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In Silico Comparative Expression Analyses of Pattern Recognition Receptors in Human Eosinophil Cell Lines and Primary Eosinophils

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Abstract

Objective: Eosinophils are one of the least abundant leukocytes in blood circulation; however, they compensate for this sparsity by highly potent content of their granules. Their involvement in numerous pathological conditions including acute and chronic infections make them an interesting research area for the field of immunology. Eosinophils play critical roles in the maintenance of immune homeostasis through their effector and modulatory functions in shaping innate and adaptive responses. Although they are mainly known for their roles in parasitic infections, it has become increasingly clear that eosinophils function not only in fungal, bacterial and viral infections, but also in tissue repair and signaling pathways regulating mast cells, Th2 and B cells. Of all the mediators of innate immunity, pattern recognition receptors (PRRs) are of great importance in the context of the stimuli. Therefore, we analyzed and cross-checked mRNA expression profiles of membrane-bound and cytosolic PRRs by comparing EoL-1 and HL-60 human eosinophil like cell lines to human primary eosinophils *in silico*.

Materials and Methods: Utilizing publicly available databases, we analyzed PRR repertoires of eosinophils to determine the most ideal cell line models for *in vitro* mechanistic studies, requiring high protein and mRNA yields.

Results: Our findings revealed that toll-like receptors 2 and NOD-like receptor 3 (NLRP3) had higher basal expressions in both cell lines than human primary eosinophils as opposed to NLRP12, laboratory of genetics and physiology 2 (LGP2), Dectin-1, whose expressions were higher in primary eosinophils than in both cell lines.

Conclusion: These data might attribute new physiological functions to these receptors of NLR, RIG-I like receptor and C-type lectin receptor families in eosinophil immunity.

Keywords: Human primary eosinophils, EoL-1, HL-60, pattern recognition receptors, *in silico* analysis, expression profiling

Introduction

The complex functions of eosinophils have made them intriguing and challenging cells to work with; however, many of their roles were elucidated by the research over the past 30 years. Findings from numerous studies have established eosinophils' immunomodulatory and homeostatic activities in host defenses and immunity (1,2). The distinct eosinophilic granules in the cytoplasm are the characteristic features of eosinophils, which are formed at various phases of eosinophil maturation (3). Charcot-Leyden crystal protein (CLC), eosinophil peroxidase (EPO), major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin are the cationic proteins found in eosinophil specific granules, in addition to a large number of pre-formed cytokines and chemokines (4,5). A variety of ligand receptors expressed by eosinophils are involved in cell proliferation, adhesion, chemotaxis, degranulation, and cell-to-cell communication. They participate in complement activation through both conventional and non-conventional routes. By interacting with B-cells, eosinophils can process antigens, stimulate T-cells, and promote humoral responses. Indeed, eosinophils can also

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serve as antigen-presenting cells and control the immune system T-helper 1 (Th1) and Th2 processes (6). The immunological responses are tightly regulated to maintain homeostasis by eosinophils (7). These cells play essential roles in conditions like asthma, chronic rhinosinusitis with nasal polyps, eosinophilic gastrointestinal problems, and hypereosinophilic syndromes (HES) (7). They help fight against parasites, bacterial and viral infections, as well as some malignancies (7). The involvement of eosinophils in many vital processes highlights the need for experimental and clinical research on eosinophilic diseases to elucidate the underlying mechanisms and to improve the success of biological therapies in slowing disease progression (7). Since mature eosinophils have low numbers in circulating blood (8), it is difficult to work with primary eosinophils (PE) due to lack of standardized experimental methods. Thus, a need for the most suitable cell line has emerged in order to perform experimental and clinical studies on eosinophils as well as novel target molecules to develop alternative strategies to treat eosinophil associated diseases (EADs).

The immune cells have been reported to depend on germ-line-encoded pattern recognition receptors (PRR) to trigger an inflammatory response and activate tissue repair mechanisms during infections (9). PRRs recognize the pathogen-associated molecular patterns (PAMPs), which are common structural motifs shared by pathogens, and the damage-associated molecular patterns (DAMPs), which identify cellular stress and death (9). The PRR families include membrane-bound toll-like receptors (TLRs) and C-type lectin receptors (CLRs); and cytosolic nucleotide-binding oligomerization domain (NOD) -like receptors (NLRs), retinoic acid inducible gene-I-like receptors (RLRs) (10). Another example for PRR is the receptor for advanced glycation end products, which is found either as a membrane-bound or soluble protein (11). Although the host depends on PRRs' ability to develop an immune response, this feature can also lead to unintended cellular responses. Indeed, our knowledge of how PRRs trigger these reactions has greatly increased for the past few decades. Several studies have reported the expression and importance of TLRs, NLRs, RLRs, and CLRs in eosinophils (10,12-14), suggesting that these receptors may be responsible for the development of EADs including eosinophilic asthma, chronic rhinosinusitis, idiopathic eosinophilia, eosinophilic leukemia, helminth infections and rare conditions such as HES and eosinophilic gastrointestinal disorders (EGIDs) (15). Thus, we analyzed the PRR expression profiles of EoL-1 and HL-60 human eosinophil like cell lines and compared them to PE *in silico*. Compilation and evaluation of such data could serve as a great resource to determine the most ideal *in vitro* cell line model for mechanistic studies that require high concentrations of cellular yield including protein and mRNA and therefore lay the groundwork for following *in vivo* studies of human eosinophils.

The present data have suggested the optimal eosinophil cell line before switching to human PE or *in vivo* animal models that could be coupled with *in vitro* studies and may be utilized as a guide to choose the ideal cells depending on the types of stimuli. Collectively, *in silico* analysis of eosinophil PRRs might provide insight on the alternative target molecules to better understand EADs as well as the receptor molecules that directly or indirectly affect eosinophil functioning.

Materials and Methods

Cell Lines

Eosinophilic leukemia cell lines including EoL-1, EoL-3, HL-60 and AML-14 are often used in *in vitro* models for characterizing eosinophilic functions and understanding the regulation of eosinophils during infections and also allergic inflammation (17,18). EoL-1 and EoL-3 were derived from a 33-year-old male patient's peripheral blood, who suffered from Philadelphia chromosome-negative eosinophilic leukemia. HL-60 cells were isolated from an adult female patient with acute promyelocytic leukemia, which was used to establish eosinophilic cell cultures by butyric acid stimulation at pH 7.6 (16) and AML14 human myeloid leukemia cell line was isolated from a 68-year-old man diagnosed with FAB M2 acute myeloid leukemia. EoL-1 cells can differentiate into mature eosinophilic-like cells after stimulation with agents such as n-butyrate, dibutyryl cyclic adenosine monophosphate (dbcAMP) and phorbol 12- myristate 13-acetate. N-butyrate has been reported to induce the expression of markers for mature eosinophils and reduce the proliferation of EoL-1 cells through hyperacetylation of histones and altered gene transcription leading to differentiation (19,20). Culturing the blast-like AML14 cells for a long time in a cocktail of cytokines, including Granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 3 (IL-3), and IL-5, showed phenotypic characteristics and advanced eosinophilic differentiation that appeared after a few weeks (21). Also, eosinophil-induced AML14 cells were shown to be expressing the mRNAs and proteins for all of the major eosinophil granule proteins (22). A subclone of the AML14 cell line, AML14.3D10, kept an advanced eosinophilic phenotype and proliferated vigorously (23). Given that the data on the EoL-3 and AML14 cell lines and their derivatives' expression patterns were incomplete, we chose male patient isolated EoL-1 and female patient isolated HL-60 eosinophil like cell lines to compare their characteristics with human PE in the context of PRRs, considering two separate databases.

Receptor mRNA Data Mining

Primary human eosinophils express several PRRs including TLRs, NLRs, RLRs and CLRs, all of which show immunomodulatory roles. The stimulation of these PRRs was reported to be associated with survival, oxidative burst, adhesion system activation, and release of cytokines, chemokines and cytotoxic granule proteins. The presence of a wide range of PRRs in eosinophils indicated that they were involved in defenses not only against parasitic helminths but also against bacteria, viruses and fungi (10). Therefore, we focused on the data mining for TLRs, NLRs, RLRs and CLRs in two cell lines.

Data on the human membrane bound TLRs and CLRs and intracellular NOD-like receptors and retinoic acid inducible gene families were collected from two separate open-source databases: 1) (Genevestigator 9.10.0 software) (release number: 2023-06-16), which contains Affymetrix Human Genome U133 Plus 2.0 array and 2) web based open-source Human Protein Atlas database of which the immune cell section contains single cell information on genome-wide RNA expression profiles of human protein-coding genes covering eosinophil cells.

Statistical Analysis

The expressions of PRR genes at mRNA level were presented as mean values of absolute expression levels at log2 scale (MV) for cells. Fold changes were calculated at log2 scale based on basal expression patterns of PRRs in cells by the formula of log2 (MVEoL-1/MVPrimary) for EoL- 1 and log2 (MVHL-60/MVPrimary) for HL-60 cells. In the second database, the expression levels of PRRs at RNA level were presented as transcription per million (nTPM), which were retrieved from the Human Protein Atlas. log2 scale fold changes based the on basal expression profiles of PRRs in PE were calculated by the formula of log2 (nTPMEoL-1/nTPMPrimary) for EoL-1 and log2 (nTPMHL-60/nTPMPrimary) for HL-60. Statistical analyses were performed using an unpaired Student's two-tailed t-test.

Results

EoL-1 and HL-60 Cell Lines were Chosen to Compare with Human Primary Eosinophils

All the data for mRNA expressions of PRRs in EoL-1, HL-60 and human PE are depicted in Tables 1 and 2. Here, we presented the basal expression profiles of eosinophilic PRRs in eosinophilic cell lines including EoL-1 and HL-60 and compared their expression levels with those of human PE using Genevestigator and Human Protein Atlas databases. EoL-1, HL-60 cell lines and human PE had basal PRR expressions at mRNA levels in the absence of stimuli (Table 1). Taken together, due to their rapid responsiveness to a broad range of stimuli and ability to express human eosinophilic pan markers, we further investigated their similarities to human primary cells to determine the ideal model for human eosinophil studies and the receptors that might have been overlooked in the immunobiology of eosinophils (Table 3).

Table 1. PRR mRNA profiles of eosinophilic cell lines and primary eosinophils retrieved from genevestigator

TLRs	Biotype	EoL-1 (mean value)	HL-60 (mean value)	Primary eosinophils (mean value)
TLR1	Protein coding	12.23	9.11	10.66
TLR2	Protein coding	15.19	11.86	11.61
TLR3	Protein coding	7.62	7.63	8.45
TLR4	Protein coding	11.07	10.61	10.82
TLR5	Protein coding	8.53	9.11	10.92
TLR6	Protein coding	10.23	9.27	9.66
TLR7	Protein coding	8.46	7.59	8.94
TLR8	Protein coding	9.72	9.01	9.33
TLR9	Protein coding	9.56	10.38	10.43
TLR10	Protein coding	8.64	9.33	10.59
NLRs				
NOD1	Protein coding	12.66	11.91	12.69
NOD2	Protein coding	12.80	8.78	11.54
NLRC3	Protein coding	8.83	9.82	9.63
NLRC4	Protein coding	10.14	9.11	9.75
NLRC5	Protein coding	12.20	13.34	14.74
NLRP1	Protein coding	10.64	9.35	11.68
NLRP2	Protein coding	9.35	8.87	9.04
NLRP3	Protein coding	12.55	10.88	9.90
NLRP6	Protein coding	8.34	8.44	8.89
NLRP11	Protein coding	8.46	8.36	9.22
NLRP12	Protein coding	10.40	10.46	13.31
NLRX1	Protein coding	12.28	11.87	13.22
RLRs				
RIG-I	Protein coding	11.83	11.15	13.67
MDA-5 (IFIH1)	Protein coding	11.24	11.14	8.48
LGP2 (DHX58)	Protein coding	9.95	9.93	10.89
CLRs				
Dectin-1 (CLEC7A)	Protein coding	10.74	10.02	11.14
MCL (CLEC4E)	Protein coding	14.90	10.38	10.96
MDL-1 (CLEC5A)	Protein coding	9.68	13.00	8.45
MRC1	Protein coding	10.31	8.11	9.11
CLEC4G	Protein coding	8.40	8.12	9.25
CLEC4A	Protein coding	9.87	8.45	9.44
CLEC4M	Protein coding	8.42	8.60	8.70

The mRNA levels are expressed as the mean values (MV) at log2 scale. TLR: Toll-like receptors, NLR: NOD-like receptor, RLR: RIG-I like receptors, CLR: C-type lectin receptors, PRR: Pattern recognition receptor

Table 2. PRR mRNA expression profiles of eosinophilic cell lines and primary eosinophils retrieved from the Human Protein Atlas

	Biotype	EoL-1 (nTPM)	HL-60 (nTPM)	Primary eosinophils (nTPM)
TLRs				
TLR1	Protein coding	16.7	2.1	8.4
TLR2	Protein coding	101.6	13.8	1.8
TLR3	Protein coding	0.0	0.9	0.0
TLR4	Protein coding	6.9	13.1	21.6
TLR5	Protein coding	0.0	0.0	0.4
TLR6	Protein coding	13.3	1.4	0.2
TLR7	Protein coding	0.9	0.0	4.5
TLR8	Protein coding	0.4	0.4	0.0
TLR9	Protein coding	4.6	4.3	0.0
TLR10	Protein coding	0.4	0.1	0.9
NLRs				
NOD1	Protein coding	21.8	7.0	13.5
NOD2	Protein coding	16.7	0.2	5.6
NLRC3	Protein coding	1	6.8	0
NLRC4	Protein coding	4.4	3	5.8
NLRC5	Protein coding	19.3	15.8	13.0
NLRP1	Protein coding	11.7	0.3	7.1
NLRP2	Protein coding	1.2	0.5	0.1
NLRP3	Protein coding	53.6	6.9	0.2
NLRP6	Protein coding	1.0	0.0	0.0
NLRP11	Protein coding	0.2	0.0	0.0
NLRP12	Protein coding	8.2	0.6	23.2
NLRX1	Protein coding	8.2	7.0	15.9
RLRs				
RIG-I	Protein coding	1.2	1.3	0.3
MDA-5 (IFIH1)	Protein coding	5.2	6.0	4.4
LGP2 (DHX58)	Protein coding	6.3	2.7	6.9
CLRs				
Dectin-1 (CLEC7A)	Protein coding	2.1	2.0	2.3
MCL (CLEC4E)	Protein coding	0.2	0.4	0.5
MDL-1	Protein coding	0.5	50.8	0.0
MRC1	Protein coding	0.2	0.0	0.0
CLEC4G	Protein coding	0.3	0.0	0.0
CLEC4A	Protein coding	1.4	2.8	1.1
CLEC4M	Protein coding	1.5	0.0	0.0

All units are expressed as the transcript per million (nTPM). TLR: Toll-like receptors, NLR: NOD-like receptor, RLR: RIG-I like receptors, CLR: C-type lectin receptors, PRR: Pattern recognition receptor

Table 3. P-values, basal mRNA expression Levels of PRR family members in EoL-1, HL-60 and primary eosinophils (PE)

Receptor	EoL vs PE	HL-60 vs PE
TLR2	0.0008	0.6
NLRP3	0.0001	0.01
NLRP12	0.0001	<0.0001
MDA5	0.0004	<0.0001
LPG2	0.004	<0.0001
DECTIN-1	0.59	0.04

PRR: Pattern recognition receptor, TLR: Toll-like receptors, NLR: NOD-like receptor, MDA5: Melanoma differentiation-associated protein 5

TLR2 mRNA Expressions were Consistently Higher in Cell Lines than Human Primary Eosinophils When Two Databases were Compared

In the present study, we investigated the mRNA expression levels by cross-checking two different databases containing the basal expression data for TLR family members, all of which are known to be expressed in EoL-1, HL-60 (Figure 1a, 1b). However, when we compared these expression data with PE, TLR2 had higher levels of transcripts in both cell lines than PE (Figure 1c, 1d) (p=0.008). According to the second database, which reported that these nTPM values for mRNA levels were also evidence for protein levels, TLR2 and TLR6 were the most abundantly expressed TLRs in both cell lines and they were markedly higher than those of PE (Figure 1e-1h). Conversely, referring to the protein levels, TLR4 and TLR10 were lower in both cell lines than PE. Surprisingly, TLR3, TLR8, and TLR9 mRNAs were not expressed in human PE, while TLR5 was missing in both cell lines. Lacking TLR3, TLR8 and TLR9 in PE raises the question of whether PE do not require these receptors' functions or they compensate their absence by expressing other PRRs. Finally, primary eosinophils do not have the basal expression of the whole set of TLRs that are vital against viral infections, except TLR7.

NLRP3 and NLRP12 Had the Highest Levels of mRNA Expression in Cell Lines and in Primary Eosinophils, Respectively

The data from both Genevestigator and Human Protein Atlas databases was collected and compared EoL-1 and HL-60 cells' mRNA expression levels to those of PE by normalizing both MV and nTPM of NLR family members of EoL-1, HL-60 cell lines to human PE (Figure 2). Both databases indicated that NLRP3 had higher mRNA expression in both cell lines as compared to PE (Figure 2c, 2d, 2g, and 2h) (p=0.03). NLRP3, having the highest expression level in both cell lines, was followed by NLRP2, except in HL-60 at mRNA level (Figure 2d). Moreover, NOD2 basal expression levels were consistently in the opposite direction for both EoL-1 and HL-60 when their MVs and nTPMs were normalized to those of PE, which

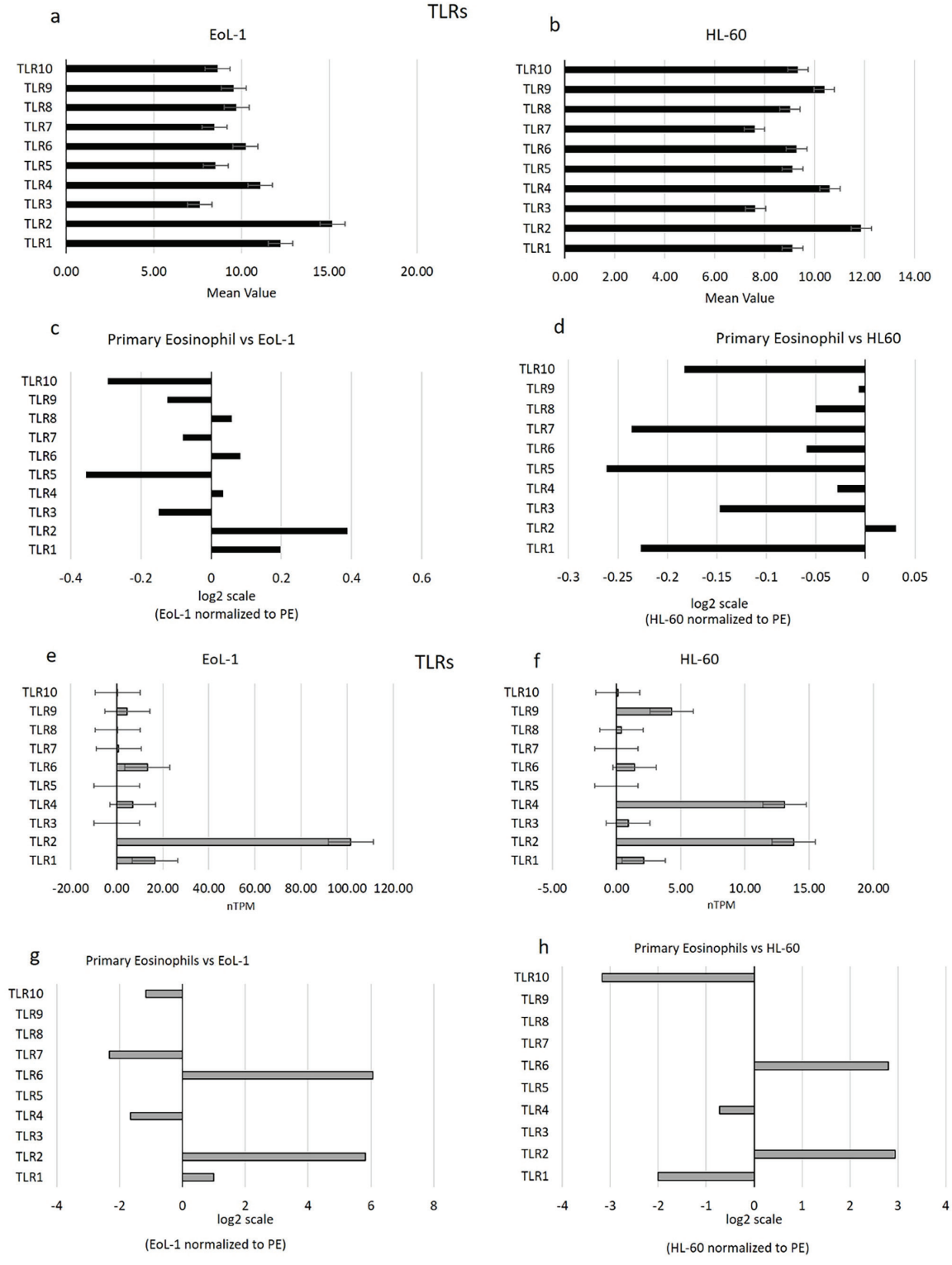

Figure 1. Expression profiles of TLRs in EoL-1 and HL-60 cell lines based on the data received from Genevestigator **(a-d)** and the Human Protein Atlas **(e-h)**. The expressions of TLRs are given at mRNA level for **a)** EoL-1 and **b)** HL-60 as mean value (MV). log2 fold change based on TLRs' expression profile of primary eosinophils was calculated by the formula: **c)** log2 (MVEoL-1/MV Primary) **d)** log2 (MVHL-60/MV Primary). Expression levels of TLRs are given at protein level for **e)** EoL-1 and **f)** HL-60 as nTPM received from the Human Protein Atlas. log2 fold changes based on TLRs' expression profile of primary eosinophils were calculated by the formula: **g)** log2 (nTPMEoL-1/nTPM Primary) **h)** log2 (nTPMHL-60/nTPM Primary). Error bars are given as standard error mean (SEM).
TLR: Toll-like receptors

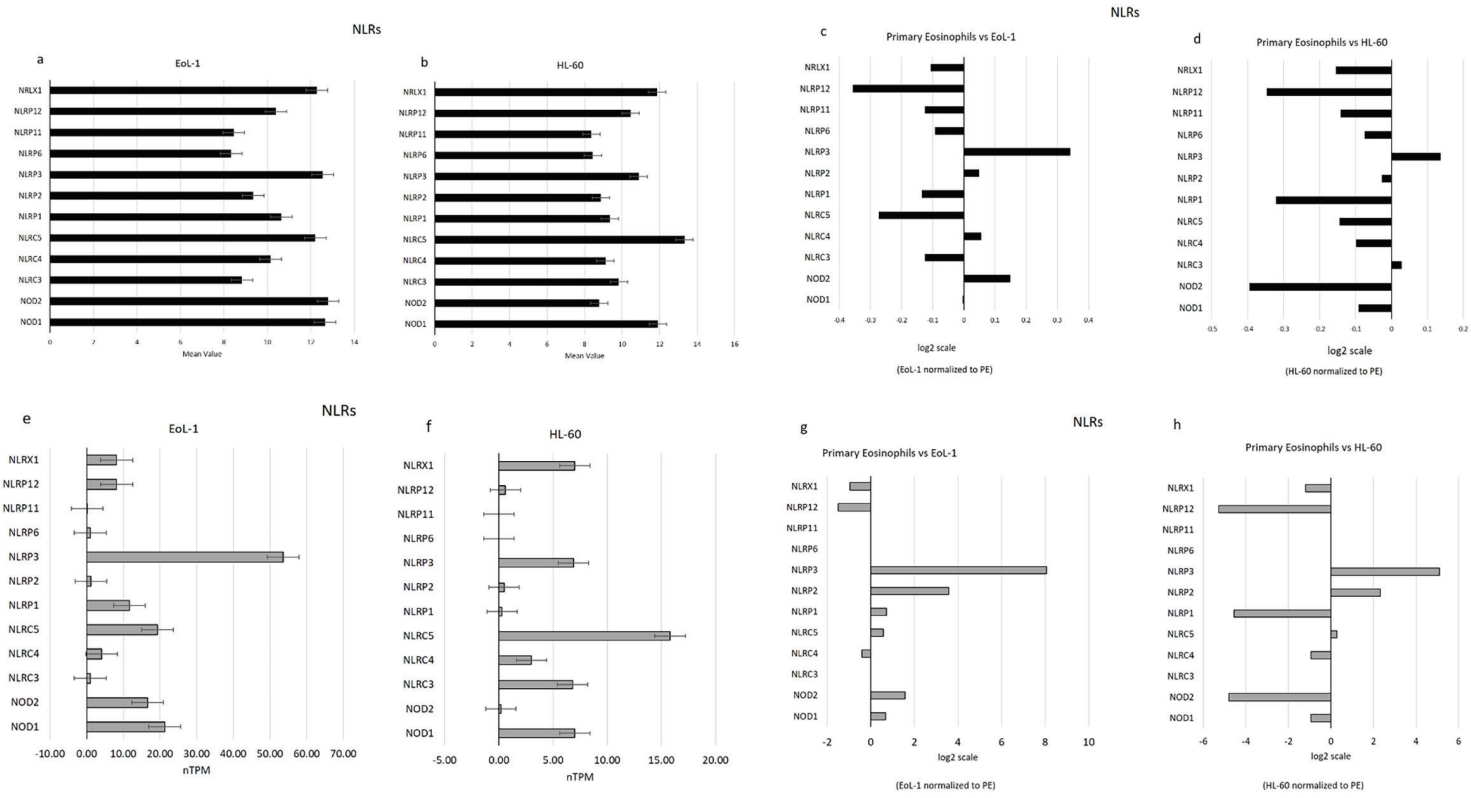

Figure 2. Expression profiles of NLRs in EoL-1 and HL-60 cell lines based on the data received from Genevestigator **(a-d)** and the Human Protein Atlas **(e-h)**. The expressions of NLR proteins are given at mRNA level for **a)** EoL-1 and b) HL-60 as mean value (MV). log2 fold change based on NLRs' expression profile of primary eosinophils was calculated by the formula: **c)** log2 (MVEoL-1/MV Primary) **d)** log2 (MVHL-60/MV Primary). Expression levels of NLRs are given at protein level for **e)** EoL-1 and f) HL-60 as nTPMs received from the Human Protein Atlas. log2 fold change based on NLRs' expression profile of primary eosinophils was calculated by the formula: **g)** log2 (nTPMEoL-1/nTPM Primary) **h)** log2 (nTPMHL-60/nTPM Primary). Error bars are given as standard error mean (SEM).
NLR: NOD-like receptor

makes this gene the most controversial among the remaining members of NLR family (Figure 2c, 2d, 2g and 2h). As the nTPMs were evidence for the protein levels as well, NLRP6 and NLRP11 (Figure 2g-h) were not expressed in neither cell lines nor PE (Table 2). In fact, we showed that EoL-1 cells did not express NLRP11 protein (data not shown), which was in agreement with the *in silico* analyses. Most importantly, NLRP12 had the highest levels of expression in PE based on both databases (p=0.0001).

MDA5 and LGP2 Had the Highest Levels of mRNA Expression in Cell Lines and in Primary Eosinophils, Respectively

Our analysis showed that mRNA levels of RLR family members were expressed at basal levels in all three types of cells included in this study based on both databases (Figure 3). When we compared the mRNA expressions of cell lines to PE, we first determined that Laboratory of Genetics and Physiology 2 (LGP2) had higher expression in primary cells than both cell lines (p=0.004) unlike MDA-5, which had lower expression of mRNA in primary cells than both cell lines (Figure 3c and d) (p=0.0001). Secondly, RIG-1 expression showed variations in its expression profile in cell lines and PE, which requires further experimental validation (Figure 3).

Human Primary Eosinophils Had Higher Dectin-1 Expression Than Both Cell Lines

We investigated the basal mRNA expressions of CLR family members in EoL-1, HL-60, and PE (Figure 4). Of all the CLR molecules included in the comparative analyses, two cell lines differed in basal mRNA expression profile of CLEC4A, which was consistently higher in EoL-1 cells than PE (Figure 4c).

Surprisingly, Dectin-1 had higher mRNA expression in PE than both cell lines across the databases utilized in this study (p=0.04). Collectively, these results may suggest new unexplored roles for Dectin-1 in human eosinophil functions.

Discussion

Eosinophil-related diseases are rather complex because of their heterogenicity (40). Low numbers of eosinophils in blood and limited number of animal models along with

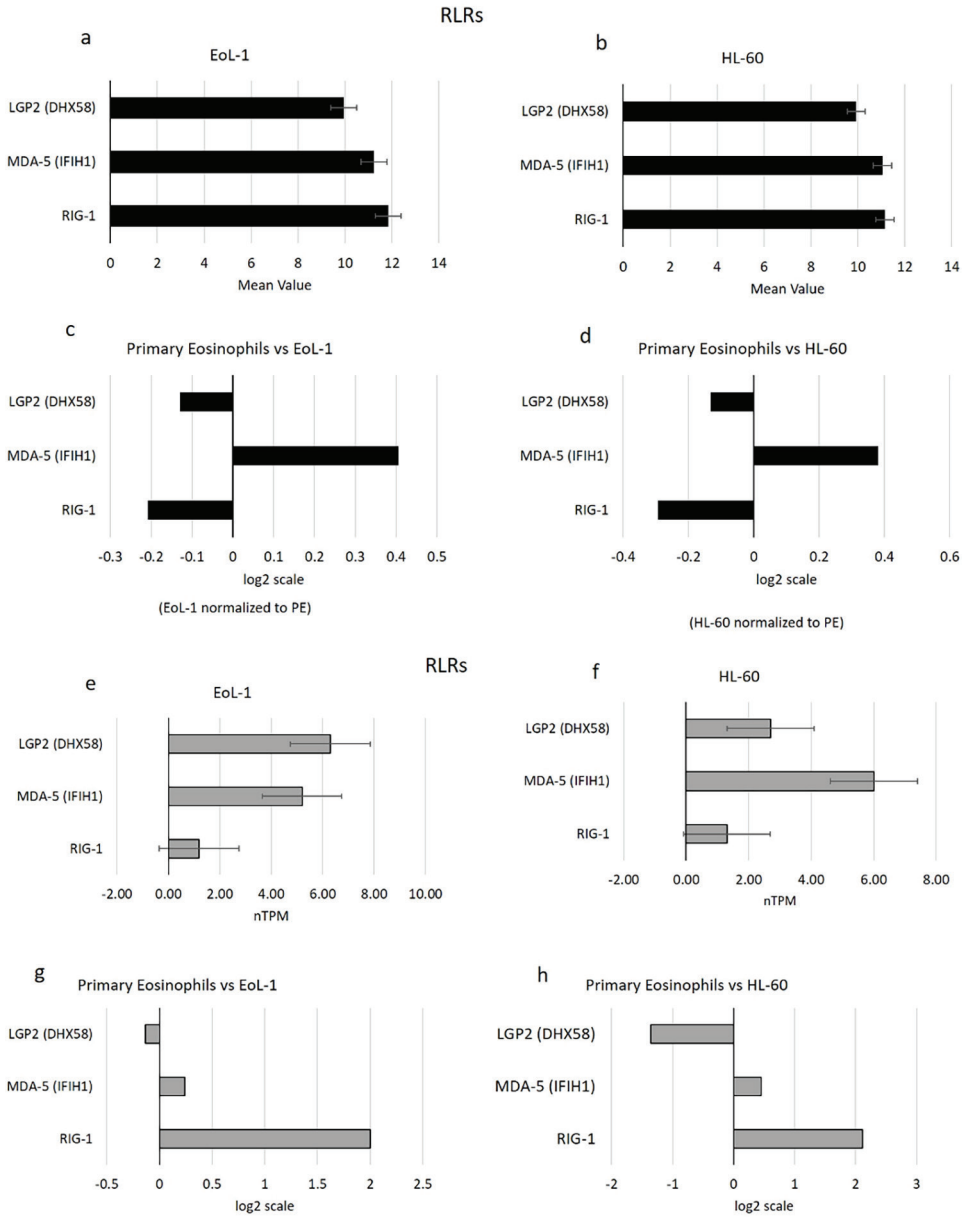

Figure 3. Expression profiles of RLRs in EoL-1 and HL-60 cell lines based on the data received from Genevestigator **(a-d)** and the Human Protein Atlas **(e-h)**. The expressions of RLRs are given at mRNA level for **a)** EoL-1 and **b)** HL-60 as mean value (MV). log2 fold changes based on RLRs'expression profile of primary eosinophils were calculated by the formula: **c)** log2 (MVEoL-1/MV Primary) **d)** log2 (MVHL-60/MV Primary). Expression levels of RLRs are given at protein level for **e)** EoL-1 and f) HL-60 as nTPM received from the Human Protein Atlas. log2 fold changes based on RLRs' expression profile of primary eosinophils were calculated by the formula: **g)** log2 (nTPMEoL-1/nTPM Primary) **h)** log2 (nTPMHL-60/nTPM Primary). Error bars are given as standard error mean (SEM).
RLR: RIG-I like receptors

technical challenges to study eosinophils, and inadequacy of standardized methods in histopathology hamper the progress in basic eosinophil research (40). Eosinophil gene profiling is crucial to better understand their roles in human diseases; therefore, we particularly chose four families of PRRs, including TLRs, NLRs, RLRs and CLRs, to determine the basal expression patterns in human eosinophil cell lines EoL-1 and HL-60 and compared their expression profiles to those of human PE to define the ideal model for *in vitro* studies, especially for optimization and mechanistic studies that necessitated higher concentrations of cellular products such as nucleic acids, proteins and organelles, which were quite challenging to obtain from PE.

TLRs are membrane-bound PRRs, which are responsible for recognizing molecular patterns of microorganisms and have crucial functions in innate and adaptive immunity. The N-terminal domain of the receptor includes leucine-rich repeats which sense PAMPs and DAMPs whereas the C-terminal domain (CTD) consists

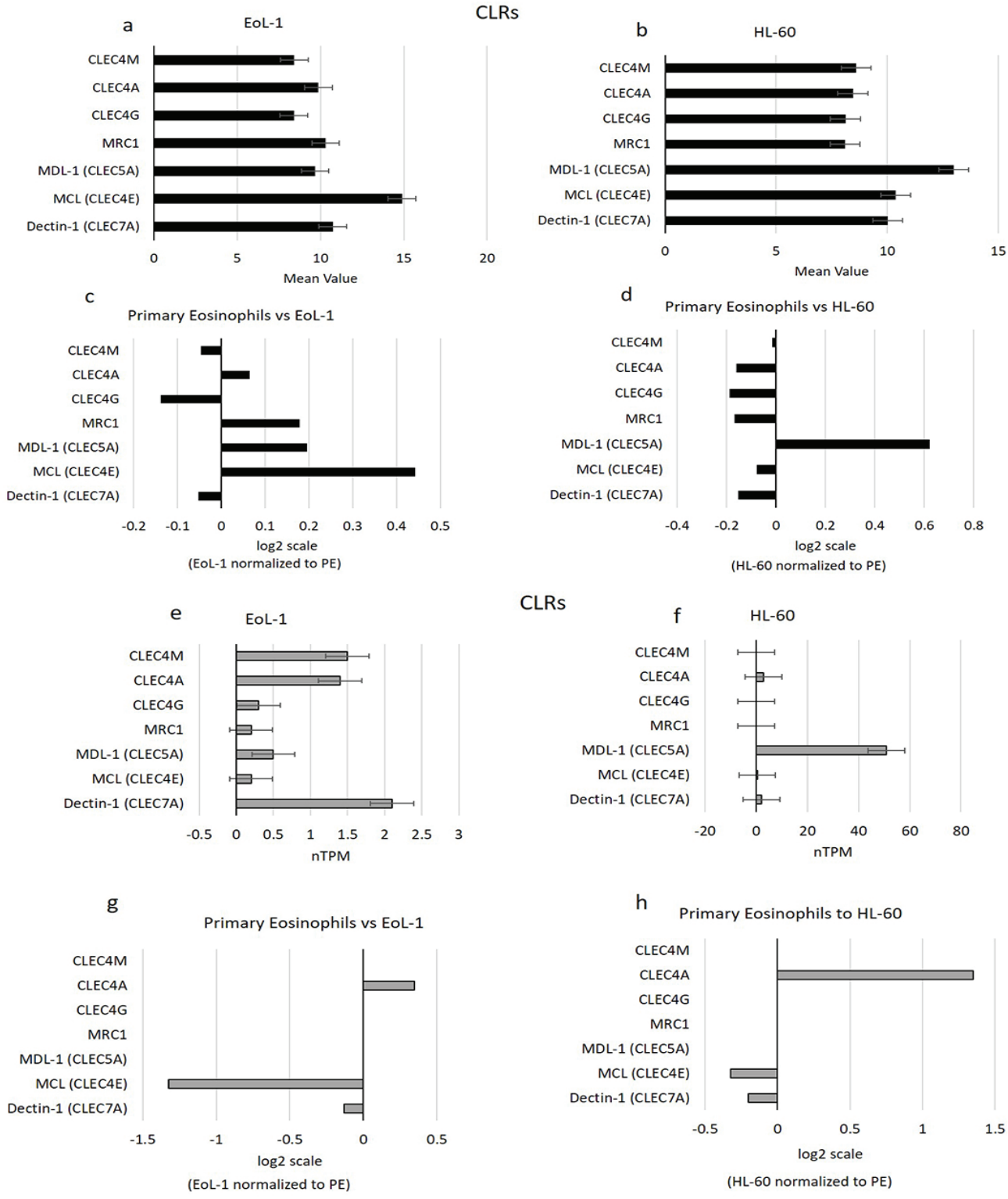

Figure 4. Expression profiles of CLRs in EoL-1 and HL-60 cell lines based on the data received from Genevestigator **(a-d)** and the Human Protein Atlas **(e-h)**. Differentially expressed CLR proteins are given at mRNA level for **a)** EoL-1 and **b)** HL-60 as mean value (MV). log2 fold changes based on CLRs' expression profile of primary eosinophils were calculated by the formula: **c)** log2 (MVEoL-1/MVPrimary) **d)** log2 (MVHL-60/MVPrimary). Expression levels of RLRs are given at protein level for e) EoL-1 and f) HL-60 as nTPM received from the Human Protein Atlas. log2 fold changes based on CLRs' expression profile of primary eosinophils were calculated by the formula: **g)** log2 (nTPMEoL-1/nTPMPrimary) **h)** log2 (nTPMHL-60/nTPMPrimary). Error bars are given as standard error mean (SEM).
CLR: C-type lectin receptors

of Toll/IL-1 receptor domain which is homologous to the IL-1 receptor intracellular domain and plays roles in downstream signaling. There are ten members of TLRs (TLRs 1-10) expressed in human cells each of which recognizes distinct structural motifs (24). Eosinophilic leukemia cell line EoL-1 was shown to express ten human TLRs at different levels (25). The functional expression of TLR4 in EoL-1 cells was displayed by a study in which the stimulation of differentiated EoL-1 cells with TLR4 ligands lipopolysaccharide (LPS) and palmitic acid resulted in the activation of eosinophilic cells and upregulation of immune mediators. Furthermore, EoL-1 cells were suggested to influence M1 and M2 macrophage polarization when treated with TLR4 ligands LPS and palmitic acid, respectively (26). In another study, TLR7 ligand R837-treated EoL-1 cells induced the secretion of inflammatory mediators and TLR7-activated eosinophils were suggested to be involved in neutrophil recruitment, activation and survival (27). In addition to EoL-1 cells, HL-60 cells were shown to express TLR1 - TLR10 (26) and their treatment with dimethyl sulfoxide (DMSO) reduced the expression of TLR3 and TLR8 (28). Given that several studies focused on these cell lines and PE, we revealed that TLR2 expression was higher in both cell lines and therefore, TLR2 ligand PAM3CSK4 was a better stimulant than TLR4 ligand LPS for induction of cells (41). As nTPMs were also evidence for protein levels, TLR10, which was missing in cell lines, seemed to be expressed by PE. To date, the true ligand for human TLR10 remains elusive (42), hence this may provide a promising view for those who focus on TLR10 function. Furthermore, lacking TLR3, TLR8 and TLR9 in PE raises the question of whether PE do not require these receptors' functions.

Among PRRs, NLRs are one of the intracellular super families of receptors responsible for the detection of bacterial as well as viral peptides in the cytosol. They are generally described as inflammatory mediators for their recognition pathways of intracellular microbes. Although PE' abilities to recognize pathogens are mostly attributed to TLRs (29), the functionality and roles of the NLRs are barely studied and there still remain as the key roles to be explored. We previously showed that EoL-1 cells expressed NLRP3 and NLRC4 as well as other inflammasome components such as caspase-1, ASC and NAIP at both mRNA and protein levels. Stimulation of EoL-1 cells with TLR2 ligand PAM3CSK4 and TLR5 ligand Flagella activated EoL-1 cells and triggered the formation of NLRC4 inflammasome which led to IL-1β secretion (13). Furthermore, according to the CCLE cell line gene expression profiles database, EoL-1 cells can express NOD1 and NOD2. Another finding from our study was the abundance of NLRP3 expression in both EoL-1 and HL-60 cell lines as compared to PE, suggesting that these cell lines could give rise to high cellular protein when induced by NLRP3 ligands including silica and nigericin (43), and therefore, could be utilized for protein interaction studies before switching to *in vivo* models.

NLRP12 has functions in the recruitment of neutrophils to the site of inflammation (30); however, no roles for NLRP12 have been reported in terms of eosinophil immunity yet. Intriguingly, NLRP12 was expressed by PE whose function yet to be elucidated in the context of eosinophil immunity. Indeed, NLRP12 and NLRP3 dependent caspase-1 activation has been reported to initiate inflammation and hypersensitivity to bacterial superinfection upon malaria induction, suggesting roles for eosinophils during pathogenesis of malaria (44,45). Data presented here suggested that these cell lines could serve as models to study endogenous proteins instead of overexpressing the NLRs of interest in cells. Overall, NLRP3 exerted the highest expression profile in cell lines and NLRP12 in human PE.

RLRs are cytosolic proteins responsible for the recognition of cytosolic RNA and are of great importance for viral infections. There are three members of RLRs: RIG-I, melanoma differentiation-associated protein 5 (MDA5) and LGP2. RIG-I and MDA5 carry a central helicase domain and a CTD, which recognize target RNA, as well as two amino-terminal caspase activation and recruitment domains (CARDs), which take part in signal transduction. LGP2 does not include CARDs and is mostly known to regulate RIG-I and MDA5. HL-60 cells have been shown to express RIG-I and MDA5 and their expressions were further upregulated by the stimulation with DMSO (28). Our analyses of mRNA expression profiling of RLR family members in three different cell subsets suggested that MDA5 had higher expression in cell lines as compared to primary cells. Interestingly, primary cells exerted higher expression of LGP2 than cell lines. There is a growing body of evidence that LGP2 has positive roles in MDA5 antiviral signaling and eosinophils might be worth studying in viral immunity via RLR signaling (46). RIG-I expression profile was rather complex and required experimental validation.

Another membrane bound PRR family includes the CLRs, which are characterized by the presence of at least one structurally homologous carbohydrate recognition domain (CRD), also known as a C-type lectin-like domain determining carbohydrate specificity. CLRs include various receptors such as selectins, collectins, proteoglycans and lymphocyte lectins (31,32). Upon ligand recognition by CLRs, most of them can induce intracellular signaling and caspase- recruitment domain-containing domain protein 9 (CARD9) signaling, which is crucial for the regulation of immune responses. Their deregulation or dysfunction can cause severe infections in humans and mice (33,34). CLR signaling is primarily mediated by the splenic tyrosine kinase (Syk)-dependent activation of MAPK and NF-κB and the subsequent production of proinflammatory cytokines (35).

Transmembrane CLRs are mainly classified into two subgroups based on CRD: Type I and type II CLR. The mannose receptor (MR) family and DEC-205 belong to the type I CLR group while the sialoglycoprotein receptor family, DC-associated C-type lectin 1 (Dectin-1) and macrophage

galactose C type lectin belong to type II CLRs (36). One of the well-known type II CLRs, Dectin-1 [human homologue termed β-glucan receptor (βGR)], recognizes β-glucans, the major fungal cell wall component, and zymosan (37). Dectin-1 can trigger several responses, including adaptive immune responses and phagocytosis, through the spleen tyrosine kinase (Syk)/CARD9 pathway, which causes cytokine production (38,39). In our study, Dectin-1 showed higher expression profile in PE than both cell lines we included in this study. Number of studies focusing on CLRs of eosinophils are very limited; however, it may potentially increase when more data become available from *in silico*, *in vitro* and *in vivo* studies (47).

Analysis of such data from separate databases is valuable for a better understanding of the roles of eosinophils in pathogenesis of human diseases. Design and screening of new drugs or treatment strategies targeting eosinophils to selectively deplete them can only be possible by the participation of multiple disciplines including immunology, microbiology, molecular biology, clinical studies, computational modeling, *in silico* analyses and so on. Overall, our findings may widen the point of view and let us re-examine the unmet need for eosinophil research and may provide new insights for new experimental designs.

Ethics

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Authorship Contributions

Concept: E.B., Y.O., E.Y.S., C.C., Design: Z.C., E.B., C.C., Data Collection or Processing: Z.C., Y.O., E.Y.S., Analysis or Interpretation: Z.C., E.B., Y.O., E.Y.S., C.C., Literature Search: Z.C., E.B., Y.O., E.Y.S., C.C., Writing: Z.C., E.B., Y.O., E.Y.S., C.C.

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