# Aberrant B-cell Subsets and Immunoglobulin Levels in Patients with Moderate-to-severe Psoriasis

Katrin KAHLERT[1], Franziska GRÄN[1], Khalid MUHAMMAD[2], Sandrine BENOIT[1], Edgar SERFLING[2], Matthias GOEBELER[1] and Andreas KERSTAN[1]

*[1]Department of Dermatology, Venereology and Allergology, University Hospital Würzburg, DE-97080 Würzburg, and [2]Department of Molecular Pathology, Institute of Pathology, Julius-Maximilians-University, Würzburg, Germany. E-mail: Kahlert_K@ukw.de*

Accepted Oct 15, 2018; Epub ahead of print Oct 15, 2018

Psoriasis is a chronic inflammatory disease affecting 2–3% of the world's population. Clinically, skin inflammation is mirrored by infiltrated erythematous papules and plaques with silver-white scaling. At the cellular level T lymphocytes are considered as the key immunological players. Commonly used and emerging therapeutic strategies accordingly rely on the inhibition of proinflammatory cytokines, amongst others tumour necrosis factor (TNF)-α and interleukin (IL)-17. Although B cells are assumed to play a role in cutaneous immunity, both in malignancy, e.g. melanoma (1, 2), and in particular autoimmune skin inflammation, e.g. lupus erythematosus (3), they have been little-studied in the pathogenesis of psoriasis (4). To elucidate the potential impact of B cells in psoriasis we performed a systematic longitudinal analysis of B-cell subpopulations and corresponding immunoglobulin (Ig) levels.

## METHODS

Heparinized peripheral blood was collected from 34 patients (18 males and 16 females, mean age 50.6 ± 15.4 years) with moderate-to-severe plaque psoriasis defined by a Psoriasis Area and Severity Index (PASI) ≥10 (mean PASI 15.6 ± 6.1). Patients were naïve to or off systemic therapy for at least 4 weeks. Longitudinal analyses were performed on 18 patients who achieved a PASI improvement of at least 75% upon treatment. Thirty-four healthy volunteers served as controls (16 men and 18 women, mean age 46.9 ± 15.7 years). Individuals with acute infections and recent vaccinations were excluded from the study.

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. B cell subsets were evaluated using flow cytometry (BD FACS Canto, Becton Dickinson GmbH, Heidelberg, Germany) and differentiated via surface immunolabelling according to the gating strategy outlined in Fig. S1[1]. IgM, IgA, IgE and IgG serum levels were measured using cytometric bead assays (BD Pharmingen Becton Dickinson GmbH).

The experimental protocol was established according to the Declaration of Helsinki guidelines and approved by the ethics committee of the University of Würzburg (#254/13). Written informed consent was obtained from patients and healthy volunteers. Human material was stored according to the standards of The Interdisciplinary Bank of Biomaterials and Data Würzburg (ibdw) at the University Hospital Würzburg (see: www.ibdw.uk-wuerzburg.de).

Statistical analyses were performed using GraphPad (Prism) software, version 5.0. Data are presented as mean ± standard deviation (SD). Unpaired *t*-test was applied for comparison of controls and patients. Paired *t*-test was applied for longitudinal data. *p*-values < 0.05 were considered statistically significant.

## RESULTS

Percentages of total B cells were lower in patients with moderate-to-severe psoriasis compared with healthy individuals (Fig. S2A[1]). Further analysis showed significantly elevated percentages of transitional B cells (trB) in line with earlier reports (5). Naïve mature B cells (NM) were moderately increased, memory B cells (M) and total plasma cells (PC) were significantly lower in patients with psoriasis. Investigation of PC in detail revealed that long-lived PC did not differ. However, plasma blasts (PB) were significantly elevated in individuals with psoriasis, indicating a pronounced activation of this short-lived Ab-producing PC subset (Fig. S2B[1]). In agreement with this, serum concentrations of IgA, but not of IgG, IgM and IgE, were significantly increased (Fig. S2C[1]). This observation hints at immunological processes in psoriasis that drive the generation of IgA.

Determination of B-cell subsets upon successful treatment (at least 75% reduction in pretreatment PASI) revealed a clear shift towards the conditions found in healthy individuals (Fig. S3A–B[1]). Most importantly, total PC were found to be unchanged, but in-depth analysis of the PC pool showed that short-lived PB decreased significantly, while long-lived PC increased moderately. Interestingly, IgA levels remained elevated upon successful treatment (Fig. S3C[1]).

## DISCUSSION

In peripheral blood of patients with moderate-to-severe psoriasis altered frequencies of B-cell subsets and altered serum IgA levels were observed. Upon successful treatment B-cell subsets normalized, whereas the IgA serum concentration remained elevated. These results favour the idea that B lymphocytes may play a role during the generation and/or maintenance of psoriasis.

B cells have largely been neglected in studies of psoriasis, potentially owing to their limited presence in involved psoriatic skin. This, however, does not necessarily exclude a role in the pathogenesis: comparative immunohistochemical analysis of psoriatic plaques and lesional skin of patients with acute SLE showed both equally low numbers of B cells (author's unpublished observation). Although SLE is undoubtedly considered a B-cell-driven disease (6–9).

TrB, characterized by $CD19^{+}CD24^{hi}CD38^{hi}$ surface expression, are assumed to play a role in autoimmune

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3069



doi: 10.2340/00015555-3069
Acta Derm Venereol 2019 Epub ahead of print

diseases, such as in SLE and psoriasis (10). In healthy individuals the regulatory capacity within the trB population has been attributed to increased secretion of IL-10, thereby controlling pathogenic Th1- and Th17-driven immune responses (3, 11).

Both in patients with active SLE (3) and in humans with psoriasis, trB were described to be significantly elevated and the IL-10-producing B-cell subset reduced (5). In addition, our group recently demonstrated the immunomodulating effect of IL-10-secreting B cells in the widely accepted Aldara (imiquimod)-induced mouse model of psoriasis-like skin inflammation (12). The importance of IL-10 is strengthened by therapeutically successful subcutaneous administration of IL-10 in patients affected by psoriasis, which resulted in clinical improvement and a shift from inflammatory (e.g. TNF-α and IL-2) to regulatory cytokine (e.g. IL-4 and IL-5) secretion in peripheral blood (13). Our analysis confirmed a significant elevation of trB in peripheral blood of patients with psoriasis. As assumed for SLE (3) a functional impairment of the active subset or the dominance of the inactive B-cell subset might affect psoriatic skin inflammation. Otherwise, interactions with either stimulatory or inhibitory cells or molecules might influence inflammatory processes. For example, NFATc1 is a transcription factor, which controls the activity of regulatory B cells by suppressing IL-10-production in the above-mentioned imiquimod-driven psoriasis-like skin inflammation mouse model (12).

Decreased peripheral blood levels of M and, in part, PC might be due to an accumulation of these cells in lymphoid tissue and psoriatic skin for the process of generating an immunological memory during the inflammation process. High levels of serum IgA refer to the existence of an unknown, but potent, stimulus that is driving activation of PB. Persistent high levels of IgA upon amelioration of skin inflammation might be due to a shift from short-lived PB to long-lived PC.

IgA is the predominant antibody of mucosal immunity (14). Due to activation and conditioning by their local microenvironment Peyer's patch-derived dendritic cells allow naïve B cells to convert into IgA-producing B cells (15). The inflamed microenvironment in psoriatic skin, in some respect (e. g. disturbed epidermal barrier) possibly resembling that of intestinal mucosa, might influence and promote activation of IgA-producing cells in psoriasis.

Although T cells might be dominant in the pathogenesis of psoriasis alteration of B-cell subsets and, in particular, up-regulation of trB, short-lived PB and IgA might be part of an inflammation-induced compensatory mechanism, which aims to: (*i*) repress detrimental and pathogenic effects of Th1- and Th17-cells (e.g. secretion of TNF-α and IL-17), (*ii*) strengthen protective, regulatory effects of T cells (e.g. secretion of IL-10), and (*iii*) counteract yet not identified antigens. However, although our data corroborate the idea of B cells being part of a complex immunological network contributing to the pathogenesis of psoriasis, larger cohort studies have to determine the precise role of B cells, preferentially by addressing potential specific antigen(s) and pathogenic autoantibodies, as well as in-depth skin analyses, to substantiate the concept of psoriasis as an autoimmune (skin) disease.

## ACKNOWLEDGEMENTS

This work was supported by Deutsche Forschungsgemeinschaft (DFG; SE 469/24-1, GO 811/5-1, KE 1342/2-1) and Interdisziplinäres Zentrum für klinische Forschung (IZKF Würzburg; K.K., Z-2/73). The authors thank Christine Hosp for feedback and discussions and Claudia Rüth, Margit Ott and Daniela Schillinger for excellent technical assistance.

*The authors have no conflicts of interest to declare.*

## REFERENCES

1. Karagiannis P, Villanova F, Josephs DH, Correa I, Van Hemelrijck M, Hobbs C, et al. Elevated IgG4 in patient circulation is associated with the risk of disease progression in melanoma. Oncoimmunology 2015; 4.
2. Saul L, Ilieva KM, Bax HJ, Karagiannis P, Correa I, Rodriguez-Hernandez I, et al. IgG subclass switching and clonal expansion in cutaneous melanoma and normal skin. Sci Rep 2016; 6: 29736.
3. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. Immunity 2010; 32: 129–140.
4. Dass S, Vital EM, Emery P. Development of psoriasis after B cell depletion with rituximab. Arthritis Rheum 2007; 56: 2715–2718.
5. Hayashi M, Yanaba K, Umezawa Y, Yoshihara Y, Kikuchi S, Ishiuji Y, et al. IL-10-producing regulatory B cells are decreased in patients with psoriasis. J Dermatol Sci 2016; 81: 93–100.
6. Sanz I, Lee FE. B cells as therapeutic targets in SLE. Nat Rev Rheumatol 2010; 6: 326–337.
7. Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol 2003; 56: 481–490.
8. Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 2001; 1: 147–153.
9. Chan VS, Tsang HH, Tam RC, Lu L, Lau CS. B-cell-targeted therapies in systemic lupus erythematosus. Cell Mol Immunol 2013; 10: 133–142.
10. Mavropoulos A, Varna A, Zafiriou E, Liaskos C, Alexiou I, Roussaki-Schulze A, et al. IL-10 producing Bregs are impaired in psoriatic arthritis and psoriasis and inversely correlate with IL-17- and IFNgamma-producing T cells. Clin Immunol 2017; 184: 33–41.
11. Flores-Borja F, Bosma A, Ng D, Reddy V, Ehrenstein MR, Isenberg DA, et al. CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. Sci Transl Med 2013; 5: 173ra123.
12. Alrefai H, Muhammad K, Rudolf R, Pham DA, Klein-Hessling S, Patra AK, et al. NFATc1 supports imiquimod-induced skin inflammation by suppressing IL-10 synthesis in B cells. Nat Commun 2016; 7: 11724.
13. Asadullah K, Sterry W, Stephanek K, Jasulaitis D, Leupold M, Audring H, et al. IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. J Clin Invest 1998; 101: 783–794.
14. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. The immune geography of IgA induction and function. Mucosal Immunol 2008; 1: 11–22.
15. Massacand JC, Kaiser P, Ernst B, Tardivel A, Burki K, Schneider P, et al. Intestinal bacteria condition dendritic cells to promote IgA production. PLoS One 2008; 3: e2588.