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Cutting Edge: Chronic NF-κB Activation in CD4⁺ T Cells in Rheumatoid Arthritis Is Genetically Determined by HLA Risk Alleles

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Of identified genetic variants, HLA polymorphisms confer the greatest risk for developing autoimmune diseases, including rheumatoid arthritis (HLA-DRB1*04). There are strong influences of HLA polymorphisms on cell type–specific gene expression in B cells and monocytes. Their influence on gene expression in CD4⁺ T cells is not known. We determined transcript and proteins levels of target genes in lymphocyte/monocyte subsets in healthy controls and rheumatoid arthritis subjects as a function of HLA-DRB1*04 haplotype. We identified gene expression dependent on HLA-DRB1*04 genotype in CD4⁺ T cells. NF-κB activity in CD4⁺ T cells was also dependent on HLA-DRB1*04 genotype, and blocking HLA-DR inhibited NF-κB activity in CD4⁺ T cells and normalized gene expression, as did pharmacologic inhibition of NF-κB. We conclude that interactions between TCR and MHC class II encoded by HLA-DRB1*04 create a proinflammatory "hum" altering CD4⁺ T cell phenotype.

RNA isolation and quantitative RT-PCR

Total RNA was purified as described (11). CDNA was synthesized from total RNA using oligo(dT) primers (SuperScript III first-strand synthesis kit, Life Technologies) and purified using Qagen PCR purification kits. PCR reactions were in 25 μl with 50 ng cDNA, TaqMan master mix, and TaqMan gene expression assays (Applied Biosystems). Expression levels were calculated using the ΔΔCt method by comparing target gene transcripts to GAPDH transcripts.

Statistical analysis

Statistical analysis was performed using a Student t test with a Welch correction. A p value of <0.05 after correction for multiple testing was considered significant.

Results

CD4+ T cell eQTL defined by RA-associated HLA-DRB1*04 SNP markers

The goal of our studies was to identify eQTL associated with RA HLA-DRB1*04 risk alleles, determine whether eQTL were confined to CD4+ T cells, and determine how a given HLA-DRB1 protein may confer alterations in CD4+ T cell–specific eQTL. Based on results from previous microarray analyses we performed, we designed a TaqMan low-density array to ascertain expression levels of 45 target genes and 3 housekeeping genes with the goal of developing tools to aid in the diagnosis of autoimmune diseases, for example, RA, inflammatory bowel disease, and multiple sclerosis (12–15). To this end, we analyzed >1500 CTRLs, subjects with different autoimmune diseases, and subjects with chronic noninflammatory diseases (disease controls). It also seemed that we could use these data to identify HLA-DRB1*04-specific eQTL localized to CD4+ T cells.

To initiate our studies we used SNP rs6457620 (chr6:32,771,829, hg18 build) known to tag the RA HLA-DRB1*04 risk allele (2) to genotype CTRL and RA subjects for which we had expression data. This polymorphism is either C or G. In our cohorts, frequencies in white CTRL subjects were ~25% C/C, 35% C/G, and 40% G/G and ~40% C/C, 60% C/G, and <1% G/G in white RA subjects (Supplemental Table I). Presence of the C nucleotide confers RA risk. From these data, we determined whether gene expression levels were associated with HLA-DRB1*04 genotype in CTRL and RA subjects (Supplemental Table II). We identified three categories, which included those genes differentially expressed in CTRL subjects with C/C genotypes compared with C/G and G/G genotypes and in RA with either C/C or C/G genotypes (Fig. 1A, Supplemental Table II), those that differed in CTRL subjects with C/C or C/G genotypes compared with G/G genotypes (Fig. 1B, Supplemental Table II), and those that were independent of genotype but were different between CTRL and RA (Fig. 1C).

FIGURE 1. Gene expression is associated with HLA-DRB1*04 SNP tags. Means ± SD of gene transcripts were determined by quantitative PCR and normalized to GAPDH. (A) Genes differentially expressed in CTRL (n = 82) dependent on HLA-DRB1*04 C/C genotype. (B) CDKN1A transcripts are reduced in CTRL subjects with C/G or C/C genotypes. (C) Genes transcripts independent of genotype. Statistical determinations were performed using a Student t test after correction for multiple testing (see Supplemental Table II).
PKGI mRNA levels were elevated in CTRL subjects with C/C genotypes with the greatest significance (p < 0.001). PKG-1 protein levels were selectively elevated in CD4+ T cells from CTRL subjects with C/C relative to G/G genotypes (Fig. 2B). PKG-1 protein levels were independent of genotype in CD8+, CD14+, and CD19+ cells. Of the mRNAs that were underexpressed in CTRL subjects with C/C versus G/G genotype, ANAPCI was the most significant (p < 0.001). ANAPCI encodes a subunit of the anaphase-promoting complex/cyclosome (APC/C, or APC1) that controls progression through mitosis and the G1 phase of the cell cycle. We found that APC1 protein levels were depressed in CD4+, CD8+, CD19+, and CD14+ subsets from subjects with C/C compared with G/G genotypes (Fig. 2C). These results demonstrate that genotype-dependent differences in γ-H2AX and PGK-1 were restricted to CD4+ T cells whereas APC1 differences were shared among CD4+, CD8+, CD14+, and CD19+ cells.

NF-κB activity and HLA-DRB1*04–associated eQTL

Given the high degree of linkage disequilibrium within the MHC genomic region and the associative nature of genetic studies, we sought to perform mechanistic studies to further establish relationships between HLA-DRB1*04 and T cell eQTL. Stimulation of the TCR by Ag presented by MHC class II activates downstream transcription factors NF-κB, NFAT, and AP-1. Therefore, we asked whether gene expression differences between subjects with G/G and C/C genotypes were reversed or “corrected” in subjects with C/C genotypes by inhibition of these transcription factors. We employed Bay 11-7085, an irreversible inhibitor of IκBα phosphorylation, to inhibit NF-κB, cyclosporine A to inhibit NFAT, and the JNK inhibitor Bi-78D3 to inhibit AP-1. Inhibition of NF-κB increased levels of ANAPCI, which is normally repressed in C/C relative to G/G genotypes (Fig. 3). In contrast, inhibition of NFAT or AP-1 did not increase levels of ANAPCI. Inhibition of NF-κB in C/C genotypes also reduced transcript levels of PKGI, B2M, and NRRAS to levels equivalent to those in subjects with G/G genotypes. Inhibition of NFAT did not alter these gene transcript levels. Inhibition of AP-1 reduced expression of PKGI to a similar degree as inhibition of NF-κB. One interpretation of these results is that these gene transcript levels are determined, at least in part, by activity of the proinflammatory transcription factor NF-κB and perhaps to a lesser degree by AP-1.

The above results suggest that basal NF-κB activity may be higher in CD4+ T cells from subjects with C/C versus G/G or G/G genotypes. To test this hypothesis we employed flow cytometry to measure NF-κB activity (P-p65) in PBMC subsets from CTRL subjects with C/C, C/G, or G/G genotypes. We found that CTRL subjects with C/C genotypes expressed elevated NF-κB activity in CD4+ T cells compared with CTRL subjects with G/G genotypes (Fig. 4A). NF-κB activity in subjects with RA is shown for reference. P-p65 activity was not increased in CD8+, CD19+, and CD14+ cells in subjects with C/C compared with G/G genotypes (Fig. 4B). We interpret these results to indicate that the type of polymorphic MHC class II molecule expressed by APC contributes to basal NF-κB activity in CD4+ T cells.

To further test this hypothesis, we employed a specific anti–HLA-DR blocking Ab to prevent interactions between HLA-DR and the TCR on CD4+ T cells. PBMC from CTRL subjects with C/C genotypes were treated with either an isotype control Ab or an HLA-DR blocking Ab overnight and intracellular levels of active P-p65 were measured by flow cytometry the next day. Ab treatment reduced detection of HLA-DR protein by >75% as measured by surface staining with a second conjugated Ab (Fig. 5A) and inhibited NF-κB activity in CD4+ T cells compared with CTRL subjects with G/G genotypes by >50% (Fig. 5B). Finally, we asked whether the genetically determined gene expression signature identified above was altered by treatment with anti–HLA-DR. We found that treatment with anti–HLA-DR increased expression of ANAPCI and decreased expression of B2M, NRAS, and PGKI (Fig. 5C). We conclude that presence of the C/C ge-
notype increases indices of inflammation, in part, by elevating NF-κB activity.

Discussion

We show that carrying HLA-DRB1*04 risk alleles as measured using surrogate SNP tags regulate gene expression in CD4+ T cells in healthy individuals. Although HLA polymorphisms are known to regulate gene expression and additional phenotypes, such as premature telomere shortening, the underlying mechanisms are not understood (7, 17). Our results demonstrate that the HLA-DRB1*04 risk allele increases activity of a key proinflammatory, prosurvival transcription factor, NF-κB, specifically in CD4+ T cells and this increase is dependent on HLA-DR. We propose that increased NF-κB activity conferred by the HLA-DRB1*04 genotype contributes to RA disease risk.

Major signaling paths activated following productive engagement of the TCR by Ag presented by MHC class II molecules culminate in stimulation of the transcription factors NF-κB, specifically in CD4+ T cells and this increase is dependent on HLA-DR. We propose that increased NF-κB activity conferred by the HLA-DRB1*04 genotype contributes to RA disease risk.

Disclosures

The authors have no financial conflicts of interest.
References


