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PEDIATRICS

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THE ROLE OF MONOCYTE CHEMOATTRACTANT PROTEIN 1 IN IMMUNE RESPONSE FORMATION IN CHILDREN WITH HEMORRHAGIC VASCULITIS

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Abstract: The goal of the investigation is to define the role of monocyte chemoattractant protein 1 in immune response formation in children with hemorrhagic vasculitis. The study included 60 patients with hemorrhagic vasculitis at the age of 1 to 18 years. To achieve the target goal of the investigation we analyzed MCP-1 concentration in blood serum and studied cellular and humoral component of immune system and phagocytosis. Increased levels of MCP-1 and cellular component of immune system indices levels show that this chemoattractive agent takes part in T-lymphocyte and natural killer cells involvement into inflammation lesion. Present inverse correlation relationships of MCP-1 levels and phagocytic activity may indicate compensation abilities decrease whereas in the presence of humoral component of immune system underactivity the synthesis of MPC-1 increases for the purpose of macrophages involvement into inflammation lesion and depletion process compensation. Resulting data indicate direct involvement of MPC-1 into hemorrhagic vasculitis immunopathogenesis as immunodefence activation marker or as a possible trigger of pathologic process activation.

KeyWords: children, hemorrhagic vasculitis, monocyte chemoattractant protein 1, immunity.

INTRODUCTION

Hemorrhagic vasculitis is a part of widespread vasculitis and represents generalizable immune complex microthrombovasculitis [1, 2, and 3]. In spite of the spectrum of known in modern times etiologic factors the diagnostics of hemorrhagic vasculitis occurs to be inadequately timed and identification of provoking factor succeeds not in all cases. Since pathogenesis of hemorrhagic vasculitis is not completely known active involvement of immune system into pathologic process occurs. Currently most of investigators conceive that in the presence of vasculitis several immune and perhaps non-immune pathogenic mechanisms simultaneously play role in the development of vascular damage [4].

Over the past decade, studies showed that one of the principal molecular markers of vasculature endothelium damage was the monocyte chemoattractant protein 1 (MCP-1) [5].

MCP-1 is produced by several cells including monocytes, T-lymphocytes, fibroblasts, vessel endotheliocytes, epithelial and smooth muscle cells of bronchi [6]. MCP-1 is effective chemoattractant for monocytes, activated CD4 and CD8 T-lymphocytes binding with them through CCR2 receptor. As a result of binding, cells draw toward focus of inflammation. Besides MCP-1 can induce integrin expression required for chemotaxis [7, 8]. MCP-1 is not only chemoattractant that provides migration and extravasation of mononuclear cells into inflammation focus but also is a mediator of inflammation at the same time activating resident cells. Consequently, involvement of above-mentioned pathological mechanisms in hemorrhagic vasculitis pathogenesis appears to be possible taking into account immune complex nature of disease that belongs to systemic diseases and develops pathologic process in endothelium.

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2 PURPOSES, SUBJECTS AND METHODS:

2.1 Purpose

To define the role of monocyte chemoattractant protein 1 in immune response formation in children with hemorrhagic vasculitis.

2.2 Subjects

Study included 77 children of 1 to 18 years old. 60 patients with hemorrhagic vasculitis (25 girls and 35 boys) who received medical treatment at Communal Health Protection Institution "Kharkiv Municipal Children's Hospital No.16". Control group included 17 apparently healthy children of similar sex and age. Diagnosis of disease was verified and determined with the help of commonly accepted clinical laboratory and instrumental findings according to protocol of Ministry of Health Care of Ukraine No. 676 of 12.10.2006 "Clinical protocol of delivery of healthcare to patients with Henoch-Schonlein syndrome (hemorrhagic vasculitis, Henoch-Schonlein purpura) (HSP)".

2.3 Methods

The activity rate of pathologic process and severity of disease course were measured according to such parameters as clinical evidence and laboratory method data of investigation i.e. leukocyte level in peripheral blood, ESR, indicators of acute phase of inflammation (seromucoids, glycoproteins), and gamma-globulins.

To achieve the target goal of the investigation we analyzed MCP-1 concentration in blood serum with the help of immunoenzymatic set for quantitative test of human MCP-1 Bender MedSystems GmbH (Austria) and studied cellular and humoral component of immune system and phagocytosis. Identification of T- and B-lymphocytes subpopulations (CD3, CD4, CD8, CD16, CD22) in absolute and relative value using the method of their identification with the help of diagnostic agent "НВЛ Гранум" (Ukraine), blood serum Ig A, Ig M and Ig G levels using method of G.Mancini (1965) with the help of Federal State Unitary Enterprise SPA "Microgen" (The Ministry of Healthcare of The Russian Federation, Russia), circulating immune complex (CIC) using method of V. Haskova et al. in modification of Yu.A.Grynevich and A.N.Alfyorov (1978), phagocytosis indices (phagocytizing neutrophils, phagocytic number and neutrophil index activity) according to ability of polymorphonucleocytes and monocytes of peripheral blood to bind on their surface, absorb and digest microbial testing culture, NST-test according to Stuart (1975) in modification of B.S. Nagoev (1983). We calculated mean cytochemical coefficient of myeloperoxidase content in neutrophils according to the Graham-Knoll's method and mean cytochemical coefficient of lysosomal cationic protein content according to M.G. Shubich method (D.V.Belokrynytskiy, 1987). Using statistical programs „EXCELL FOR WINDOWS" and „STATISTICA 7.0. FOR WINDOWS" received data underwent statistical processing. For samples other than Gaussian sample we calculated median (Me) and interquartile range (Lq – low quartile; Uq – up quartile). By comparison of values that involved comparison of more than 2 points, we used H criterion of Kruskal-Wallis dispersion analysis (KW). Level of significance was identified taking into account Bonferonni adjustment. To analyze statistical significance of differences between two independent groups we used nonparametric U-test of Mann-Whitney (MW). Analysis of indices range connection was performed according to Spearman rank correlation method (r). Results had statistical significance when $p < 0.05$.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

Classification of children by sex in total number of patients did not show statistical significant difference: 35 boys (58.3%), 25 girls (41.7%). Analysis of age peculiarities of children from main group showed that 29 children (48.3±6.42%) got into the group of 1-6 year old patients, 23 children (38.3±6.2%) got into the group of preschool age and only 8 patients (13.3±4.3%) got into the group of puberty. Now, therefore, 86.6±4.3% (p=0.003) of children suffer from hemorrhagic vasculitis at the age under 12 that indicates significant "youthification" of this pathology.

Study showed that 30 (50±6.4%) patients from main group had allergic disposition of the body, that is 11 (18.3±4.9%) children had gastrointestinal allergy, 5 (8.3±3.5%) children had medicamentous allergy, and 14 (23.3±5.4%) children had combined allergy.

Frequent acute respiratory diseases (4 and more times a year) occurred in 44 (73.3±5.7%) children, chronic center of nasal infection occurred in 27 (45±6.4%) children, frequent inflammatory airway disease occurred in 6 (10±3.8%) patients and persistent herpetic infection occurred in 8 (13.3±4.3%) children.

While investigating nasal structure of examined children we found that dermic form of the disease occurred in 8 (13.3±4.3%) patients, dermic-articular form of the disease occurred in 24 (40±10%) patients, 19 (31.6±6%) patients had combined form of hemorrhagic vasculitis and 9 (15±4.6%) children had combined form of the disease with renal syndrome. 40 (66.6±6.2%) children were registered with acute course of the disease, 3 (5±2.8%) children had chronic course of the disease and 17 (28.3±5.8%) had recidivating course of hemorrhagic vasculitis. Taking into account severity of the course and activity rate of pathologic process all children were divided into following groups: 1st group included patients with mild disease or I degree of activity (n=19), 2nd group included children with moderate course of the disease or II degree of hemorrhagic vasculitis activity (n=22) and 3rd group included children with severe course of disease or III degree of activity (n=19). T-lymphocytes content in blood in children with I degree of pathologic process activity significantly did not differ from values in control group. Children with II degree of activity were registered with increase of total count of absolute and relative T-lymphocytes number (2.50 (2.20; 3.20) *109/L, 73 (68; 80) %) versus values in control group. In addition, we detected increase of correlation index of T-helpers and T-suppressors at the cost of increase of the former and decrease of the latter ones versus values in control group. The level of natural killer cells was twice higher than data levels in control group.

In children with III degree of hemorrhagic vasculitis activity, we detected similar changes of T-cellular component but more expressive. CD8 function decrease provides prevalence of stimulating effect of T-helpers as well as B-lymphocytes that produce antibodies (Table 1).

Table 1.

Values of the cellular component of immune system according to degree of hemorrhagic vasculitis activity in children in acute episode of disease (Me (Lq; Uq)).

Value	Degree of activity			Control group (n=17)
	I (n=19)	II (n=22)	III (n=19)	
CD_3 (T total), $*10^9$/L	1.60 (1.56;1.68)	2.50 (2.20; 3.20)*	5.70 (4.00; 6.90)*	1.60 (1.40; 1.80)
%	67 (65; 68)	73 (68; 80)	79 (76; 80)*	69 (65; 72)
CD_4 (T helper), $*10^9$/L	0.90 (0.90; 1.00)	1.75 (1.50; 2.00)*	3.90 (3.20; 4.50)*	0.90 (0.80; 1.00)
%	36 (34; 40)	37 (36; 39)	53 (51; 60)*	37 (35; 40)
CD_8 (T count), $*10^9$/L	0.75 (0.65; 0.75)	0.75 (0.70; 0.80)	1.70 (1.00; 2.00)*	0.74 (0.70; 0.80)
%	32 (30; 34)	26 (22; 30)	27 (24; 30)	30 (27; 32)
CD_4/ CD_8	1.28 (1.20; 1.53)	2.31 (1.50; 2.85)*	2.38 (1.85; 2.73)*	1.17 (1.12; 1.25)
CD_{16} (NK), $*10^9$/L	0.34 (0.30; 0.35)	0.60 (0.50; 0.90)*	1.80 (0.90; 2.00)*	0.30 (0.20; 0.30)
%	12 (11; 12)	18 (15; 19)*	23 (18; 25)*	12 (10; 14)

Note.*-p<0.01- probable difference with values in children from control group.

Represented data are confirmed by analysis of cellular effectors levels of humoral component of immune system

(CD22) which indicates their increase and direct dependence on severity of hemorrhagic vasculitis course (Table 2). Statistical processing of received data indicates significant difference of circulating immune complex levels in children with minimal to maximal degree of hemorrhagic vasculitis activity (67 (59; 74) c.u., 81 (76; 86) c.u., 136 (100; 162) c.u., respectively).

Table 2.

Values of the humoral component of immune system according to degree of hemorrhagic vasculitis activity in children in acute episode of disease (Me (Lq; Uq)).

Value	Degree of activity			Control group (n=17)
	I (n=19)	II (n=22)	III (n=19)	
CD_{22}(B-lymph) $*10^9$/L	0.60 (0.50; 0.65)*	1.21 (1.10; 1.32)*	1.90 (1.60; 2.40)*	0.50 (0.40; 0.60)
%	18 (17; 20)	26 (22; 29)*	35 (27; 37)*	17 (13;19)
Ig A, g/L	2.29 (2.12; 2.43)*	3.25 (2.90; 3.50)*	2.70 (2.20; 3.30)*	1.22 (0.90; 1.62)
Ig M, g/L	1.70 (1.10; 2.00)*	2.15 (2.00; 2.60)*	1.55 (1.26; 2.40)*	0.98 (0.82; 1.11)
Ig G, g/L	10.70 (9.98; 12.30)	14.35 (13.00; 15.70)*	9.98 (8.00; 12.70)	9.95 (8.16; 11.24)
CIC, c.u.	67 (59;74)*	81 (76; 86)*	136 (100;162)*	29 (25; 34)

Note.*-p<0.01- probable difference with values in children from control group.

While analyzing Ig A levels in blood serum in acute episode of hemorrhagic vasculitis we detected statistically significant differences with values in control group. The most expressive differences were detected in children with II degree of activity (3.25 (2.90; 3.50) g/L). Concerning Ig A levels in blood serum, significant increase was registered in children with moderate course of hemorrhagic vasculitis (14.35 (13.00; 15.70) g/L). Ig M level in blood serum was significantly increased in acute episode of the disease in children of all degrees of activity.

Patients with minimal degree of activity in acute episode of the disease appeared to have significant increase of values of phagocytizing neutrophils and phagocytic number (88 (85; 94) %, 6.0 (4.3; 6.5), respectively).

In children with II and III degree of hemorrhagic vasculitis activity we registered systemic decrease of these values which may indicate low reserve of compensative capacity of phagocytosis in patients of given groups and their exhaustion associated with their involvement into elimination of inflammation products and circulating immune complex from the body. In all patients with different degrees of activity of the disease, we registered significant increase of NST-test values versus values in control group. The highest points were registered in children with II degree of activity (35 (30; 38), %).

Activity of leukocytes myeloperoxidase according to mean cytochemical coefficient data in acute episode was decreased in patients of all groups versus values of control group particularly in children with II degree of hemorrhagic vasculitis activity (2.00 (1.88; 2.50), mean cytochemical coefficient). Lysosomal cationic protein values in children with I and II degrees of activity did not statistically differ from values of control group, significant increase of them was registered in children with III degree of activity (1.24 (1.12; 1.26), mean cytochemical coefficient) (table 3).

Table 3

Values of phagocytosis according to degree of hemorrhagic vasculitis activity in children in acute episode of disease (Me (Lq; Uq)).

Value	Degree of activity			Control group (n=17)
	I (n=19)	II (n=22)	III (n=19)	
Phagocytizing neutrophils, %	88 (85; 94)*	67 (64; 77)*	50 (47; 55)*	82 (78; 86)
Phagocytic number	6.0 (4.3; 6.5)*	3.2 (3.1; 5.0)	2.4 (2.2; 3.1)*	4.2 (3.8; 4.8)
Neutrophil activity index	0.98 (0.90; 1.00)*	1.10 (0.90; 1.30)	0.92 (0.78; 0.92)*	1.09 (1.00; 1.15)
Myeloperoxidase, mean cytochemical coefficient	2.30 (2.25; 2.45)*	2.25 (2.25; 2.34)*	2.00 (1.88; 2.50)*	2.56 (2.55; 2.57)

Lysosomal cationic protein, mean cytochemical coefficient	1.26 (1.20; 1.30)	1.27 (1.22; 1.35)	1.24 (1.12; 1.26)*	1.23 (1.22; 1.24)
NST-test, %	26 (24; 30)*	35 (30; 38)*	18 (14; 20)*	9 (7;12)

Note.*-p<0.01- probable difference with values in children from control group.

Analysis of MCP-1 values according to pathologic process activity in children who suffer from hemorrhagic vasculitis indicates a significant increase of its values particularly the dependence of MCP-1 levels in blood serum on degree of hemorrhagic vasculitis activity should be noted. The remarkable thing is that criterion of Kruskal-Wallis of MCP-1 levels among represented groups equals to 70.53 where p=0.0000, that indicates occurrence of differences in represented groups. Data obtained by pairwise comparison indicate a significant difference in children with different degrees of hemorrhagic vasculitis activity versus values of control group as well as among each other (table 4).

Table 4

Values of MCP-1 in blood serum in children according to degree of hemorrhagic vasculitis activity in acute episode of the disease

Value	Statistical value	Degree of activity			Control group (n=17)
		I (n=19)	II (n=22)	III (n=19)	
MCP-1, pg/ml	Me	870.4	1280.6	2350.6	370.6
	Lq	786.5	1127.4	2050.2	350.3
	Uq	955.3*	1430.2*	2780.6*	400.0
KW H=70.53, p=0.0000, p_{I-II}=0.0000**, p_{I-III}=0.0000**, p_{II-III}=0.0000**, p_{c-I}=0.0000*, p_{c-II}=0.0000*, p_{c-III}=0.0000*					

Notes:* - p<0.01- probable difference with values in children from control group; ** - statistical significance level according to Bonferonni adjustment versus values of different groups – p<0.01; I, II, III – degree of hemorrhagic vasculitis activity.

It is important to note that in children with I degree of activity a significant correlation relationship was not found. In children with II degree of hemorrhagic vasculitis activity, direct significant coefficients of correlation of MCP-1 levels in blood serum and absolute and relative CD4 levels, by CD4/CD8 ratio (rxy = +0.87, rxy = +0.72, rxy = +0.84 respectively, n=22), and inverse correlation relationship of MCP-1 levels and absolute and relative CD8 levels (rxy = -0.65, rxy = -0.91, respectively, n=22) indicate MCP-1 involvement into activation of T-cellular component of immune system. Present positive correlation relationship of MCP-1 levels and CD16 levels indicates significant role of this chemoattractive agent in Th1 way formation of immune response in patients with hemorrhagic vasculitis. Irreversible correlation relationship of MCP-1 levels and absolute and relative CD22 amount likely indicates expression of MCP-1 by B-lymphocytes (rxy = +0.50, rxy = +0.50, respectively, n=22). Analyzing correlation relationship of MCP-1 and phagocytosis values we noted inverse correlation coefficients of phagocytizing neutrophil levels, phagocytic number and MCP-1 levels in blood serum (rxy = -0.51, rxy = -0.53, respectively, n=22). In addition, we registered direct correlation relationship of Ig A, Ig M, Ig G levels and MCP-1 levels (rxy = +0.45, rxy = +0.48, rxy = +0.45 respectively, n=22).

In the process of correlation analysis of immunological indicators and indicators of MCP-1 in blood serum in children with III degree of hemorrhagic vasculitis we received similar data. Direct coefficients of correlation registered between absolute, relative amount of CD4, absolute, relative amount of CD16, absolute, relative amount of CD22, Ig A, Ig M and MCP-1 (rxy = +0.71, rxy = +0.74, rxy = +0.80, rxy = +0.64, rxy = +0.76, rxy = +0.50, rxy = +0.72, rxy = +0.55 respectively, n=19). Inverse correlation relationship was registered between absolute, relative amount of CD8, phagocytic neutrophils, phagocytic number and MCP-1(rxy = -0.68, rxy= -0.72, rxy = -0.83, rxy = -0.54 respectively, n=19).

4 CONCLUSIONS

MCP-1 in the presence of hemorrhagic vasculitis serves as chemoattractant not only for monocytes and for basophils but also involves T-lymphocytes and natural killers into focus of inflammation. Present inverse correlation relationship of MCP-1 levels and phagocytic

activity may indicate compensative capacity decrease of phagocytosis that in the presence of active inflammation reaction focused on elimination of inflammation product and circulating immune complex. Whereas in the presence of insufficient activity of phagocytic component of immune system a MCP-1 synthesis increases for the purpose to involve macrophages into focus of inflammation and compensate exhaustion process. Thus, MCP-1 high levels and present correlation relationship with immune response indicators can be from one side as a marker of activation of immunodefences and from another side as a possible trigger of pathologic process activation. Received data indicate direct involvement of MCP-1 into hemorrhagic vasculitis immunopathogenesis.

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