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The correlation between activity and a circulating lymphocyte pattern in plasma of patients with psoriasis preceded by an infection

Korelacja pomiędzy aktywnością choroby a obrazem krążących limfocytów plazmy pacjentów z łuszczycą bakteryjną

Psoriasis is a common and enigmatic skin disease that involves the interplay of recruited blood--derived immunocompetent cells and endogenous dermal and epidermal cell types (12). Norris et al. (12) describe the following steps in the development of lymphocyte activation in psoriasis. They consist of systemic lymphocyte activation, local accumulation of activated CD4+ lymphocytes, recruitement of nonspecific CD4+ lymphocytes and monocytes and clonal intraepidermal CD8+ lymphocytes (14). Psoriasis may not be a simple inflammatory skin disease with local T–cell activation but may be a disorder associated with systemic T–cell activation (19). Although T–cell proliferation is thought to occur in the skin, no specific triggering antigens have yet been identified (11). There are many reports suggesting that bacterial infection caused by a superantigen can trigger this illness (12, 16).

Superantigens (Sags), including molecules derived from bacteria, viruses or mycoplasma, activate T–cells by interacting with specific variable regions of the T–cell receptor (TcR) β chains and MHC (major histocompatibility complex) class II molecules on antigen–presenting cells (4). It may be hypothesized that patients with latent psoriasis develop guttate psoriasis lesions after superantigens have been released from the streptococcal infection, transported with the blood, and bound to antigen–presenting cells in the skin (epidermis and dermis), which in turn activates T–cells bearing specificT–cell receptor V β chains. (17). In skin, interferon -γ–activated class II–positive keratinocytes (KC) as well as Langerhans cells function as accessory cells, and release T–cell activating cytokine(s) in response to bacterial superantigens (21). Expansion of T lymphocyte populations with limited TCR V β diversity in clinically exacerbated skin lesion suggests a potential role for bacterial and possibly fungal superantigens in the pathogenesis of psoriasis (21).

Staphylococcus aureus is carried on the skin of 50% of patients (21). Cellular immunity has been less well studied, although enhanced responses of blood lymphocytes to beta–heamolytic streptococci have been found both in acute and chronic psoriasis (20). Many recent studies concerning T lymphocytes in peripheral blood and skin were conducted by means of flow cytometry (3, 18). Less information concerns the possible links between the PASI SCORE (Psoriasis activity and severity index) and the percentage of lymphocytes and their expression.

OBJECTIVE

The aim of this study was to analyse if the activity and severity index (PASI) has any influence on the subpopulation of the activated lymphocytes in peripheral blood in patients with psoriasis preceded by an infection.

MATERIAL AND METHODS

SUBJECTS

32 non–treated psoriasis patients (17 males and 15 females) with disease induced by an infection (3–8 weeks prior to admission) and 22 (13 males and 9 females) healthy controls were studied. The means (±SD) of patients' age was 34.5 ± 13.9 years. The mean age of the control group was 31.7 ± 11.1 years. The type of psoriasis was pint point, guttate and guttate and nummular. Bacteriological examinations were not performed because all the patients had previously received anti–infectious treatment.

METHODS

Percentages of the following cells were studied: CD3+ (T lymphocytes), CD19+ (B lymphocytes), CD4+8+ (double positive lymphocytes CD4+, CD8+), CD4+ (T helper lymphocytes), CD8+ (T suppressor lymphocytes), NK (natural killer cells), CD25+ 3+ (T lymphocytes with alpha chain of IL–2R), CD25+ (alpha chain of IL–2R on all lymphocytes), CD45 RO+ (memory cells on all lymphocytes), CD45 RO+ CD4+ (memory cells on T lymphocytes) and expression of the antigens mentioned above on the same cells.

Peripheral blood samples were obtained at diagnosis from 32 patients with psoriasis and 22 healthy donors. The cell surface antigens in each case were determined on fresh cells at the time of sample submission. Mononuclear cells were isolated by density centrifugation on Lymphoprep (Ny–comed, Norway) and washed twice in phosphate buffered saline (PBS) containing 1% bovine serum albumin. Double colour immunofluorescence studies were performed using combinations of phycoerythrin (PE) and fluorescein isothyocyanate (FITC) conjugated monoclonal antibodies. Monoclonal antibodies were obtained from Ortho Diagnostic Systems (Germany), Becton Dickinson (Germany), or Dako (Denmark). The following antibody combinations were used: FITC IgG1/IgG2a PE negative control antibody Ortho Diagnostic Systems, Goat Anti–Mouse FITC, Ortho Diagnostic Systems; CD3PE, Isotype

Ig G2a (Ortho Diagnostic System); CD19PE, Isotype Ig 01, Clone 407, Becton–Dickinson; CD45FITC/CD14 PE, Isotype Ig Gl/IgG2a, Clone IMMU 19.2/RM052, Ortho Diagnostic Systems, CD45RA FITC/CD4 PE, Isotype Ig Gl/IgGl, Ortho Diagnostic Systems, CD45RO FITC, Isotype Ig G2a, Ortho Diagnostic Systems, CD4 FITC/CD8 PE, Isotype Ig G2a/Ig G2a, Ortho Diagnostic Systems, CD4PE, Isotype Ig 2b, Ortho Diagnostic Systems, CD8, Isotype Ig G2a, Ortho Diagnostic Systems; CD25FITC, Isotype Ig G2a, Ortho Diagnostic Systems; CD3FITC/CD19PE, Isotype Ig G2/IgG1, Ortho Diagnostic Systems, CD3FITC/CD16+CD56PE, Isotype IgG1, Clone SK7/B73.1+MY31 Becton–Dickinson; CD56 PE, Isotype IgG1, Ortho Diagnostic Systems. 10^6 cells were incubated with monoclonal antibodies for 30 min. at 40°C and washed twice with PBS afterwards.

All samples were measured on a Cytoron flow cytometer (Ortho Diagnostic Systems). 10,000 cells were analysed per test. In order to quantitate the levels of fluorescence, the mean fluorescence intensity and fluorescence signal strength of the study antigens were calculated. The mean fluorescence intensity and fluorescence signal strength of histogram were measured from the upper limit of the negative control.

In the PASI scoring system, the area of psoriatic involvement of the head, trunk, upper extremities and lower extremities is given by a numerical value from 0 to 6, and in each area erythema (E), inflitration (I) and desquamation (D) are assessed on a scale of 0–4. The PASI score is calculated from the mathematical formula (21). The means (±SD) of psoriasis area and severity index (PASI) score were 25.7 (±7.6), from 10.0 to 48.0 respectively.

Statistical analysis. Data are expressed as mean ±SD. Mann–Whitney U–Test was used for the comparison of the groups. Differences were considered significant at $p < 0.05$. Correlations between the percentage and expression of lymphocytes and PASI score were assessed by Spearman's Rank test.

RESULTS

In all the studied subpopulations of lymphocytes (CD3+, CD4+, CD8+) a decrease in percentage of these cells in comparison with the controls is found; a significant decrease occurs in CD3+, CD4+ ($p < 0.05$). At the same time the percentage and expression of CD3, CD4 were decreased with statistical significance. The only parameter which is significantly increased is the percentage of memory cells CD45 RO+CD4+ ($p < 0.05$). There were contradictory tendencies observed which concerned the percentage of CD19+ cells and expression of CD 19 antigen on these cells. A decrease in expression of CD19 ($p < 0.05$) was observed.

Among the studied correlations only two were statistically significant, i.e. a positive correlation between the CD16+ antigen on NK cells and a PASI score ($R = 0.401175$, $p < 0.03$) and a negative correlation between the percentage of lymphocytes bearing a receptor for IL–2 (%CD25) and a PASI score ($R = -0.378372$, $p < 0.05$). In all the remaining parameters there was no correlation between them and a PASI score observed.

DISCUSSION

A study of acute guttate psoriasis patients by Leung et al. (8) has convincingly linked pharyngeal streptococcal toxins with the lymphocyte activation seen in guttate psoriasis lesions by demonstrating that streptococci isolated from the pharynx of patients with guttate psoriasis produced the superantigenic toxin SPEC, a potent stimulator of $V\beta2+$ T lymphocytes and that all patients studied

had local expansion of Vβ2+ CD4 and CD8 lymphocytes in biopsies of skin taken from normal–appearing skin at the advancing border of new skin lesions, consistent with SPEC activation (10). In addition, lymphocytes were cloned from the guttate skin lesions and the sequences of their TCR were analyzed, leading to the demonstration that there was polyclonal expansion of TCR Vβ2, consistent with superantigenic activation by the SPEC toxin identified in the patients. Studies by Leung and colleagues (8, 9, 12) have also demonstrated that bacterial superantigens activate the expression of the skin homing receptor CLA in lymphocytes.

There was reported an increase as well as hyporesponsiveness of peripheral blood lymphocytes to the streptococcal superantigens and the presence of serum inhibitors that specifically inhibit T–cell response to the superantigens in psoriatic patients (2, 7). The increased percentages of lymphocytes CD45 RO+CD4+ found in our psoriatic group may indicate an increased number of memory cells that have been in contact with the streptococcal antigen.

In our studies expression of CD3, CD4 on T lymphocytes and CD19 on B lymphocytes are significantly decreased, which indicates that the signals coming from the antigens may be less clearly received by these cells.

Krasowska et al. (7) pointed out the existence of correlation between serum level of sELAM–1 and the extent of skin involved in acute psoriasis measured by the psoriatic severity index (PASI) as well as Bonifati et al. (8). They suggested that there may be a relationship between the extent of endothelial cell activation and disease severity (8).

The magnitude of the proliferative responses in Yokote et al. (19) results was correlated with the severity of skin lesions (PASI score). The authors suggest that bacterial superantigens play a role in the activation of T–cells in lesional skin. They suggest that their results indicate that the difference in the responsiveness to superantigens stems from different reactivities of individual T–cells and not from a difference in the number of reactive T–cells.

The study of Pita et al. (13) reports the presence of a clear relation between the cells in skin and the cells in peripheral blood stating that these data strongly support the possibility that circulating lymphocytes could share the same activation markers with skin infiltrating lymphocytes. The rise of cell membrane–associated and soluble form of CD25, as well as the similar feature observed for sCD4 and sCD8 molecules, suggest a chronic T–cell activation.

The results obtained by our group are consistent with the results of the Fornalczyk – Łódź group (5) with reference to CD3, CD4, CD19 and are the same as the studies conducted by Asadullah et al. (1) with reference to the behaviour in peripheral blood of the subpopulation of lymphocytes T CD3+ cells as well as CD4+ – decrease in percentage was observed (statistically insignificant) and they are in agreement with reference to percentage of CD19+B cells, however, we observed a statistically significant decrease in the expression of CD19MFI ($p < 0.05$).

CD16 antigen is a receptor for a Fc constant part of Ig immunoglobulin molecule. NK cells with CD16 receptor take part in a cytotoxic reaction dependent on an antibody (Ig). An increase in CD 16 expression on NK cells and a positive correlation with PASI also suggest a response of NK cells to a present inflammatory process. The presence of a positive correlation between CD 16 antigen expression on NK cells and disease activity can indirectly confirm the role of discovered by Nickoloff et al. (11, 17) killer cell inhibitory receptors and killer cell activating receptors on NK cells. An α chain receptor of IL–2 (CD25) is a lymphocyte activation marker. Thus a negative correlation observed between lymphocyte percentage bearing an α chain receptor of IL–2 and a PASI can testify to the translocation of activated CD25+ lymphocytes from peripheral blood to skin to the area where there is an inflammatory process.

CONCLUSIONS

The obtained results testify to clear activation of T lymphocytes in peripheral blood at the time of dissemination of pathological changes after a bacterial infection.

REFERENCES

1. Asadullah K. et al.: Enhanced expression of T–cell activation and natural killer cell antigens indicates systemic anti–tumor response in early primary cutaneous T–cell lymphoma. J. Invest. Dermatol., 108, 743, 1997.
2. Baker B.S. et al.: Decreased T cell reactivity to trypsinized group A, Type M22 Streptococci in psoriasis. Acta Derm. Venereol. (Stockh.), 74, 276, 1994.
3. de Boer O.J. et al.: Cellular interactions and adhesion molecules in psoriatic skin. Acta Derm. Venereol. (Stockh.) Suppl., 186, 15, 1994.
4. Bour H. et al.: *In vitro* T cell response to staphylococcal Enterotoxin B superantigen in chronic plaque type psoriasis. Acta Derm. Venereol. (Stockh.), 75, 218, 1995.
5. Fornalczyk–Wachowska E. et al.: Niektóre parametry odporności komórkowej u chorych na łuszczycę pospolitą we wczesnym, wysiewnym okresie choroby. Przegl. Dermatol., 1, 33, 1996.
6. Horiuchi N. et al.: Peripheral blood lymphocytes from psoriatic patients are hyporesponsive to beta–streptococcal superantigens. Br. J. Dermatol., 138, 229, 1998.
7. Krasowska D. et al.: Serum level of sELAM–1 in psoriatic patients correlates with disease activity. J. E. A. D. V. 12, 140, 1999.
8. Leung D.Y. et al.: Evidence for a streptococcal superantigen–driven process in acute guttatae psoriasis. J. Clin. Invest., 96, 2106, 1995.
9. Leung D.Y. et al.: Bacterial superantigens induce T–cell expression of the skin–selective homing receptor, the cutaneous lymphocyte–associated antigen, via stimulation of interleukin 12 production. J. Exp. Med., 181, 747, 1995.
10. Moss P. et al.: The repertoire of T cell antigen receptor β–chain variable regions associated with psoriasis vulgaris. J. Invest. Dermatol., 109, 14, 1997.
11. Nickoloff B.J. et al.: Superantigens, autoantigens, and pathogenic T cells in psoriasis. J. Invest. Dermatol., 110, 459, 1998.
12. Norris D.A. et al.: Lymphocyte activation in the pathogenesis of psoriasis. J. Invest. Dermatol., 109, 1, 1997.
13. De Pita O. et al.: Psoriasis: comparison of immunological markers in patients with acute and remission phase. J. Dermatol. Sci., 13, 118, 1996.
14. Sigmundsdottir H. et al.: Circulating T cells of patients with active psoriasis respond to streptococcal M–peptides sharing sequences with human epidermal keratins. Scand. J. Immunol., 45, 688, 1997.
15. Skov L. et al.: Superantigens. Do they have a role in skin diseases? Arch. Dermatol., 131, 829, 1995.

16. Szabo S.K. et al.: Identification and quantitation of interferon-gamma producing T cells in psoriatic lesions: localization to both CD4+ and CD8+ subsets. J. Invest. Dermatol., 111, 1072, 1998.
17. Lecewicz-Toruń B. et al.: The peripheral blood lymphocyte pattern in psoriasis preceded by an infection. Submitted to J. E. D. V.
18. Valdimarsson H. et al.: Psoriasis: a T-cell mediated autoimmune disease induced by streptococcal superantigens?. Immunol. Today 16, 145, 1995.
19. Yokote R. et al.: Susceptible responsiveness to bacterial superantigens in peripheral blood mononuclear cells from patients with psoriasis. Arch. Dermatol. Res., 287, 443, 1995.

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STRESZCZENIE

Wyniki niektórych prac sugerują, że łuszczyca może być chorobą autoimmunologiczną, w której aktywacja komórek T w łuszczycy kroplistej jest inicjowana przez superantygeny. Celem pracy było sprawdzenie, czy aktywność i ciężkość przebiegu choroby wyrażona w skali PASI ma jakikolwiek wpływ na subpopulację aktywowanych limfocytów we krwi obwodowej pacjentów, u których wystąpienie łuszczycy poprzedzone było infekcją. U 32 osób z łuszczycą uogólnioną typu pin-point i 22 osób z grup kontrolnej metodą cytometrii przepływowej oznaczano odsetek i ekspresję wybranych antygenów. Wykazano spadek odsetka limfocytów CD3+ i CD4+ oraz ekspresji antygenów CD19 i CD4. Stwierdzono również istotny statystycznie wzrost odsetka CD45RO+CD4+, co może sugerować wzrost liczby komórek pamięci immunologicznej. Stwierdzono również istnienie statystycznie istotnie dodatniej korelacji ($R = 0{,}401175$, $p < 0{,}03$) pomiędzy wskaźnikiem PASI i ekspresją komórek NK oraz ujemnej korelacji pomiędzy PASI a odsetkiem limfocytów CD25 wiążących łańcuch α interleukiny 2 ($R = -0{,}378372$, $p < 0{,}05$)

Table 1. Comparison of parameters between psoriasis preceded by an infection and the control

Parameter	Controls	Psoriatics	p
% of CD3+ lymphocytes	70.38 ± 10.02	58.46 ± 14.56	p < 0.05
CD3MI	120.86 ± 4.22	113.51 ± 7.57	p < 0.001**
% of CD19+ lymphocytes	4.314 + 2.3	5.47+3.6	p > 0.2
CD 19MI	102.82 ± 13.95	83.04±22.45	p < 0.05
% of CD 4+ 8 lymphocytes	0.88 ± 0.72	1.26 ± 1.14	p > 0.2
CD 4+ 8 MI	89.48±8.45	87.53 ± 14.4	p > 0.3
% of CD4 lymphocytes	50.75 ± 6.59	44.06 ± 15.66	p < 0.05
CD4MI	107.75 ± 4.76	101.16 ± 10.38	p < 0.05
% of CD8 lymphocytes	27.58 + 4.6	24.60 + 11.46	p > 0.2
CD8MI	113.73 + 8.18	110.22 + 19.78	p > 0.2
CD4/CD8 RATIO	1.9 + 0.52	2.43 + 1.8 (1.28)	p > 0.6
% of NK	18.54	19.5 + 17.52	p > 0.
NKMI	88.21 + 23.78	99.95 + 18.01	p > 0.3
% of CD25 3	3.27 + 2.88	3.68 + 3.91	p > 0.6
CD25 3MI	73.64 + 7.19	86.06 + 22.65	p > 0.2
% of CD25	0.53 + 0.37	1.56 + 2.88	p > 0.2
CD25 MI	74.5 + 8.26	78.1 + 20.5	p > 0.5
% of CD45 RO4	1.51 ± 1.96	3.46 ± 3.74	p < 0.05
CD45 RO4	75.82 + 18.78	79.56 + 23.75	p = 1.0
% of CRO	1.59 + 2.1	3.0 + 5.6	p > 0.08
CDCROMI	72.74 + 14.05	77.57 + 22.32	p > 0.9

Table 2. Correlations between parameters in patients with psoriasis preceded by an infection and the PASI score

Parameter	Spearman R	p
% of CD3+ lymphocytes	–0.187758	p > 0.3
CD3MI	–0.167921	p > 0.3
% of CD19+ lymphocytes	0.089248	p > 0.6
CD 19MI	–0.045329	p > 0.8
% of CD 4+ 8 lymphocytes	0.051664	p > 0.7
CD 4+ 8 MI	–0.124977	p > 0.4
% of CD4 lymphocytes	–0.077029	p > 0.6
CD4MI	–0.191962	p > 0.2
% of CD8 lymphocytes	–0.142058	p > 0.4
CD8MI	0.170123	p > 0.3
% of NK	–0.046064	p > 0.8
NKMI	0.401175	p < 0.03*
% of CD25 3	0.210598	p > 0.2
CD25 3MI	–0.172980	p > 0.3
% of CD25	–0.378372	p < 0.05*
CD25 MI	–0.345443	p > 0.07
% of CD45 RO4	0.068687	p > 0.7
CD45 RO4	–0.201138	p > 0.2
% of CRO	–0.156284	p > 0.3
CDCROMI	–0.267389	p > 0.1