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# 129/SvJ mice have mutated CD23 and hyper IgE

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ABSTRACT

CD23, the low affinity IgE receptor, is hypothesized to function as a negative regulator of IgE production. Upon discovering reduced CD23 surface levels in 129/SvJ inbred mice, we sought to further investigate 129/SvJ CD23 and to examine its influence on IgE levels. Five amino acid substitutions were found in 129/SvJ CD23. Identical mutations were also observed in CD23 from New Zealand Black and 129P1/ReJ mice. 129/SvJ B cells proliferated more rapidly than those from BALB/c after stimulation with IL-4 and CD40 ligand trimer. However, *in vitro* IgE levels in supernatants from stimulated 129/SvJ B cells were significantly reduced. Contrary to the *in vitro* findings, the 129/SvJ CD23 mutations correlated with a hyper IgE phenotype *in vivo* and 129/SvJ were able to clear *Nippostrongylus brasiliensis* infection more rapidly than either BALB/c or C57BL/6. Overall, this study further suggests that CD23 is an important regulatory factor for IgE production.



## 1. Introduction

The low affinity IgE receptor, CD23 (FcεRII), is a type II transmembrane protein and a member of the calcium-dependent (C-type) lectin family [1]. In the mouse system, CD23 is expressed on the B lymphocyte, the follicular dendritic cell and the gut epithelium [2] whereas in the human system, CD23 can be found on B cells, monocytes, eosinophils, and Langerhans cells. CD23 consists of four domains, including a carboxy-terminal lectin head, a stalk region, a transmembrane domain, and a short, cytoplasmic tail. Based on molecular modeling studies, Gould and colleagues proposed a model whereby CD23 oligomerizes, with two of the lectin heads of the trimer binding to one IgE molecule, resulting in a high avidity interaction of CD23 with IgE [3]. CD23 contains two cleavage sites within its stalk domain and is cleaved from the cell surface by the metalloprotease ADAM10 [4], resulting in soluble CD23 (sCD23)[2] fragments [5,6]. sCD23 consists primarily of the lectin domain, with varying amounts of stalk, and is thus capable of binding to IgE, albeit with a lower avidity than that of oligomeric CD23 [6,7].

Since its discovery, CD23 has been proposed to play a role in regulating IgE levels. The finding that IL-4 increased both the expression of IgE as well as that of CD23 led to the hypothesis that CD23 could serve as a natural means to dampen IgE responses. Recently, CD23's role as a negative regulator of IgE production *in vivo* has been demonstrated through studies utilizing CD23 transgenic and CD23 knockout mice as well as antibodies targeting CD23. CD23 deficient mice, on a C57BL/6 background, produce higher amounts of IgE than littermate controls after stimulation with Ag–alum [8] while mice overexpressing CD23, regardless of background strain make significantly less IgE in response to the same stimuli [9,10]. Injection of mice with a polyclonal rabbit anti-stalk CD23 results in increased serum IgE levels [11]. We have recently shown that destabilization of CD23 via injection of a rat anti-stalk CD23 (19G5) enhanced the cleavage of CD23 from the cell surface and increased serum IgE levels [12]. Collectively, these data demonstrate that CD23 is an important control factor for IgE production *in vivo*.

129/SvJ mice are frequently used for gene targeting studies due to the availability of several embryonic stem cell lines that have been derived from them (reviewed in [13]). Over the past 10 years, several immunological defects have been uncovered in the 129 strains, thus questioning their usefulness in gene targeting studies without sufficient backcrossing. Corcoran and Metcalf [14] observed that B cells from 129/Sv and 129/Ola mice were defective in signaling downstream of the IL-5 receptor, which could be explained at least in part by reduced expression of the IL-5R alpha chain. McVicar et al. [15] found that natural killer cells were defective in their ability to signal through the receptor DAP12. Recently, Kaminski and Stavnezer [16] showed that 129/Sv spleens had more marginal zone B cells compared with those from C57BL/6 mice. In addition, they observed that 129/Sv B cells were defective in their class switching to IgG3 *in vitro*. 129/Sv mice have also recently been reported to have defects in their mast cell signaling downstream of the FcεRI. Yamashita et al. [17] found that 129/Sv mice

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[2] *Abbreviations used:* sCD23, soluble CD23; CD40LT, CD40 ligand trimer; *Nb, Nippostrongylus brasiliensis*; KLH, keyhole limpet hemocyanin.

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had greater expression levels of FcεRI and that ligation of the FcεRI resulted in increased mast cell degranulation. They also noted that the 129/Sv mice had increased anaphylaxis as compared to C57BL/6 mice.

In this study we demonstrate that CD23 is mutated in 129/SvJ mice. This mutated CD23 is associated with reduced CD23 surface levels and increased serum IgE production in the 129/SvJ strain as compared to the BALB/cJ strain. Overall, the data lend further support to CD23's role as a negative regulator of IgE production and demonstrate that the 129/SvJ strain may be beneficial for the study of allergic diseases.

## 2. Materials and methods

### 2.1. Animals

BALB/c and C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD). BALB/cJ, C57BL/6J, 129/SvJ, 129P1/ReJ, and NZB mice were purchased from the Jackson Laboratory (Bar Harbor, ME). CD23 deficient mice [18] were generated as described and maintained at VCU. All mice used were between 6 and 16 weeks of age at the start of the experiment and were housed in an accredited and pathogen-free animal facility. Female mice were primarily used. All studies were approved by the VCU IACUC.

### 2.2. Preparation and purification of monoclonal antibodies

The monoclonal antibodies 19G5, 2H10, B3B4, B1E3, R1E4, 2.4G2, and mouse IgE anti-DNP were prepared from cell culture supernatants using CL-1000 Adhere CELLine flasks (Integra Biosciences, Switzerland). Cells were grown in complete RPMI (RPMI 1640 supplemented with 10% heat inactivated FBS, 100 U/ml penicillin and streptomycin, 2 mM L-glutamine, 10 mM Hepes, and $5 \times 10^{-5}$ M 2-mercaptoethanol) supplemented with 2 μg/ml gentamicin. Supernatants harvested from CELLine flasks were centrifuged at 2000 RPM for 5 min, filtered through a 0.8 μm filter (PALL Life Sciences, Ann Arbor, MI), and stored at −20 °C until purification. Just prior to purification, cell supernatants were further clarified by centrifugation at 20,000*g* for 30 min. Antibody purification was performed by hydrophobic charge induction chromatography using MEP HyperCel sorbent (PALL Life Sciences, East Hills, NY) [19].

### 2.3. B cell purification and culture

B cells were purified from spleens by negative selection as previously described [20]. Briefly, T cells in single cell suspensions were coated with antibodies and depleted by complement lysis. The remaining spleen cells were layered over a discontinuous Percoll (GE Healthcare) gradient [21]. Resting B cells selected from the 66% to 70% interface were used for *in vitro* proliferation studies and Ig analyses. For some experiments (*i.e.* RNA isolation and Scatchard analysis), total B cells (50–70% Percoll interface) were used. B cells were cultured in B cell media consisting of RPMI 1640 supplemented with 10% heat inactivated FBS, 100 U/ml penicillin and streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM Hepes, 100 mM non-essential amino acids and $5 \times 10^{-5}$ M 2-mercaptoethanol.

To examine IgE, IgG1, and IgM production, resting B cells were cultured in 96-well plates (Corning Life Sciences, Lowell, MA) at varying concentrations (50,000–250 cells/well) in 200 μl B cell media containing 10,000 U/ml recombinant mouse IL-4 (kindly provided by William Paul), 5 ng/ml mouse IL-5 (eBioscience, San Diego, CA), 25 ng/ml mouse CD40LT, and 100 ng/ml M15 (anti-leucine zipper). Both CD40LT and M15 [22] were generous gifts from Amgen, Seattle, WA. For IL-4 dose response studies, 5000 cells per well were stimulated as above with IL-5, CD40LT, and M15 in the presence of increasing concentrations of IL-4. Supernatants were harvested after 8 days of culture and analyzed by ELISA.

For proliferation studies, purified resting B cells were cultured as above and were pulsed with 1 μCi per well $^{3}$H-thymidine (MP Biomedicals, Irvine, CA) during the last 8 h of a 72 h incubation. Plates were harvested using a Filtermate 196 harvester onto a Unifilter 96-well plate. Thirty microliters of Microscint-20 scintillation fluid was added to each well and plates were counted using a Top Count Scintillation Microplate Reader. Harvester, scintillation fluid, and counter were all from Perkin-Elmer (Boston, MA). Alternatively, purified B cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) as described [23]. Labeled cells were cultured as above and on indicated days, the amount of CFSE labeling remaining was determined by FACS analysis.

For stimulation of B cells for RNA isolation, flow cytometry, and Scatchard analyses, purified total B cells were incubated in 24-well plates (Greiner Bio-One North America, Monroe, NC) containing 1 ml of B cell media supplemented with 10,000 U/ml recombinant mouse IL-4, 25 ng/ml mouse CD40LT, and 100 ng/ml M15. Cells were harvested at either 24 or 48 h depending on the assay.

### 2.4. RNA isolation and analysis

Total RNA was isolated from unstimulated total B cells or IL-4 and CD40LT activated total B cells using Trizol reagent as recommended by the manufacturer (Invitrogen). For quantitative PCR analysis (qPCR), the use of the TaqMan® One Step PCR Master Mix Reagent Kit was employed. All the samples were tested in triplicate under the conditions recommended by the fabricant. The cycling conditions were as follows 48 °C/30 min, 95 °C/10 min, 40 cycles of 95 °C/15 s, and 60 °C/1 min. The cycle threshold was determined to provide the optimal standard curve values. The experiments were performed in the ABI Prism® 7900 Sequence Detection System (Applied Biosystems, Foster City, Ca). The probes and primers were designed using the Primer Express® 2.0 version. The probes were labeled in the 5′ end with FAM (6-carboxyfluoresceine) and in the 3′ end with TAMRA (6-carboxytetramethylrhodamine). Ribosomal RNA (18 S rRNA) from the Pre-developed TaqMan®Assay Reagents was used as endogenous control. The reactions and the synthesis of the probes and primers were performed in the VCU Nucleic Acid Research Facilities.

For RT-PCR analysis, reactions were performed using the EPICENTRE MasterAmp High Fidelity RT-PCR Kit (EPICENTRE, Madison, WI) according to the manufacturer's protocol. Briefly, total RNA (1000 ng/reaction) was mixed with mouse CD23 specific primers (12.5 pmoles/reaction) and sterile nuclease-free water in a reaction mix containing 1× MasterAmp RT-PCR PreMix, 40 Units MMLV-RT (Moloney Murine Leukemia Virus Reverse Transcriptase) Plus, and 1 Unit MasterAmp TAQurate DNA Polymerase Mix to a final volume of 50 μl. The forward (CTGCCATGGAAGAAAATG) and reverse (TGAGCAGAAGTTTGTCAGG) primers were designed to amplify the entire coding sequence of CD23. Reactions were also set up using primers specific for mouse actin [24] as an internal control. The following cycle was used: 65 °C for 5 min, 37 °C for 30 min, 95 °C for 1 min, 95 °C for 30 s, 45 °C for 30 s, 70 °C for 1 min 10 s, 72 °C for 7 min.

### 2.5. Cloning and sequence analysis

Purified total B cells isolated from the spleens of 129/SvJ, 129P1/ReJ, and NZB mice were stimulated for 48 h at $1 \times 10^{6}$ cells per well in 1 ml B cell media containing IL-4, CD40LT, and M15. RNA was isolated and RT-PCR was carried out as described above using murine CD23 sense and murine CD23 antisense primers. The 1.1 Kb PCR