

**Immunoprecipitation and SDS-PAGE**

Thymocytes (0.5 × 10^7 cells) were labeled with [3H]Thymidine (NEN Life Science Products, Boston, MA) (30) and lysed in 500 μl of lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1% of Nonidet P-40). Immunoprecipitation were conducted as previously described (31). Briefly, cell lysates were first precleared by incubation with control mAb P3 followed by protein A/G-agarose (Sigma). Pre-cleared cell lysates were the incubated with CD45, CD45RA, or control mAbs for 4 h followed by incubation with protein A/G-agarose (Sigma). Immune complexes were washed five times with buffer (10 mM Tris-HCl (pH 8.0), 140 mM NaCl, and 0.025% NaN3), resuspended in SDS-PAGE sample buffer, boiled for 5 min, and subjected to SDS-PAGE on 7% polyacrylamide gels.

**Indirect immunofluorescence staining and flow cytometry**

Serial frozen 5-μm sections of thymus were cut and incubated with saturating amounts of mAbs against CD3, CD4, CD8, CD45RA, CD45RO, CD45RB, and CD45RC or control mAb P3 in PBS containing sodium azide for 30 min at room temperature, washed twice with PBS, and then incubated with saturating amounts of goat anti-mouse IgG-FITC for 30 min at room temperature. These sections were washed three times with PBS, then viewed under fluorescence microscopy. Thymocytes in suspension (0.5 × 10^7 cells/tube) before and after activation with PHA were incubated for 30 min at 4°C with various mAbs against the cell surface molecules, including CD4, CD8, CD45RA, CD45RO, CD45RB, CD45RC, and CXCR4 and control mAb P3 in μl of PBS containing 0.2% BSA and 0.1% sodium azide. Cells were washed twice with PBS with BSA/sodium azide, incubated with goat anti-mouse IgG-FITC for 30 min at 4°C followed by washing twice with PBS with BSA/sodium azide. In some experiments in which three-color flow cytometric analysis was performed, thymocytes were incubated with PE-conjugated mouse anti-CD4, and Cy5-conjugated mouse anti-CD8 after completion of incubation with the goat anti-mouse IgG-FITC. After staining, thymocytes were washed, fixed in 4% paraformaldehyde in PBS, and protected from light at 4°C until analysis by a flow cytometer. Three-color flow cytometry was performed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Data acquisition and analysis were conducted with CellQuest software. For each cell sample, a total of 10^4 cell events were analyzed. Data were expressed as the mean fluorescence channel (MFC), an indication of the intensity of cells stained with specific mAbs.

**Statistical analysis**

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keept for patients regarding race or ethnic background, and there-

To characterize thymuses with the heterozygous and homozygous CCR5 genotypes as reported for a randomly selected population notype in the thymus tissue bank was similar to the distribution of CCR5 genotypes, and there were no differences in the level of expression of CD45RO, CD45RB, and CD45RC between thymocytes isolated from thymus tissues with the CD45RA normal vs the abnormal phenotype (Fig. 3).

CD45 is comprised of at least five different isoforms, ABC, AB, BC, B, and O, with molecular masses of 220, 200 (AB and BC), 190, and 180 kDa, respectively. On the protein level, the hetero-
geneity of different CD45 isoforms can be resolved by mAbs that react with restricted CD45 epitopes (19). In immunoprecipitation assays, protein bands immunoprecipitated by pan-CD45 Abs are 220, 200, 190, and 180 kDa, and those immunoprecipitated by CD45RA Abs are 220 and 200 kDa. Immunoprecipitation assays were conducted to characterize the CD45 isoforms using cell lysates of thymocytes from CD45RA normal and abnormal phenotype individuals. CD45 molecules from cell lysates of thymocytes were first immunoprecipitated using the pan-CD45 mAb F10-89-4 that is directed against a common epitope shared by all CD45 isoforms. We found that CD45 mAb F10-89-4 immunoprecipitated protein bands of 200 and 180 kDa in the CD45RA normal thymocytes, whereas the pan-CD45 mAb immunoprecipitated a large 220-kDa band in addition to 200- and 180-kDa bands in CD45RA abnormal thymocytes (Fig. 4). When normal thymocytes were analyzed with the CD45RA mAb F8-11-13, there were no major protein bands identified in normal control thymocytes (because only ~7% of normal thymocytes were CD45RA⁺; Fig. 4), indicating that the 200-kDa band seen by the pan-CD45 mAb in normal thymocytes was either the 200-kDa CD45BC isoform or the 190-kDa CD45B isoform. However, in cell lysates of thymocytes of CD45RA normal individuals, in which ~90% of thymocytes are CD45RA⁺, CD45RA mAb F8-11-13 immunoprecipitated two

| Thymocyte development in CCR5Δ32⁺⁻, CCR5Δ32⁻⁻ and CCR5 wild-type human thymocytesa |
|-----------------------------------------------|
| Thymus Genotype                  | CD4⁺CD8⁻ DN thymocytes | CD4⁺CD8⁺ DP thymocytes | CD4⁻CD8⁺ SP thymocytes | CD4⁻CD8⁻ SP thymocytes |
|-----------------------------------------------|
| CCR5 wild type                  | 10.7 ± 1.3              | 42.6 ± 14.7             | 31.3 ± 13.5              | 15.6 ± 1.3              |
| CCR5Δ32⁺⁻                  | 11.3 ± 3.1              | 46.9 ± 14.1             | 26.9 ± 13.8              | 15.1 ± 1.8              |
| CCR5Δ32⁻⁻                  | 8.8 ± 3.3               | 31.7 ± 18.8             | 37.1 ± 10.9              | 22.4 ± 1.2              |

* There were no significant differences in percentage of positive cells in each subset of CD4⁺CD8⁻, CD4⁺CD8⁺, CD4⁻CD8⁺, and CD4⁻CD8⁻ thymocytes among groups of CCR5 wild-type thymocytes and the CCR5Δ32⁺⁻ (p > 0.05) thymocytes, or among the groups of CCR5 wild-type thymocytes and the combination of the CCR5Δ32⁺⁻ or the CCR5Δ32⁻⁻ (p > 0.05).
major isoforms of 220 and 200 kDa, demonstrating that the CD45RA reactivity in the CD45RA abnormal phenotype thymuses was due to the lack of normal down-regulation of 200- and 220-kDa CD45RA isoforms during thymocyte development.

To analyze the subsets of thymocytes that expressed the CD45RA abnormal phenotype, three-color flow cytometry was performed. As previously noted in Table II, CD45RA abnormal thymuses had normal percentages of CD4<sup>+</sup>/CD8<sup>-</sup> DN, CD4<sup>+</sup>/CD8<sup>-</sup> DP, CD4<sup>+</sup>/CD8<sup>-</sup> SP, and CD4<sup>+</sup>/CD8<sup>-</sup> SP populations (Fig. 5A). In CD45RA normal thymuses, about 7% of all thymocytes were CD45RA<sup>-</sup>; CD45RA<sup>-</sup> thymocytes were primarily present in DN CD4<sup>+</sup>/CD8<sup>-</sup> (28% of DN thymocytes were CD45RA<sup>-</sup>) and SP CD4<sup>+</sup>/CD8<sup>-</sup> (31% CD45RA<sup>-</sup>) subsets. In the SP CD4<sup>-</sup>/CD8<sup>-</sup> population, only 9% of thymocytes were CD45RA<sup>-</sup> (Fig. 5B). In contrast, in those thymuses found to express the CD45RA abnormal phenotype, all subsets of thymocytes expressed CD45RA, including DN CD4<sup>+</sup>/CD8<sup>-</sup>, DP CD4<sup>+</sup>/CD8<sup>-</sup>, SP CD4<sup>+</sup>/CD8<sup>-</sup>, and SP CD4<sup>+</sup>/CD8<sup>-</sup> thymocyte populations (Fig. 5). Thus, ~90% of all CD45RA abnormal thymocytes were CD45RA<sup>-</sup>.

Striking differences in CD45RA expression were observed between the CD45RA normal and abnormal thymuses in all thymocyte populations. For example, while 90% of DP CD4<sup>+</sup>/CD8<sup>-</sup> cells and 98% of CD4<sup>+</sup>/CD8<sup>-</sup> cells were CD45RA positive in the CD45RA abnormal phenotype isoforms, only 0.2% of DP CD4<sup>+</sup>/CD8<sup>-</sup> and 9% of CD4<sup>+</sup>/CD8<sup>-</sup> thymocytes were CD45RA positive in CD45RA normal thymocytes (Fig. 5B).

**Relationship of CD45RA abnormal phenotype in thymocytes with a previously described genetically determined lack of CD45RA-negative lymphocytes in PBMC**

It has been previously reported that a genetically determined lack of CD45RA-negative PB T cells is present in 8% of healthy individuals (35). No loss of CD45RA expression in PB T cells of these individuals was observed after in vitro activation with PHA due to selective lack of down-regulation of the 200-kDa CD45RA isoform (35, 36). To address whether the CD45RA abnormal phenotype identified in thymus in our study was similar to the genetically determined lack of CD45RA-negative T cells found in peripheral blood lymphocytes (35, 36), CD45RA normal and abnormal thymocytes were activated in vitro with 1 μg/ml of PHA, and analyzed for surface expression of CD45RA and CD45RO.

As shown in Fig. 6A, most unactivated CD45RA normal thymocytes were CD45RA<sup>low</sup> or negative with an MFC of 101, and CD45RO<sup>+</sup> with an MFC of 224. In contrast, most thymocytes from the CD45RA abnormal thymus were CD45RA<sup>+</sup> with an MFC of 409. Activation of thymocytes in vitro with PHA down-regulated CD45RA expression in thymocytes with the CD45RA normal phenotype (as reflected by a lower MFC of 50 in Fig. 6B). However, CD45RA expression in thymocytes with the CD45RA abnormal phenotype was not down-regulated after PHA activation, but, rather, was increased by almost 200% to an MFC of 832 (Fig. 6B). After PHA activation, expression of CD45RO was up-regulated in both CD45RA normal and CD45RA abnormal phenotype thymocytes, and no differences in CD45RO expression after in...
Expression of CD45 isoforms in human thymocytes. Thymocytes were isolated from thymus tissues identified as either normal CD45RA phenotype (A) or abnormal CD45RA phenotype (B). Thymocytes (10^6 cells/tube) were incubated with saturating amounts of mAbs against CD45RA (F8-11-13), CD45RB (N-L 162), CD45RC (N-L 121), and CD45RO (UCHL-1) or were incubated with the control mAb P3 and analyzed by flow cytometry. Shown in dotted lines are the flow cytometric profiles of thymocytes stained with control mAb P3, and shown in solid lines are thymocytes stained with mAbs against specific CD45 isoforms as indicated at the bottom of each panel. MFCs of thymocytes stained with mAbs against specific CD45 isoforms are also indicated. Data are representative of three separate experiments.

Analysis of linkage of abnormal thymocyte expression of CD45RA with the CCR5Δ32 mutation

Four hundred and six of the 571 original thymus tissues were available to screen for the CD45RA abnormal phenotype. We found statistically significant higher numbers of the CD45RA abnormal phenotype in thymuses with either the heterozygous and homozygous CCR5Δ32 mutation than in CCR5 wild-type thymuses (Table III). We identified the abnormality of CD45RA expression in 1 of 2 thymuses homozygous for the CCR5Δ32 mutation, in 5 of 64 thymuses heterozygous for the CCR5Δ32 mutation, and in 6 of 340 thymocytes with wild-type CCR5 genes with no CCR5Δ32 mutations. The CCR5Δ32 allele was found in 50% of those expressing the CD45RA abnormal phenotype compared with 15% in those not expressing it. Using two-tailed Fisher’s exact test to test for association between CD45RA abnormality and CCR5Δ32 mutation, a statistically significant association between CD45RA abnormality and CCR5Δ32 mutation was demonstrated (p = 0.00258). We next performed three pairwise comparisons of the CCR5Δ32 genotypes. Pairwise comparison of the homozygous and heterozygous CCR5Δ32 genotypes with the wild-type CCR5 genotype detected statistically significant association of both the homozygous and heterozygous CCR5Δ32 genotypes with the CD45RA abnormal phenotype (Table IV).

Differences in HIV-1 infectivity have been previously suggested in normal PBMC CD45RA+ naive vs CD45RO+ memory CD4+ T cells (37), and the functional capacity of CD45RO+ CD4+ T cells is affected by HIV-1 more than that of CD45RA+ CD4+ T cells (37). However, to date, CCR5Δ32+/− thymocytes have not been studied regarding HIV-1 infectivity. Considering the importance of CCR5 in HIV-1 infection (38) taken together with the association between the CD45RA abnormal phenotype and CCR5Δ32 genotype in thymocytes described here, it was also of interest to determine whether the CD45RA abnormal phenotype could influence the infectability of thymocytes with different CCR5 genetic backgrounds. Individuals who are homozygous for the CCR5Δ32 allele are highly resistant to infection by R5 strains of HIV-1, and their PBMC cannot be infected with R5 strains in vitro (8, 39). Individuals who are heterozygous for the CCR5Δ32 allele, on the other hand, are susceptible to infection with R5 strains, but progress to AIDS at a slower rate relative to infected individuals who have wild-type CCR5 alleles (12, 13, 40). Likewise, PBMC from heterozygous CCR5Δ32 individuals are infectable by R5 strains, although not always to the same extent as PBMC with normal CCR5 alleles (8, 41). Lower levels of infection...
in heterozygous CCR5Δ32 PBMC might in some cases be due to decreased amounts of CCR5 on the cell surface (41, 42).

Sufficient cell numbers were not available to test thymocytes homozygous for the CCR5Δ32 mutation in HIV-1 infectivity assays. However, thymocytes with the CD45RA normal and abnormal phenotypes were tested in HIV-1 infectivity assays in combination with either wild-type CCR5 alleles or alleles that were heterozygous for the CCR5Δ32 mutation. Cells in each group were tested for infectability with five primary R5 isolates, two primary R5X4 isolates, and one X4 T cell line-adapted strain of HIV-1. Infection was compared with the infectability of PBMC from a healthy, HIV-1-negative individual who had normal wild-type CCR5 alleles. Equal numbers of viable cells of each cell type were used so that direct comparisons of levels of infection could be made. Viral replication was measured by p24 production on days 4 and 8 of infection.

Thymocytes in each group were infectable by all strains of HIV-1 tested, although infection proceeded at a slow rate in all thymocyte samples compared with PBMC (Table 5). For example, HIV-1 p24 production in PBMC increased from 6.1 to 496 ng of p24/ml on day 4 of incubation, whereas infection in thymocytes was lower (0.2–2.0 ng of p24/ml) or undetectable (<0.1 ng of p24/ml) on day 4. The medium was replaced on day 4, and p24 again was again measured on day 8. Higher levels of p24 were seen in the thymocyte cultures at this time, in some cases approaching the levels detected in PBMC (Table V). Although the results varied among cell types and viruses, at least two general observations were made. First, R5 primary isolates were capable of producing a productive infection regardless of the CCR5 genotype and CD45RA phenotype. The level of infection was lower in thymocytes that were heterozygous for the CCR5Δ32 mutation compared with CCR5 wild-type thymuses (p < 0.01). Second, the level of HIV-1 infection of thymocytes was independent of the CD45RA expression phenotype. We saw no difference in the level of p24 production on day 4 or 8 in CD45RA normal vs CD45RA abnormal thymocytes (p > 0.5). R5 viruses replicated in thymus as well as, if not better than, X4 and R5X4 viruses in most cases. One possible exception was thymocytes from thymus T-690 that was CCR5Δ321/2 and CD45RA normal phenotype. Here, no infection with R5 strains was detectable on days 4 and 8 of incubation. However, infection with X4 and X4R5 strains in these cells was also low, suggesting that in this case the lack of detectable infection with R5 strains may be a quantitative issue related to slow replication rather than a qualitative aspect of the infectability of the thymocytes.

Discussion
In this study we have demonstrated the association of the CCR5Δ32 mutation with a functional polymorphism of CD45RA...
expression in thymocytes. Moreover, we have shown that thymocytes, like PB T cells, when heterozygous for the CCR5Δ32 mutation, produced lower HIV p24 in vitro after infection with HIV. However, we could determine no effect of the CD45RA polymorphism on in vitro HIV infectivity of thymocytes.

A number of mutations in HIV-1 coreceptor genes have been found that modify HIV-1 infectivity in vitro, modify the clinical course HIV-1 infection, or both. These include CCR2 (43), stem cell-derived factor-1 (44), and CXCR4 (45) mutations as well as the CCR5Δ32 mutation (7–16). The identification of a functional polymorphism of CD45RA expression in association with the CCR5Δ32 mutation is of potentially great interest. The presumption is that whatever evolutionary advantage conferred on humans by the CCR5Δ32 mutation may have been potentially impacted in an as yet unknown way by linkage to the CD45RA abnormal phenotype.

The family of human CD45 leukocyte common Ags is comprised of five glycoprotein members with Mr of 180 kDa (O), 190 kDa (B), 200 kDa (AB and BC), and 220 kDa (ABC) that are derived from alternative splicing of a single gene. The heterogeneity of CD45 isoforms can be identified by mAbs that specifically react with epitopes of the distinct isoforms. The expression of CD45RA and CD45RO isoforms has, in general, defined complementary subsets of T cells that differ in naive and memory functional properties (46, 47).

Because CD45RA− PB CD4+ T cells when infected with HIV-1 produce lower levels of p24 (37), we postulated that abnormal expression of CD45RA isoforms in thymus would confer on thymocytes some degree of HIV-1 resistance as well. That this was not the case suggests that factors other than CD45RA expression per se in CD45RA− PB native CD4+ T cells are responsible for the relative resistance to HIV-1 infection of the naive T cell subset.

Given the recent appearance of HIV-1 in man and the ancient nature of the CCR5Δ32 mutation, it has been postulated that the CCR5Δ32 mutation confers a selective advantage to humans in an

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**Table III.** CD45RA expression abnormality in human thymuses of the CCR5 wild type, and in thymuses heterozygous and homozygous for the CCR5Δ32 mutation

| CCR5Δ32 Status | No. with abnormal CD45RA expression | No. with normal CD45RA expression | % with abnormal CD45RA expression |
|---------------|----------------------------------------|-----------------------------------|----------------------------------|
| Homozygote (−/−) | 1                                      | 1                                 | 50                               |
| Heterozygote (+/−) | 5                                      | 59                                | 7.8                              |
| Wild type (+/+ )  | 6                                      | 334                               | 1.8                              |
| **Total** | **12**                                  | **394**                           | **3.0**                          |
as yet unknown way that may be unrelated to modulating host resistance to HIV-1 infection. Functionally, CCR5Δ32 PB T cells are normal in in vitro T cell proliferation assays (8). We have found CCR5Δ32−/− and CCR5Δ32+/− thymocytes undergo normal T cell maturation (Table II and Fig. 5A) and proliferate normally in vitro in response to TCR-mediated triggering (unpublished observations). Thus, as yet, no while signaling with CCR5 ligands is, as expected, absent (H.-X. Liao and B. F. Haynes, unpublished observations). Given the similarity of the defect of activation-induced CD45RA isofrom down-regulation in thymocytes described in this report to that of the previously described lack of CD45RA down-regulation in PB T cells (35), we suggest that the defects are identical, and that we have now observed the consequence of the previously described PB T cell CD45RA abnormality in thymocytes (35, 36). However, this question remains unresolved until PB and thymocyte specimens are available from the same person with the CD45RA abnormality for study.

It is of interest that in the CD45RA abnormal phenotype described here, there is lack of down-regulation of both the 220- and 200-kDa CD45RA isofoms during thymocyte development. In contrast, in the previously described polymorphism of persistent CD45RA expression on memory PB T cells (34), in the transition from naive to memory T cells only the 200-kDa CD45RA isoform is down-regulated in activated thymocytes, the levels of p24 produced in thymocyte cultures infected with the X4 strain, IIIB, and the R5X4 strain, 89.6, were lower than those in thymocyte cultures infected with R5 HIV-1 strains. Thus, as postulated by others there may be thymotropic strains of HIV-1 that are more destructive to thymuses than other less thymotropic HIV-1 strains (52).

Although CXCR4 is expressed on fresh thymocytes and up-regulated in activated thymocytes, the levels of p24 produced in thymocyte cultures infected with the X4 strain, IIIB, and the R5X4 strain, 89.6, were lower than those in thymocyte cultures infected with R5 HIV-1 strains. Thus, as postulated by others there may be thymotropic strains of HIV-1 that are more destructive to thymuses than other less thymotropic HIV-1 strains (52).

Given the similarity of the defect of activation-induced CD45RA isofrom down-regulation in thymocytes described in this report to that of the previously described lack of CD45RA down-regulation in PB T cells (35), we suggest that the defects are identical, and that we have now observed the consequence of the previously described PB T cell CD45RA abnormality in thymocytes (35, 36). However, this question remains unresolved until PB and thymocyte specimens are available from the same person with the CD45RA abnormality for study.

### Table IV. The association between abnormal CD45RA expression and the CCR5Δ32 mutation

| CCR5 Genotype Pairwise Comparison | CCR5Δ32 | CCR5 WT | p Valueb |
|----------------------------------|---------|---------|----------|
| Homozygous vs wild type          | 1/2     | 6/340   | 0.041    |
| Heterozygous vs wild type        | 5/64    | 6/340   | 0.018    |
| Combined homozygous and heterozygous vs wild type | 6/66 | 6/340 | 0.006 |

a WT, wild type; +/+ for CCR5 gene expression; CCR5Δ32, cells carry either one (-/-, heterozygous) or two (-/-, homozygous) CCR5 alleles with the CCR5Δ32 mutation.

b Statistical significance determined by the Fisher exact test.

### Table V. Assay on day 8 for p24 to determine HIV-1 infection in human thymuses and PBMC by various strains of HIV-1

| Cellsa | p24 Production by Virus (phenotype)b |
|--------|-------------------------------------|
|        | IIB (X4) | 89.6 (R5X4) | V67970 (R5X4) | Ba-L (R5) | P15 (R5) | P46 (R5) | JR-FL (R5) |
| CCR5+/+ , CD45RA NL | | | | | | | |
| T-324 | 2.0 ± 1.0 | 1.3 ± 0.8 | 1.3 ± 0.5 | 25.5 ± 15 | 26.5 ± 34.0 | 1.3 ± 1.0 | 2.0 ± 0.9 |
| T-310 | 2.7 ± 1.8 | 10.4 ± 3.1 | 32.7 ± 11.3 | 37.0 ± 9.5 | 62.4 ± 18.1 | 28.3 ± 3.5 | 42.8 ± 21.2 |
| T-471 | 1.1 ± 0.3 | 1.8 ± 0.8 | 18.8 ± 15.6 | 56.3 ± 31.0 | 14.8 ± 1.9 | 6.6 ± 3.0 | 5.6 ± 2.5 |
| CCR5+/− , CD45RA NL | | | | | | | |
| T-684 | 3.4 ± 0.5 | 4.6 ± 2.6 | 12.2 ± 2.2 | 27.1 ± 15.2 | 3.1 ± 2.8 | 1.7 ± 0.9 | 1.8 ± 1.9 |
| T-698 | 2.1 ± 1.1 | 1.1 ± 0.9 | 6.2 ± 6.6 | 3.2 ± 1.5 | 2.0 ± 2.3 | 1.8 ± 2.7 | 1.2 ± 1.1 |
| T-690 | 1.4 ± 0.7 | 0.9 ± 0.6 | 2.0 ± 0.5 | <0.1 | <0.1 | <0.1 | <0.1 |
| CCR5+/− , CD45RA ABNL | | | | | | | |
| T-205 | 1.1 ± 0.7 | 2.1 ± 0.9 | 11.9 ± 5.1 | 28.5 ± 12.6 | 81.3 ± 80.5 | 7.6 ± 3.1 | 5.8 ± 1.0 |
| T-592 | 0.3 ± 0.1 | 1.6 ± 0.6 | 32.0 ± 12.1 | 24.6 ± 14.8 | 47.4 ± 20.6 | 30.0 ± 9.8 | 41.4 ± 6.5 |
| T-668 | 1.7 ± 0.4 | 1.4 ± 0.7 | 5.8 ± 2.4 | 6.8 ± 2.4 | 4.7 ± 5.6 | 0.6 ± 0.3 | 0.9 ± 0.3 |
| CCR5−/− , CD45RA ABNL | | | | | | | |
| T-223 | 6.8 ± 6.2 | 3.8 ± 3.3 | 15.1 ± 9.2 | 9.1 ± 7.2 | 12.2 ± 14.3 | 0.2 ± 0.1 | 0.8 ± 0.6 |
| T242 | 0.9 ± 0.1 | 1.3 ± 0.4 | 12.5 ± 3.0 | 5.4 ± 3.0 | 17.9 ± 6.5 | 3.9 ± 1.7 | 3.7 ± 0.4 |
| PBMC | 111.8 ± 29.4 | 76.7 ± 12.4 | 83.1 ± 50 | 133.3 ± 9.7 | 114.26 ± 2.1 | 143.5 ± 17.4 | 150.3 ± 15.5 |

a CCR5+/+, wild-type alleles; CCR5+/−, heterozygous for the CCR5Δ32 allele; CD45RA NL, normal CD45RA phenotype; CD45RA ABNL, CD45RA abnormal phenotype.
b Values are the average concentration of p24 (ng/ml) ± SD (n = 4 wells).
PB T cell conversion (lack of down-regulation of only the 200-kDa isoform).
Thus, we have shown a new association of the CCR5Δ32 mutation with an as yet uncharacterized gene mutation responsible for the inability to appropriately down-regulate CD45RA isoforms during thymocyte development and activation. Because CD45 is encoded on chromosome 1 (53, 54), and CCR5 is encoded on chromosome 3 (12), this association must be mediated by an unidentified gene product that modifies CD45 processing and is located on chromosome 3 at a distance from CCR5. It will be of interest to determine in population studies whether the presence of the CD45RA abnormal polymorphism in association with the CCR5Δ32 mutation has any effect on the clinical outcome of HIV-1 infection.

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