

Expression of TLR2 and TLR4 on peripheral blood monocytes during exacerbation of atopic dermatitis

Namjil N. Tsybikov, M.D., Ph.D.,¹ Irina Petrisheva, M.D., Ph.D.,¹ Elena V. Fefelova, M.D., Ph.D.,¹ Boris I. Kuznik, M.D., Ph.D.,² and Eli Magen, M.D.³

ABSTRACT

Background: In atopic dermatitis (AD), monocytes, which accumulate in the inflamed skin, are characterized by a significantly impaired Toll-like receptors (TLR) expression and TLR2-mediated cytokine secretion. However, data on expression of TLR on monocytes of peripheral blood (PB) in AD are not available.

Objective: To investigate TLR2 and TLR4 expression on PB monocytes during AD exacerbation and to assess the relationships between TLR expressions with AD clinical severity and with serum interleukin (IL) 4, IL-10, and IL-17a levels.

Methods: The objective Scoring Atopic Dermatitis index, TLR2 and TLR4 expression on CD14⁺ human leukocyte antigen-DR (HLA-DR⁺) PB monocytes by flow cytometry, serum IL-4, IL-10, IL-17a (enzyme-linked immunosorbent assay) and total immunoglobulin E levels were measured at study entry and after 4 months in patients with AD and healthy controls.

Results: Eighty-two patients with AD, 35 women (45.1%) and 47 men (54.9%), mean (standard deviation [SD]) age, 42.2 ± 11.5 years, were included. Thirty healthy volunteers served as controls. We observed a significant difference in the levels of TLR2 expression in the CD14⁺ HLA-DR⁺ PB monocytes of patients with AD (mean [SD], 51.6 ± 23.1% and 264 ± 118 cells/mm³) at exacerbation (but not at the end of the 4-month postexacerbation period) compared with the healthy control subjects (mean [SD], 22.3 ± 10.6% and 105 ± 50 cells/mm³; $p < 0.001$). TLR4 expression in PB monocytes was significantly greater in AD (mean [SD], 50.1 ± 20.9% and 275 ± 114 cells/mm³) than in the healthy subjects (mean [SD], 31.2 ± 8.7% and 147 ± 41 cells/mm³; $p < 0.001$) both at exacerbation and at the 4-month postexacerbation period. Significant correlations between TLR2⁺ (but not TLR4⁺) PB monocytes and the objective Scoring Atopic Dermatitis index ($r = 0.604$, $p < 0.001$), serum levels of IL-17a and TLR2⁺ PB monocytes ($r = 0.416$, $p = 0.027$), and IL-4 and TLR2⁺ PB monocytes ($r = -0.307$, $p = 0.014$) were observed during AD exacerbation.

Conclusion: PB CD14⁺ HLA-DR⁺ TLR2⁺ monocytes might have a role in the skewing of a T-helper 2/T-helper 17-mediated immune response during AD flare.

(Allergy Asthma Proc 36:e140–e145, 2015; doi: 10.2500/aap.2015.36.3901)

Atopic dermatitis (AD) can be exacerbated by microbial infections, and microbial products activate the innate immune system through interaction with receptors that recognize pathogen-associated molecular patterns.^{1–3} Although the role of Toll-like receptors (TLR) in the pathophysiology of AD is not entirely understood, these receptors were found to be involved in the pathogenesis and flare-ups of the disease.⁴ TLR2 and TLR4 are localized to the cell membrane and recognize lipoteichoic acid of Gram-positive bacteria and lipopolysaccharide of Gram-negative bacteria, respectively.⁵ *Staphylococcus aureus*, which provides potent TLR2 ligands, frequently colonizes AD skin.⁴ TLR2 signals convert transient T-helper

(Th) 2 cell-mediated dermatitis into persistent inflammation, through interleukin (IL) 4-mediated suppression of IL-10.⁶ TLR4 is also implicated in the pathogenesis of allergic diseases.^{7,8} For instance, dust-mite allergen Der p 2 induces allergic inflammation in a TLR4-dependent manner through increased expression of the transcription factor GATA Binding Protein 3 and production of Th2-associated cytokines.⁹

In human skin, TLRs are expressed by many different cell types.⁴ In addition to keratinocytes, which express functional TLRs, there are resident and trafficking immune system cells in the skin that express TLRs, including monocytes and/or macrophages.¹⁰ In AD, macrophages, which accumulate in the inflamed skin, are characterized by a significantly impaired TLR2 expression and TLR2-mediated cytokine secretion.¹¹ However, data on expression of TLR on monocytes and/or macrophages of peripheral blood (PB) in AD are not available.

Generally, TLR2 and TLR4 activation can promote an activation of Th17 cells, which leads to enhanced IL-17a and IL-22 production.¹² Nevertheless, AD is characterized by the reduced skin expression of IL-17a, which has been postulated to result from the inhibition

From the ¹Pathophysiology Department, Chita Medical Academy, Chita, Russia, ²Normal Physiology Department, Chita Medical Academy, Chita, Russia, and ³Clinical Immunology and Allergy Unit, Medicine B Department, Barzilai Medical Center, Ben Gurion University of the Negev, Ashkelon, Israel

The authors have no conflicts of interest to declare pertaining to this article

Address correspondence to Eli Magen, M.D., Clinical Immunology and Allergy Unit, Medicine B Department, Ben Gurion University of the Negev, Zionut 21/33, Ashdod, 77456, Israel

E-mail address: allergologycom@gmail.com

Copyright © 2015, OceanSide Publications, Inc., U.S.A.

of IL-17a production by the Th2 cytokines.^{13,14} Recently, Leonardi *et al.*¹⁵ showed that serum IL-17a levels were significantly higher and that serum IL-10 levels were significantly lower in the subjects with AD than in the healthy controls, with a positive correlation between the Scoring Atopic Dermatitis (SCORAD) index and IL-17a, and an inverse correlation between the SCORAD index and IL-10. Therefore, we studied expression of TLR2 and TLR4 on PB monocytes of patients with AD during AD exacerbation and at the 4-month postexacerbation period to assess the relationships between their expressions with clinical severity of AD and with serum IL-4, IL-10, and IL-17a levels.

MATERIALS AND METHODS

Subjects

The subjects were recruited during the period of 2011 to 2014 from the dermatology dispensary at the Chita Medical Academy (Chita, Russia). Consecutive patients with AD, ages between 15 and 40 years, were enrolled in this study. AD was diagnosed by the criteria of Hanifin and Rajka, with a duration of ≥ 1 year. The disease severity was graded by the objective Severity SCORAD index. The objective SCORAD index has a range of 0–83 (plus an additional 10 points for severe disfiguring eczema on the face and hands). An objective SCORAD score of <15 was classified as mild, 15–40 as moderate, and >40 as severe. The subjective itch intensity was obtained by the visual analog scale (ranging from 0 to 10) by the same dermatologist (I.P.).

Exclusion criteria were as follows: any other chronic disease, acute infection, oral intake of any medications, ultraviolet therapy conducted <2 months before the study entry, pregnancy, and lactation. A group of age- and sex-matched healthy volunteers served as controls. The study was approved by the ethics committee at the Chita Medical Academy (Chita, Russia), and all the participants gave informed consent.

There were two main reasons for choosing subjects with AD of ages 15–40 years old: (1) in our region, the prevalence of asthma in children with moderate-to-severe AD ranges from 25 to 50%, so all our patients had AD without any other chronic disease; and (2) the children with AD experience increased rates of infectious, especially during cold seasons in Siberia, therefore, we believed that it would be easier to find subjects with AD and without comorbidities in those ages 15–40 years to eliminate the potential influence of comorbidities on laboratory investigations.

The patients with AD were included during exacerbation, manifested by flares of eczema at ≥ 3 sites or by generalized skin lesions. Every recruited patient was assessed at two time points: (1) during the exacerbation (study entry), and (2) at the 4-month postexacerbation period, after using emollients, antihistamines, and top-

ical steroids. None of the patients had used oral corticosteroids and/or immunomodulatory medications during the study.

Flow Cytometry for Detection of CD14, TLR2, TLR4, and HLA-DR Surface Expression

Flow cytometry was applied to freshly isolated whole-blood samples to investigate the expression levels of CD14, human leukocyte antigen-DR (HLA-DR), TLR2, and TLR4 on PB monocytes of patients with AD and of healthy controls. For staining, 100 μL of whole-blood samples were incubated with the following antihuman antibodies (10 μL per 100 μL of blood) for 15 minutes in the dark at 4°C: Phycoerythrin cyanin 5 labeled anti-CD14 (catalog no. 45–0149-42; eBioscience, San Diego, CA), antihuman CD282 (TLR2) fluorescein isothiocyanate (catalog no. 11–9922-42; eBioscience), antihuman CD284 (TLR4) phycoerythrin (catalog no. A07765; Beckman Coulter, Miami, FL), anti-HLA DR–phycoerythrin cyanine 7 (catalog no. A40579; Beckman Coulter, Miami, FL). Isotype-matched antibody controls were used to detect nonspecific staining (eBioscience). Consequently, red blood cells were lysed with lysing solution (fluorescence-activated cell sorting; BD Biosciences, San Jose, CA) and removed by washing two times with cell wash solution (fluorescence-activated cell sorting; BD Biosciences). To compare and evaluate the gating, a control sample stained with CD14–phycoerythrin cyanin 5, HLA DR–phycoerythrin cyanine 7 and mouse immunoglobulin G2a (IgG2a) isotype control fluorescein isothiocyanate (catalog no. 11–4724-42; eBioscience), and mouse IgG2a isotype control phycoerythrin (catalog no. 12–4724-42; eBioscience) antibodies was used.

Beckman Coulter FC-500 flow cytometer (two-laser, six-color configuration) with CXP software (Beckman Coulter, Fullerton, CA) was used for data acquisition. Quality control included regular checkups with Flow-Check Pro Fluorospheres (Beckman Coulter, Fullerton, CA). Fluorescence compensation was automatically assessed. Amplifier settings for forward scatter and side scatter were used in linear mode, and those for fluorescence channels were used in the logarithmic mode. The forward scatter area scaling factor and threshold rate were accordingly adjusted. The samples stained for CD282, CD284, CD14, and HLA-DR were acquired by using a forward scatter–A/side scatter–A gate around the monocyte population, with 10,000 gated events recorded. Monocytes were identified and gated according to their characteristic forward- and side-scatter flow cytometry profiles, and their expression of CD14 and HLA-DR. Natural killer cells and neutrophils were excluded by their lack of HLA-DR expression; B cells were

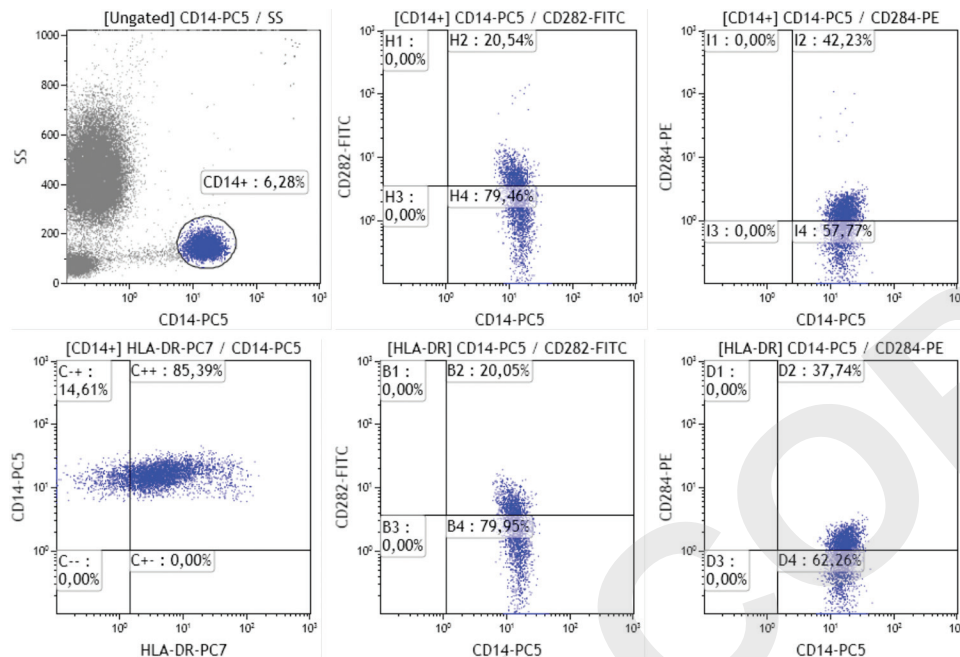


Figure 1. The representative results of flow cytometry for cluster of differentiation 14 (CD14), Toll-like receptor (TRL) 2, TRL4, and human leukocyte antigen-DR (HLA-DR).

excluded by their lack of CD14. The intra- and inter-assay variability was 1.6% and 4.5%, respectively. Routinely, a total of 10,000 CD14⁺ monocytes were read for each sample. All results are expressed either as percentages of TLR2, TLR4, and HLA-DR⁺ monocytes in the total monocyte population (a threshold was defined with the isotype control). The representative results of flow cytometry for CD14, TLR2, TLR4, and HLA-DR are presented in Fig. 1.

Serum IgE and Cytokines Assays

Serum samples were analyzed for total IgE levels by using the immunometric assay of the Immulite 2000 system (Diagnostic Products Corporation; Global Siemens Health Care, Erlangen, Germany). Serum IL-4, IL-10, and IL-17a levels were measured by using enzyme-linked immunosorbent assay (Vector-Best, Novosibirsk, Russia) according to the manufacturer's instructions.

Statistical Analyses

Results are presented as mean (standard deviation [SD]). Differences in clinical and laboratory characteristics between the groups were analyzed by using the Wilcoxon signed rank test for nonparametric variables, and χ^2 test or Fisher's exact test (when appropriate) for parametric variables. Correlations between the variables were analyzed by the Pearson correlation and Spearman rank test, respectively, depending on the distribution of the data. Statistical significance was defined as a two-sided p value of

<0.05 . All statistical analyses were performed by using the software Statistica 6 (StatSoft, Inc. Tulsa, OK).

RESULTS

Clinical and Anthropometric Characteristics

The clinical characteristics of the patients and controls are presented in Table 1. The study included 82 patients with AD, 35 women (45.1%) and 47 men (54.9%), and mean (SD) age, 24.5 ± 7.9 years. A group of 30 healthy volunteers, matched for age and sex served as controls. The mean (SD) objective SCORAD score during the exacerbation of AD was 48.8 ± 19.4 and during remission was 16.1 ± 8.3 ($p < 0.01$).

TLR2- and TLR4-Surface Expression on CD14⁺ HLA-DR⁺ PB Monocytes

We observed a significant difference in the mean (SD) levels of TLR2 expression on the CD14⁺ HLA-DR⁺ PB monocytes of patients with AD ($51.6 \pm 23.1\%$, 264 ± 118 cells/mm³) at exacerbation (baseline) compared with the healthy control subjects ($22.3 \pm 10.6\%$; 105 ± 50 cells/mm³; $p < 0.001$). At the 4-month postexacerbation period, there was no significant difference in CD14⁺ CD282⁺ between the study groups (Table 1).

The mean (SD) TLR4 expression on PB monocytes was significantly greater in AD ($50.1 \pm 20.9\%$ and 275 ± 114 cells/mm³) than in the healthy subjects ($31.2 \pm 8.7\%$ and 147 ± 41 cells/mm³; $p < 0.001$) at exacerbation. By contrast, we found no significant re-

Table 1. Demographic and laboratory data of the study population

	AD (N = 82)	Healthy Controls (N = 30)	p
Age, mean (SD), y	24.5 ± 7.9	24.9 ± 6.2	0.803
Sex (female) no. (%)	37 (45.1)	14 (46.7)	1
Mean (SD) duration of AD, y	17.1 ± 11.2	0	<0.001
Body mass index, mean (SD), kg/m ²	25.2 ± 2.2	25.4 ± 1.9	0.684
Objective SCORAD, mean (SD)			
Exacerbation (baseline)	48.8 ± 19.4	0	<0.001
Mean (SD) 4-mo postexacerbation period	16.1 ± 8.3*	0	<0.001
Mean (SD) serum IgE, kU/L			
Exacerbation (baseline)	355.6 ± 331.2	49.2 ± 27.9	<0.001
The 4-mo postexacerbation period	281.5 ± 194.7	45.7 ± 32.1	<0.001
Mean (SD) monocytes (CD14 ⁺ HLA-DR ⁺), cells/mm ³			
Exacerbation (baseline)	549 ± 207	471 ± 241	0.094
The 4-mo postexacerbation period	524 ± 219	462 ± 237	0.197
Monocytes/TLR2 ⁺ (CD14 ⁺ HLA-DR ⁺ CD282 ⁺), mean (SD), %			
Exacerbation (baseline)	51.6 ± 23.1	22.3 ± 10.6	<0.001
The 4-mo postexacerbation period	22.3 ± 9.3*	22.2 ± 13.1	0.964
Monocytes/TLR2 ⁺ (CD14 ⁺ HLA-DR ⁺ CD282 ⁺), mean (SD), cells/mm ³			
Exacerbation (baseline)	264 ± 118	105 ± 50	<0.001
The 4-mo postexacerbation period	117 ± 49*	103 ± 61	0.213
Monocytes/TLR4 ⁺ (CD14 ⁺ HLA-DR ⁺ CD284 ⁺), mean (SD), %			
Exacerbation (baseline)	50.1 ± 20.9	31.2 ± 8.7	<0.001
The 4-mo postexacerbation period	56.7 ± 23.7	30.1 ± 11.5	<0.001
Monocytes/TLR4 ⁺ (CD14 ⁺ HLA-DR ⁺ CD284 ⁺), mean (SD), cells/mm ³			
Exacerbation (baseline)	275 ± 114	147 ± 41	<0.001
The 4-mo postexacerbation period	297 ± 125	139 ± 53	<0.001
Serum IL-4, mean (SD), kU/L			
Exacerbation (baseline)	7.2 ± 3.5	0.1 ± 0.4	<0.001
The 4-mo postexacerbation period	0.2 ± 0.4*	0.2 ± 0.3	1
Serum IL-10, mean (SD), kU/L			
Exacerbation (baseline)	0.6 ± 0.3	0.7 ± 0.5	0.201
The 4-mo postexacerbation period	1.4 ± 0.9*	0.8 ± 0.5	<0.001
Serum IL-17a, mean (SD), pg/mL			
Exacerbation (baseline)	176.4 ± 49.2	71.3 ± 24.1	<0.001
The 4-mo postexacerbation period	79.1 ± 19.4	74.1 ± 15.7	0.207

AD = Atopic dermatitis; SD = standard deviation; SCORAD = Scoring Atopic Dermatitis; IgE = immunoglobulin E; HLA-DR⁺ = human leukocyte antigen-DR; TLR = toll-like receptor; IL = interleukin.

**p* < 0.001 (exacerbation vs the 4-mo postexacerbation period time point).

duction in the levels of TLR 4 expression on the PB monocytes at the 4-month postexacerbation period in subjects with AD (Table 1). There was a statistically significant strong positive correlation between CD14⁺ HLA-DR⁺ TLR2⁺ PB monocytes and an objective SCORAD score during AD exacerbation (Fig. 2). No significant correlation (*r* = 0.207, *p* = 0.061) was observed between CD14⁺ HLA-DR⁺ TLR4⁺ PB monocytes and objective SCORAD index.

Serum Levels of IL-4, IL-10, and IL-17a

During AD exacerbation, the mean (SD) serum IL-4 (7.2 ± 3.5 kU/L) and mean (SD) IL-17a (176.4 ± 49.2 pg/mL) levels were significantly higher in patients with AD than in healthy controls (0.1 ± 0.4 kU/L and 71.3 ± 24.1 pg/mL, respectively; *p* < 0.001). There was no significant difference in mean (SD) serum IL-10 level (0.6 ± 0.3 kU/L) between the two groups at AD

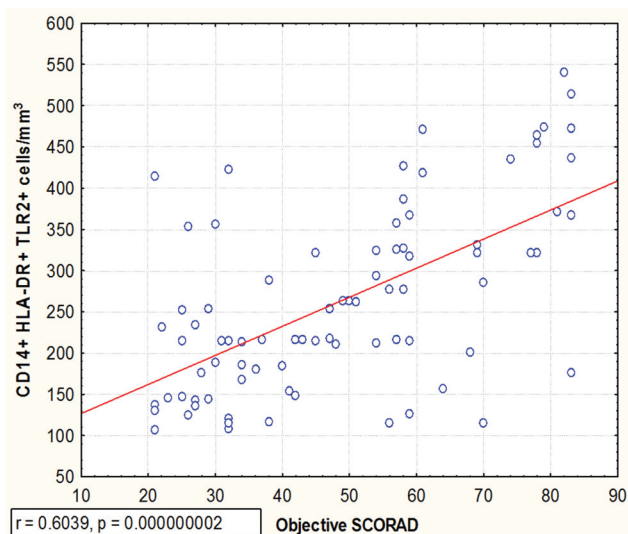


Figure 2. Correlation between CD14⁺ HLA-DR⁺ Toll-like receptor 2⁺ peripheral blood monocytes and objective Scoring Atopic Dermatitis (SCORAD) score during atopic dermatitis exacerbation.

flare, but, at the 4-month postexacerbation period, the mean (SD) serum IL-10 level (1.4 ± 0.9 kU/L) in the AD group was higher than in healthy controls (0.8 ± 0.5 kU/L; $p < 0.001$). No statistically significant correlations were observed between the SCORAD index and serum cytokine levels. There was a significant positive correlation ($r = 0.416$, $p = 0.027$) between serum levels of IL-17a and a negative correlation ($r = -0.307$, $p = .014$) between serum levels of IL-4 and the CD14⁺ HLA-DR⁺ TLR2⁺ PB monocytes during AD exacerbation.

DISCUSSION

The present study revealed higher expression of TLR2 and TLR4 on circulating CD14⁺ HLA-DR⁺ monocytes of patients with AD compared with healthy control subjects. The detectable differences in TLR2/TLR4 surface expression on PB monocytes allowed us to hypothesize about possible involvement of circulating monocytes in the pathogenesis of AD.

So far, research of TLRs in AD has mainly focused on the relationship between the TLR polymorphism and AD severity.^{16–19} Previously, monocytes from patients with AD were shown to have a significant impairment in the TLR2-mediated production of proinflammatory cytokines.²⁰ In this study, we observed a strong statistical correlation of TLR2 expression on blood pressure monocytes with AD clinical severity (measured by the objective SCORAD score) and the Th17-dominated inflammatory profile during AD exacerbation. Although higher expression on blood pressure monocytes was found for both TLR2 and TLR4 in AD, there was no

downregulation of TLR4 at the 4-month postexacerbation period in contrast with TLR2.

During AD flares, monocytes sense *S. aureus* secreted toxins and contribute to priming of naive T cells into Th2 cells through activation and overexpression of TLR2.^{21,22} Although the upper inflamed dermis in AD is characterized by massive infiltration of FcεRI⁺ (high-affinity IgE receptor, or Fc ε RI) monocytes, with high TLR2 expression levels, these monocytes cannot adequately respond to repeated TLR stimulation (a phenomenon referred to as endotoxin tolerance).^{20,23} Although we observed higher serum levels of IL-4 and IL-17a cytokines during AD flare, a positive correlation was detected only between serum levels of IL-17a and TLR2 expression on PB monocytes. There is growing evidence that IL-17a plays an important role in the pathophysiology of AD, particularly during AD flares.²⁴ Furthermore, an increase of Th17 cells in the PB of patients with AD was shown to be positively correlated with disease severity.²⁴

Certain myeloid-origin antigen presenting cells are likely to influence immune responses toward those dominated by the Th17 phenotype.²⁵ In humans, various populations of monocytes act at the crossroad of regulatory T cell–Th17 differentiation, and, although CD14⁺ HLA-DR[−] monocytes induce forkhead box P3⁺ regulatory T cells, CD14⁺ HLA-DR⁺ monocytes induce a generation of IL-17-secreting Th17 cells when cocultured with autologous CD4⁺ T lymphocytes.^{26,27} Our findings can signify that preferential activation of TLR2 rather than TLR4 on CD14⁺ HLA-DR⁺ monocytes are prone to induce Th17-dominated inflammatory responses during a flare of AD. The exact mechanism and biologic relevance of our findings still need to be elucidated, although it is reasonable to suggest that TLR2 expression on monocytes can play some role in immune regulation during AD flares.

Several limitations of our study warrant consideration. First, during 4 months of the study period, patients with AD were under the influence of several medications, including topical steroids, antihistamines, and emollients, although the control subjects were not. Due to ethical limitations, it was impossible to expose the healthy control subjects to the same medications. Therefore, this did not allow us to determine whether any of these medications may have contributed to the changes seen in the results of the patients with AD. Second, it has to be considered that blood monocytes and serum cytokines levels may not accurately reflect the pathophysiologic situation at the site of skin inflammation. Third, the contributions of PB monocytes and their TLRs to the pathophysiology of AD is unclear and deserve further investigation. When taking into consideration the development of new TLR-targeted immunotherapies of allergic diseases, this might have great impact on the development of new

pharmaceuticals to act on these receptors and their associated signaling pathways in AD.

REFERENCES

1. Lacour M, and Hauser C. The role of microorganisms in atopic dermatitis. *Clin Rev Allergy* 11:491–522, 1993.
2. Leung DY. Atopic dermatitis and the immune system: The role of superantigens and bacteria. *J Am Acad Dermatol* 45(suppl.): S13–S16, 2001.
3. Biedermann T. Dissecting the role of infections in atopic dermatitis. *Acta Derm Venereol* 86:99–109, 2006.
4. Kang SS, Kauls LS, and Gaspari AA. Toll-like receptors: Applications to dermatologic disease. *J Am Acad Dermatol* 54:951–983; quiz 983–986, 2006.
5. Ong PY, Ohtake T, Brandt C, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 347:1151–1160, 2002.
6. Kaesler S, Volz T, Skabytska Y, et al. Toll-like receptor 2 ligands promote chronic atopic dermatitis through IL-4-mediated suppression of IL-10. *J Allergy Clin Immunol* 134:92–99, 2014.
7. Hussein YM, Awad HA, Shalaby SM, et al. Toll-like receptor 2 and Toll-like receptor 4 polymorphisms and susceptibility to asthma and allergic rhinitis: A case-control analysis. *Cell Immunol* 274:34–38, 2012.
8. Smit LA, Siroux V, Bouzigon E, et al. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. *Am J Respir Crit Care Med* 179:363–368, 2009.
9. Trompette A, Divanovic S, Visintin A, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 457:585–588, 2009.
10. Miller LS, and Modlin RL. Toll-like receptors in the skin. *Semin Immunopathol* 29:15–26, 2007.
11. Niebuhr M, Lutat C, Sigel S, and Werfel T. Impaired TLR-2 expression and TLR-2-mediated cytokine secretion in macrophages from patients with atopic dermatitis. *Allergy* 64:1580–1587, 2009.
12. Zuo A, Liang D, Shao H, et al. In vivo priming of IL-17(+) uveitogenic T cells is enhanced by Toll ligand receptor (TLR)2 and TLR4 agonists via $\gamma\delta$ T cell activation. *Mol Immunol* 50: 125–133, 2012.
13. Eyerich K, Pennino D, Scarponi C, et al. IL-17 in atopic eczema: Linking allergen-specific adaptive and microbial-triggered innate immune response. *J Allergy Clin Immunol* 123:59–66.e4, 2009.
14. Margolis DJ, Apter AJ, Gupta J, et al. The persistence of atopic dermatitis and filaggrin (FLG) mutations in a US longitudinal cohort. *J Allergy Clin Immunol* 130:912–917, 2012.
15. Leonardi S, Cuppari C, Manti S, et al. Serum interleukin 17, interleukin 23, and interleukin 10 values in children with atopic eczema/dermatitis syndrome (AEDS): Association with clinical severity and phenotype. *Allergy Asthma Proc* 36:74–81, 2015.
16. Oh DY, Schumann RR, Hamann L, et al. Association of the toll-like receptor 2 A-16934T promoter polymorphism with severe atopic dermatitis. *Allergy* 64:1608–1615, 2009.
17. Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K, et al. The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol* 113:565–567, 2004.
18. Weidinger S, Novak N, Klopp N, et al. Lack of association between Toll-like receptor 2 and Toll-like receptor 4 polymorphisms and atopic eczema. *J Allergy Clin Immunol* 118:277–279, 2006.
19. Schimming TT, Parwez Q, Petrasch-Parwez E, et al. Association of toll-interacting protein gene polymorphisms with atopic dermatitis. *BMC Dermatol* 7:3, 2007.
20. Hasanejad H, Takahashi R, Kimishima M, et al. Selective impairment of Toll-like receptor 2-mediated proinflammatory cytokine production by monocytes from patients with atopic dermatitis. *J Allergy Clin Immunol* 120:69–75, 2007.
21. Hoeger PH, Lenz W, Boutonnier A, et al. Staphylococcal skin colonization in children with atopic dermatitis: Prevalence, persistence, and transmission of toxigenic and nontoxigenic strains. *J Infect Dis* 165:1064–1068, 1992.
22. Mandron M, Ariès MF, Brehm RD, et al. Human dendritic cells conditioned with *Staphylococcus aureus* enterotoxin B promote TH2 cell polarization. *J Allergy Clin Immunol* 117:1141–1147, 2006.
23. Horelt A, Belge KU, Steppich B, et al. The CD14⁺CD16⁺ monocytes in erysipelas are expanded and show reduced cytokine production. *Eur J Immunol* 32:1319–1327, 2002.
24. Toda M, Leung DY, Molet S, et al. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol* 111:875–881, 2003.
25. Paustian C, Taylor P, Johnson T, et al. Extracellular ATP and Toll-like receptor 2 agonists trigger in human monocytes an activation program that favors T helper 17. *PLoS One* 8:e54804, 2013.
26. Koga C, Kabashima K, Shiraishi N, et al. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 128: 2625–2630, 2008.
27. Sergejeva S, Ivanov S, Lötvall J, Linden A. Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. *Am J Respir Cell Mol Biol* 33:248–253, 2005. □

Copyright of Allergy & Asthma Proceedings is the property of OceanSide Publications Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.