ORIGINAL ARTICLE

# Relation of an Interleukin-10 Promoter Polymorphism to Graft-versus-Host Disease and Survival after Hematopoietic-Cell Transplantation

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## ABSTRACT

### BACKGROUND

Polymorphisms in cytokine genes can influence immune responses, inflammation, and tissue injury and may affect the outcome of hematopoietic stem-cell transplantation.

### METHODS

We analyzed single-nucleotide polymorphisms in the genes for interleukin-1$\beta$, interleukin-1–receptor antagonist, interleukin-6, interleukin-10 (*IL10*), and tumor necrosis factor $\alpha$ in 570 transplant recipients and their HLA-identical sibling donors. Genotypes were tested for an association with graft-versus-host disease (GVHD) by multivariable analysis. A second cohort of 423 transplant recipients was independently analyzed for the genotype associations identified in the first cohort.

### RESULTS

The recipient's *IL10* promoter region genotype was significantly associated with the risk of acute GVHD in the first cohort. Analysis of all 993 transplant recipients showed that, as compared with the C/C genotype, the *IL10* −592A/A genotype was associated with a decreased risk of grade III or IV acute GVHD (hazard ratio, 0.4; 95 percent confidence interval, 0.2 to 0.9; P=0.02) and death in remission (hazard ratio, 0.6; 95 percent confidence interval, 0.3 to 1.0; P=0.05). A haplotype analysis showed that the *IL10* −592A allele was a specific marker for a promoter haplotype, T-C-A-T-A, defined by five polymorphisms at positions −3575, −2763, −1082, −819, and −592, respectively.

### CONCLUSIONS

Among recipients of hematopoietic cells from an HLA-identical sibling, the *IL10* −592A allele is a marker of a favorable outcome after transplantation.

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HEMATOPOIETIC STEM-CELL TRANS-plantation can be lifesaving for patients with otherwise fatal diseases. However, mature T cells in the graft can initiate immune reactions that cause graft-versus-host disease (GVHD), a potentially fatal complication.[1] Matching of donor and recipient for HLA[2] and minor histocompatibility antigens[3] is important to optimize the outcome of transplantation, because mismatches increase the risk of GVHD. Certain genes may also affect the outcome by modulating the intensity of inflammation and tissue injury associated with the alloimmune reaction and other transplantation-related complications.

Cytokines and other regulators of the immune response may have an important role in the pathogenesis of GVHD.[4] Nucleotide variations in the genes encoding these molecules may affect the transcription or translation of the genes or the secretion or function of the corresponding proteins.[5] We analyzed seven single-nucleotide polymorphisms located in five cytokine genes — interleukin-1 beta (*IL1B*), interleukin-1–receptor antagonist (*IL1RA*), interleukin-6 (*IL6*), interleukin-10 (*IL10*), and tumor necrosis factor α (*TNFA*) — in a group of 570 HLA-identical donor–recipient sibling pairs designated cohort 1. These polymorphisms correlate with gene function and susceptibility to disease.[5] A statistically significant finding in this cohort led to independent testing in a second cohort. Results of a combined analysis demonstrated a significant association between polymorphisms in the promoter region of the recipient's *IL10* gene and the risk of acute GVHD and death.

## METHODS

### PATIENTS

The entire study population consisted of 993 transplant recipients and their HLA-identical sibling donors. All patients received grafts containing T cells. The HLA genotypic identity of each recipient and donor was established as previously described.[6] Inclusion criteria were the availability of pretransplantation blood samples, the use of methotrexate and cyclosporine for prophylaxis against GVHD, and the availability of acute GVHD grading scores before the study began. The study was divided into two phases and involved separate cohorts. The initial cohort consisted of 570 HLA-A2–positive donor–recipient pairs previously assembled for a different study.[7] We used this cohort to screen for an associ-ation between GVHD and seven single-nucleotide polymorphisms in five cytokine genes. The second cohort included 423 recipients, most of whom were HLA-A2–negative. This cohort was used for confirmatory analysis. There were significant differences between these groups in the patients' ages, years of transplantation, and use or nonuse of total-body irradiation (Table 1). A final analysis of clinical end points included both cohorts. All recipients and donors gave written informed consent according to protocols approved by the institutional review board of the Fred Hutchinson Cancer Research Center.

### NOMENCLATURE OF SINGLE-NUCLEOTIDE POLYMORPHISMS

Seven single-nucleotide polymorphisms were studied in five genes: *IL1B*, *IL1RA*, *IL6*, *IL10*, and *TNFA*. Six of these single-nucleotide polymorphisms — −511C or T of *IL1B*, +3954C or T of *IL1B*, −174C or G of *IL6*, −592A or C of *IL10*, −1082A or G of *IL10*, and −308A or G of *TNFA* — are identified by a number that refers to its position in the nucleotide sequence upstream (indicated by a minus sign) or downstream (indicated by a plus sign) of the start of the transcription site, followed by a letter indicating the polymorphism, adenine (A), cytosine (C), guanine (G), or thymine (T). The upstream single-nucleotide polymorphisms (at position −511 of *IL1B*, at position −174 of *IL6*, at position −592 of *IL10*, at position −1082 of *IL10*, and at position −308 of *TNFA*) are presumed to be located in the promoter region. The +3954 single-nucleotide polymorphism of *IL1B* is located in the fifth exon of the gene. One of the single-nucleotide polymorphisms — 9261A or G of *IL1RA* — maps to the second intron of the gene. The number 9261 refers to the locator number of Genbank sequence accession number X64532 (http://www.ncbi.nlm.nih.gov).

### GENOTYPING OF SINGLE-NUCLEOTIDE POLYMORPHISMS

A multiplex polymerase-chain-reaction–restriction-fragment–length polymorphism (PCR-RFLP) assay was developed for simultaneous typing of the polymorphisms involving position −511 of *IL1B*, position +3954 of *IL1B*, and position 9261 of *IL1RA* by *Alw*NI-based and *Taq*I-based RFLP; polymorphisms involving positions −1082 and −592 of *IL10* by *Bsl*I-based RFLP, and polymorphisms involving position −174 of *IL6* and position −308 of *TNFA* by *Bsl*I-based RFLP.[8-10] Reagent specificity was confirmed by testing DNA samples from the International His-

tocompatibility Working Group cytokine gene polymorphism reference panel (http://www.ihwg.org/components/cytokine/cytover.htm). Genotypes of two recently identified single-nucleotide polymorphisms (−3575 and −2763) located in the distal promoter region of the IL10 gene were determined by PCR-RFLP with the use of Tsp509I endonuclease.[11]

### STATISTICAL ANALYSIS

Acute GVHD and chronic GVHD were diagnosed and graded according to standard criteria.[12,13] Death in remission was defined as any death occurring before the recurrence of the underlying disease. The cumulative rates of incidence of acute GVHD, chronic GVHD, and death in remission and overall survival were estimated according to the methods of Andersen et al.[14] Death was considered to be a competing risk in the analysis of acute and chronic GVHD, and relapse was considered to be a competing risk in the analysis of chronic GVHD and death in remission.

The relations between single-nucleotide–polymorphism genotypes and outcome were evaluated with proportional-hazards regression models, after adjustment for known risk factors. Data on outcomes were censored at the time of a competing event as defined above. Analysis of acute GVHD was adjusted for age at transplantation (as a continuous variable), presence or absence of sex mismatch between donor and recipient, use or nonuse of total-body irradiation in the conditioning regimen, and disease risk group (reason for transplantation). The year of transplantation (1981 through 1991 vs. 1992 through 2000) was also included in the multivariable analysis of acute GVHD because the sensitivity of methods for diagnosing GVHD of the gut increased after 1991. Analysis of chronic GVHD was adjusted for the same risk factors, except for year of transplantation. The analysis of death in remission and overall survival was adjusted for the age at transplantation, the time from diagnosis to transplantation (as a continuous variable), and the disease risk group.

All P values are two-sided and derived from likelihood-ratio statistics from the proportional-hazards regression models. Hazard ratios for the M/m and m/m genotypes were compared with the M/M genotype, the designated reference group, where M represents the more frequent (major) allele and m represents the less frequent (minor) allele in the studied population. Tests for trend were also carried out by assigning the ordinal values 1, 2, and 3 to the

| Table 1. Characteristics of the Transplant Recipients in the First and Second Cohorts.* | | | |
|---|---|---|---|
| Characteristic | First Cohort (N=570) | Second Cohort (N=423) | P Value |
| Age — yr | | | <0.001 |
|   Median | 35.8 | 41.4 | |
|   Range | 1.3–67.8 | 0.6–65.5 | |
| Transplantation year — no. (%) | | | <0.001 |
|   1981–1991 | 334 (59) | 57 (13) | |
|   1992–2000 | 236 (41) | 366 (87) | |
| Sex of recipient–donor pair — no. (%) | | | 0.08 |
|   Male/male | 196 (34) | 136 (32) | |
|   Male/female | 140 (25) | 110 (26) | |
|   Female/male | 120 (21) | 69 (16) | |
|   Female/female | 114 (20) | 108 (26) | |
| Total-body irradiation — no. (%) | | | <0.001 |
|   Yes | 346 (61) | 191 (45) | |
|   No | 224 (39) | 232 (55) | |
| Reason for transplantation — no. (%)† | | | 0.18 |
|   Nonmalignant disease | 87 (15) | 83 (20) | |
|   Low-risk cancer | 295 (52) | 202 (48) | |
|   High-risk cancer | 188 (33) | 138 (33) | |
| Cumulative incidence of grade III or IV acute GVHD on day 100 — % | 18 | 17 | 0.59 |
| Cumulative incidence of clinically extensive chronic GVHD at 3 yr — % | 37 | 41 | 0.79 |
| Cumulative incidence of death in remission at 3 yr — % | 27 | 22 | 0.11 |
| Cumulative overall survival at 3 yr — % | 55 | 61 | 0.11 |

\* GVHD denotes graft-versus-host disease.
† Nonmalignant diseases included aplastic anemia, myelodysplastic syndrome, and paroxysmal nocturnal hematuria. Low-risk cancers included acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), and non-Hodgkin's lymphoma (NHL) in remission and chronic myelogenous leukemia (CML) in chronic phase. High-risk cancers included ALL, AML, chronic lymphocytic leukemia, and NHL in relapse; CML in other than chronic phase; multiple myeloma, and Hodgkin's disease.

genotypes M/M, M/m, and m/m, respectively, and testing the association of the resulting variable with the outcome. Any variable associated with a hazard ratio of 2.0 or greater or of 0.5 or less or a P value for trend of 0.05 or less in the first cohort was reevaluated in the second cohort. No adjustments have been made for multiple comparisons.

Haplotype frequencies and the $\chi^2$ test for linkage disequilibrium among pairs of alleles were calculated with use of the Estimating Haplotype-frequencies (EH) program from Rockefeller University (ftp://linkage.rockefeller.edu/software/eh/).[15]

## RESULTS

### FIRST COHORT

In the first cohort of 570 recipients, the *IL10* −592A allele was significantly associated with a lower incidence of severe (grade III or IV) acute GVHD in both homozygous recipients (those with the A/A genotype) and heterozygous recipients (those with the A/C genotype) (P for trend=0.003; hazard ratio among patients with the −592A/A and A/C genotypes, as compared with the C/C genotype, 0.5 and 0.5, respectively) (Table 2). A possible association was observed for the *IL10* −1082 genotype of the recipient (P for trend=0.05; hazard ratio associated with the −1082G/G and A/G genotypes, as compared with the A/A genotype, 1.7 and 1.2, respectively) (Table 2). No significant association with severe acute GVHD was detected for *IL10* genotypes of the donors, and no significant association was detected for the *IL1B* −511, *IL1B* +3954, *IL1RA* 9261, *IL6* −174, or *TNFA* −308 genotypes in either donors or recipients (Table 2).

### SECOND COHORT

In the second cohort of 423 recipients, the hazard ratio for severe GVHD among the 34 recipients with the A/A genotype at the *IL10* −592 locus was 0.3 (P for trend=0.12). Although the small number of recipients with the −592A/A genotype precludes a statistical analysis, the consistency of the effects between the two independent cohorts suggested that the *IL10* −592A/A genotype in the recipient was associated with a reduced risk of severe acute GVHD.

### *IL10* PROMOTER-REGION GENOTYPE AND OUTCOMES OF TRANSPLANTATION

Analysis of polymorphisms in the *IL10* promoter region of the recipients and the risk of acute GVHD was next performed in the combined cohort of 993 recipients. Two additional polymorphisms in the *IL10* promoter region (−2763 and −3575)[11] were evaluated (Table 3). A significant association with a reduced incidence of severe acute GVHD was observed for the *IL10* −592A/A genotype, and weak associations with lower incidence were observed for the *IL10* −1082A/A and −2763C/C genotypes. Homozygosity for the *IL10* −592A allele was associated with the lowest risk of grade III or IV acute GVHD (hazard ratio, 0.4; 95 percent confidence interval, 0.2 to 0.9; P=0.02) (Fig. 1A). No significant differences were detected in the distribution of known risk factors for GVHD, including age at transplantation, year of transplantation, presence or absence of sex mismatch between donor and recipient, use or nonuse of total-body irradiation in the conditioning regimen, and disease risk group among patients with the various *IL10* −592 genotypes (data not shown).

No significant association was found between the recipient's *IL10* promoter genotype and the risk of extensive chronic GVHD. Cumulative rates of incidence of extensive chronic GVHD at three years for patients with the *IL10* −592A/A, A/C, and C/C genotypes were 38 percent, 38 percent, and 41 percent, respectively.

Death in remission and overall survival among the combined cohort of recipients was also associated with the genotype of the *IL10* promoter region. The lowest risk of death in remission was found among recipients who were homozygous for the −592A allele (hazard ratio for the comparison with the C/C genotype, 0.6; 95 percent confidence interval, 0.3 to 1.0; P=0.05). The cumulative rates of incidence of death in remission at three years for recipients with the *IL10* −592A/A, A/C, and C/C genotypes were 13 percent, 26 percent, and 25 percent, respectively (Fig. 1B). The cumulative rates of incidence of death in remission at three years among recipients with grades 0, I, II, III, and IV acute GVHD were 22 percent, 19 percent, 20 percent, 46 percent, and 96 percent, respectively. The hazard ratio for death in remission after the diagnosis of grade III or IV GVHD was 3.7 (95 percent confidence interval, 2.9 to 4.8; P<0.001). Results for overall survival were similar to those for death in remission. Among recipients with the *IL10* −592A/A, A/C, and C/C genotypes, the probability of surviving three years was 71 percent, 56 percent, and 57 percent, respectively.

### *IL10* PROMOTER-REGION HAPLOTYPE

We stratified patients according to *IL10* promoter region haplotypes in order to examine the relative effect of the individual haplotype on the risk of severe acute GVHD. Haplotypes are clusters of genetic variants that are inherited as a unit on the same chromosome. Within a population, the individual variants in the *IL10* promoter region within such clusters are not randomly distributed; this phenomenon is known as linkage disequilibrium. These nonrandom distributions make possible the identification of haplotypes in the *IL10* promoter region. Previous studies have identified at least two clusters of single-nucleotide polymorphisms in the 5′

**Table 2.** Association of Cytokine Gene Polymorphisms in Recipients and Donors and Grade III or IV Acute Graft-versus-Host Disease (GVHD) in the First Cohort of 570 Transplant Recipients.

| Gene, Position, and Genotype* | Recipients | | | | Donors | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of Recipients | Incidence of GVHD | Hazard Ratio† | P Value for Trend‡ | No. of Donors | Incidence of GVHD | Hazard Ratio† | P Value for Trend‡ |
| | | *percent* | | | | *percent* | | |
| ***IL1B*** | | | | | | | | |
| Position +3954 | | | | 0.54 | | | | 0.94 |
| T/T | 35 | 20 | 1.0 | | 41 | 17 | 0.9 | |
| C/T | 218 | 16 | 0.8 | | 197 | 19 | 1.0 | |
| C/C | 317 | 20 | Reference | | 332 | 18 | Reference | |
| Position −511 | | | | 0.88 | | | | 0.66 |
| T/T | 69 | 22 | 1.2 | | 76 | 17 | 0.9 | |
| C/T | 260 | 17 | 0.9 | | 258 | 18 | 0.9 | |
| C/C | 241 | 19 | Reference | | 236 | 19 | Reference | |
| ***IIL1RA*, position 9261** | | | | 0.25 | | | | 0.14 |
| G/G | 40 | 15 | 0.7 | | 41 | 20 | 0.9 | |
| A/G | 228 | 17 | 0.9 | | 216 | 14 | 0.6 | |
| A/A | 302 | 20 | Reference | | 313 | 21 | Reference | |
| ***IL6*, position −174** | | | | 0.89 | | | | 0.22 |
| C/C | 72 | 18 | 1.0 | | 69 | 26 | 1.7 | |
| C/G | 259 | 17 | 0.9 | | 279 | 17 | 0.9 | |
| G/G | 239 | 19 | Reference | | 222 | 18 | Reference | |
| ***IL10*** | | | | 0.003 | | | | 0.20 |
| Position −592 | | | | | | | | |
| A/A | 52 | 12 | 0.5 | | 48 | 13 | 0.6 | |
| A/C | 222 | 13 | 0.5 | | 224 | 17 | 0.8 | |
| C/C | 296 | 23 | Reference | | 298 | 20 | Reference | |
| Position −1082 | | | | 0.05 | | | | 0.19 |
| G/G | 117 | 24 | 1.7 | | 128 | 22 | 1.4 | |
| A/G | 270 | 19 | 1.2 | | 252 | 19 | 1.2 | |
| A/A | 183 | 14 | Reference | | 190 | 15 | Reference | |
| ***TNFA*, position −308** | | | | 0.47 | | | | 0.47 |
| A/A | 12 | 25 | 1.5 | | 12 | 25 | 1.5 | |
| A/G | 132 | 21 | 1.1 | | 132 | 21 | 1.1 | |
| G/G | 426 | 17 | Reference | | 426 | 17 | Reference | |

* Each single-nucleotide polymorphism is a dialleleic nucleotide polymorphism. The two observed alleles are indicated (A, C, G, or T). The three possible genotypes for each single-nucleotide polymorphism are indicated (e.g., T/T, C/T, or C/C). With one exception, the genotype distributions were consistent with the existence of Hardy–Weinberg equilibrium. The distribution of the donor interleukin-10 −1082 genotype did not meet the requirements for Hardy–Weinberg equilibrium, possibly reflecting the racial admixture of the population. *IL1B* denotes interleukin-1 beta, *IL1RA* interleukin-1–receptor antagonist, *IL6* interleukin-6, *IL10* interleukin-10, and *TNFA* tumor necrosis factor α.

† Individual genotypes were compared and hazard ratios for GVHD in the recipients were calculated with the use of homozygosity for the more frequent (major) allele as the reference group (hazard ratio, 1.0).

‡ P values were calculated with the use of multivariable logistic-regression analysis, adjusted for age at transplantation, year of transplantation, presence or absence of sex mismatch between donor and recipient, use or nonuse of total-body irradiation in the conditioning regimen, and disease risk group.

§ TNF-α denotes tumor necrosis factor α.

flanking region of the *IL10* gene, one at the distal positions −3575 and −2763, and the other at the proximal positions −1082, −819, and −592[11,16] (Fig. 2). There is linkage disequilibrium between the −819 single-nucleotide polymorphism (T or C allele) and the −592 single-nucleotide polymorphism (A or C allele), with only two haplotypes observed at reasonable frequencies. In one, −819T is linked to −592A (the T-A haplotype), and in the other, −819C is linked to −592C (the C-C haplotype).

Haplotype frequencies among the combined population of 993 transplant recipients were estimated as previously described.[15] The results were consistent with previous findings.[11,16] In the result-

**Table 3.** Association of Interleukin-10 Promoter-Region Genotypes and Grade III or IV Acute Graft-versus-Host Disease (GVHD) in Both Cohorts of 993 Transplant Recipients.

| Interleukin-10 Single-Nucleotide Polymorphism and Genotype | No. of Recipients | Incidence of GVHD | Hazard Ratio (95% CI)* | P Value for Trend† |
|---|---|---|---|---|
| | | % | | |
| −592 | | | | 0.001 |
| A/A | 86 | 9 | 0.4 (0.2–0.9) | |
| A/C | 382 | 15 | 0.7 (0.5–0.9) | |
| C/C | 525 | 21 | Reference | |
| −1082 | | | | 0.08 |
| A/A | 319 | 14 | Reference | |
| A/G | 469 | 19 | 1.4 (0.9–1.9) | |
| G/G | 205 | 20 | 1.4 (0.9–2.2) | |
| −2763 | | | | 0.07 |
| C/C | 444 | 15 | Reference | |
| A/C | 441 | 20 | 1.3 (1.0–1.8) | |
| A/A | 108 | 21 | 1.4 (0.9–2.3) | |
| −3575 | | | | 0.31 |
| T/T | 426 | 16 | Reference | |
| A/T | 441 | 19 | 1.2 (0.9–1.7) | |
| A/A | 126 | 19 | 1.2 (0.7–1.9) | |

* Individual genotypes were compared and hazard ratios were calculated with the use of homozygosity for the more frequent (major) allele as the reference group (hazard ratio, 1.0). CI denotes confidence interval.

† P values were calculated with the use of multivariable logistic-regression analysis, adjusted for age at transplantation, year of transplantation, presence or absence of sex mismatch between donor and recipient, use or nonuse of total-body irradiation in the conditioning regimen, and disease risk group.

ing model (assuming complete linkage disequilibrium between −819 and −592), we found that the five single-nucleotide polymorphisms segregated as six distinct haplotypes with frequencies of 4 percent or greater: T-C-A-T-A, T-C-A-C-C, T-C-G-C-C, A-C-G-C-C, T-A-G-C-C, and A-A-G-C-C (Fig. 2). The proximal A-T-A haplotype (or the −592A allele) and the A-C-C haplotype were associated exclusively with the distal T-C haplotype to form the extended IL10 promoter T-C-A-T-A and T-C-A-C-C haplotypes, respectively. The proximal G-C-C haplotype is found together with the distal T-C, A-C, T-A, and A-A haplotypes to form the extended T-C-G-C-C, A-C-G-C-C, T-A-G-C-C, and A-A-G-C-C haplotypes (Fig. 2).

Associations with severe acute GVHD were first evaluated in patients classified according to three proximal promoter haplotypes (A-T-A, A-C-C, and G-C-C), defined by single-nucleotide polymorphisms in positions −1082, −819, and −592. Among the six resulting genotypes, the incidence of severe acute GVHD was lowest in the group that was homozygous for A-T-A (9 percent for genotype A-T-A/A-T-A), intermediate in the two groups that were

heterozygous for A-T-A (13 percent and 16 percent for genotypes A-T-A/A-C-C and A-T-A/G-C-C, respectively), and highest in the three groups without A-T-A (20 percent, 21 percent, and 22 percent for genotypes G-C-C/G-C-C, A-C-C/A-C-C, and A-C-C/G-C-C, respectively) (Table 4).

When the three genotypes defined by the A-T-A and A-C-C haplotypes were compared, the A-T-A/A-T-A genotype was associated with a lower risk of severe acute GVHD than were the A-T-A/A-C-C and A-C-C-/A-C-C genotypes (P for trend=0.03). When the three genotypes consisting of the A-T-A and G-C-C haplotypes were compared, the A-T-A/A-T-A genotype was associated with a lower risk of severe acute GVHD than were the A-T-A/G-C-C and G-C-C/G-C-C genotypes (P for trend=0.03). Together with the extended haplotype analysis (Fig. 2), these data suggest that the proximal A-T-A haplotype (extended T-C-A-T-A haplotype) conveys a lower risk of severe acute GVHD than the proximal A-C-C haplotype (extended T-C-A-C-C haplotype) or proximal G-C-C haplotype (extended T-C-G-C-C, A-C-G-C-C, T-A-G-C-C, or A-A-G-C-C haplotype).

### DOMINANT EFFECT OF *IL10* −592 GENOTYPE

When the analysis was limited to recipients with the *IL10* −592C/C genotype, the incidence of severe acute GVHD in recipients with −1082G/G, A/A, or A/G was similar (20 to 22 percent) (Table 4). When the analysis was limited to recipients with the −1082A/A genotype, the incidence of severe acute GVHD was 9 percent among recipients with the −592A/A genotype, 13 percent among those with the −592A/C genotype, and 21 percent among those with the −592C/C genotype. Results were similar when analyses were stratified for −592 and either the distal −2763 or −3575 polymorphism (data not shown).
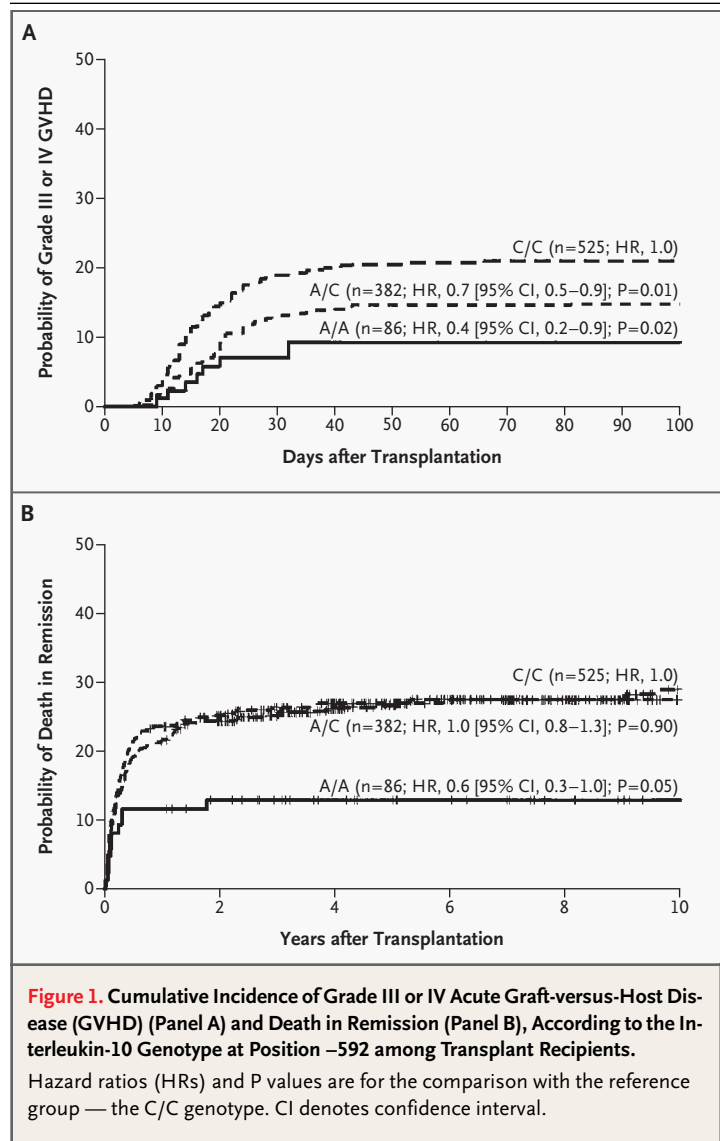
A multivariable analysis adjusted for the −592 genotype indicated that polymorphisms at −1082, −2763, or −3575 were not independent risk factors for severe acute GVHD (P for trend=0.98, 0.67, and 0.59, respectively). In contrast, the P values for trend for −592 remained significant when the analysis was adjusted for the −1082, −2763, or −3575 genotype (P=0.005, 0.005, and 0.002, respectively). Although these results are consistent with the hypothesis that the −592 polymorphism in this population has a dominant effect on the outcome of transplantation, the data cannot be used to distinguish whether this single nucleotide (−592A allele) or an extended sequence (T-C-A-T-A haplotype) accounts for the observed functional effect.

## DISCUSSION

Previous studies have suggested an association between polymorphisms in the *IL1RA*, *IL6*, *IL10*, *TNFA*, and interferon-γ genes and the outcome of hematopoietic stem-cell transplantation.[17-21] The results, however, have been inconsistent, probably because of heterogeneity among the patients, the relatively small numbers of patients in individual studies, and potential statistical problems associated with multiple comparisons. We were able to confirm and extend the previous findings that polymorphisms in the *IL10* promoter region in transplant recipients have a significant effect on the outcome of hematopoietic-cell transplantation.
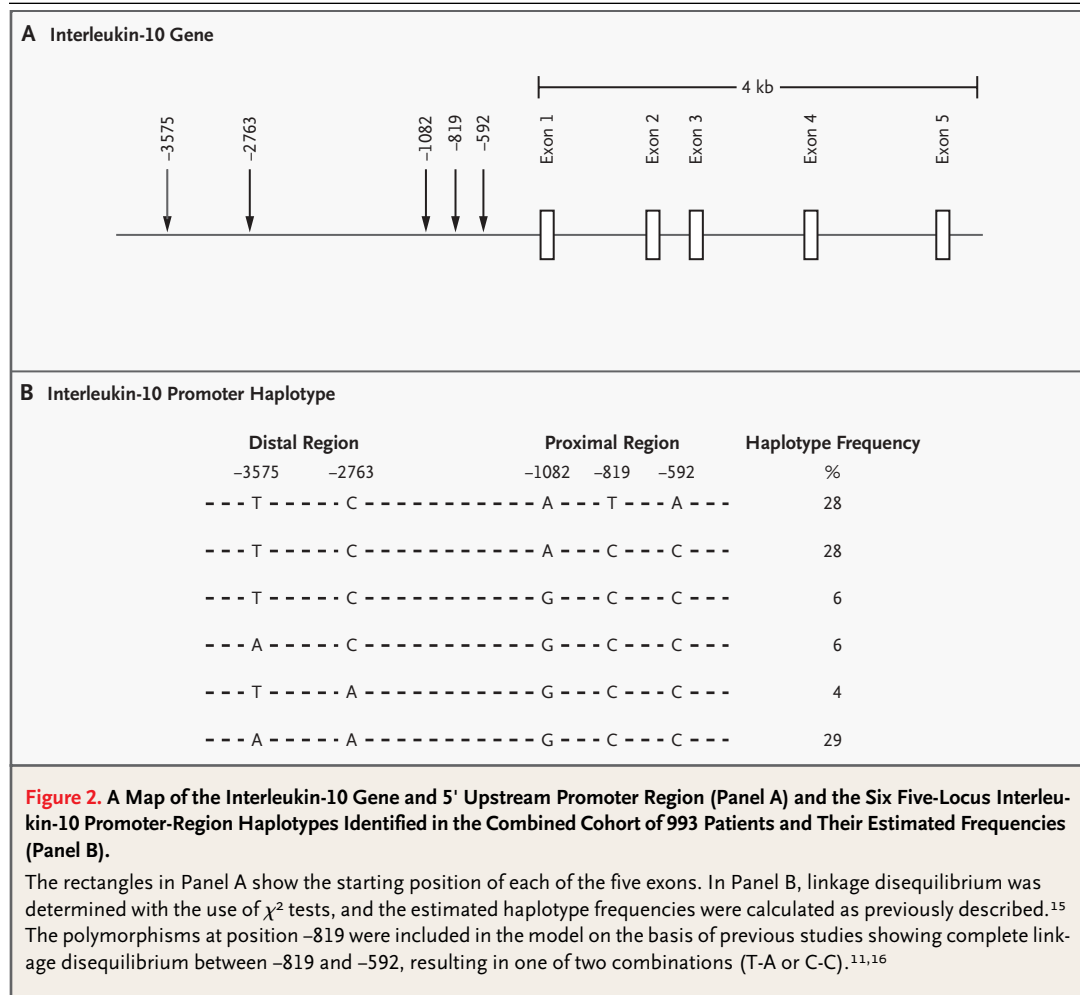
We found that a specific *IL10* promoter-region haplotype had a protective effect. The lowest incidence of severe acute GVHD and death in remission was associated with homozygosity for the T-C-A-T-A (−3575/−2763/−1082/−819/−592) haplotype. The −592A allele was found to be in complete linkage disequilibrium with the rest of the T-C-A-T-A haplotype, allowing this single-nucleotide polymorphism to serve as a convenient "marker tag" for the genetic elements associated with the observed clinical outcomes.[22] The mechanism underlying the lower incidence of acute GVHD and death in remission among recipients who were homozygous for the T-C-A-T-A haplotype and the molecular effects of the nucleotide variation in the *IL10* promoter region are not precisely known. A full understanding of gene function will require more detailed knowledge of the interactions between specific *IL10* transcription factors and the various *IL10* promoter-region haplotypes.

Interleukin-10 is a potent suppressor of TNF-α, interleukin-1α, interleukin-1β, interleukin-6, interleukin-12, and interferon-γ production and may facilitate the induction of tolerance after allogeneic transplantation.[23-29] The administration of exogenous interleukin-10, however, has variable effects in murine models of GVHD.[30,31] Blazer et al. have reported a dose-dependent effect of interleukin-10 on the lethality of GVHD in mice. High doses potentiated GVHD, whereas lower doses were protective.[32] Clinical studies, however, suggest that elevated levels of endogenous interleukin-10 may be associated with immunologic tolerance. Bacchetta et al.[33] observed an association between increased expression of the *IL10* gene in recipient-derived monocytes and the absence of GVHD after the receipt of HLA-mismatched fetal hematopoietic cells



**Figure 1. Cumulative Incidence of Grade III or IV Acute Graft-versus-Host Disease (GVHD) (Panel A) and Death in Remission (Panel B), According to the Interleukin-10 Genotype at Position −592 among Transplant Recipients.**

Hazard ratios (HRs) and P values are for the comparison with the reference group — the C/C genotype. CI denotes confidence interval.

among patients with severe combined immunodeficiency. Holler et al.[34] have reported that elevated levels of endogenous interleukin-10 before transplantation are associated with a reduced risk of GVHD. These data suggest that endogenous interleukin-10 production soon after transplantation may facilitate the suppression of alloimmune responses.

Conflicting results have been reported regarding the genetic control of interleukin-10 production.[11,16,35-37] Increased production of interleukin-10 by peripheral-blood mononuclear cells has been associated with the −1082G allele or the G-C-C (−1082/−819/−592) haplotype[16,35]; however, Keij-

**A** Interleukin-10 Gene



**B** Interleukin-10 Promoter Haplotype

| Distal Region | | Proximal Region | | | Haplotype Frequency |
|---|---|---|---|---|---|
| −3575 | −2763 | −1082 | −819 | −592 | % |
| - - - T - - - - - · C - - - - - - - - - - - - - A - - - · T - - - A - - - | | | | | 28 |
| - - - T - - - - - · C - - - - - - - - - - - - - A - - - · C - - - C - - - | | | | | 28 |
| - - - T - - - - - · C - - - - - - - - - - - - - G - - - · C - - - C - - - | | | | | 6 |
| - - - A - - - - - · C - - - - - - - - - - - - - G - - - · C - - - C - - - | | | | | 6 |
| - - - T - - - - - · A - - - - - - - - - - - - - G - - - · C - - - C - - - | | | | | 4 |
| - - - A - - - - - · A - - - - - - - - - - - - - G - - - · C - - - C - - - | | | | | 29 |

**Figure 2. A Map of the Interleukin-10 Gene and 5' Upstream Promoter Region (Panel A) and the Six Five-Locus Interleukin-10 Promoter-Region Haplotypes Identified in the Combined Cohort of 993 Patients and Their Estimated Frequencies (Panel B).**

The rectangles in Panel A show the starting position of each of the five exons. In Panel B, linkage disequilibrium was determined with the use of $\chi^2$ tests, and the estimated haplotype frequencies were calculated as previously described.[15] The polymorphisms at position −819 were included in the model on the basis of previous studies showing complete linkage disequilibrium between −819 and −592, resulting in one of two combinations (T-A or C-C).[11,16]

sers et al.[36] reported that the −1082G allele, or G-C-C haplotype, was associated with a decreased production of interleukin-10. This finding has been supported by a more recent report by Gibson et al.[11] that the distal promoter region A-A (−3575/−2763) haplotype was associated with the proximal G-C-C (−1082/−819/−592) promoter haplotype. They also found decreased production of interleukin-10 in vitro among patients with the distal A-A haplotype. The results of these two studies suggest that the A-A-G-C-C haplotype is associated with decreased production of interleukin-10 (Fig. 2). The estimated haplotype frequencies in our study population were essentially the same as those described by Gibson et al.[11] The distal A-A promoter haplotype was linked to the proximal G-C-C haplotype, and the proximal A-T-A and A-C-C haplotypes were each linked to the distal T-C haplotype. The −592A allele was found only in recipients with the T-C-A-T-A hap-

lotype. In our study, recipients homozygous for the *IL10* T-C-A-T-A haplotype had a lower risk of acute GVHD than recipients who were heterozygous for the T-C-A-T-A or T-C-A-C-C haplotype or recipients who were homozygous for the T-C-A-C-C haplotype. Together with the reported biologic functions of interleukin-10 in vitro and in vivo,[23,34] these data suggest that the T-C-A-T-A haplotype is defined by high levels of interleukin-10.

Japanese patients have a lower incidence of acute GVHD than do white patients after the receipt of cells from either HLA-matched siblings or unrelated donors.[38,39] This difference has been assumed to reflect a lower degree of diversity for HLA and minor histocompatibility antigens among Japanese. However, the frequency of the *IL10* −592A allele is 67 percent in the Japanese population[40] and 70 percent in the Taiwanese populations (Tseng L-H: unpublished data), both of which are much higher

**Table 4.** Analysis of the Risk of Graft-versus-Host Disease (GVHD) According to the Proximal Interleukin-10 Promoter-Region Haplotypes of the Recipients.

| Observed −1082 Genotype | Observed −592 Genotype | Haplotype for Assigned −1082, −819, −592* | No. of Recipients | Grade III or IV GVHD | |
|---|---|---|---|---|---|
| | | | | Incidence % | Hazard Ratio (95% CI)† |
| A/A | A/A | A-T-A/A-T-A | 86 | 9 | Reference |
| A/A | A/C | A-T-A/A-C-C | 146 | 13 | 1.4 (0.6–3.2) |
| A/G | A/C | A-T-A/G-C-C | 236 | 16 | 1.7 (0.8–3.6) |
| G/G | C/C | G-C-C/G-C-C | 205 | 20 | 2.2 (1.0–4.8) |
| A/A | C/C | A-C-C/A-C-C | 87 | 21 | 2.3 (1.0–5.4) |
| A/G | C/C | A-C-C/G-C-C | 233 | 22 | 2.5 (1.2–5.4) |

* The −819 single-nucleotide polymorphism is assumed on the basis of the known complete linkage disequilibrium between the −819 (T or C) and −592 (A or C) polymorphisms, resulting in one of two haplotypes (T-A or C-C). The estimated frequency of the G-T-A haplotype was 0 percent in this population.

† Hazard ratios were calculated with the use of homozygosity for the A-T-A haplotype as the reference group (hazard ratio, 1.0). CI denotes confidence interval.

than the frequency of 23 percent and 24 percent previously reported in two white populations.[35,37] These data suggest that the higher frequency of the −592A allele in the Japanese population may explain the lower incidence of GVHD among Japanese transplant recipients. Variation in the *IL10* gene polymorphisms may be one of the factors that account for differences in the incidence and severity of disease among various populations.

Despite recent advances in supportive care, severe grade III or IV GVHD remains a serious complication of transplantation and contributes to transplantation-related mortality. On the basis of the results of previous in vivo and in vitro studies and our own clinical correlations, we hypothesize that a high level of interleukin-10 production by recipients' cells during the early post-transplantation period mitigates the intensity of the alloimmune response and GVHD-induced inflammation, thereby reducing the clinical manifestations of GVHD and associated mortality. Knowledge of the *IL10* promoter-region genotypes and, possibly, of polymorphisms in other immune regulatory genes, could be incorporated into the pretransplantation risk-assessment process and serve as a guide for the planning of treatment. The use of alternative approaches, such as nonablative conditioning regimens, might reduce morbidity and mortality in selected high-risk patients. Further insight into the mechanism underlying the association between the *IL10* promoter-region genotype and GVHD could prompt new strategies for modulating the intensity of the alloimmune response and reducing the toxicity of GVHD.

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