

THE ROLE OF CD23 IN IgE DEPENDENT SIGNALING: IMPLICATIONS FROM PHARMACOGENETICS

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ABSTRACT

The association of immunoglobulin E (IgE) with allergic diseases and asthma is well established. IgE binds to two receptors on various immune and inflammatory cells. The lower-affinity IgE Fc receptor, CD23, has multiple functions in enhancing the regulation of IgE production itself, and that of various pro-inflammatory activities and mediators. The data in this report are derived from an analysis of variation in the CD23 gene that leads to a coding exchange and to a single nucleotide polymorphism (SNP) associated with the substitution of an arginine residue for a tryptophan residue in the protein structure of CD23. This genetic variation is associated with three findings identified in this report. First, the tryptophan exchange is associated with greater expression of RNA for the interleukin (IL)-4 receptor alpha chain and greater expression of RNA for *egr-1* transcription factor, both of which are proinflammatory gene products that influence allergy-related immune functions. Second, the exchange is associated with cell surface expression of IL-4R. Third, an analysis of potential arginine-to-tryptophan exchanges in the entire human genome has identified a number of interesting exchanges in immunologic genes of interest for their role in allergic responses. A discussion of these three findings is presented.

Immunoglobulin E (IgE) plays a critical role in the allergic inflammatory process in diseases such as allergic rhinitis and asthma. Cross-linking by multivalent allergens of IgE bound to its receptor on cells initiates a chain of events resulting in allergic immune responses. Mast cells and basophils are involved in the early, immediate response, which is marked by cellular deregulation and the release of pro-inflammatory mediators, including histamine. Antigen-presenting cells

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(APC) are also activated by allergen-loaded IgE, resulting in immunomodulation of T-cell responses.

IgE binds to two types of receptor: a high-affinity (Fc ϵ R1) receptor, expressed on mast cells, basophils, and APC, and a low-affinity (Fc ϵ R2 or CD23) receptor, expressed on airway smooth-muscle cells and several types of hematopoietic cells, including monocytes, macrophages, dendritic cells (DC), lymphocytes, and eosinophils.

Genetic polymorphisms of *fc ϵ r2*, the gene that encodes the low-affinity IgE receptor (Fc ϵ R2 CD23), have recently been linked to the severity of asthma and to a lack of success in its treatment. We found that a single nucleotide polymorphism (SNP) in exon 4 of *fc ϵ r2*, encoding a non-synonymous amino-acid change (R62W), was associated with severe exacerbations of asthma in asthmatic children. Stable human B-cell transfectants expressing either wild-type (WT) CD23b or the genetic variant CD23bR62W were established. When these transfectants were stimulated through CD23, those expressing the genetic variant CD23bR62W (compared with WT CD23b) exhibited higher levels of RNA expression of the RNAs for *egr-1* and interleukin-4 receptor α chain (IL-4R α). Flow-cytometric analyses confirmed the increase in cell-surface expression of the IL-4R α chain after anti-CD23 treatment of CD23bR62W-expressing transfectants. Our data suggest that the SNP R62W within CD23 that appears to be associated with severity of asthma symptoms in individuals having this genotype may favor increased IgE production through increased responsiveness to IL-4.

SNPs in *fc ϵ r2* are associated with an increased risk of exacerbations of asthma in asthmatic individuals treated with inhaled corticosteroids (1), but the functional relevance of these SNPs in *fc ϵ r2* remains unknown. One such SNP encodes a non-synonymous amino acid change (R62W). In an epidemiologic study of 42 human subjects, the allelic frequency of this mutation was 15%, suggesting that the SNP was not a rare event (2).

CD23 has been proposed to participate in both a positive and a negative feedback mechanism in the regulation of IgE synthesis by B cells (3, 4). Human B cells undergo isotype class switching and begin to secrete IgE when stimulated with IL-4 plus anti-CD40. In that process, germ-line C transcripts appear before the mature C ϵ transcript is made (5). In contrast, when a monoclonal antibody to CD23 is added at the time human B cells are stimulated with IL-4 plus anti-CD40, germ-line C ϵ RNA expression (6) and total IgE production are diminished (6, 7), suggesting regulation by CD23. However, the specific mechanism by which CD23 might regulate IgE production remains unknown.

Non-synonymous SNPs occur in the coding regions of genes. A mis-sense SNP that results in the substitution of one amino acid, for another in the protein encoded by the gene and is therefore likely to change the structure and function of the protein.

The R62W CD23 SNP is located in exon 4 of the *fcεr2* gene (2). CD23 is a type-II integral membrane protein with a single transmembrane region and its carboxy-terminal region is therefore extracellular, while its amino-terminal domain is intra-cytoplasmic (8). The R62W SNP is in the extracellular portion of the protein, close to the transmembrane domain. It was suggested that variations in the CD23 molecule resulting from an SNP could adversely affect the role of CD23 in the control of IgE synthesis (9), possibly by influencing the tertiary and quaternary structure of the molecule.

In the present study we show that the CD23 R62W SNP is functionally relevant and leads to the up-regulation of IL-4 receptor expression on human B cells, and that SNPs involving substitutions of thymine for cytosine (C→T) can cause amino-acid exchanges of R-W in the coding regions of many other genes.

RESULTS AND DISCUSSION

Gene expression by PCR array. DG75 cells derived from a Burkitt's lymphoma and which do not express any Fc receptors, including CD23 (10) were transfected by electroporation with a lentivirus-expressing plasmid containing either full-length CD23b or the genetic variant CD23bR62W, and the level of CD23 expression was monitored through flow cytometry. Both of the transfected cell lines expressed comparable levels (> 94%) of CD23 (Figure 1). Authenticity of the CD23b and CD23bR62W was confirmed by DNA sequencing (data not shown).

Because protein produced by the R62W SNP was located extracellularly and close to the transmembrane domain, we sought to determine whether a difference in gene expression in the two transfectants that was generated might occur after stimulation through CD23. We extracted cytoplasmic RNA and prepared cDNA from RT-PCR products. Figure 1 shows that the genes for *egr-1* and IL-4R α (CD124) were differentially expressed in the CD23b and CD23bR62W transfectants. After anti-CD23 treatment, the CD23bR62W transfectant expressed a 16-fold and 14-fold increase in *egr-1* and IL-4R, RNA expression, respectively, as compared to the non-stimulated CD23b-expressing transfectants. Of interest also was that the non-stimulated and isotype control-treated CD23bR62W transfectants had constitutively higher

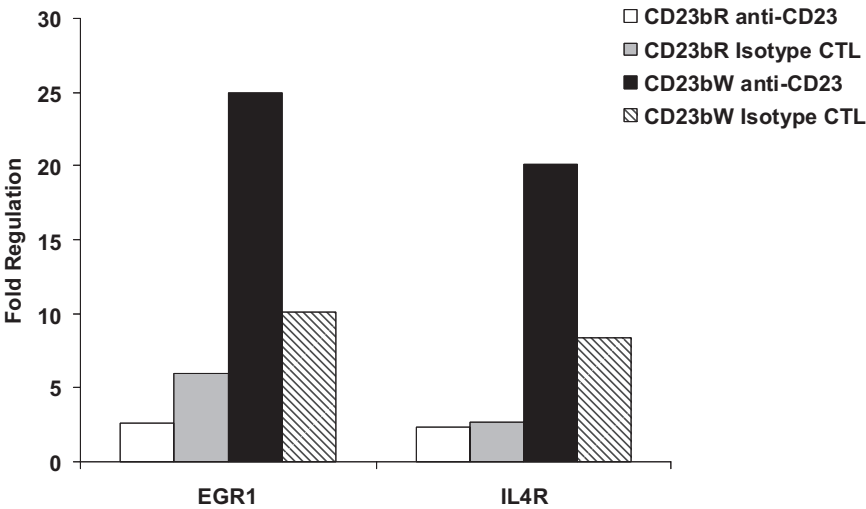


FIG. 1. Differential expression of *egr-1* and IL-4R α . CD23b ($\square$) and CD23bR62W ($\blacksquare$) transfectants were not stimulated or treated with anti-CD23 or isotype control. cDNA was prepared from these cells after 6 hours and subjected to quantitative RT-PCR array analysis. A change of more than 20-fold in the *egr-1* RNA level and IL-4R α RNA level is seen. Results were normalized relative to non-stimulated CD23b transfectants

levels of RNA expression for IL-4R than did the non-stimulated CD23b cells (Figure 1).

Surface expression of IL-4R. Having observed a difference in IL-4R RNA expression by the two types of transfected DG75 cells in our study, we wanted to confirm whether this difference in expression was reflected at the protein level. For this, the transfectants were left unstimulated or treated with anti-CD23 or isotype control. After 48 hours the cells were stained with anti-IL-4R (CD124) antibody IL-4R α and examined flow cytometrically. In a representative experiment, a modest increase in IL-4R α expression was observed in the CD23bR62W transfectants after treatment with anti-CD23, in contrast to a lack of such expression in the CD23b WT transfectants treated in a similar manner (Figure 2).

We conducted a whole-genome evaluation of C $\rightarrow$ T exchanges that could potentially result in a coding SNP. There are approximately 5,000 SNP exchanges of C $\rightarrow$ T that could potentially code for an amino acid exchange of R $\rightarrow$ W (arginine $\rightarrow$ tryptophan) according to a bipad algorithm for potentially identifying SNP changes in coding regions within the genome. Table 1 analyzes known genes on chromosome 22 with an coding regions R $\rightarrow$ W exchange in their that could have an effect on asthma immunology. These SNP-induced R $\rightarrow$ W exchanges,

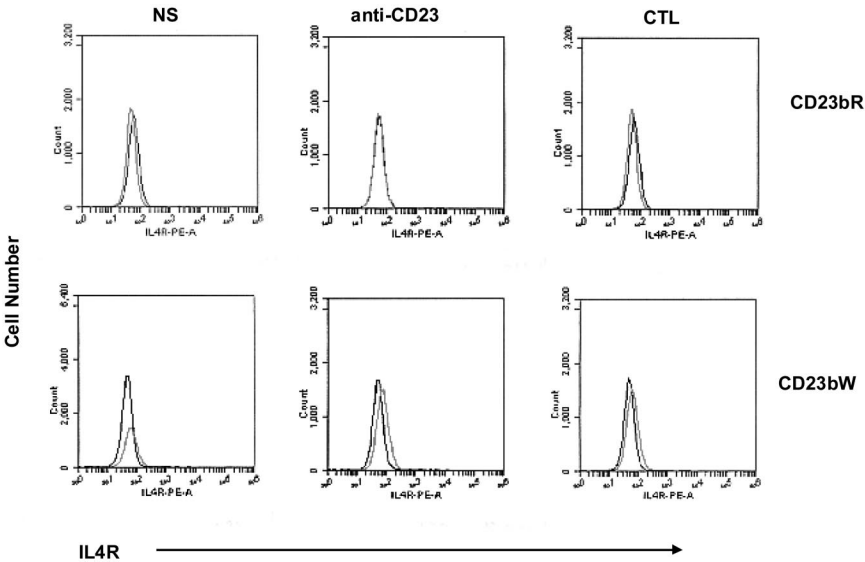


FIG. 2. IL-4R α surface expression. CD23b and CD23bR62W transfectants were left unstimulated (NS) or were treated with anti-CD23 ($\square$ CD23) antibody or isotype control (Iso) for 48 hours. Cells were analyzed with flow cytometry after staining with either anti-IL-4R α (CD124) (solid line) or isotype control (stippled line) directly conjugated to phycoerythrin. Depicts mean fluorescence intensity (MFI) for CD23b ($\square$) and CD23bR62W ($\blacksquare$).

which could affect the biology of the resulting proteins, were noted in the genes for the IL-17 receptor precursor, the adenosine A₂ receptor, and a number of immunologically relevant genes have this R $\rightarrow$ W exchange, which could be associated with a change in protein function consistent with what has been seen with the CD23 gene. Examination of a potential association of each of these variants with asthma and allergic disease is underway.

TABLE 1
Coding SNPs with R $\rightarrow$ W Potential Exchanges on Chromosome 22 for Immune Genes

IL-17r (NM_014339)	Interleukin-17 receptor precursor
GGTL4 (NM_199127)	Gamma-glutamyltransferase-like 4, isoform 1
BCR (NM_004327)	Breakpoint cluster region isoform 1
ADORA2A (NM_000675)	Adenosine-A2a receptor
VPREB1 (NM_007128)	Immunoglobulin iota-chain pre-proprotein
P2RXL1 (NM_005446)	Purinergic receptor P2X-like 1, orphan receptor
COMT (NM_000754)	Catechol-O-methyltransferase isoform MB-COMT
DGCR6(NM_005675)	DiGeorge syndrome critical region protein 6
BCL2L13 (NM_015367)	BCL2-like 13 (apoptosis facilitator)
GGT2 (NM_002058)	Gamma-glutamyltranferase 2

CONCLUSION

SNPs have been identified in *fce2*, encoding CD23. The genetic variants are associated with increased number of severe exacerbations of asthma among asthmatic individuals and with increased serum IgE levels (1). We established stable human B-cell transfectants expressing the R62W SNP located in exon 4 of the *fcer2* gene. Our data demonstrated an increase in IL-4R type I expression associated with the genetic variant CD23bR62W as compared with its expression by WT CD23b transfectants with stimulation through CD23 (12, 13, 14). This would suggest that the R62W SNP results in a possible change in the role of IgE regulation by CD23. Thus, a predisposition to allergic asthma may occur in patients having this genotype, through its favoring increased IgE production via increased responsiveness to IL-4. The link between increased *egr-1* and IL-4R α gene expression observed in the genetic variant remains unknown. An Egr-1 binding site has not been described in the promoter region of the human gene for IL-4R α .

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DISCUSSION

Sacher, Cincinnati: During the treatment of hairy-cell leukemia, CD23 is released, especially when interferon is used. Have you looked at any of the phenotypic variants in that disease?

Rosenwasser, Kansas City: No. We haven't looked at hairy-cell leukemia. Let me just say that when lumiliximab was dropped as a potential intervention, for a variety of business reasons related to the merger of Biogen and Idec and some Japanese partners that they had, we did a pretty extensive but somewhat limited, in terms of population number, steroid reduction trial in asthma with lumiliximab. We found that at the lower doses, there was a clear trend toward a treatment effect that was positive, but that at the higher doses of the antibody that effect went away; and that made them a little bit nervous about contributing or starting a very large campaign. I was also told that lumiliximab was being held in reserve for those various conditions in which resistance to Rituxan might occur. Thus, many of the cells that are targeted for CD20 also express CD23. People have reported that in asthma, circulating soluble CD23 does correlate with asthma exacerbation and treatment with steroids.