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Immunological profile of erythema nodosum and their relationship of C-reactive protein level and erythrocyte sedimentation rate: A retrospective analysis of 61 patients

Chunyu Cao*, Lihua Chen, Shu Li, Yishan Wang, Lili Yuan*

Department of Dermatology and Venerology, The Affiliated Taizhou People's Hospital of Nanjing Medical University, Taizhou, Jiangsu225300, China

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ABSTRACT

This experiment was carried out to investigate changes in lymphocyte subpopulation, immunoglobulins (Igs), and complements, and also to explore the relationship between these immune indices and C-reactive protein and erythrocyte sedimentation rate in 61 patients with erythema nodosum. For this aim, a 4-year, retrospective study contained 61 patients with erythema nodosum, and 61 healthy control subjects were included from the out-patient clinic. The subpopulation of the T, B and natural killer lymphocytes and levels of IgA, IgG, IgM, complement C3, complement C4, C-reactive protein, and erythrocyte sedimentation rate from peripheral blood of them were detected. A correlation analysis was done between lymphocyte subpopulation, levels of IgA, IgG, and IgM, complement C3, complement C4 and C-reactive protein level and erythrocyte sedimentation rate in the patient group. Results showed that the percentage of $CD4^+$ cells, $CD4^+/CD8^+$ ratio, the level of C-reactive protein and the erythrocyte sedimentation rate in the patients were higher than in controls ($P<0.05$). While the percentage of $CD8^+$ cells and the serum levels of complement C3 were lower than in controls ($P<0.05$). There were no differences in the percentages of $CD3^+$, B and natural killer cells and the serum levels of IgA, IgG and IgM, and complement C4 between the patients and the controls ($P>0.05$). IgM level was positively correlated with C-reactive protein ($P<0.05$) but did not correlate with an erythrocyte sedimentation rate ($P>0.05$). There was no correlation between lymphocyte subpopulation, levels of IgA, IgG, complement C3, complement C4 and C-reactive protein level and erythrocyte sedimentation rate ($P>0.05$). In conclusion, there was dysregulation of both cellular immunity and humoral immunity in patients with erythema nodosum. IgM level has a positive correlation with C-reactive protein.

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Introduction

Erythema nodosum (EN) is the most frequent type of panniculitis clinically, which typically manifests with tender nodules and plaques located predominantly over the extensor surface of the lower limbs. The ascertainable aetiological factors of EN include infections, sarcoidosis, malignancies, autoimmune diseases, drugs, and pregnancy (1). However, the cause of EN in many cases is impossible to identify, resulting in about one-third to one-half of EN being considered idiopathic (2-5). The exact pathogenesis of EN remains unclear, in view of many conditions associated with EN relate to immunopathogenesis, and the lesions have inflammatory infiltrate composed of lymphocytes according to histopathological examination, the immunological characterization in the pathogenesis of EN needs more investigation (6-8). The pathogenesis of erythema nodosum relates to immunological features needs more research, and studies involving the Immunological profile of erythema nodosum are scarce. We designed a retrospective survey of 61 patients with EN to evaluate the immunophenotype of peripheral blood lymphocytes, serum levels of immunoglobulins (Ig), complement (C), which are involved in the immunoreactions and their relationship with C-reactive protein (CRP) and erythrocyte sedimentation rate(ESR) were investigated.

Materials and Methods

Subjects

A total of 61 patients with EN presented at the dermatology department of our hospital from October 2013 to October 2017 were recruited in the study. Of the patients, 13 were men and 48 were women, with an average age of 40.62±13.83 years (age range 17-76 years), each patient received a comprehensive medical examination to exclude any other disease, such as sarcoidosis. The diagnostic criteria for EN were (i) tender, red, and painful nodules or plagues with a diameter≥1 cm, and (ii) histopathology represents septal panniculitis without vasculitis. All patients had not received any treatment dealing with cutaneous lesions for less than two months. The clinical characteristics of patients with EN were shown in Table 1. 61 healthy volunteers from out-patient department served as controls, containing 11 men and 50 women, with an average age of 41.95±10.97 years(age range 21-73 years). There was no statistically significant difference in sex and age between the patients and controls.

* Corresponding author. Email: caochunyu830@126.com

Table 1. Clinical characteristics of patients with EN (values are expressed in percentage)

Clinical features	values
Duration from onset to the hospital(days)	
1≤and< 21	68.9%
21≤and< 42	18.0%
42≤and< 60	13.1%
Number of lesions(%)	
1≤and<5	13.1%
5≤and<10	23.0%
≥10	63.9%
Distribution of lesions	
upper and lower extremities	19.7%
lower extremities	75.4%
torso and lower extremities	4.9%
Nonspecific systemic illness	
fever	66%
malaise	59%
arthralgias	52%
arthritis	24%
abdominal pain	3.3%

Instruments and reagents

Lymphocyte subset analysis was tested by flow cytometry which was performed by FC500 (Beckman Coulter, Dallas, TX, USA), CD45⁄CD14, IgG1⁄IgG2a, CD3⁄CD19, CD3⁄CD4, CD3⁄CD8, CD3/CD16+CD56, bi-color straight bidding sheet clonal antibody and hemolytic agents were bought from Beckman Coulter. $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+56^+$ represented total T cells, Th cells, Tc cells or destructive T cells, B and natural killer(NK) cells. IgG, IgA, IgM, C3, C4 and CRP levels were determined by turbidimetric inhibition immunoassay which was accomplished by Siemens BNIIautomatic protein analyzer (Siemens, Am Lustgarten, Berlin, GER). Examination reagent boxes were bought from Siemens. ESR was detected according to the Westergren by Automated Erythrocyte Sedimentation Rate Analyzer (China).

Experimental methods

The blood specimens were tested according to the reagent instruction manual. The FC500 flow cytometer was used to determine and analyze. It was first prepared for sample analysis using Flow-Check™ Fluorospheres beads and autocomp software. Whole blood samples were first stained with Leucogate (CD45/CD14) through lysing solution, and then run on the flow cytometer and analyzed with CXP Analysis software. The Leucogate tube was used to gate lymphocytes. The software automatically collected a sufficient number of events to obtain 5000 lymphocytes within the lymphocyte gate. Turbidimetric inhibition immunoassay and the Westergren was used strictly according to the instruction manual.

Statistical analysis

The quantitative data normally distributed were expressed as mean ± standard deviation and statistically analyzed using a personal computer with the Statistical Package Social Sciences (SPSS) version 19 program. An independent sample Student's *t*-test was applied to compare the data groups. Analysis of the relevancy among the indices used Pearson correlational analysis method. $P < 0.05$ was taken as significant.

Results

Results of flow cytometry

The percentage of $CD4^+$ cells was significantly higher in patients compared to controls($P < 0.05$), as well as the ratio of $CD4^+$ cells to $CD8^+$ cells($P < 0.05$), the percentage of $CD8^+$ cells was significantly lower in patients compared to controls($P < 0.05$), but there were no differences in the percentage of $CD3^+$, B or NK lymphocyte between the patients and the controls($P > 0.05$)(Table 2).

Results of turbidimetric inhibition immunoassay

In patients with EN, the serum level of C3 was found to be significantly lower($P < 0.05$). However, the serum levels of IgA, IgG, IgM and C4 were not significantly different when patients and controls were compared($P > 0.05$)(Table 3).

Results of the Westergren

CRP level and ESR were significantly higher in patients compared to controls($P<0.05$) (Table 4).

Results of correlation analysis

There was a positive correlation between the serum IgM level and the serum CRP level ($P<0.05$) (Table 5) (Fig. 1), while there was no correlation between lymphocyte sub-

Table 2. Comparison of percentage of lymphocyte subpopulation between patients with EN and controls ($\bar{x} \pm S$).

	Patients	Controls	*P*-value**
$CD3^+$ cells (%)	67.70±8.71	66.41±7.17	0.374
$CD4^+$ cells (%)	39.93±6.27	37.75±5.43	0.042
$CD8^+$ cells (%)	22.97±5.89	25.07±4.99	0.035
$CD4^+$ / $CD8^+$	1.91±0.76	1.58±0.45	0.004
B cells (%)	11.95±4.57	10.46±4.20	0.063
NK* cells (%)	15.36±7.26	15.42±6.58	0.964

* NK: natural killer, ** *P*-value: result of the Student's *t*-test.

Table 3. Comparison of serum levels of IgA, IgG, IgM, C3 and C4 between patients with EN and controls ($\bar{x} \pm S$).

	Patients	Controls	*P*-value
IgA* (g/L)	2.48±1.40	2.35±0.75	0.523
IgG** (g/L)	12.67±3.34	12.70±2.51	0.953
IgM † (g/L)	1.35±1.16	1.11±0.55	0.139
C3‡ (g/L)	1.04±0.23	1.14±0.12	0.007
C4 § (g/L)	0.25±0.11	0.22±0.07	0.093

*IgA: immunoglobulin A, **IgG: immunoglobulin G, †IgM: immunoglobulin M, ‡C3: complement C3, §C4: complement C4.

Table 4. Comparison of serum levels of CRP and ESR between patients with EN and controls ($\bar{x} \pm S$).

	Patients	Controls	*P*-value
CRP* (mg/L)	19.39±25.51	8.25±8.47	0.002
ESR** (mm/h)	40.13±25.93	8.00±6.04	0.000

*CRP: C-reactive protein, **ESR: erythrocyte sedimentation rate.

Table 5. Correlation analysis between lymphocyte subpopulation, levels of IgA, IgG, IgM, C3, C4 and CRP level in the patient group (n=61).

	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+$ / $CD8^+$	B	NK	IgA	IgG	IgM	C3	C4
					CRP						
r-value*	0.089	0.043	0.124	0.099	-0.030	-0.138	-0.111	-0.087	0.259	0.043	-0.147
p-value	0.493	0.743	0.342	0.448	0.816	0.290	0.396	0.505	0.044	0.744	0.257

*r-value: the Pearson correlation coefficient.

Table 6. Correlation analysis between lymphocyte subpopulation, levels of IgA, IgG, IgM, C3, C4 and ESR in the patient group (n=61).

	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+$ / $CD8^+$	B	NK	IgA	IgG	IgM	C3	C4
					ESR						
r-value	0.171	0.083	0.194	-0.188	0.135	-0.036	-0.002	0.126	0.113	0.237	0.151
p-value	0.188	0.525	0.133	0.147	0.300	0.784	0.990	0.333	0.388	0.066	0.246

population, levels of IgA, IgG, C3, C4 and CRP level ($P > 0.05$) (Table 5), as well as no correlation between lymphocyte subpopulation, levels of IgA, IgG, IgM, C3, C4 and ESR ($P > 0.05$) (Table 6).

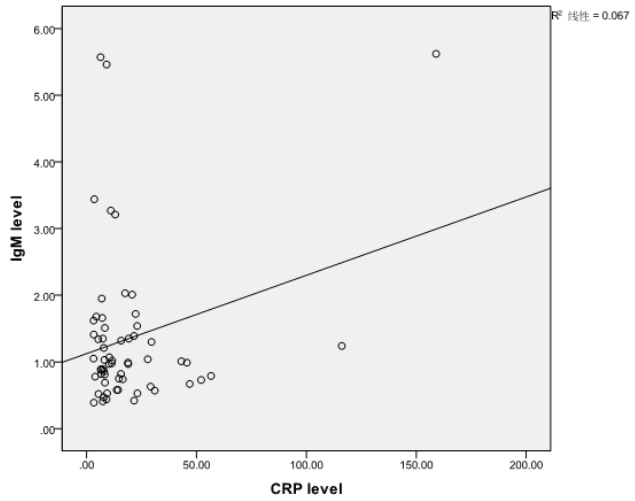

Figure 1. The relationship of serum IgM level with CRP level in the patient group (n=61).

Discussion

The exact mechanisms causing erythema nodosum are not known and the involvement of cellular immunity and humoral immunity in the pathogenesis of erythema nodosum is subject to discussion (9). The immunological profile of patients with erythema nodosum has been estimated in a few studies but depended on patients with erythema nodosum leprosum (ENL) who were tested for a few immunological parameters (10-14). Our study is based on patients who were assessed in an extensive way comprising a broad lymphocyte subpopulation testing, serum levels of Ig and complement determination.

T-lymphocytes play a role in the adaptive immune response which helps to clean out bacterial, viral, parasitic infections or malignant cells (15). There are two major T-cell lineages, namely, CD4 and CD8.[16] $CD4^+$ cells produce cytokines as effector T helper (Th) cells when they are activated, whereas $CD8^+$ cells form effector cytotoxic T lymphocytes(CTL). $CD4^+$ cells can differentiate into Th1, and Th2 cells (16). Th1 cells are the typical cell type took part in cell-mediated inflammation and delayed-type hypersensitivity reaction, they are critical to immunity to intracellular pathogens; Th2 cells have effects on host defense against multi-cellular parasites and their involvement in allergies and atopic illnesses (17). Erythema nodosum is thought to be a nonspecific cutaneous reaction of type IV delayed hypersensitivity to various antigens (18). We may naturally deduce that $CD4^+$ cells are a critical factor in forming the lesions of EN. Some scholars identified with the thesis that cell-mediated immunity has taken part in the pathogenesis of EN (19,20). In the study, we found that the percentage of $CD4^+$ cells from the patients was significantly increased as compared with the controls ($P < 0.05$), the reason might be that a list of etiologic factors of EN triggered the innate immune response thus leading to the clonal expansion and maturation of $CD4^+$ cells finally. In the present study, we found the percentage of $CD8^+$ cells was significantly lower in patients compared to controls ($P < 0.05$), consequently, the ratio of $CD4^+/CD8^+$ was found to be highly significant ($P < 0.05$). Nearly almost observations of an increased $CD4^+/CD8^+$ ratio in ENL patients were reported by several studies (21,22), although they had a significantly lower percentage and an absolute number of T cells compared to healthy volunteers (23). It seems that EN is associated with viral diseases according to the lower percentage of $CD8^+$ cells, but further researches are needed. Interestingly, although the percentage of $CD4^+$ and $CD8^+$ cells showed differences, there were no differences in the percentage of $CD3^+$, B or NK lymphocytes between the patients and the controls ($P > 0.05$).

We also examined the serum levels of IgA, IgG, IgM, C3 and C4 and found that C3 levels were significantly lower in patients compared to controls. Our results also indicated that serum levels of IgA, IgG, IgM and C4 were of no significant difference between patients and controls ($P > 0.05$), which implies that the serum level of C3 was closely related to the pathogenesis of EN. Evidence for the role of complement activation in EN has been proven, there were deposits of immunoglobulins and complement in the walls of the affected vessels observed by direct immunofluorescence of the lesions (24), which indicated that EN may be caused by the formation of immune complexes(ICs) and their deposition in and around the vessels of connective tissue septa of the subcutaneous fat (25). It is unknown how exactly ICs affect the pathogenesis of EN, but they may result in tissue damage through the activation of the complement system and promotion of inflammatory cells infiltration into the tissue, which aggravates destroying of the tissue by releasing inflammatory mediators (1). All of this leads to the histopathology of EN presented as a lymphohistiocytic infiltration and an inflammation that is typically concentrated at the periphery of the septae and

extends to neighboring fat lobules between adipocytes (26-29). The ICs or antigen-antibody complexes are the results of the binding of one or more antibody molecules (30). The ICs have the ability to activate the complement system, they activate complement pathways that opsonize or coat antigen-antibody complexes with large numbers of C3 molecules (31,32). In the immune response process, massive C3 molecules were depleted which brings about declining in the serum level of C3. This was coincident with Saha′s research that the serum level of C3 decreased during ENL while increasing after remission of the lesions (33).

Furthermore, we found that patients with EN had higher CRP levels and ESR, interestingly, although there was no significant difference in IgM level between patients and controls, when we did a correlation analysis between lymphocyte subpopulation, levels of IgA, IgG, IgM, C3, C4 and CRP level and ESR, we found a positive correlation between the serum IgM level and the serum CRP level, CRP is an acute phase protein which is used as a marker of the acute phase response (34), and can help find clinical infections (35-37). IgM appears early in the course of an infection and can be useful in the diagnosis of infectious diseases. Taking into account the above clinical significance of CRP and IgM in infectious diseases, the positive correlation between them may suggest the possible underlying causes of EN were a series of infections, which is consistent with the finding that most of the EN patients in the study had a fever (66%) which is common in infectious diseases.

The major limitations of our study were the small sample size and the levels of circulating immune complex (CIC) were not detected. In summary, according to our findings, we believe that there exists immunological dysregulation in EN, reflected by the cellular immunity profile in association with an imbalance of $CD4^+$ and $CD8^+$ cells percentage, and the humoral immunity profile in association with an imbalance of C3 levels, the imbalance may participate in the pathogenesis of EN. We propose that a successful treatment plan for EN may aim for a regulatory pathway linking $CD4^+$ cells, $CD8^+$ cells and C3, and as the elevated levels of CRP and IgM and their positive correlation may be associated with infection, we think anti-infective therapies in the treatment of EN may be actively considered. While our study is based on retrospective clinical observations, we suggest that further studies elucidate the association and potential pathogenesis.

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Conflict of interest
There is no conflict of interest between any of the authors with the results of this study.

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